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**THE ROLE OF DIET
IN FELINE
INFLAMMATORY BOWEL DISEASE**

by

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A thesis submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

in Veterinary Clinical Science

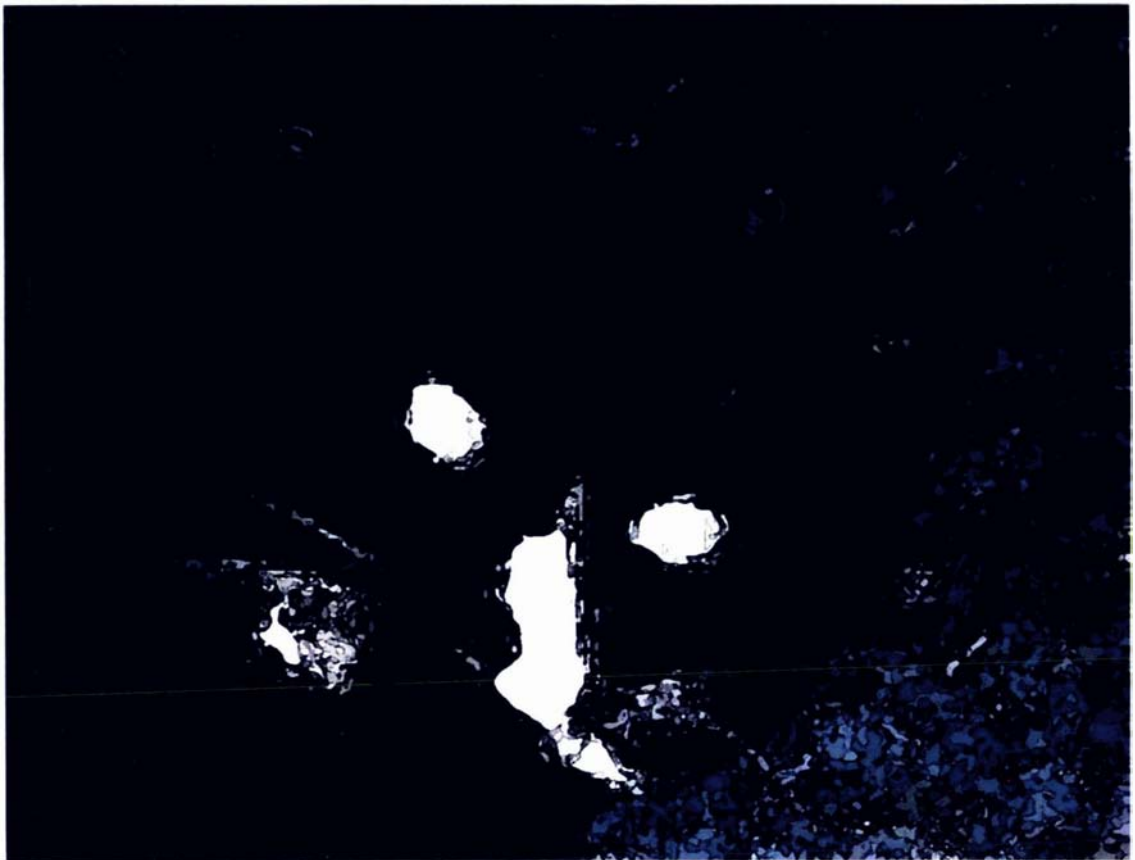
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*The Role of Diet in Feline
Inflammatory Bowel Disease*

Volume II



Dedicated to the
LOVING MEMORY
of my father

MASSEY UNIVERSITY**ABSTRACT****THE ROLE OF DIET IN FELINE
INFLAMMATORY BOWEL
DISEASE**

Inflammatory bowel disease (IBD) is a chronic, idiopathic inflammatory condition of the gut mainly studied in man but also recognized in the cat. The main constraint of working with IBD is that diagnosis cannot be reached with absolute certainty. A role for diet in the initiation and/or maintenance of chronic inflammation in IBD has long been suspected among a number of other possible risk factors. On the other hand, diet is also recognized as a very important mode of therapy in IBD. There is a paucity of information on feline IBD and the effects of dietary components on gastrointestinal inflammation in the cat. This is partly due to the lack of practical and effective techniques to improve diagnosis and easily monitor therapy in a clinical patient. To test the hypothesis that diet does have a role in feline IBD, a retrospective multicentre epidemiological study and two prospective dietary clinical trials examining the influence of different sources of carbohydrates and a dietary fibre equivalent were conducted. In addition a device for easy and simple collection of colo-rectal mucosal fluid was developed and tested in a small number of cats. This device may help to improve diagnosis or to measure responses to treatment, including the response to dietary management of feline inflammatory bowel disease.

Since IBD diagnosis is a deciding factor in the inclusion criteria for both the retrospective and prospective study, it is of pivotal importance to define how this diagnosis was reached. In this thesis a diagnosis of IBD was restricted to cats with chronic clinical signs of gastrointestinal disease (anorexia, vomiting, diarrhoea, weight loss, haematochezia) and signs of leukocytic infiltration in the lamina propria of the gastrointestinal tract. In addition these cats had negative serology for

FIV/FelV, negative faecal flotation, normal haematology and biochemistry, normal serum thyroxine concentration, a lack of response to a week-long dietary trial and a lack of abnormalities detected either by abdominal radiography or ultrasound. Other tests were included in the prospective studies as considered necessary to establish the diagnosis.

The epidemiological study investigated diet and other risk factors associated with the presence of IBD in cats. Data on the signalment, stress factors (sexual activity, length of ownership, change of address, number of cats in the house, other pets in the house, frequency of boarding and cats show attendance), environment, temperament, lifestyle, presence of disease and diet prior to the diagnosis of IBD were collected. Matched (by age, gender and breed) and random control groups were included. Cats with IBD were commonly females, 7 to 15 years old. Exotic breeds were over-represented. In addition, having only one dog in the household appeared to be associated with the disease, while more than one did not. Overall, a higher frequency of potential stress factors appeared to significantly predispose to the disease. Vomiting, diarrhoea, anorexia and dental disease were found to be common in cats with IBD before diagnosis. Skin (acne, reaction to insecticide spray, eosinophilic plaque, plasma cell pododermatitis, otitis externa) and respiratory problems (sneezing or coughing) were also more prevalent among cats with IBD. Lifestyle, veterinary care and diet were very variable between countries but none of them proved to be significantly associated with feline IBD.

The retrospective nature of epidemiological studies does not allow all possible nutritional associations to be studied. This fact along with the known nutritional idiosyncrasies of feline nutrition provided the logic to investigate diet as a mode of treatment in two prospective dietary trials. One trial involved a comparison of different sources of starch (rice, barley, tapioca and corn 18.4 – 25.8%ME) by healthy cats and cats with IBD. The rationale for this comparison was that cats do not possess all the tools to deal with dietary carbohydrates when compared to other species, and that carbohydrate malabsorption can occur during gastrointestinal inflammation and contribute to the clinical signs of gastrointestinal disease. The other prospective dietary trial tested diets supplemented with different amounts of inulin (0, 0.1 or 0.2%DM). Inulin is an oligosaccharide which effects

in the gastrointestinal tract of man and other species resemble the actions of dietary fibre. The products of fermentation of dietary fibre are considered beneficial for colonic health and have been used for the treatment of idiopathic colitis in several species.

The study on carbohydrate tolerance was a crossover study that included a control group of healthy cats from a research facility and cats diagnosed with IBD (according to the criteria mentioned above). Breath hydrogen collection, faecal grade and water content, faecal sodium, faecal potassium, faecal osmolar gap and gastrointestinal clinical signs were used to compare carbohydrates. IBD cats showed a higher area under the curve (AUC) of breath hydrogen ($p=0.0001$) indicating malabsorption of carbohydrate, irrespective of starch source, when compared with healthy controls. No deleterious effects on faecal characteristics, clinical signs or body weight were observed. The faecal osmolar gap did not prove to be useful to identify cats with IBD. Rice increased faecal sodium/potassium ratio when compared with the other starches. In summary, carbohydrate malabsorption seems to be a feature of gastrointestinal inflammation in the cat but in the short term it does not seem to be detrimental in terms of clinical signs or body weight. On the other hand the feline colon appears to have an amazing capacity to maintain water absorption in the midst of an increased load of fermentable material. Hence carbohydrate malabsorption cannot be judged by faecal characteristics in the cat. In addition, the use of rice as the preferred carbohydrate for dietary management of feline IBD may need to be further examined since the AUC when consuming rice was similar or higher than with the other sources of starch. The significance of the finding that the rice based diet was associated with an increase in faecal sodium is uncertain.

The inulin study was conducted in healthy cats (20 belonging to a research facility and 10 owned by the public) and a small number of cats with IBD. Changes in microdissection parameters (number of dividing cells per crypt, number of epithelial cells per crypt cell column, crypt length, crypt width and crypt area) in the duodenum, colon and rectum were studied as well as changes in histological preparations, transit of radiopaque markers and macronutrient digestibility. The addition of inulin to feline diets was associated with an increase in the number of

epithelial cells per colonic crypt ($p=0.006$) and colonic crypt length ($p=0.025$) after the cats ate the diets supplemented with inulin for four weeks with no indication of a dose response to inulin. There was also a trend towards a greater number of dividing cells in duodenal ($p=0.07$) and colonic ($p=0.07$) crypts in publicly owned cats consuming the diet with 0.2% inulin. Much variation was found between research colony cats and publicly-owned cats before the trial started. The addition of inulin was not detrimental to faecal characteristics, macronutrient digestibility and did not cause any change in the transit of radiopaque markers in healthy cats. The increase in crypt cellularity in healthy cats is a potentially beneficial effect for the treatment of colitis but further research in cats with clinical colitis is required.

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At the start of this degree I was told that it would be a ‘sink or swim experience’. Staying afloat was, at times, very difficult and I have swallowed a fair amount of water in the process. Little did I know when I started that besides the academic challenge, life would have a few surprises in store. However, the support of family and friends and the generosity of many people I did not know carried me through and were fundamental to my finishing of this thesis. Although this thesis will bare my name, it is the result of the time, effort and good will of many people.

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**THE ROLE OF DIET IN FELINE
INFLAMMATORY BOWEL DISEASE
VOLUME 1**

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CHAPTER 3

MULTICENTRE EPIDEMIOLOGICAL STUDY OF FELINE INFLAMMATORY BOWEL DISEASE

Feline Inflammatory Bowel Disease (IBD) is an idiopathic disease. The diagnostic criteria for IBD are controversial but are based on the presence of chronic gastrointestinal signs (anorexia, vomiting, diarrhoea, weight loss, etc.), the identification of gastrointestinal inflammation in bowel biopsy specimens of the small and/or large bowel and the exclusion of other diseases known to cause chronic gastrointestinal inflammation¹⁻³. In man, IBD is believed to be a multifactorial disease⁴ that results in gastrointestinal signs and occasionally extra-intestinal symptoms⁵. Many of the possible contributing pathophysiological factors have been studied (e.g. disturbance of oral tolerance, gastrointestinal permeability, gastrointestinal flora, gastrointestinal immune system function- refer to Chapter 2). However, which factors, if any, contribute to the aetiopathogenesis remains to be elucidated.

The incidence of IBD in humans started increasing in the 1950's⁶. Since that time, many epidemiological studies have attempted to identify the factors associated with the presence of the disease. Factors investigated to date have included: diet⁷⁻¹⁵, smoking habits^{16,17}, stress^{18,19}, infant feeding practices^{20,21}, cow's milk sensitivity²²,

season ²³, ethnicity ²⁴, familial association ²⁵⁻²⁷ marital status and socio-economic background ⁶, infectious agents and oral contraceptives ²³. Many of the earlier studies were conducted as descriptive studies without comparisons of the IBD patient population with other populations (e.g. healthy individuals or patients with other gastrointestinal diseases) ⁶. Later studies have included control groups and have been multicentre in nature ^{28,29}. Multicentre trials are advantageous in diseases with an uneven geographic distribution (like human IBD) because a greater number of affected individuals can be studied and because comparisons between different populations assist the elimination of regional confounding factors ³⁰.

IBD has been associated with the presence of several other diseases or clinical signs referable to body systems and tissues other than the gastrointestinal tract. Some of these can accompany IBD, others may occur prior to the diagnosis of IBD. A long list of diseases including skin, liver, joint, ocular, bronchopulmonary and renal diseases has been reported in humans with IBD ⁵. Veterinary scientists have suggested associations between IBD and other diseases of dogs and cats, namely joint disease ³⁷ and hepatic disease ¹. One paper has shown an association between IBD and hepatic, pancreatic and renal disease in cats ³⁸.

Several retrospective studies and case reports have described the clinical presentation of feline IBD and the signalment of the affected animals ³¹⁻³⁶. However, to the author's knowledge, as yet there have been no studies comparing the frequency of factors potentially associated with the aetiopathogenesis of IBD between populations of cats with and without the disease.

Diet has been considered a possible trigger or maintenance factor for the chronic inflammation seen in patients diagnosed with IBD. Alternatively, diet is also recognized as an important mode of therapy (see Chapter 2). However, taken as a whole IBD is considered a multifactorial disease (See Chapter 1) in man just as it is thought to be in veterinary patients. The objective of this study was to investigate the association between dietary factors, lifestyle, veterinary care and potential stressors with the frequency of feline IBD with the intention of obtaining information that could help guide or direct further research.

Materials and Methods

Institutions

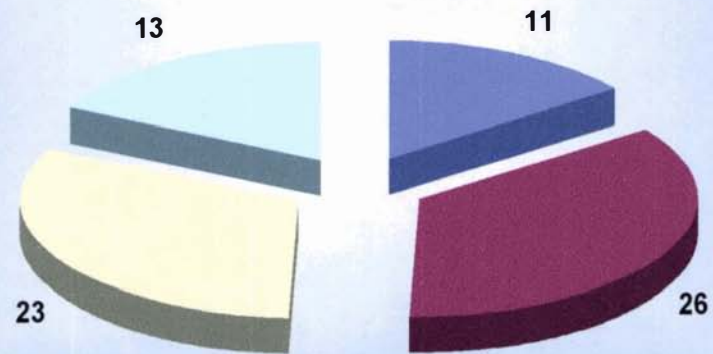
Several Veterinary Teaching Hospitals provided a total of 73 clinical cases of feline IBD. These hospitals were located at Massey University (11), the University of California at Davis (23), and the University of Pennsylvania (13). Additional cases of cats with IBD were procured from private veterinary practices (26) in New Zealand (**Figure 1**). Most of these practices were located in Auckland but two were from the Wellington area. The cases from private practices were identified by a search of the histopathology records of the Animal Health Laboratories of the Ministry of Agriculture and Fisheries. The owners of the cats with IBD were contacted only after permission had been received from their local veterinarian.

Cases

The computerized clinical files of the Veterinary Teaching Hospitals were searched for the records of cats that had been diagnosed with IBD within the period a

Distribution of IBD cases collected at different sites

MASSEY NZ DAVIS PENN



Note: Massey=Massey University Veterinary Teaching Hospital; NZ=Private Practices in New Zealand; Davis=Veterinary Teaching Hospital at the University of California at Davis; Penn=Veterinary Teaching Hospital at the University of Pennsylvania.

spanning 1994 to 1999. Cats were included in the study only if they had undergone a thorough diagnostic work-up. The complete set of diagnostic tests varied according to the clinical case but the minimum diagnostic procedures required for inclusion were a faecal flotation, a haemogram, a serum chemistry profile, an assay of serum thyroxine concentration (in cats over 5 years old), FIV and FeLV serology, a dietary trial of minimum length of a week (exclusive feeding of a highly digestible diet containing a novel protein), and either abdominal radiography or abdominal ultrasound. Faecal culture for enteropathogens was not a requirement for inclusion unless the clinical presentation, history and environmental situation were highly suggestive of the possibility of infectious disease since a positive faecal culture by itself is not diagnostic of infectious gastroenteritis [McDonough, 1996].

Additional requirements were the willingness of the local veterinarian to allow contact with their client and the desire of the cat owner to participate in a phone interview regarding the affected animal. An experienced pathologist with expertise in IBD diagnosis (Dr L. Roth) reviewed all histopathological slides used

Grading system for endoscopic biopsies

Grade	Morphologic characteristics
Normal	Lymphocytes and plasma cells among up to 75% of glands. No more than 3-4 cells in array between glands. Normal epithelium
Mild inflammation	Diffuse distribution of lymphocytes and plasma cells. More than 5 -8 cells in array between glands. Occasional intraepithelial lymphocytes. Normal epithelium
Moderate inflammation	Diffuse distribution of lymphocytes and plasma cells. More than 5 -8 cells in array between glands. Occasional to moderate numbers of intraepithelial lymphocytes. Slight flattening of absorptive epithelial cells and decreased numbers of goblet cells. Multifocal erosions
Severe inflammation	Diffuse distribution of lymphocytes and plasma cells. More than 5 -8 cells in array between glands. Moderate numbers of intraepithelial lymphocytes. Cuboidal appearance of absorptive epithelial cells and marked decrease to absence of goblet cells. Multifocal to coalescing erosions to ulcers

for the diagnosis of the disease from all institutions and practices. Cats without convincing histopathological evidence of gastrointestinal inflammation were eliminated from the study. The assessment was based on the cellularity of the lamina propria according to type of cells and distribution between glands, the general appearance of the epithelial cells, the presence of intraepithelial lymphocytes, distribution of goblet cells and the presence of erosions, ulcers or fibrin (see insert in the previous page). In addition, morphological alterations of villi or crypts were assessed. Details on each cat included in the study can be found at the end of the Appendix to this Chapter.

Controls

A random and a matched control were chosen for each cat with IBD from the same institution or private practice from which the clinical case had originated. Random controls were obtained by searching the whole log of clients that had visited the clinic during the same period as the cats with IBD (the last five years). The matched controls were cats that were the same sex, age (\pm 3 years) and breed as the IBD cases.

Random controls were chosen using a random list of codes including a letter of the alphabet and a number. There were 999 codes for each alphabetic letter (i.e. A001, A002, etc). The code list had all codes 'scrambled' so that they followed a random order. To make use of the code list, the list of cat owners attending a certain clinic was modified so as to obtain similar codes to the random code list. For example, when computerized lists of client names were available, the surnames of the clients were alphabetically ordered and each entry under each letter of the alphabet was given a

sequential number starting at one. Thus, each client was given a code that contained the first letter of their surname and their numeric order among the other clients whose surnames started with the same alphabetic letter. Then codes were randomly picked from the random code list and paired with the same code from the client list. If this client's cat was unavailable for the study the process was repeated.

The same list of random codes was used for the private practices without computerized records. These practices kept an alphabetic card filing system. Several codes were picked at random from the random code list and a list of the first letter of the codes picked was sent to the practices. The staff of the practice used the first letter of the list sent to choose the alphabetic group of cards from which to randomly pick one client card. If the cat or the client were unavailable for the study the process was repeated with the following letters in the order provided.

Matched controls were obtained in the Teaching Hospitals using the computerized log of clients of the last five years. The cats were separated according to age, sex and breed; and control cats were chosen to match the signalment of the IBD cats provided by that institution for the present study. In the private practices, the matched controls were identified by the nursing staff.

Questionnaire

All cat owners were phoned and after their consent to participate in the trial was obtained a time was arranged to call back to go over the questionnaire. On average each questionnaire took 90 minutes to complete. The transcript of the questionnaire is included in Appendix 1. At the start of the questionnaire, owners of

cats with IBD were reminded that the questions referred to the time before their cats had been diagnosed with IBD. Each question was read verbatim from the questionnaire to minimize variability and avoid leading questions. If the question was not answered appropriately, it was repeated or modified slightly for the sake of clarity. The latter was most frequently required when the question was open-ended.

The majority of the questions included in the questionnaire were aimed at identifying potential risk factors for IBD (particularly those that had been previously identified in studies of humans with IBD). Some questions were asked to ascertain the reliability of the information being gathered from the owner (length of ownership, who feeds the cat, etc). On most occasions the rationale behind the questions will be obvious to the reader but a brief explanation is provided in the results section whenever this may not be clear.

Statistical analysis

All data were first tabulated and appraised as entered. Some variables were separated in categories or groups with the purpose of better describing the data and making the analysis more manageable. Categories for each variable are described in the results. The data set was divided into signalment, stress, life-style, disease history and dietary variables as shown in **Table 1**.

Proc FREQ in SAS version 6.12 was used for the statistical analysis of most variables (SAS/STAT software: changes and enhancements through release 6.12, 1997). The data were separated into IBD, random and matched control groups. It was then further stratified into two geographic sources (America and NZ) and 4

Table 1
Risk factors investigated in this study

Signalment	Sex
	Hair Length
	Hair Colour
	Breed
	Weight
	Body Condition
	Age
Stress	Age at Neutering
	Length of Ownership
	Area of Residence
	Address Changes
	Number of Cats in the Household
	Number of Other Pets in the Household
	Frequency of Boarding
	Attendance at Cat Shows
Environment, Temperament and Lifestyle	Exposure to Cigarette Smoke
	Presence of a cat door
	Time Spent Outdoors
	Fighting Frequency
	Physical Activity
	Sleeping Quarters
	Veterinary Visits
	Vaccination and Worming
	Temperament
Presence of Disease	Clinical Signs of Gastrointestinal Disease
	Urinary Disease
	Skin Disease and Flea Allergy Dermatitis
	Dental Disease
	Adverse Reactions to Food
	Joint Disease
	Respiratory Disease
	Other Diseases
Diet, Eating Behaviour and Hunting Activity	Frequency of Feeding
	Rapidity of Food Consumption
	Fastidiousness Towards Food
	Hunting Activity
	Dietary History

institutional sources (Massey, NZ Private Practices, Davis and Pennsylvania). Comparisons were made between IBD and random control cats, and IBD and matched control cats to eliminate the confounding effects of sex, age and breed between geographical sources and institutional sources. Significant findings were reported.

Contingency tables were generated and differences in cell frequencies analysed. Most of the variables examined were categorical and if the data was independent (IBD and random controls) the variables were analyzed by the Chi-Square method or by the Fischer's Exact test (contingency table frequencies below 5 in more than 20% of the cells). The Cochran-Mantel-Haenszel Statistic was used to analyze differences between institutional sources. McNemar's test, based on the number of discordant pairs, was used for the analysis of related (non-independent) groups (e.g. IBD and matched control cats)³⁹. For all statistical analysis, a p value <0.05 was considered significant.

These measures of association were complemented by calculating the appropriate Odds Ratio (OR) for independent (ad/bc) and related samples (s/t) to gain an insight into the strength of the association³⁹. Odds ratio confidence intervals were also calculated to determine the significance of the OR.

The Kruskal-Wallis test was used to determine if there was a significant difference between a 'total stress score' of the IBD, random controls and matched controls. The stress score was calculated from the 'stress variables' (see **Table 1**) by the addition of the scores of each of the suspected stress factors studied.

Once the univariate analysis of all factors was completed, statistically significant variables, variables that tended toward statistical significance or variables that were considered important because they could be involved in the pathogenesis or clinical presentation were included in a multivariate analysis. The multivariate analysis was carried out using PROC CATMOD in SAS vs. 6.12 for the independent samples (IBD and random controls) and PROC LOGISTIC for the related samples (IBD and matched controls). For the independent samples several models were fitted progressively until only the significant variables were included.

The analysis of the related samples (IBD and matched controls) was performed using a stepwise conditional logistic regression analysis which automatically and progressively selects variables into the final model according to their significance ($p \leq 0.25$ was used to include factors and keep them in the model). The data for this step was prepared by the method of Hosmer and Lemeshow⁴⁰.

The multivariate analysis was also complemented by the calculation of OR from the maximum likelihood estimates (b) produced by PROC CATMOD. The base of the natural log was raised to the power of twice these estimates i.e. $OR = e^{2b}$ ⁴¹. In contrast, OR were directly obtained from the output of the statistical software when using PROC LOGISTIC. Odds Ratio confidence intervals (CI), needed to determine significance, were calculated using the standard error of the estimate ($b \pm (1.96 \times bSE)$)³⁹.

Results

SIGNALMENT VARIABLES

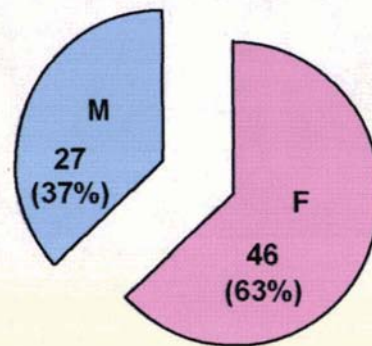
SEX

Most cats selected for the study had been neutered. Only one entire female and 3 entire males were included in the study. As a result, this variable was analyzed in just two categories – female and male. Female cats were over represented in the IBD population when compared to males (**Figure 2**). Females had an Odds Ratio (OR) of 1.85 (CI 0.955 to 3.582) for developing IBD. Further analysis of the data according to the geographic source of the cats showed that the preponderance of females in the combined data was due to the high number of female cats from NZ included in the study (**Figure 2**). If all data from NZ (Massey University and Private Practices) were grouped together and analyzed on their own, the difference in the frequency of IBD between the sexes was significant ($p=0.017$, OR=3.284, CI= 1.22 - 8.836).

HAIR LENGTH

This variable was classified into three categories: long, short and medium length hair. There were no significant differences in hair length between the IBD and random selected cats. However, a test of independence showed that the separation between breed and hair length was poor ($p=0.01$). This is not surprising when considering that the majority of the cats in this study belong to breeds named according to hair length i.e. domestic short hair (DSH) and domestic long hair (DLH).

Sex distribution in a multicentre population of cats diagnosed with IBD



Sex distribution of feline IBD cases in different institutions

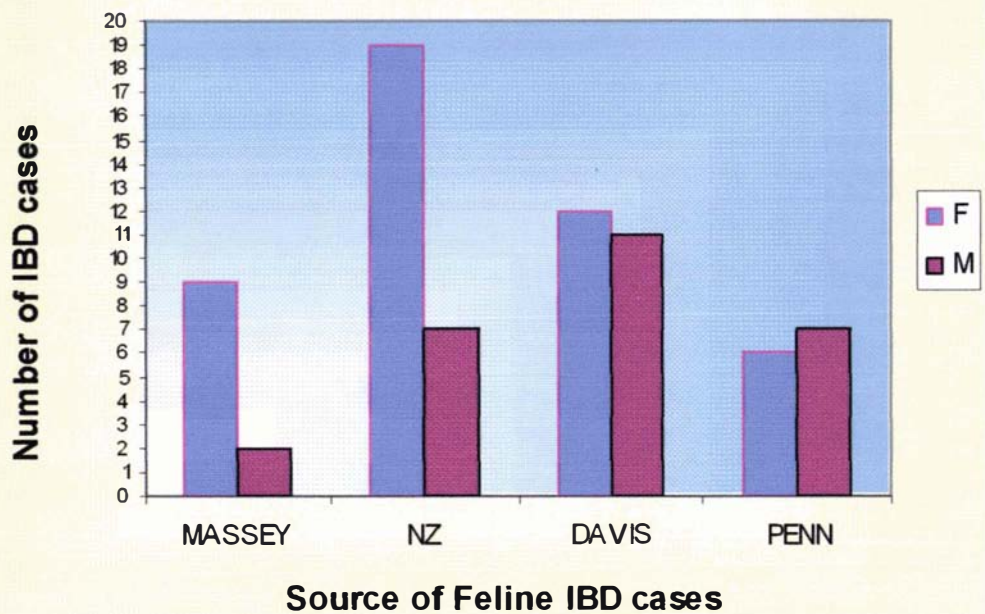


Figure 2.- Sex distribution in a multicentre population of cats diagnosed with IBD. F = female; M = male.

COAT COLOUR

Coat colour was classified into the following categories: solid colour, broken colour and tabby. Further division of the data into specific coat colours was not attempted because of the low frequencies present in each colour category. A test of independence indicated that the separation of the breed and colour variables was poor ($p=0.001$) with most exotic breeds been categorized as having a solid colour coat (24 out of 32) and most DLH falling into the broken colour coat category (11 out of 21). In contrast, categorization of the coat colour in the above manner was successful in separating the coat colour and sex variables (test of independence $p=0.22$).

There were no significant differences in the frequency of the different categories of coat colour between the cats with IBD and the random control cats. Since the separation between coat colour and breed (a matching factor) was poor the comparison between matched controls and IBD cats was not carried out.

BREED

The cat breeds represented in the study were classified into the following categories: DSH, DLH, Birman, Oriental, Exotic and Siamese. The specific breeds included in each of these categories are shown in **Table 2** along with the OR and confidence intervals for each category (See also **Figure. 3A**). Significant differences were found in the frequency of IBD between breed categories ($p=0.02$). 'Exotic' breeds were significantly over-represented. Other breeds that were not significantly different between the groups but may have an increased predisposition to feline IBD

based on their relatively high OR are ‘Birman’ and ‘Siamese’. If the latter two breeds are pooled into the ‘exotic’ category along with the ‘oriental’ breeds, statistical significance is maintained ($p=0.001$; OR= 3.98, CI 1.65 – 9.61). The frequency of

Table 2
Breeds frequency among IBD cats and their odds ratios

Category	Breeds Included	Frequency	Odds Ratio
Birman	<i>Birman</i>	4/5	4.17 (0.45-38.28)
DLH	<i>DLH</i>	7/21	0.45 (0.17-1.18)
DSH	▪ <i>DSH</i>	39/85	0.58 (0.29-1.16)
	▪ <i>DMH</i>	3/8	
Exotic*	▪ <i>Himalayan</i>	2/3	8.86 (1.079-72.78)
	▪ <i>Main Coon</i>	1/1	
	▪ <i>Main Coon Cross</i>	1/1	
	▪ <i>Manx</i>	1/1	
	▪ <i>Abyssinian</i>	1/1	
	▪ <i>Devon Rex</i>	2/2	
Oriental	▪ <i>Persian</i>	2/3	1.67 (0.52-5.38)
	▪ <i>Persian Cross</i>	3/5	
	▪ <i>Burmese</i>	3/4	
	▪ <i>Oriental</i>	0/1	
Siamese	<i>Siamese</i>	4/5	4.17 (0.45-38.28)

Note: the frequency is the number of cats with IBD of that particular breed over the total number of cats of that breed amongst the cats with IBD and the healthy random control cats.

*OR statistically significant.

cats with IBD from the various breed categories presented to each of the institutions was not significantly different (**Figure 3B**).

Fig. 3A.- Frequency of different breeds among IBD cats and a healthy random population of cats

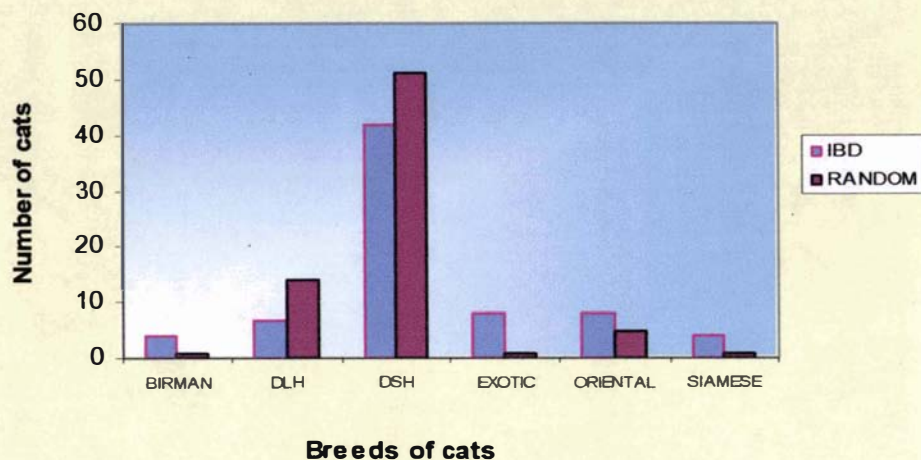
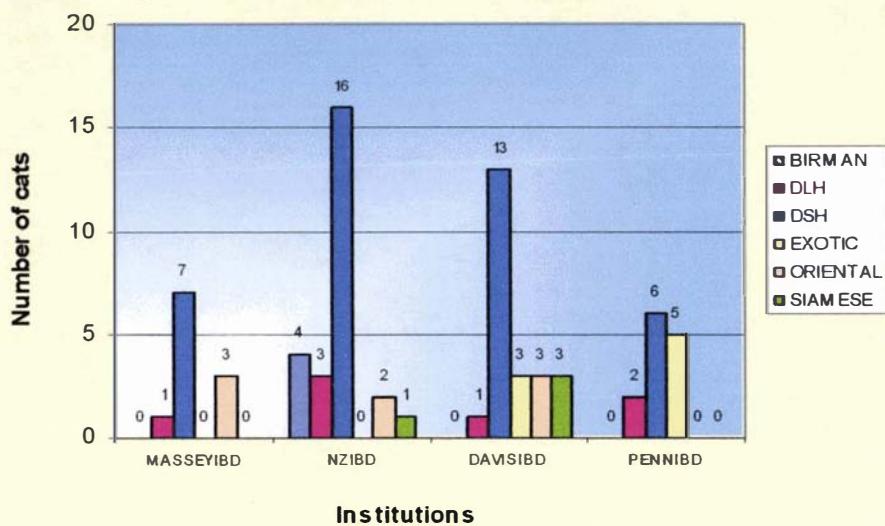


Fig. 3B.- Frequency of different breeds among IBD cats in different institutions



WEIGHT

The cats were classified into 3 bodyweight categories: <3 Kg, 3 to 5 Kg and >5Kg. Most cats diagnosed with IBD weighed between 3 to 5 Kg (38/59) as did the healthy random and matched controls (18/36). No significant differences were found in bodyweight between any of the groups.

BODY CONDITION

Cat owners were asked to classify the body condition of their cats as lean, average, slightly overweight or moderately overweight. Approximately 70% of the cats with IBD were categorized as lean or average body condition. No significant differences in body condition, as assessed by the owners, were found between groups.

AGE

The cats were classified into six age-categories: <3 years, 3 to 6 years, 6 to 9 years, 9 to 12 years, 12 to 15 years, and >15 years. The frequency distribution curves of these age-categories amongst the cats with IBD and the random controls can be seen in **Figure 4**. The frequencies and corresponding OR are shown in **Table 3**. The age frequency distribution in IBD tended to be different ($p=0.07$) to that of the random control cats. Hence, the data was then re-classified into three age-categories in accordance with the odds ratios listed in **Table 3**. The three categories chosen were 0 to 7 years, 8 to 15 years and >15 years. The frequency of the cats in each of these age-categories is shown in **Table 4**. There were significantly ($p=0.02$) higher number of IBD cats than random controls between 7 and 15 years of age.

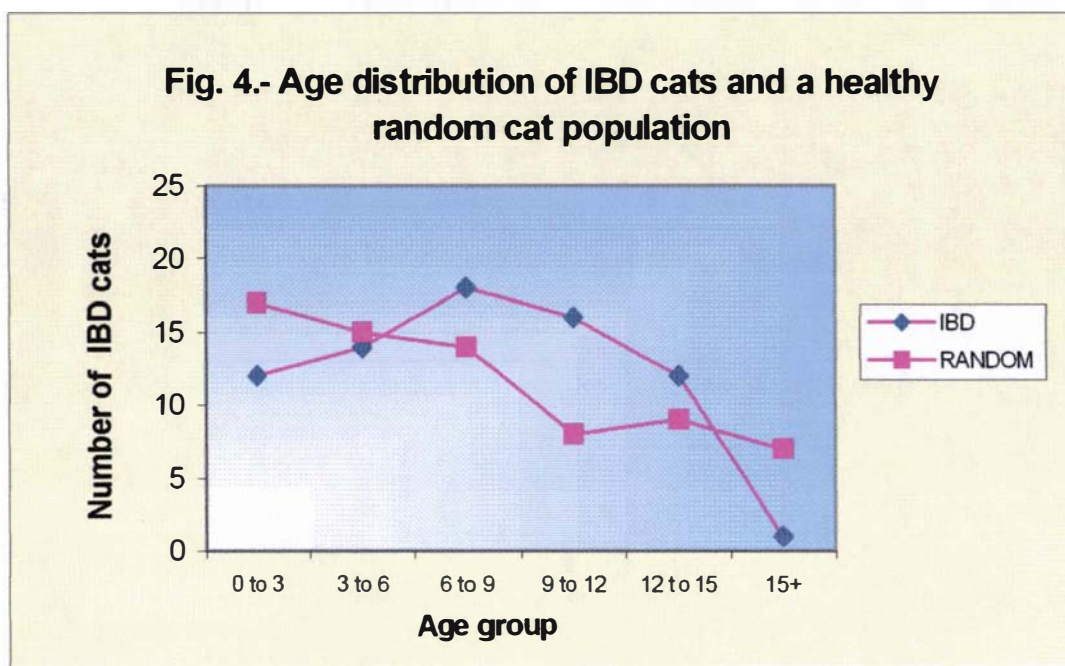


Table 3
Frequency of age groups and odds ratios of IBD cats

Age group	Frequency	Odds Ratio (CI)
0-3	12/29	0.61 (0.27-1.40)
3-6	14/29	0.87 (0.38-1.97)
6-9	18/32	1.31 (0.59-2.88)
9-12	16/24	2.17 (0.86-5.47)
12-15	12/21	1.33 (0.524-3.39)
15+	1/8	0.125 (0.015-1.04)

Note: The frequency is the number of IBD cats of the specified age group over the total number of cats of that particular age group among both IBD and healthy random cats included in this study.

Table 4
Frequency of age groups and odds ratios of IBD cats

Age group	Frequency	Odds Ratios
0-7	30/65	0.7 (0.36-1.35)
7-15 *	42/70	2.03 (1.04-3.95)
15+	1/8	0.125 (0.015-1.04)

Note: The frequency is the number of IBD cats of the specified age group over the total number of cats of that particular age group among both IBD and healthy random cats included in this study. * Statistically significant OR.

STRESS VARIABLES

AGE AT NEUTERING

The intention of including 'age at neutering' as a variable in the analysis was to explore the influence of sexual hormones and sexual activity on the epidemiology of feline IBD. Five categories were established for this variable: neutered at an unknown age, neutered as a kitten, neutered between 7 months and 2 years of age, neutered between 2 and 5 years of age, and neutered after 5 years of age. The last category included very few cats (n=8) as did the category in which the age of neutering was unknown (n =19). For this reason, these categories were removed from the analysis.

Most cats (162 out of 193) had been neutered as kittens. The majority of the remaining cats had been neutered before 2 years of age (23 out of 193). No significant differences of age at neutering were demonstrated between IBD, matched and random control groups.

LENGTH OF OWNERSHIP

Two categories were established for this variable: ownership of less than 5 years and ownership of more than five years. The duration of cat ownership was similar amongst the clientele of all institutions. No differences in length of ownership were found between IBD, random and matched control cats. Most cats had been owned since being a kitten (181 out of 219). This long duration of ownership prevented exploration of the influence of stress caused by changing owners on the prevalence of feline IBD.

AREA OF RESIDENCE

Area of residence was included as a stress variable because it determines cat lifestyle in many ways. Presence of cats in close proximity to defend a territory, size of territory, availability of prey, type of owner-cat relationship, contact with other people than the owner, type of relationship with other pets, presence of traffic, noises, etc. are different between rural and city environments and relate to different types and degree of stressful situations. Two categories were established for this variable: rural and city dwellings. Most of the cats lived in city residential areas (144 of 219). Only 49 of 219 lived in strictly rural areas (farms, small rural towns, countryside). No differences in the area of residence between IBD, random and matched control cats were demonstrated.

Analysis of the data shown in **Figure 5** suggests that American institutions have a slightly higher number of rural cats in their IBD population. Hence, all cats were divided into just two sources, America (108) and NZ (111). No significant differences were found between American and NZ cats regarding their area of residence, but the odds ratio indicates a higher approximate relative risk of IBD in rural American cats when compared with the random control cats (See **Figure 5** and **Table 5**).

ADDRESS CHANGES

Address changes were included as a source of stress because they result in a change of territory and potentially interaction with new animals and people. The variable was originally classified into three categories: no moves, 1 to 3 moves, and

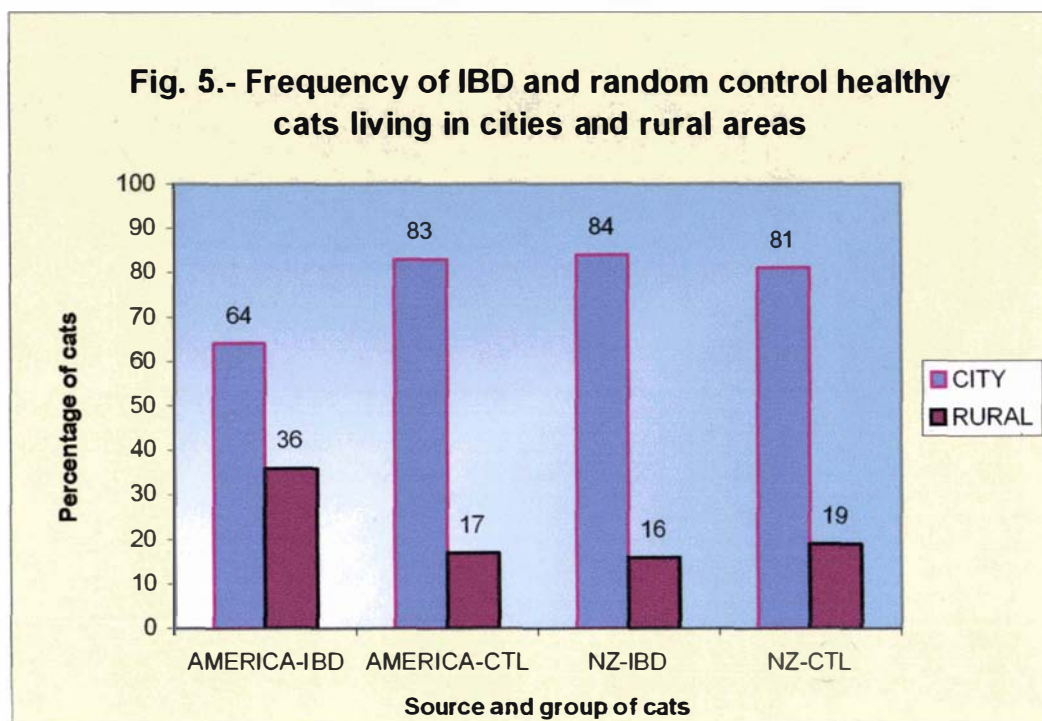


Table 5
Odds Ratios of cats with IBD vs. random control cats living in different areas

Source of cats	Area	Odds Ratio (CI)
American Institutions	<i>City</i>	0.35 (0.12 - 1.07)
	<i>Rural</i>	2.82 (0.93 - 8.57)
NZ	<i>City</i>	1.2 (0.56 - 4.0)
	<i>Rural</i>	0.83 (0.25 - 2.75)

more than 4 moves in the last 5 years and subsequently reclassified into only two categories: no moves or one or more moves. No significant differences in the number of cats that ever moved or the number of address changes were demonstrated between the IBD, random and matched control groups.

Inconsistencies between institutions were observed. IBD cats from the private practices in NZ moved less than their random controls ($p=0.026$) while the opposite was true for cats belonging to clients of Massey University ($p=0.08$). There were no significant differences between cats belonging to the clients of the American

Table 6
Odds ratios of IBD cats whose owners changed address sometime during the cat's life.

Institutions	OR (CI)	Source	OR (CI)
NZ*	0.28 (0.08 – 0.87)	NZ	0.72 (0.29 – 1.80)
Massey*	7.9 (1.1 – 56.12)		
Davis	1.7 (0.53 – 5.47)	America	2.2 (0.86 – 5.64)
Penn	3.89 (0.71 – 21.0)		

Note: * Statistically significant OR

institutions in the number of address changes they experienced, although IBD cats tended to have had more address changes than the random control cats (see **Table 6**).

NUMBER OF CATS IN THE HOUSEHOLD

This variable was classified into two categories: no other cats or one or more other cats in the household. This classification was chosen after separation of the multiple cat household data into the precise number of cats in the household showed

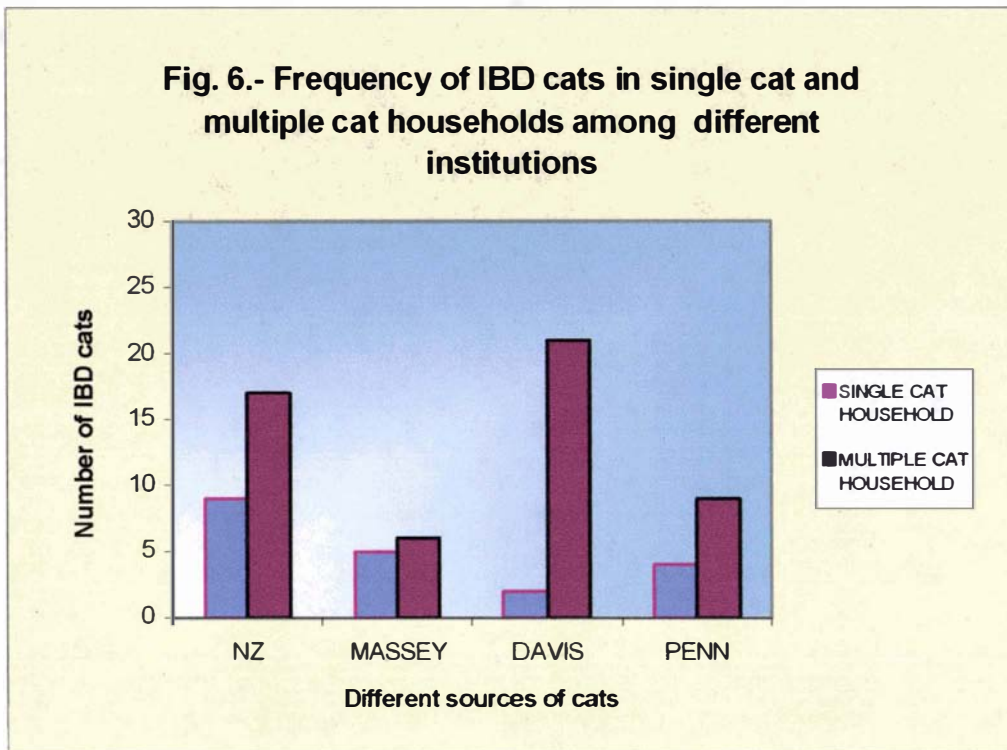


Table 7
Odds Ratios of feline IBD in multiple cat households

Source	OR (CI) - IBD / Random Controls	OR (CI) - IBD/ Matched Controls
NZ	1.89 (0.62 – 5.76)	1.16 (0.33 – 2.98)
Massey	1 (0.13 – 7.77)	-
Davis	1 (0.187 – 5.36)	6 (0.12 – 8.26)
Penn	1.4 (0.27 – 7.13)	-

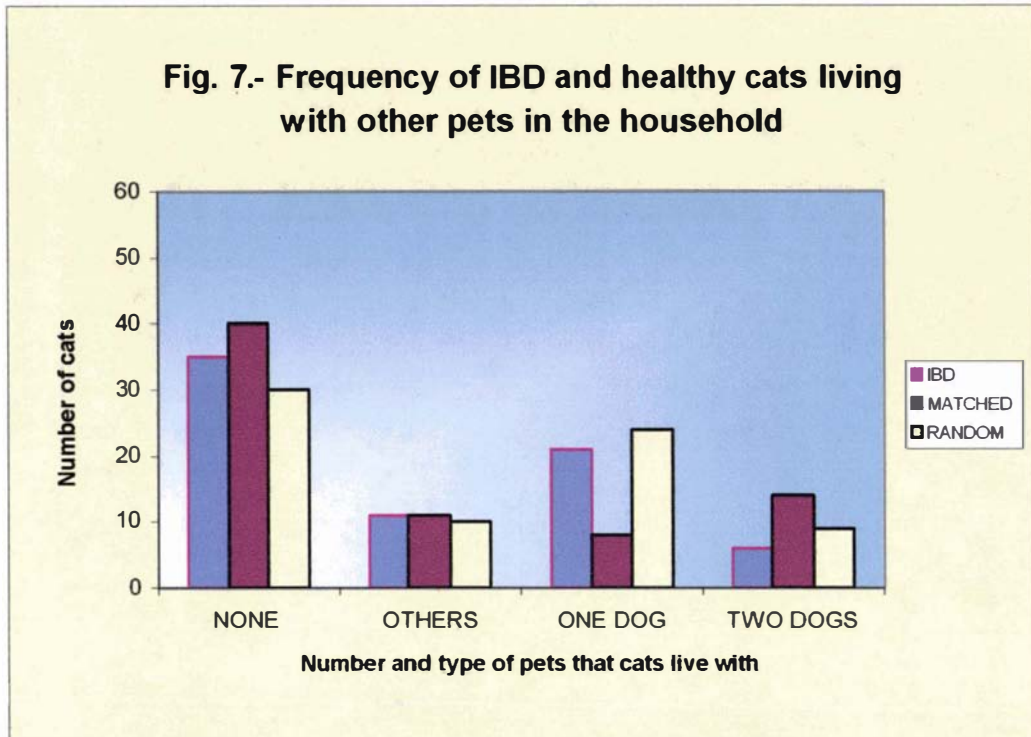
Note: (-) No discordant pairs were present so OR could not be calculated.

low frequencies in the contingency table. No significant differences were detected in the number of cats living singly or in a multiple-cat household between the cats with IBD and the random and matched controls. Similarly, there were no statistically significant differences in the frequency of multiple-cat households between the institutions.

However, the approximate relative risk (OR) of cats having IBD if they lived in a multiple-cat household varied between the different institutions (See **Figure 6** and **Table 7**). In particular, the approximate relative risk of IBD was high in Davis cats living in a multiple-cat household when compared to their matched controls.

PRESENCE OF OTHER (NON-FELINE) PETS IN THE HOUSEHOLD

This variable was first classified into 4 categories: no other pets, pets not expected to compete with cats (fish, birds, lizards, mice, etc), households with one dog (+/- other pets) and households with two or more dogs (+/- other pets). However, further analysis showed that most of the difference in the presence of feline IBD originated between the matched controls and IBD cats ($p=0.007$) specifically with reference to the number of dogs in the household. Thus, all cats were separated in two categories, i.e.: households with only 1 dog and other households. Calculation of OR demonstrated an increased risk of IBD in cats belonging to households with only one dog (OR=3.6, CI 1.33 – 9.57), while there was a decreased relative risk of IBD if the household had more than one or no dog (OR=0.28; 0.1 – 0.74). This difference was apparent only when considering the IBD cats against their matched controls, and not when comparing them to the general cat population at large (random controls). Please see **Table 8** and **Figure 7**.



Note: None = no other pets; Others = pets that are not expected to compete with cats (small mammals, birds, fish, etc); One dog = households with only one dog +/- pets not expected to compete with cats; Two dogs = households with more than one dog +/- pets not expected to compete with cats. Other cats in the household are excluded from this analysis

Table 8
Odds Ratios in cats living with other pets

Cat Group	Other pets	Odds Ratio
IBD vs. Random Controls	Households with only 1 dog	0.82 (0.41 – 1.67)
	Other households	1.21 (0.6 – 2.45)
IBD vs. Matched Controls	Households with only 1 dog*	3.6 (1.33 – 9.57)
	Other households*	0.28 (0.10 – 0.74)

Note: * Statistically significant OR

FREQUENCY OF BOARDING

Most cats in this study never used boarding facilities (146 out of 219). The cats that did use boarding services were then classified into the following categories: frequent boarders (more than once a year), regular boarders (once a year only) and occasional boarders (sometimes but not regularly). No significant differences were found in the frequency of boarding between IBD, random and matched controls. Although overall there were only few cats that were classified as frequent boarders

Table 9
Odds ratios in healthy and IBD cats according to their use of boarding facilities

Group	Boarding frequency	Odds Ratio (CI)
IBD vs. Random Controls	Frequent	1.54 (0.42 – 5.72)
	Infrequent	0.65 (0.175 – 2.4)
IBD vs. Matched Controls	Frequent	6 (0.72 –49.99)
	Infrequent	0.16 (0.019 – 1.33)

(n=11), many of these cats were in the IBD group (n=6). If the cats were reclassified into two categories as frequent boarders and infrequent boarders, the difference between IBD cats and their matched controls tended towards statistical significance ($p= 0.059$). Thus, the OR of IBD cats compared with their matched controls also indicated a higher relative risk for IBD in cats that frequently stay in boarding facilities (See **Table 9**).

ATTENDANCE AT CAT SHOWS

More than 95% of all cats in all groups (IBD, matched and random controls) had never attended cat shows. As a result, it was not possible to examine the influence of this variable on the frequency of IBD.

STRESS SCORE

The results from the analysis of all variables considered to relate to stress were used to calculate an individual “stress score”. However, ‘stress’ per se was not measured. The assumption underlying this analysis was that certain situations (variables studied) will cause stress in many cats. The null hypothesis to be tested by the analysis was that stress was not related to the presence of feline IBD. As a corollary, it was considered that a summation of these stressful situations may be more closely associated with the presence of the disease than might individual stressors. The scoring system is shown in **Table 10** and was applied to all cats in all groups to obtain a “Total Stress Score”.

The IBD cats had higher Total Stress Score than matched and random control cats ($p=0.0027$). See **Figure 9**.

ENVIRONMENT, TEMPERAMENT AND LIFESTYLE VARIABLES

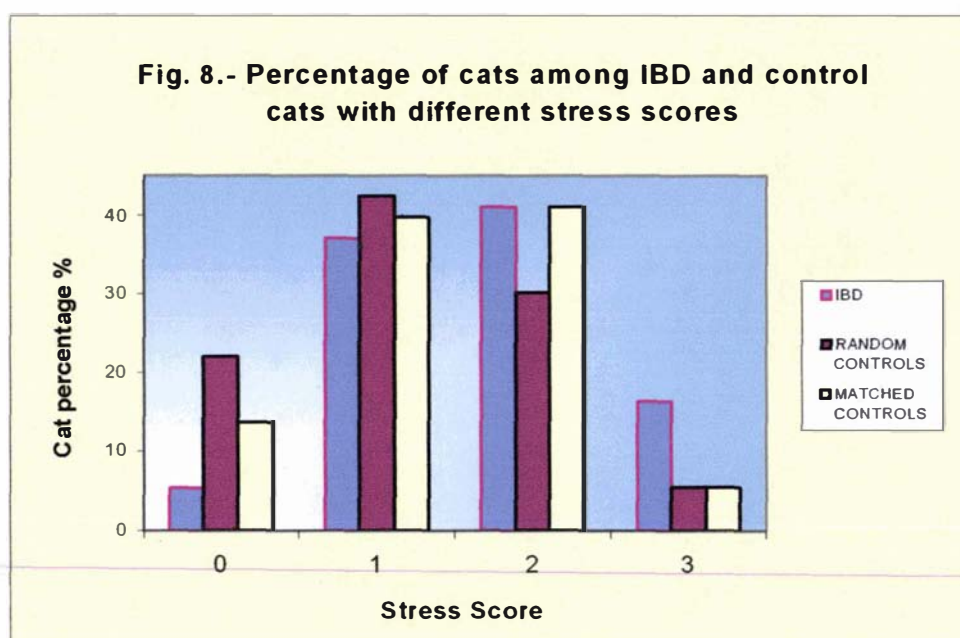
EXPOSURE TO CIGARETTE SMOKE

The exposure to cigarette smoke was classified into three categories, namely frequent exposure (if the exposure occurred daily, weekly or monthly for two years or

Table 10
Stress Scoring System

Variable	Score	Details
Sexual activity	0	No score
Length of Ownership	0	No score
Change of address	0	No moves
	1	Moved
Number of cats in the house	0	No other cats
	1	Other cats present
Other pets in the house	0	None, others or 2 or more dogs
	1	One dog
Frequency of Boarding	0	Never or occasionally
	1	More than once a year
Cats show attendance	0	No score
Range of Total Stress Score 0 to 4		

Note: Each animal was individually scored, all scores added and the Total Stress Score analysed. Variables that could not be analysed in this study (sexual activity, length of ownership and cats show attendance) were given a zero score. All other possible sources of stress were given 1 point if present or zero points if absent. Area of residence was difficult to score because of lifestyle differences between cats in different countries and was therefore eliminated from the analysis.



more), occasional exposure (if the exposure occurred weekly or monthly for two years or less, or the smoking was regular but occurred outside near the house) or no significant exposure (includes no exposure and rare episodes of short term exposure). Most cats with IBD had never been exposed to cigarette smoke (52 out of 73 or 71%). No differences in cigarette smoke exposure were present between IBD cats, matched and random controls.

PRESENCE OF A CAT DOOR

Thirty seven percent of cats could gain access to an outdoor environment when they wished to do so by way of a cat door or via a window or door left ajar for their use. No differences in independent access to the outdoors were found between IBD, matched and random controls. However, significantly ($p= 0.001$) more NZ cats (60%) had independent access to the outdoors (by way of cat doors etc.) than American cats (14%). Free access to the outdoors 'conditions' other cat activities. For example, a test of independence found poor separation between fighting ($p=0.012$), hunting ($p=0.004$) and spending time outside the house ($p=0.005$) from the variable 'free access to the outdoors'.

TIME SPENT OUTDOORS

The time cats spent outdoors was classified into three categories: mostly indoors, mostly outdoors, or variable time spent outdoors (mostly depending on season weather or availability of prey). The cats in the 'variable time spent outdoors' category were considered by their owners to spend on average half of their time indoors and half outdoors.

Overall half of the cats spent most of the day inside the house and only 15% spent most of the day outside. The remaining third of all cats spent half of their time inside and half outside. Only 9 cats of the 73 cats with IBD (12.3%) spent the majority of their time outside. There were no significant differences in the time spent outdoors between IBD, matched and random controls.

However, differences between institutions existed. Cats from the Veterinary Teaching Hospital of the University of Pennsylvania were mainly indoor cats (34 out of 39) whereas cats from NZ private practices were mainly outdoor cats (52 out of 78). Overall, a significantly ($p=0.001$) higher number of American cats (67%) than NZ cats (39%) lived indoors.

FIGHTING FREQUENCY

Fighting frequency was classified into three categories: frequent fighting (defined as more than 5 fights a year), infrequent fighting (less than five fights per year), and no fighting at all. Unfortunately most owners never observed the fighting and could not give any further insight into how the fight developed or determine which cat was the aggressor.

Most cats with IBD were never involved in cat fights (48 out of 73). The majority of the remaining IBD cats fought frequently (18 out of 73). No significant differences were found in the frequency of fighting between cats with IBD, matched and random controls overall. However, when the cats that were frequently involved in fights and the occasional fighters were pooled and compared against the cats that were never involved in fights, American cats (28%) were found to fight significantly

($p=0.001$) less than NZ cats (57%). Furthermore, American cats with IBD fought significantly less ($p=0.013$) than American matched control cats (see **Table 11** for the OR).

Table 11
Odds ratios of IBD cats regarding fighting behaviour

Source	Fighting	IBD/Random Controls OR (CI)	IBD/Matched Controls OR (CI)
NZ	Yes	0.72 (0.28 – 1.81)	1.12 (0.44 – 2.89)
	No	1.4 (0.55 – 3.53)	0.9 (0.35 – 2.32)
America	Yes	0.48 (0.14 – 1.62)	0.18 (0.04 – 0.81)*
	No	2.06 (0.62 – 6.92)	5.5 (1.21 – 24.8)*

Note: * Statistically significant OR

PHYSICAL ACTIVITY

The physical activity of the cats was classified into two categories: cats that were active to very active, or cats that were relatively inactive to inactive.

Most cats with IBD were classified in the active category (50 out of 72). No significant differences in physical activity were found between cats with IBD and

Table 12
Odds ratio of IBD cats regarding physical activity

Source	Physical Activity	IBD / Random Controls OR (CI)	IBD / Matched Controls OR (CI)
NZ	Active	3.31 (1.2 – 9.16)*	3.25 (1.07 – 9.94)*
	Inactive	0.3 (0.11 – 0.83)*	0.31 (0.10 – 0.95)*
America	Active	0.89 (0.34 – 2.31)	1 (0.42 – 2.40)
	Inactive	1.12 (0.43 – 2.92)	1 (0.42 – 2.40)

Note: * Statistically significant OR

control cats overall. However, cats with IBD in NZ were significantly, more active than random control cats ($p=0.018$) and matched control cats ($p=0.029$). Cats with IBD in America were also more numerous in the active category but not significantly more so than the controls. OR are presented in **Table 12**. There were no differences in physical activity between American and NZ cats.

SLEEPING QUARTERS

The sleeping quarters of the cats were included as a variable in the analysis as an indirect way of explaining the closeness of the owner-cat 'bond'. The assumption was that cats sleeping in or on the bed of their owner would have a closer relationship with their owner than cats sleeping outside. Most cats slept in or on the bed of someone in the household (183 out of 219 cats). There were no differences in the place chosen to sleep between IBD, matched and random control cats, nor among cats from different institutions.

VETERINARY VISITS

Cats were classified into three categories: those that were never or infrequently taken to the veterinarian; those that were taken to the veterinarian once a year on average; and those that were frequently taken to the veterinarian (twice or more per year). Most IBD cats were taken to the veterinarian at least once a year (50 out of 72). Only 25% of IBD cats were frequently taken to veterinarians. No significant differences in the frequency of veterinary visits were present between IBD, random and matched control cats or between institutions. However, NZ owners visited their veterinarian significantly less often than American owners ($p=0.001$). This same general trend could be observed also among IBD cats, namely 36% of American cats

with IBD were often taken to visit their vets whereas only 14% of NZ cats with IBD were frequently taken to their veterinarian. The OR for each category can be seen in **Table 13**.

Table 13
Odds ratio of IBD cats according to how often they visit their veterinarian

Source	Veterinary Visits	Frequency	Odd Ratio
NZ	Frequent	5/36	0.8 (0.23 – 3.02)
	Regular	29/36	1.99 (0.68 – 5.82)
	Never	2/36	0.3 (0.06 – 1.62)
America	Frequent	13/36	0.56 (0.22 – 1.45)
	Regular	21/36	2.2 (0.85 – 5.64)
	Never	2/36	0.47 (0.08 – 2.75)

VACCINATION AND WORMING

More than 80% of cats with IBD and the random and matched control cats were vaccinated regularly (i.e. every 1 to 3 years) against feline calicivirus, rhinotracheitis virus and panleukopenia virus. There were no significant differences in vaccination frequency against these diseases between the cats with IBD and the control groups or between institutions or countries.

Vaccination for feline viral leukaemia was common among American cats (60 to 70% in IBD and control groups) but rare in NZ (8 to 20 % in IBD and control groups). Vaccination for rabies was also very common in American cats (77 to 83% in IBD and control groups). Vaccination for rabies in NZ is not currently practised because the country is free of the disease.

There were no significant differences in the frequency of worming between the cats with IBD and the control groups. However, cats were wormed significantly ($p=0.001$) more regularly in NZ than in America. In NZ, 92% of cats (from IBD and control groups) were wormed regularly (once every one or two years) to frequently (several times a year). This practice was uncommon among American cats (10%). Only 33 out of 219 cat owners knew what anthelmintic was being administered to their cats.

TEMPERAMENT

Owners were asked to classify their cats into the following categories: nervous, placid, aggressive, affectionate, assertive, grumpy, and independent. These categories were chosen after a trial questionnaire presented to several cat owners indicated that most people could easily relate to these terms and use them meaningfully. None of these categories were exclusive and the owners were permitted to characterize their cat by more than one temperament. Each temperament was analyzed on its own and not as part of a combination of temperaments.

Significantly fewer cats in the IBD group were classified as placid by their owners than in the random control group ($p=0.006$; OR= 0.38, CI= 0.19 – 0.77) and the matched control group ($p=0.024$; OR= 0.43, CI= 0.20 – 0.90). Most of this difference originated in the cats from NZ. There were significantly fewer placid cats among NZ cats with IBD than in the NZ random control group ($p=0.01$; see **Table 14** for OR) and matched control group ($p=0.008$; OR= 0.43, CI= 0.2 – 0.9).

Table 14
Odds Ratio of Temperament in IBD cats compared with the random controls

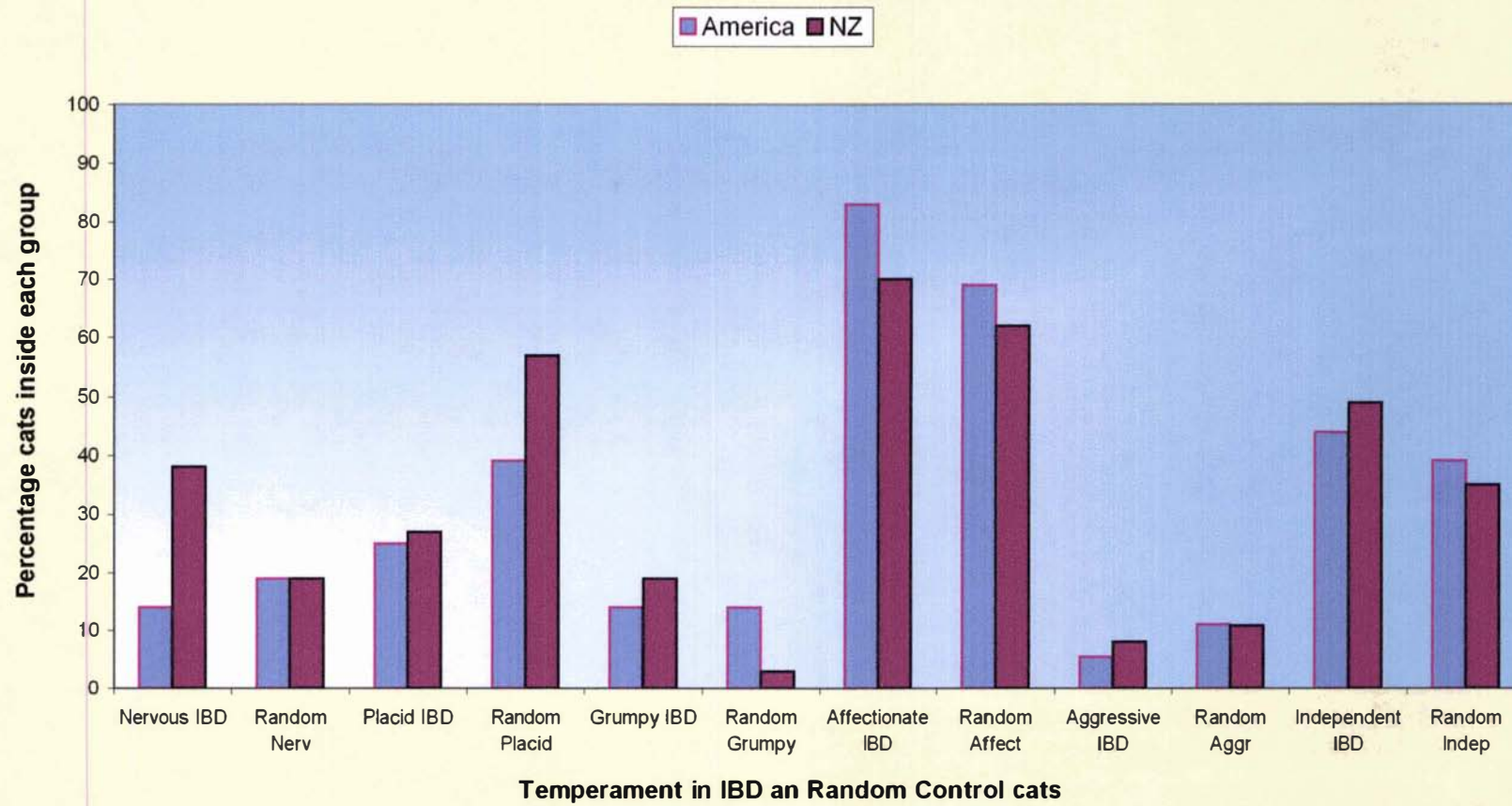
Source	Temperament	OR (CI)
America	Nervous	0.67 (0.19 – 2.34)
	Grumpy	1 (0.26 – 3.30)
	Placid	0.52 (0.19 – 1.44)
	Independent	1.26 (0.49 – 3.21)
	Affectionate	2.2 (0.71 – 6.79)
	Aggressive	0.47 (0.08 – 2.75)
NZ	Nervous	2.6 (0.91 – 7.51)
	Grumpy	8.4 (0.98 – 72.15)
	Placid *	0.28 (0.11 – 0.75)
	Independent	1.75 (0.69 – 4.45)
	Affectionate	1.44 (0.55 – 3.79)
	Aggressive	0.73 (0.15 – 3.50)

Note: Not significant unless stated.

*Statistically significant Odds Ratio in NZ between IBD and Random controls.

Another difference seen among NZ cats was that ‘grumpy’ cats were over represented in the IBD group when compared to the random controls ($p=0.05$ although the OR does not indicate a significant difference). However, in other regions grumpy cats were not significantly more frequent amongst cats with IBD than amongst

Fig. 9.- Temperament distribution among IBD and random control cats in NZ and America



controls. Nervousness is another temperament that was more frequent in NZ cats with IBD ($p=0.071$) than in the random control cats. However, this temperament was not significantly different between IBD and matched control cats ($p=0.6$). **Figure 9** shows the distribution of different temperaments among IBD cats and random control cats in the two geographical locations. **Table 14** contains the OR for each group.

HISTORY OF DISEASE

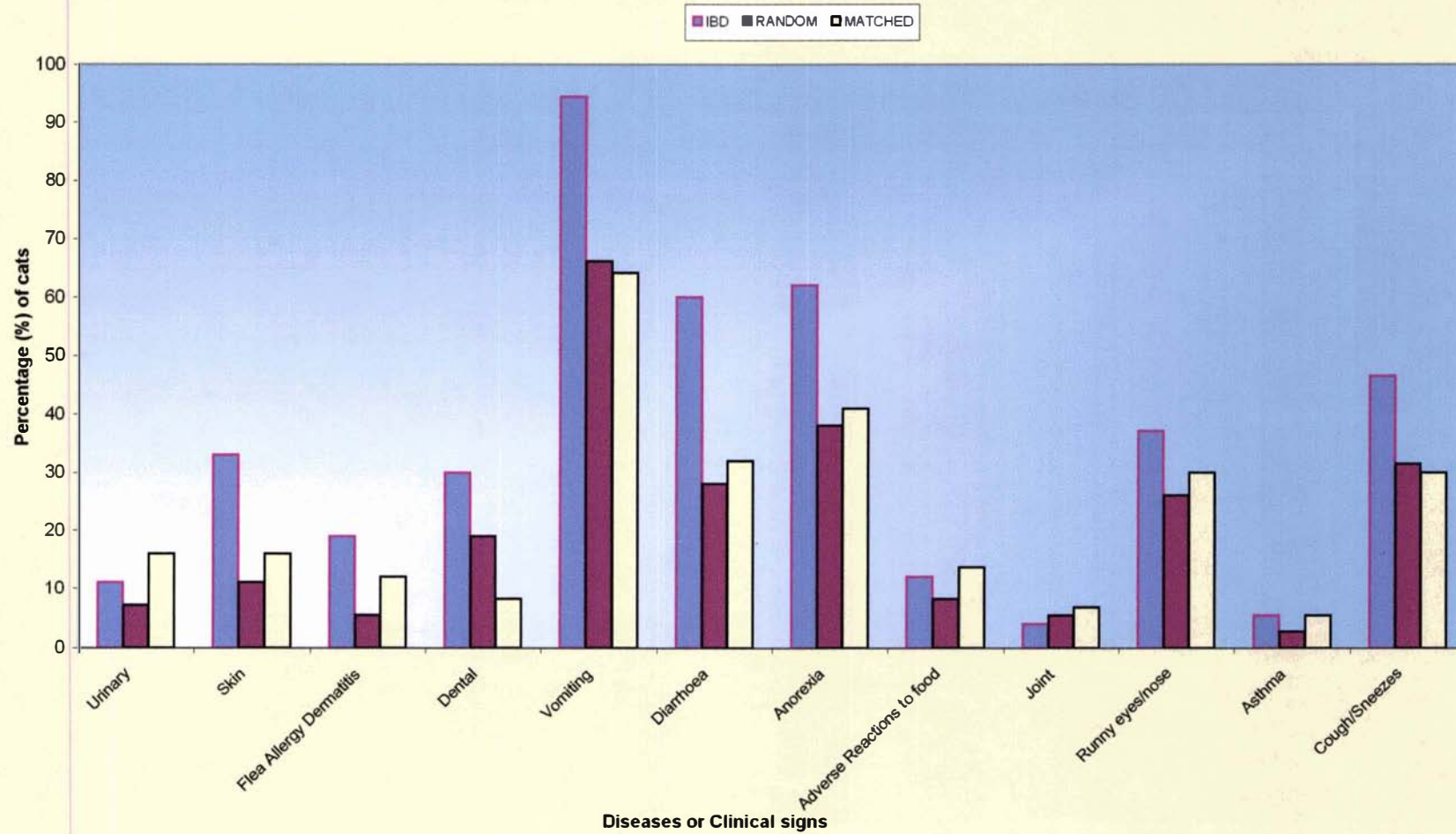
The percentage of IBD and control cats showing various clinical signs before the diagnosis of IBD was made is shown in **Figure 10**.

CLINICAL SIGNS OF GASTROINTESTINAL DISEASE

a. - Anorexia

Periodic anorexia (total or partial loss of appetite) was reported prior to diagnosis in more than 60% of cats with IBD. Anorexia was significantly more common in IBD cats prior to diagnosis ($p=0.005$; OR= 2.58, CI= 1.32 – 5.03) than in random controls (38%). Most of this difference originated in the NZ cats ($p=0.004$; OR= 4.08, CI= 1.51 – 11.03). The proportion of anorectic cats among the IBD American cats prior to diagnosis was similar than in IBD cats in NZ. However, the American random controls were reported more often to suffer from anorexia than the NZ random control cats ($p=0.23$; OR= 1.79, CI= 0.69 – 4.64) and hence the difference between American IBD cats and their random controls was not significant. Anorexia prior to diagnosis was reported more frequently in IBD cats than in matched control cats also ($p=0.014$; OR=2.36, CI= 1.15 – 4.77).

Fig. 10.- Presence of gastrointestinal signs and other diseases among control and IBD cats before diagnosis



b.- Vomiting

Vomiting was reported more frequently in cats with IBD prior to diagnosis (94% of cats) than in random control cats (66% of cats; $p=0.001$; OR=7.08 CI= 2.53 - 19.8) or matched control cats (64% of cats; $p=0.001$; OR=22, CI= 2.86 – 163.02).

The content of the vomit was infrequently recorded (57 out of 218 cats). Food, grass and fur were the most commonly reported contents. The presence of bile (yellow fluid) was noticed by many owners. The act of vomiting was rarely observed by the owners but the description of the vomit did not indicate regurgitation (i.e. cylindrical shape of eliminated material). Most owners were unable to determine the frequency of vomiting but mentioned that vomiting happened regularly. A proportion of cats (20%) that vomited did so between once a week and twice a month. Only a few vomited more often than that (2 to 3 times a week). One random control was reported as having periodic bouts of vomiting every year without its health being affected. Owners of control cats were not concerned by their cat's vomiting and a few of them considered it a regular 'normal' activity of cats. The comparison of the frequency of vomiting and the content of the vomit in cats with IBD prior to diagnosis and the control groups could not be assessed in this study but the personal impression of the principal investigator is that there were no differences present.

c.- Diarrhoea

Diarrhoea was a common clinical sign in the cats with IBD prior to diagnosis and was reported significantly more frequently than in the random ($p=0.001$; OR= 3.94, CI= 1.96 – 7.92) and matched ($p=0.001$; OR= 6.0, CI= 2.08 – 17.3) control cats. Overall, the proportion of cats that had a history of diarrhoea prior to diagnosis was

60% for the IBD cats, 28% for the random controls and 32% for the matched controls. Diarrhoea occurred mostly on an occasional basis and it was usually temporally related to the consumption of some foods (e.g. dairy products, fish, some biscuits brands). Consequently, these foods were not given to the cats often and were specifically avoided. Only a handful of control cats were reported by the owners to have loose stools regularly without changes in their demeanour or ill effects on their health.

Interestingly, the reported frequency of diarrhoea prior to diagnosis varied widely between countries. In NZ, the frequency of diarrhoea reported in cats with IBD (38% of cats) was not significantly different from that of random control cats (24%). In contrast, the frequency of diarrhoea reported in American cats (83% of cats) was significantly higher ($p=0.001$) than that of the random control cats (31% of cats). The corresponding odds ratio for this clinical sign was 1.9 (CI 0.69 - 5.16) in NZ cats and 10.9 (CI 3.52 – 33.78) in American cats.

American IBD cats also had a significantly higher frequency of diarrhoea prior to diagnosis than matched controls (40% of matched control cats had a history of diarrhoea, $p=0.001$; OR=16, CI= 2.08 – 120.64).

URINARY DISEASE

Clinical signs of urinary disease were uncommon in cats with IBD. No significant differences were found in the frequency of urinary disease between IBD cats, random controls and matched controls.

SKIN DISEASE AND FLEA ALLERGY DERMATITIS

The owners of 33% of IBD cats reported that their cats had skin disease prior to diagnosis of IBD. Overall, skin disease was significantly more common in IBD cats than in random control cats ($p=0.001$) and matched control cats ($p=0.023$). Similarly, NZ IBD cats had significantly more skin disease reported than their random controls ($p=0.003$). American IBD cats showed a similar but not significant trend. Odds ratios for skin disease was $OR=2.5$ ($CI= 0.81 - 7.64$) for the American IBD cats and $OR=8.4$ ($CI= 1.7$ to 40.8) for the NZ IBD cats compared to their random controls.

Several specific skin diseases were mentioned by the owners of IBD and control (random and matched) cats. These included ringworm ($n=4$ control cats), reaction to insecticide spray ($n=1$ IBD cat), acne ($n=1$ IBD cat), acne + allergy ($n=1$ control cat), allergy ($n=1$ IBD cat), eczema ($n=3$ control cats), eosinophilic plaque ($n=1$ IBD cat), excessive grooming ($n=1$ control cat), plasma cell pododermatitis ($n=1$ IBD cat), squamous cell carcinoma ($n=3$ control cats) and otitis externa ($n=1$ IBD cat).

Flea allergy dermatitis was not commonly reported amongst the cats in this study but was slightly and significantly more frequent among IBD cats (19%) when these were compared with the random controls (5%; $p=0.012$; $OR= 4.1$; $CI= 1.28 - 13.1$). No significant differences were found between IBD cats and matched controls (14%) in the frequency of flea allergy dermatitis. Not surprisingly, the presence of flea allergy dermatitis was not independent of the presence of skin disease ($p=0.001$).

DENTAL DISEASE

The frequency of dental disease was higher in the cats with IBD (30%) than the random control cats (19%) and matched control cats (8%). The latter was statistically significant ($p=0.001$; OR= 6.33, CI= 1.873 –21.36).

ADVERSE REACTIONS TO FOOD

Adverse reactions to foods were reported to have occurred in 25 of the 219 cats (11%) included in the study and 9 of the 73 cats with IBD (12%). The foods to which adverse responses were reported included: fish, turkey, venison, red meat, mutton, milk, condensed milk, cream, ‘cheap cat food’, ‘dry food’ and ‘coloured dry food’. No significant differences were found between IBD, matched and random control cats in the reported frequency of adverse reactions to foods.

JOINT DISEASE

Joint disease was infrequently reported. Three of 73 cats with IBD (4%) were reported to have a history of joint pain, swelling or arthritis. This frequency was not significantly different from that of the random control cats (5%) or the matched control cats (6.8%).

RESPIRATORY DISEASE

a.- Upper respiratory tract signs

Many of the cats with IBD (37%) were reported to have suffered from “runny eyes” or a “runny nose” sometime during their life. The frequency of these clinical

signs was not significantly different from that of random control cats (26%) or matched control cats (28%).

b.- Sneezing or Coughing

Approximately 47% of cats with IBD had been observed to cough or sneeze during their lives in comparison to 31% of random controls and 28% of matched controls. The difference in the frequency of these signs between cats with IBD and the random control cats ($p=0.06$; OR= 1.89, CI= 0.96 – 3.72) and the matched control cats ($p=0.058$; OR= 1.86, CI= 0.97 – 3.55) verged on statistical significance.

c.- Asthma or Wheezing

Owners reported that their veterinarians had clinically diagnosed their cat with feline asthma in 4 of 73 cats with IBD, 1 of 73 random control cats and 4 of 73 matched control cats. Owners confirmed that wheezing was the main clinical sign. No significant differences were found between the groups.

OTHER DISEASES

Cats with IBD suffered from significantly fewer other diseases than the matched and random controls ($p=0.028$; OR= 0.47, CI= 0.24 – 0.93). In America, 50% of the cats with IBD had been diagnosed with a disease other than IBD at some stage in their life compared to 70% of the random controls ($p=0.093$) and 80% of the matched control cats ($p=0.008$). In NZ the trend was similar but of a lesser magnitude. Only 16% of IBD cats in NZ had been diagnosed with a disease other than IBD compared to 30% of the random controls ($p=0.09$) and 37% of matched control cats ($p= 0.059$).

Other diseases reported in cats with IBD in the USA included: autoimmune gingivitis (n=1), inappropriate defecation (n=1), chronic renal failure (n=1), diabetes mellitus (n=1), eosinophilic disease (n=1), feline lower urinary tract disease (n=1), entropion (n=1), ear mites (n=1), heart defects (n=1), heart disease (n=2), pancreatic insufficiency (n=1), squamous cell carcinoma (n=1) and urinary tract infection (n=1). In NZ, other diseases reported in cats with IBD were chronic active pancreatitis (n=1), eczema (n=1), feline lower urinary tract disease (n=2) and hyperthyroidism (n=1).

DIET, EATING BEHAVIOUR AND HUNTING ACTIVITY

WHO FED THE CAT?

This question was included to gain an insight into the reliability of the nutritional information obtained. More than 80% of cats in all three groups were fed exclusively by the person interviewed for the present study. In the remaining households, different family members shared the task of feeding the cat.

FREQUENCY OF FEEDING

Sixty five to seventy percent of cats in all groups were fed ad lib. The rest of the cats were fed two or more times a day. No significant differences existed in the frequency of feeding between groups, countries or institutions.

RAPIDITY OF FOOD CONSUMPTION

Between 55 and 65% of cats in all groups were considered by their owners to eat their food at a speed similar to that of other cats (i.e. an 'average speed'). The speed at which cats with IBD consumed their food was not significantly different to that of the random control cats. However, cats with IBD were considered to eat significantly ($p=0.034$) faster than their matched controls.

FASTIDIOUSNESS TOWARDS FOOD

Thirty percent of cat owners did not know if their cat was a fastidious eater. There were no differences in fastidiousness between IBD, random and matched control cats. However, cats with IBD in NZ were significantly more fastidious than their random controls ($p=0.016$ OR=3.87). No differences were present in NZ between IBD and matched controls cats.

HUNTING ACTIVITY

Approximately 70% of cats in the IBD and control groups were known to hunt. There was no significant difference between the frequency of cats with IBD that were reported to hunt and the frequency of the random or matched control cats that were reported to hunt. However, significantly more ($p=0.001$) cats from NZ (89%) were reported to engage in hunting than American cats (52%).

Between 20 to 30% of cats in the IBD and control groups were known to frequently catch prey (i.e. the cats had been observed to catch prey more than 3 times per year). Another 30% of the cats in the IBD and control groups would hunt only occasionally (i.e. 2-3 times per year). The owners of the remaining 8 to 15% of cats which hunted (in the IBD and control groups) did not know how often their cats engaged in this activity.

The prey captured by the cats consisted mainly of birds (thrushes, ducks, pheasants and other small wild birds), small mammals (mice, rats, rabbits, gophers, squirrels, moles, chipmunks, bats, stoats), cold-blooded animals (geckos, snakes and fish), and insects (flies, bumble-bees, bees, blowflies, cockroaches, butterflies, moths, spiders, praying mantises, wetas, cicadas, locusts, crickets, beetles, dragon flies). There were no differences between the IBD, random and matched control groups in the type of prey captured.

Interestingly, although overall 70% of cats in the IBD and control groups were known to catch prey, only approximately 40% of the cats in each group were observed to consume their prey. No differences were found between IBD, matched and random control groups either in the frequency with which cats consumed their prey or in the type of prey (birds, small mammals, cold-blooded animals or insects) most often consumed. However, regional differences were present. American hunting cats consumed their prey more often than New Zealand hunting cats ($p=0.07$). New Zealand cats ate more birds ($p=0.001$) and small mammals ($p=0.02$) than American cats. Similarly, cats from Davis ate more birds ($p=0.05$) and slightly more small

mammals ($p=0.7$) than cats from the Philadelphia region. The latter consumed mainly insects and some small mammals. Most of the cold-blooded animals were consumed in NZ. Insects were consumed to the same extent in all locations.

DIETARY HISTORY

a.- Petfoods

The cats were separated in three categories according to the proportion of dry commercial food consumed in their daily meal and again in three categories for the proportion of canned commercial cat food. The categories were 0 to 30% of their daily intake as dry cat food or canned food, 30 to 70% of their daily intake as these foods, and 70% or more of their daily intake as these foods. No significant differences were present between the IBD, random and matched control groups in the daily amounts of dry or canned food consumed (See **Figure 11**). However, regional differences were identified. American cats consumed a significantly greater proportion of their diet as dry pet food than NZ cats ($p=0.001$). Dry cat food comprised more than 70% and canned food less than 30 % of the daily intake of 60% of American cats. In contrast, 50% of NZ cats consume between 30 and 70% of their daily intake as canned food and less than 30% of their daily intake as dry food (See **Figure 12**).

Most owners regularly changed the brands and flavours of food they fed to their cats. Very few owners fed only one pet food brand and more than 50% of owners fed their cat all flavours that were readily available within a brand. The varied

Fig. 11.- Proportion of different types of commercial cat foods (dry and canned) in the daily meal of IBD and control cats

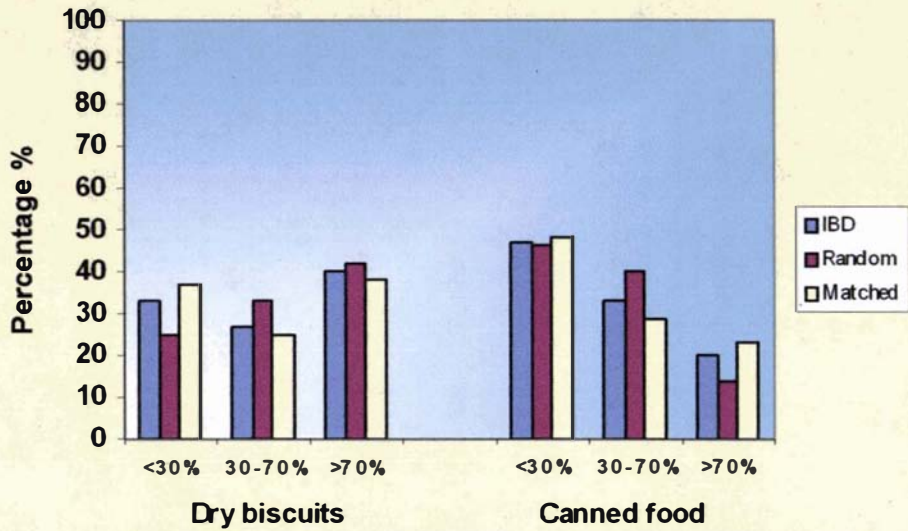
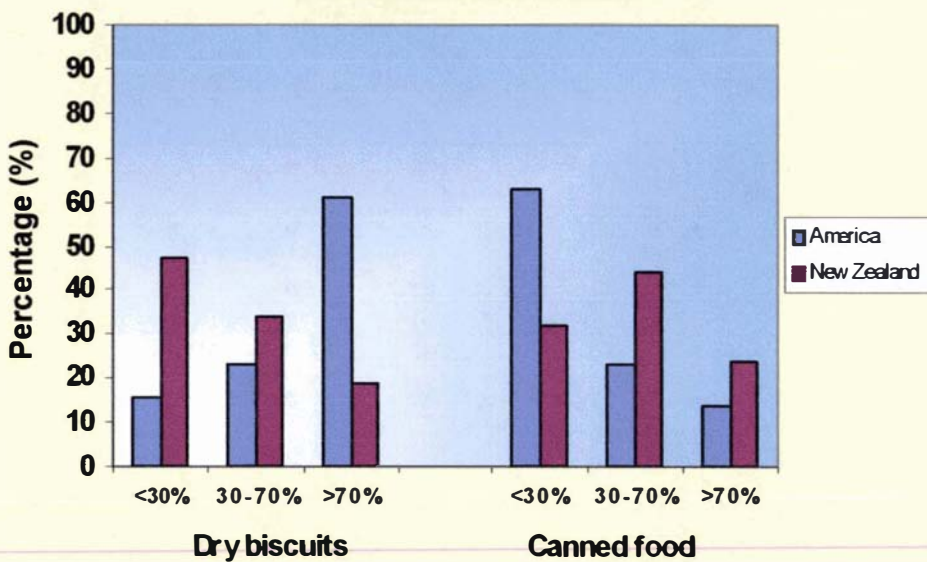


Fig. 12.- Proportion of different types of commercial cat foods (dry and canned) in the daily meal of cats in America and New Zealand



diets of the cats prevented a comparison of the feeding frequency of specific brands and flavours between the IBD and control groups

Sixty four percent of American cat owners and 78% of NZ cat owners everyday discarded uneaten cat food remaining in their pets' food bowls. This observation, along with the regular changes to the brands of pet food fed, prevented attempts to quantify and compare the intake of the macronutrients between the groups.

b.- Fresh or cooked meat meals

Approximately 60 to 65% of cats in the IBD and control groups consumed meals of poultry meat (chicken, turkey, giblets). Nearly 50% of cats in all groups ate poultry more than once a month. Between 42 to 52 % of cats in all groups consumed beef in the form of steak, sausages, liver or ox heart. Approximately 36% of these cats would consume beef more often than once a month. Other meats (lamb, mutton, pork, venison) were consumed by fewer cats (33 to 40% of cats in all groups). These meats were eaten principally as steak, sausages, ham, bacon, lambs fry, and lamb kidney. Around 20 to 30% of cats in all groups consumed these other meats regularly. Just above 50% of cats in all groups consumed fish intended primarily for human consumption (salmon, sardines, tuna, and fresh fish in NZ). Approximately 30 to 40 % of cats would do this regularly. No significant differences were found overall in the frequency of consumption of any of these meats or fish between the IBD and control groups. Most cats consumed these meats fresh or cooked without differences between IBD, random or matched controls.

However, differences between countries existed. Significantly more NZ cats consumed chicken meat ($p=0.021$), beef meat ($p=0.001$), other meats ($p=0.001$) and fish ($p=0.001$) than their American counterparts. Similarly, more IBD cats owned by the clients of the private practices in NZ consumed fish regularly ($p=0.07$) than the control cats.

c.- Table foods

All cats consumed a wide variety of table foods. This type of food was usually given as a treat in smaller quantities than the meat meals described above. Most of the table foods listed below were fed more than once per month. The foods were classified as: dairy products (ice cream, cheese, cream, yoghurt, cream cheese); foods containing egg (egg whites and egg yolks, cakes, pies, omelettes); starchy foods such as cereals, potatoes, legumes or nuts (e.g. wheat in pasta, bread, flour, gravy, biscuits and cakes; potatoes mashed or in chips and French fries; rice, corn, tofu; beans; and peanuts); vegetables and fruits (broccoli, carrots, pumpkin, green beans, peas, tomatoes, melon, orange, banana); and commercial cat treats.

Dairy products were consumed by 41-53% of cats in the all groups. Egg-containing table foods were eaten by 7- 9% of cats in all groups. Starchy foods were eaten by 20-27% of cats in all groups (wheat-containing foods being the most frequent). Fruits and vegetables were consumed by 9-12% of cats in all groups and 10-20 % of cats were given cat treats (dry pellets or dry fish). No significant differences were found in the frequency of consumption of these table foods between the IBD, random and matched control groups.

Differences between American and NZ cats were present. Significantly more NZ than American cats consumed dairy products ($p=0.001$), egg-containing foods ($p=0.016$) and wheat-containing products ($p=0.04$). In contrast, American cats received more cat treats than NZ cats ($p=0.001$).

d.- Scavenging

Eleven percent of American cats and 20% of NZ cats were reported to scavenge (eating rubbish or obtaining food from other places than the owner's house). No differences in the number of cats that scavenged were detected between the IBD, random and matched control groups.

e.- Fresh milk

Nearly 60% of the cats in all groups consumed liquid milk occasionally. There were no significant differences between groups. However, significantly more American cats with IBD drank milk than their random controls ($p=0.032$) but not matched controls ($p=0.16$). American cats mostly drink low fat milk, while NZ cats mostly drink regular milk ($p=0.001$).

d.- Water

Approximately 75% of all the cats drank city water. Significantly fewer NZ cats with IBD drank water from wells than did the random controls ($p=0.043$).

RESULTS OF THE MULTIVARIATE ANALYSIS

a.- IBD cats vs. matched controls

The variables included in the multivariate analysis were: veterinary visits, area of residence, time spent outdoors, other pets present in the household, frequency of boarding, 'Total Stress Score', frequency of fighting with other cats, physical activity, placid, nervous or grumpy temperament, history of respiratory disease, history of vomiting and the presence of other cats in the household. History of dental disease was not included because it separated IBD cats from matched controls fully and would not allow the inclusion of other variables other than vomiting in the final model. The results of the stepwise conditional logistic regression that compared IBD cats with matched control cats and factors kept in the final model are presented in **Table 15**.

Table 15
Stepwise Conditional Logistic Regression Results

Factors	<i>p</i>	OR	Lower CI	Upper CI
Vomiting History	0.0001	51.4*	7.35	999
Presence of 1 dog	0.0131	5.95*	1.73	27.1
Cat Fights	0.0127	0.185*	0.046	0.585
Placid Temperament	0.051	0.35*	0.097	1.033
Veterinary Visits	0.1	0.43	0.116	1.4
History of Respiratory Disease	0.17	2.0	0.74	5.95
Anorexia	0.18	2.57	0.82	9.68
Total Stress Score	0.22	0.6	0.24	1.33

Note: * Indicates OR significant difference

b.- IBD cats vs. random controls

The multivariate analysis using a log-linear model between IBD and random controls was originally fitted with the same variables as above in addition to the matching variables (sex, breed and age). The significant factors included in the final model are listed in **Table 16**. In this final model the factor ‘age’ was divided into the 3 groups used in the univariate analysis, with the first and second age groups compared to the third (over 15 years old); ‘breed’ was separated into domestic breeds (DSH and DLH) and rare and/or exotic breeds and the analysis represented the risk associated with the latter. The risk of females having IBD was compared with the risk of males having the disease and the presence of respiratory disease or vomiting with no history of these events. The risk associated with a high Total Stress Score (2 or 3) was compared with the risk of a low one (0 or 1).

Table 16
Log-linear Model Results

Factors	<i>p</i>	OR	Lower CI	Upper CI
Age (1 to 7y)	0.8999	0.92	0.25	3.33
Age (7 to 15y)	0.0482	3.50*	1.01	12.16
Breed	0.0001	8.65*	2.86	26.13
Sex	0.0053	3.74*	1.48	9.44
Indoors/Outdoors	0.0813	2.18	0.91	5.24
Physical Activity	0.0253	2.79*	1.14	6.87
Total Stress Score	0.0428	2.55*	1.03	6.30
History of Respiratory Disease	0.0571	2.25	0.98	5.18
History of Anorexia	0.0181	2.70*	1.18	6.14
History of Vomiting	0.009	3.56*	1.36	9.26

Note: * Indicates OR significant difference

Discussion

The main constraint of observational studies such as the present study, is that most of the information is based on human perceptions and recollections - both of which can be mistaken or misleading. Fortunately, over 80% of the cats in the present study had been owned since they were kittens and had been fed exclusively by the person interviewed. These observations suggest that most owners participating in this study were in a position to provide reliable information for the survey. In spite of these drawbacks, epidemiologic studies such as the present study can guide and narrow the focus of further research.

This study demonstrated several apparent differences in risk factors for IBD amongst the different geographic and institutional sources of the cats in the present study. These differences lend credit to the decision to undertake a multicentre methodology. Many of the factors studied could have appeared as significant associations with IBD if it had not been for the participation of IBD and control cats from widely different areas. The univariate analysis showed that marked differences existed regarding time spent outdoors, fighting frequency, physical activity, veterinary visits, vaccination and worming, sign of gastrointestinal disease, presence of other diseases and diet between American and NZ IBD cats. However, many of these differences appeared to relate to differences of lifestyle or custom between countries and were not associated with the disease overall.

The predominance of females among IBD cats has not been previously reported. Most of the published studies to date have reported equal number of both sexes or a predominance of males^{33,35,36,42,43}. This female predominance appeared to be primarily a characteristic of the IBD cat population in NZ, since most of the difference in the frequency of IBD between males and females in the pooled data originated from the NZ cats. Nevertheless, the predisposition to IBD between the sexes in people has varied according to location⁶ and to date there is no known sexual predisposition for human IBD.

Exotic breeds of cats showed a higher relative risk of IBD when compared with the cat population at large. Similar observations were made in previously reported studies of IBD in spite of smaller sample sizes^{33,34,36,43}. In humans, ethnic differences in susceptibility to IBD have been known for a long time⁶. It is unknown if the apparent increased risk of IBD in exotic breeds of cats is due to their genetic make-up (e.g. deleterious recessive genes in pure-bred cat populations) or environmental and/or socio-economic factors (clients that want and can afford better diagnosis and veterinary medical care). The search for genetic markers continues in the human IBD field. However, exciting as these findings are, there is yet inconclusive information to link disease and genetics in a simple and direct way⁴⁴. Furthermore, the ethnic predisposition to IBD has not been attributed to a specific genetic make up as yet⁴⁵.

The predisposition of middle-aged to older cats to IBD noted in the present study is supported by previous reports of IBD in which middle-aged to older cats are

well represented^{31,33,35,36,42,43}. However, it is the subjective impression of the author that in the present study, IBD cats were noted by their owners to have a long history of vomiting and diarrhoea prior to diagnosis, an observation which suggests that the disease may have been present for a considerable period before presentation of the cats to their veterinarians. It is also possible that as cats get more sedentary with age, clinical signs become more obvious to the owner. If we consider IBD as a multifactorial disease, it is also feasible that the threshold for the manifestation of disease may take several years to be reached.

None of the 'stress variables' examined in this study were significantly more frequent in cats with IBD except for the likelihood of sharing a house with a single dog. The stress created for a cat by living with a dog might be due to the natural fear of cats for dogs (however, paradoxically, two or more dogs did not seem to constitute a risk factor) or for competition for the attention of the owner, territory, or food. Since the significant difference was found between the IBD cats and the matched controls, the interplay between sex, age and breed (matching factors) of the IBD population and the presence of a single dog in the household is interesting. It is possible that the age, breed, or sex of a cat (or any combinations of these variables) may make a cat more sensitive to stressors such as having a dog in the household. For example, perhaps exotic cat breeds may be more susceptible to stress of this type because of a nervous predisposition or a stronger owner-animal bond. Alternatively, perhaps middle aged cats may be more assertive and set in their ways making them less tolerant of living with dogs than younger more adaptable cats.

More conclusive evidence for an influence of stressors on the risk of feline IBD was provided by the Total Stress Score. If all suspected causes of stress were scored and added together, IBD cats had a significantly higher average score than the matched and random controls. An important role for stress in the pathogenesis of feline IBD has been previously proposed⁴⁶. Stress is also considered an important factor in precipitating new bouts of clinical signs in humans with IBD⁴. If we apply this observation to the veterinary field, stressful events may just make the disease overt to cat owners and not be related to it in any other more direct way.

The temperament of cats with IBD, specifically the lack of 'placidity' and the increase in 'grumpiness' noticed in the IBD cats of the present study, may contribute to the effect of stress on the pathogenesis of feline IBD. For example, a 'grumpy' temperament may indicate a predisposition to over-respond to changes or other stressors and a placid disposition may suggest the opposite. Alternatively, these temperaments may be a result of the disease or a direct result of stress per se.

Feline lifestyle proved to be very different between countries regarding access to the outdoors, time spent outdoors, physical activity, fighting activity and veterinary care. Cats with access to the outdoors may appear healthy to their owners because vomiting and diarrhoea are not observed, weight loss may not be present and the cat may still be eating. Also the secretive nature of some cats will make observation of stools or the presence of vomiting difficult to assess. Interestingly fighting, which may be considered a source of stress, was decreased in frequency in IBD cats compared to control cats. It is possible that IBD cats are more lethargic as a result of their disease and so don't attempt to fight as frequently as healthy control cats.

Alternatively, cats with IBD may be closer and more dependent on their owner for their needs and may not worry about the presence of other cats in their surroundings.

This study confirms other reports^{32,33,43 2,3,35,36,42,46-48} in the literature that vomiting and diarrhoea are important clinical signs in cats with IBD. Furthermore, it indicates that even prior to diagnosis vomiting and diarrhoea were significantly more common among cats that would eventually be diagnosed with IBD than healthy cats. Anorexia, another common sign of gastrointestinal disease was also more prominent prior to diagnosis in the IBD cats than in the control groups.

Skin disease was found to be more common in cats with IBD prior to diagnosis than control cats in the present study. This association was strongest in NZ cats with IBD. However skin disease could not be separated from flea allergy dermatitis in the analysis. As a result, the stronger association between IBD and skin disease in NZ may be another reflection of cat lifestyle differences between America and NZ (i.e. flea allergy dermatitis is likely to have a higher prevalence in outdoors as opposed to indoor cats). It is noteworthy, however, that a higher prevalence of skin disease has been reported among people with IBD⁵ and recent studies have shown a plausible pathophysiological pathway by which IBD and skin disease can be linked. Specifically, a cross-reaction between cells involved in contact hypersensitivity and effector cells of the IBD immune response have been found in an hapten model of murine IBD⁴⁹ suggesting that the skin and the gut share information and reactivity.

Dental disease (described by the cat owners as more serious than routine periodontal care) was more frequent in the cats with IBD prior to diagnosis than in

matched control cats without the disease. Thus, the age distribution of IBD cats (i.e. the predominance of middle-aged to older cats) does not explain this finding. Similarly, differences in diet do not account for this difference. It is possible the frequent vomiting of IBD cats may play a role in the pathogenesis of the dental disease. Stomach and duodenal fluid in the vomit might be capable of damaging the oral mucous membranes. The pH of this fluid will depend on the presence of food and the degree of admixture of saliva, stomach fluid and duodenal fluid. However, both, acid and alkaline pH can be harmful to mucous membranes in some situations⁵⁰. Vomit can also contain digestive enzymes and biliary acids which can be damaging to oesophageal tissues⁵¹. However, it is uncertain if a similar process to that which can occur in the oesophagus can damage the gums and teeth of cats and it is noteworthy that the vomiting reflex is also associated with hypersalivation and frequent swallowing⁵² – both of which are likely to protect the mouth against the deleterious effects of vomitus. When periodontal disease is present there is reportedly a shift in buccal flora towards that containing more gram negative, anaerobic, motile rods – a flora that matches gut flora⁵³. It would seem possible that frequent vomiting of gastrointestinal content might contribute to the shift of oral bacteria towards the flora more common in the gut. However, the role these changes to oral flora play in the pathogenesis of periodontal disease is unknown.

Adverse reactions to food were not reported frequently in any group. The diagnosis of adverse reactions to food is not easy from the point of view of detection by the owner and of definitive diagnosis by the veterinarian. The cats with IBD included in this study had undergone dietary trials during their diagnostic work up to eliminate the possibility that adverse reactions to food were the sole cause of the

gastrointestinal signs. Nevertheless, adverse reactions to food could have co-existed with a diagnosis of IBD. The possible link between IBD, diet and individual reactions to food ingredients has been discussed in Chapter 2. This study does not support an association between feline IBD and adverse reactions to food from the perspective of clinical history.

Signs of upper respiratory disease tended to be more common in cats that developed IBD prior to diagnosis than in control cats in the present study. The widespread nature of viral respiratory diseases in the feline population makes it difficult to demonstrate its significance in IBD pathogenesis. Respiratory infections during infancy have been associated with IBD in humans ²⁸. However, the pathogenesis of this association has not been forthcoming and although viral infections have been blamed in the past for IBD in humans ^{4,6,54}, no objective evidence ever has been presented to support this claim.

Cats with IBD appeared to be taken less often to see their veterinarians than control cats in the present study. This is surprising considering the chronic nature of the disease. Regular visits (once or twice per year) were the norm for IBD cats. However, there were more American cats that visited their vets 'often', yet less that visited it 'regularly' when compared with the NZ cats. Differences in the types of vaccines to which the cats were exposed to and in the frequency of worming were observed but were another consequence of regional differences and a reflection of the different lifestyle of cats in America and NZ. Lifestyle could have also played a part in how often cats are taken to see their veterinarian. For example, indoor cats are easy to supervise (presence of vomiting and diarrhoea) and disease bouts can be easier to

detect, which may induce owners to take the cat to the veterinarian more often. On the other hand, indoor cats may have a closer relationship with their owners than cats that only spend sometime indoors and their owners may look for veterinary advice more often.

The finding that most IBD cats only visit their vet regularly correlates well with the fact that the owners of IBD cats report less presence of other diseases than the rest of the cat population. There was a high proportion of American random cats that have had other diseases at some time in their life. These results have to be considered in light of the source of controls (vet clinics) and the differences between cats that visit Teaching hospitals in NZ and in America. American Teaching Hospitals have more referral cases sent to them than check-ups and vaccinations hence a random control group from this population will contain by default more diseased animals. The fact that more diseased animals visit these hospitals also confounds the finding that more cats in America visit the vet often. However, the presence of other diseases in cats diagnosed with IBD was similar in both countries and this indicates that in general IBD cats tend to have only one disease regardless of how often they visit their veterinarian.

Diet has always been considered an important factor in gastrointestinal homeostasis and derangements as seen by the numbers of studies including diet as an epidemiological factor in human IBD (see introduction to this chapter and Chapter 2). Diet has also been part and parcel of IBD therapy in humans⁵⁵ and cats^{1,2,31,33}. In the present study the similarities between groups regarding the consumption of pet-foods, prey and table-foods were remarkable. Regional differences in diet were large but

dietary differences between IBD and healthy cats from the same region were not apparent. The lack of differences in the types of food eaten (table foods, prey, animal flesh, petfoods), the categories of petfoods offered and the types of food proteins to which cats in this study were exposed suggest that these dietary factors per se are not important in the aetiopathogenesis of feline IBD. However, the observations in the present study do not rule out a role for diet in the pathogenesis of feline IBD. The presence of food in the gut lumen modifies the bacterial flora in composition and metabolic activity⁵⁶. Relatively recent research has shown that individual responses to bacterial flora⁵⁷ may modify the presence of IBD. The influence of bacterial flora on the expression of genes involved in nutrient absorption and other gut functions has also been shown⁵⁸. Furthermore, individual immune reactions to dietary components, as in the case of celiac disease, have been suspected for years and only recently uncovered⁵⁹. All this work indicates that an interaction between individuals and their diet, either directly or indirectly through the bacterial flora, could modify the risk of developing IBD or its course and severity. Hence, the focus on diet and its relationship with IBD may need to be shifted from what is being consumed to how whatever is being consumed interacts with a particular individual and how it modifies the gut environment in health and disease. Only then would we gain insight into the role of diet in the cause, pathogenesis and treatment of IBD and other chronic gastrointestinal ailments.

Conclusions

This epidemiological study has described the typical age, breed and sex distribution of cats with IBD in two countries. It has shown that vomiting, diarrhoea,

anorexia and dental disease are common clinical signs before diagnosis and that stress appears to be associated with the disease. The higher prevalence of skin disease and respiratory disease in cats with IBD than control cats follows the human IBD pattern. The study has also shown a considerable variation between the United States and New Zealand in the lifestyle, veterinary care and diet of the cat populations in these countries. However, none of these factors proved to be significantly associated with the disease.

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APPENDIX

TO CHAPTER 3

FELINE IBD EPIDEMIOLOGY QUESTIONNAIRE

ID No:.....

Owner's name:.....

Address:.....
.....
.....

Telephone No.:.....

Cat name:.....

Date:.....

Please if your cat is affected by IBD remember that the following set of questions relate mainly to the year BEFORE your cat was diagnosed with the disease or the time immediately preceding a diagnosis of IBD if your cat is younger than 1.5 years old.

1.- **How old is your cat now?** Estimate if you do not know.

Age:..... Indicate if this is an estimation []

2.- **What weight is your cat?**

.....

3.- **Do you consider your cat to be**

- Markedly overweight? []
- A little overweight? []
- Average []
- Lean []

4.- **Do you personally feed your cat?**

Yes No

5.- **How many times a day do you feed your cat?**

Once
Twice
Three or more
Food is always available

6.- **How often and what does your cat hunt?** e.g. moths, birds, cicadas, flies, mice, rats, other

.....
.....
.....

7.- **Does your cat eat the prey?**

No
Only part of it Specify
Completely

8.- **Is your cat** (more than one answer allowed)

A fast eater?
Average?
A slow eater?
A fussy eater?
Not fussy at all?

9.- **a)Indicate the type (canned, biscuits, roll, other), brand and flavour of the commercial foods that your cat eats in decreasing order of importance according to the amount consumed per week**

b) Indicate the type of meat or meat by-products (kidney, liver, meat, lung, heart, etc.), the animal it comes from (beef, chicken, lamb, mutton, pork, fish, etc.), and the amount. If cooked specify.

c) Indicate other foods like cheese, butter, fat trimmings, ice cream, eggs, casserole, battered fish, grease/gravy, vegetables, cat treats, etc. that your cat eats.

10.- **a) Considering all the food that your cat receives IN ONE DAY, could you indicate the amount of the following food types that make up what your cat eats per day. Use a scale of less than 1/4 (less than 25 %), 1/4 (25 %), 1/2 (50 %), 3/4 (75 %) or all.**

b) Estimate the daily quantities of foods.

11.- a) Does your cat suffer from any disease or problem?.

Yes No

b) If yes, does your cat require a special diet or medicine for this condition?

Yes Specify No

.....
.....
.....

12.- Do you give your cat any extra vitamins, mineral, kelp, or extracts or add salt, oil or any other substance to your cat's diet? Please specify how much and how often

Yes No

.....
.....

13.- Does your cat obtain food from rubbish bin/trash cans?

Yes No

14.- Does your cat drink water?

Very often (more than 3 times a day)
Often (2-3 times a day)
Sometimes (2-3 times a week)
I am unsure. I don't know

15.- From where does your cat drink water? e.g. bowl, pots, shower, toilet, rain puddles, swimming pool, garden pond, etc.

.....
.....

16.- What type of water does your cat drink (city, well, filtered, rain, etc.)?

.....
.....

17.- Does your cat drink milk? If yes, what type (cow, lactose reduced, etc)?

Yes [] No []

.....
.....

18.- If yes, how much (in cups) and how often does your cat drink milk?

.....
.....

19.- If no, why don't you give milk to your cat?

.....
.....

20.- How do you store your cat's food?

.....
.....

21.- For how long on average do you store your cat's food once the container is opened?

- Dry.....
- Canned Food.....
- Pet Roll.....
- Plastic tab.....
- Fresh meat.....
- Cat treats.....

22.- Do you throw away any old food left in the dish before adding new food?

Yes [] No []

23.- What breed is your cat?

- Purebred Specify.....
Crossbred Specify.....
Domestic
-

24.- a) How long is the hair of your cat?

- Long
Medium
Short

b) What colour is your cat's coat? Please refer to the main colour

- Black
White
Grey
Tabby
Tortoise shell
Ginger
Mixed Specify
Other Specify
-

25.- What sex is your cat?

- Male Female
-

26.- Has your cat been desexed? At what age?

- Yes
No
-

27.- How long have you owned your cat?

- Since being a small kitten
Other (specify years)
-

28.- **Where do you live?**

- | | | | |
|-----------------------|--------------------------|-------------|--------------------------|
| City | <input type="checkbox"/> | Industrial | <input type="checkbox"/> |
| | | Residential | <input type="checkbox"/> |
| | | Perimeter | <input type="checkbox"/> |
| | | Rural | <input type="checkbox"/> |
| Town | <input type="checkbox"/> | | |
| Country or small town | <input type="checkbox"/> | | |
| Farm | <input type="checkbox"/> | | |

29.- **How long have you been living at your present address?**

.....

30.- **How many times have you moved house with your cat in the last five years?**

.....

31.- **Have you owned any other cat/s at the same time as this cat? Specify**

.....

32.- **Have you owned any other (non cat) pets at the same time as this cat? Specify**

.....

33.- **Has your cat been boarding in a cattery?**

- | | |
|------------------------------|--------------------------|
| Never | <input type="checkbox"/> |
| Once a year or less | <input type="checkbox"/> |
| Two or three times a year | <input type="checkbox"/> |
| More than 2 - 3 times a year | <input type="checkbox"/> |

34.- **Do you show your cat?**

No
Once a year or less
Two or three times a year
More than 2 - 3 times a year

35.- a) **Is your cat or (has it been) exposed to cigarette smoke?**

Yes No

b) **If yes, how often did it occur?**

Weekly or monthly
Daily

c) **For how long has the exposure to cigarette smoke occurred?**

All the cat's life
For up to two years
For two to five years
For more than five years

36.- **Do you have a cat door?**

Yes No

37.- **Do you consider your cat to be...?**

Predominantly an outside cat
Predominantly an inside cat
Varies with season

38.- **Does your cat fight? How often?**

Never
Once a month or less
Once a week or more

39.- **Do you consider your cat to be mostly** (more than one answer is allowed)

- Aggressive?
- Placid?
- Nervous?
- Affectionate?
- Grumpy?
- Independent?

40.- **Is your cat**

- Inactive?
- Relatively inactive?
- Active?
- Very active?

41.- **Does your cat sleep in/on your bed?**

- Yes
- No

42.- **How many times has your cat been at the vet clinic in the last 2 years?** If only for vaccination specify.

.....

43.- **How often is your cat vaccinated?**

- Never
- Every year
- Every 2 years
- Other Specify.....

44.- **What vaccinations has your cat received?**

- Routine (Rhinitis/Enteritis)
- Chlamydia
- Leukaemia
- Rabies

45.- **How often do you worm your cat?**

- Never
- Every 2 years
- Every year
- Twice a year
- Other Specify.....

46.- **What product do you use to worm your cat?**

.....

47.- **Has your cat ever had any of the following signs of disease ?**

- Vomiting
- Diarrhoea
- Loss of appetite
- Adverse reaction to a food
- Dental/Gum disease
- Skin problems
- Was this flea allergy? NA
- Joint disease
- Runny eyes/nose
- Asthma
- Cough/sneezes
- Excessive urination (if known)
- FIV/FeLV test Do not know

FOR IBD CASES ONLY

Date of diagnosis:.....

Age:.....

A.- a) **Do you understand what *Inflammatory Bowel Disease (IBD)* means?**

- Yes
- No

b) **What do you believe is the cause of IBD?**

B.- Could you relate the start of IBD in your cat to some special event?

Yes Specify No

.....

C.- If your cat had signs of disease for a while before being diagnosed, what age was your cat when you observed the first signs? Specify

Age:.....

Signs:.....

.....

D.- a) What season was it when your cat had the first signs of IBD?

Winter

Spring

Summer

Autumn

b) When do signs more often reoccur?

Winter

Spring

Summer

Autumn

E.- a) Do you know about the health of any of your pet's litter mates?

Yes No

b) If yes, have any other litter-mates been affected with IBD or chronic vomiting or diarrhoea?

Yes No

F.- How many bouts of IBD has your cat had?

.....

G.- How was your cat treated?

a) Diet Specify ingredients or brand, type and flavour

1st bout:.....

2nd bout:.....

3rd bout:.....

b) Prednisone Specify dose

1st bout:.....

2nd bout:.....

3rd bout:.....

- c) Sulphasalazine 1 2 3
- d) Cisapride 1 2 3
- e) Sucralfate 1 2 3
- f) Metoclopramide 1 2 3
- e) Bismuth subsalicylate 1 2 3
- e) Other Specify

.....

H.- How long did it take for the treatment to take effect?

- Within 24 hs 1 2 3
- Within 4 days 1 2 3
- Within 7 days 1 2 3
- Within 14 days 1 2 3
- More 1 2 3

I.- What was the eventual outcome?

Cured

Controlled but requires special diet	<input type="checkbox"/>
Controlled but requires medication	<input type="checkbox"/>
Controlled but requires medication + special diet	<input type="checkbox"/>
Not controlled (signs persist)	<input type="checkbox"/>
Died/euthanasia because of IBD	<input type="checkbox"/>
Died of unrelated cause	<input type="checkbox"/>

J.- What signs did your cat have?

a) Diarrhoea Yes 1 2 3

Colour: 1.....2.....3.....

Volume: 1.....2.....3.....

Consistency: 1.....2.....3.....

Motions/day: 1.....2.....3.....

GRADE: 1.....2.....3.....

b) Vomiting Yes 1 2 3

Colour: 1.....2.....3.....

Volume: 1.....2.....3.....

What: 1.....2.....3.....

Vomits/day: 1.....2.....3.....

c) Appetite change Yes 1 2 3

Increased: 1.....2.....3.....

Decreased: 1.....2.....3.....

d) Weight loss Yes 1 2 3

e) Flatulence Yes 1 2 3

f) Straining Yes 1 2 3

g) Blood in faeces Yes 1 2 3

h) Mucus in faeces Yes 1 2 3

i) Increased defecation
frequency Yes 1 2 3

j) Abdominal pain Yes 1 2 3

k) Urge to defecate Yes 1 2 3

l) Abdominal bloating Y 1 2 3

m) Changed colour of
faeces Yes 1 2 3

n) Borborygmus Yes 1 2 3

K.- Had your pet been treated with any drugs within the 6 months prior to IBD?

Yes Specify No

.....
.....
.....

L.- How long is your cat healthy between bouts of IBD?

.....
.....

M.- Has medication and/or diet changed the time in between bouts?

Increased
Decreased
No change

N.- What special diet and medication does your cat need today (if any)?

.....
.....
.....
.....
.....

Add BIOCHEMISTRY TESTS, ENDOSCOPY and BIOPSY results

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
1 Stomach, 4 pieces: no significant lesions. Small Intestine, 8 pieces: There is moderate, diffuse expansion of the lamina propria by a mixture of plasma cells with fewer lymphocytes. Individual intraepithelial lymphocytes are common. Colon, 1 piece: no significant lesions.	Moderate	8y	NUMEROUS	NOT CONTROLLED	N	Food (minutes after eating) once a day, liquid or grass once a week	Increased appetite. Increased fluid content in the gut	Gastric granularity (Mild-Grade 1)
2 Stomach, 7 pieces: Spiral shaped, Warthin Starry positive organisms are entrapped in the mucus at the surface. Small Intestine, 7 pieces: There is moderate, diffuse expansion of the lamina propria by an infiltrate of plasma cells, lymphocytes, and eosinophils. There are few, scattered intraepithelial lymphocytes. Colon, 1 piece: There is a slight infiltrate of plasma cells with fewer lymphocytes in the lamina propria.	Moderate	6.6y	NUMEROUS	CONTROL WITH DIET	N	Food or fluid once a day. Scavenges for dry food	Decreased appetite and weight loss. Thickened area in mid-abdomen	Increased granularity and friability of the intestinal mucosa
3 Stomach, 8 pieces: Few individual spiral shaped organisms are en-trapped in the superficial mucus. No significant lesions. Small Intestine, 10 pieces: There is slight to moderate expansion of the lamina propria by plasma cells and lymphocytes. Individual and small aggregates of intraepithelial lymphocytes are common. Anorectal Junction, 1 piece: no significant lesions	Moderate	9y	ONE	CONTROL WITH DIET	N	Fluid. food (hours after eating), grass, fur, milk 2-3 times per week	Mildly depressed-irritable. Depressed appetite	Mild gastric erosions and mild duodenal granularity

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>4 Stomach, 5 pieces: In two pieces there is a slight to moderate, dif-fuse infiltrate of small lymphocytes with fewer plasma cells among them. In one piece the infiltrates are multifocal. The remaining two pieces are not affected. Small Intestine, 4 pieces: There is moderate, diffuse expansion of the lamina propria by a mixture of plasma cells and lymphocytes. Intraepithelial lymphocytes are common, both individually and in small aggregates. There are few regions of locally extensive infil-tration. Colon, 1 piece: There is mild edema and a focal aggregate of lym-phoid cells.</p>	Moderate	13y	ONE	CONTROL WITH DIET	N	Bile or food, small volume, hours after eating	Weight loss	Stomach and duodenum friable
<p>5 Stomach, 6 pieces: no significant lesion. Small Intestine, 6 pieces: There is moderate, diffuse expansion of the lamina propria by a mixture of plasma cells with fewer small lym-phocytes. There are scattered, individual intraepithelial lympho-cytes. Colon, 2 pieces: There is moderate, diffuse expansion of the lamina propria by plasma cells, lymphocytes, and fewer eosinophils and neu-trophils. There are aggregate of gut associated lymphoid tissue. Colon, 2 pieces: There is slight to moderate expansion of the lamina propria by edema and an infiltrate of plasma cells, lymphocytes, and few neutrophils.</p>	Moderate	12y	6 PER YEAR	DIED/ RELATED TO IBD	Yellow Brown. grade 1. 4-5 per day	N	Thickened bowel loops & gaseousness. Polydipsia. Mucus and blood. Weight loss	Pronounced antral mucosal folds and narrowed pyloric cord- colon friable

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>6. Stomach, 6 pieces: There are two discrete aggregates of lympho-cytes in the deep mucosa. Small Intestine, 5 pieces: There is minimal expansion of the lamina propria due to an infiltrate of plasma cells and lymphocytes. There are scattered individual intraepithelial lymphocytes and globule leu-kocytes. Colon, 1 piece: There is moderate, diffuse expansion of the lamina propria by an infiltrate of plasma cells with fewer lymphocytes, and scattered neutrophils. There are individual, intraepithelial lympho-cytes.</p>	Moderate	6m	ONE	DIED/ UNRELATED CAUSE	Large volume, watery, 6 per day	N	Polydipsia. Enlarged submandibular lymph nodes. Hepatomegaly. Abdominal pain. Flatulence. Weight loss. Increased appetite. Depressed.	NAF
<p>7. Stomach, 4 pieces: There is mild, diffuse separation of glands by loose connective tissue. There are small aggregates of lymphocytes and plasma cells in the superficial lamina propria. Small Intestine, fragments: The lamina propria of fragments of mucosa is expanded by a mixture of plasma cells and lymphocytes with few eosinophils and neutrophils. Colon, 2 piece: There is mild diffuse edema and few, small aggre-gates of plasma cells, lymphocytes, and neutrophils in the lamina pro-pria.</p>	Moderate	12y	CONTINUOUS	EUTHANIZED BECAUSE OF IBD	Grey, soft-liquid, 4-5 per day	Food or foam, hours after eating. 2-3 times a day	Polydipsia. Depressed. Abdominal bloating. Weight loss and increased appetite	Gastric Granularity

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
8 Stomach, 8 pieces: no significant lesions. Small Intestine, 7 pieces: There is moderate to severe expansion of the lamina propria by a mixture of lymphocytes and plasma cells with few neutrophils and rare eosinophils. Intraepithelial lymphocytes are very common. There is regionally extensive infiltration, as well as small aggregates or individual cells. Colon, 2 pieces: There is slight to moderate expansion of the lamina propria by edema and a mixture of plasma cells, lymphocytes, and few neutrophils.	Severe	14y	CONTINUOUS	EUTHANIZED BECAUSE OF IBD	N	N	Weight loss. Increased defecation frequency. Changed colour of faeces to grey	Fine gastric granularity and erosions-moderate duodenal granularity and mild erosions
9 Stomach, 7 pieces: no significant findings. Small Intestine, 3 pieces: There is moderate to severe expansion of the lamina propria by an almost homogeneous population of lymphocytes with scattered eosinophils and few plasma cells among them. Multifocal infiltration of the overlying epithelium is common. Colon, 1 piece: There is slight, diffuse edema and scattered plasma cells, lymphocytes, eosinophils, and lymphocytes in the lamina propria.	Severe	9y	NUMEROUS	CONTROL BY DIET AND MEDICATION	N	Fluid or fur. large volume, hours after eating. 3-5 a week. Progressed to include food (hours after eating), once a day	Thickened bowel loops. Thyroid nodule. Upper respiratory wheezes. Mildly depressed-irritable. Decreased appetite and weight loss	Cobbled stoned appearance of duodenum and mildly friable

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
10 Stomach, 5 pieces: Granular debris and few spiral shaped organisms are entrapped in the mucus adhered to the surface. Small Intestine, 2 pieces: There is expansion of the lamina propria due to moderate, diffuse edema and an infiltrate composed mostly of plasma cells with fewer lymphocytes. Intraepithelial lymphocytes are common. Colon, 1 piece: There is a slight, diffuse infiltrate of plasma cells and fewer lymphocytes in the lamina propria. There is a focus of gut associated lymphoid tissue.	Moderate	2y	NUMEROUS	NOT CONTROLLED	Fluid diarrhoea	Vomit minutes after eating	Increased defecation frequency. Blood in faeces	NAF
11 Stomach, 8 pieces: no significant lesions. Small Intestine, 4 pieces: There is slight to moderate expansion of the lamina propria by a mixture of plasma cells and fewer lymphocytes. There are scattered individual intraepithelial lymphocytes. Anorectal Junction, 1 piece: no significant lesions	Moderate		NUMEROUS	NOT CONTROLLED	N	Food or fluid (hours after eating) 2-3 times a day	Increased appetite. Weight loss. Poor coat. Straining	NAF
12 Intestine, fragments: There is moderate, diffuse expansion of the lamina propria by a mixture of plasma cells and lymphocytes. Eosino-phils are common in some pieces and less numerous in others. There are scattered individual intraepithelial lymphocytes.	Moderate	8Y10M	NUMEROUS	NOT CONTROLLED	N	Vomit but unsure of contents, fir?	Slight dehydration. Weight loss	Duodenum: erythema, friable, cobblestone/mosaic appearance

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
13 Stomach, 2 pieces: no significant lesions. Small Intestine, 1 piece: There is moderate, diffuse expansion of the lamina propria by an infiltrate of plasma cells with fewer lymphocytes and occasional neutrophils. Intraepithelial lymphocytes are common.	Moderate	13Y	10	CONTROL WITH DIET	Y	Vomit but unsure of contents, water?, four times a week	Weight loss. Decreased appetite. Abdominal pain. Thyroid nodule. Heart murmur	NAF
14 Pylorus, 4 pieces: There is slight, diffuse edema. Few small focal aggregates of mixed inflammatory cells in the mucosa. There is also a small "pinch" of small interstitial villi in which the mucosal lamina propria is heavily infiltrated with inflammatory cells, primarily eosinophils.	Mild	12Y	6	CONTROL WITH DIET	N	Bile or food (variable time after eating), once a week	Weight loss	Thickening and redness at pylorus-hard to pass endoscope
15 Stomach, 2 full sickness: few aggregates of lymphocytes are in the lamina propria. Small Intestinal, 5 full thickness: there is slight to moderate expansion of the lamina propria by plasma cells and lymphocytes with occasional lymphocytes among the epithelial cells. In one section villi appear slightly blunted, but this could be an artefact.	Moderate	9Y	ONE	EUTHANIZED BECAUSE OF IBD	Large volume, watery, smelly. 3-4 per day	Food (30 minutes after eating)	Weight loss. Faecal incontinence	NAF
16 Small intestine, 2 pieces: there is slight widening of the lamina propria by lymphocytes and fewer plasma cells. Individual lymphocytes are scattered among the overlying epithelial cells. There is slight edema.	Mild	14Y	Don't know	CONTROL WITH DIET	N	Food immediately after eating. Reduction in frequency occurred with time	Abdominal pain. Weight loss.	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
17 Stomach, 1 piece: there are prominent lymphoid aggregates in the deep mucosa. Many bacteria (most appear to be rods, but some could be coiled (i.e. <i>Helicobacter</i> sp.). Small Intestine, 2 pieces: there is slight widening of the lamina propria by edema and a cellular infiltrate consisting mostly of plasma cells with fewer lymphocytes and scattered eosinophils. Occasional lymphocytes are among the overlying epithelial cells.	Moderate	4Y	Don't know	EUTHANIZED BECAUSE OF IBD	N	Fur, food, fluid, grass at variable times after eating. 1 in 3-4 months	Weight loss. Decreased appetite. Less active. Gaseous intestines in the past	NAF
18 Small Intestine, 6 pieces: there is slight widening of the lamina propria by edema and a slight increase in plasma cells and lymphocytes. There is moderate edema of the submucosa.	Mild	9Y	Don't know	EUTHANIZED BECAUSE OF IBD	N	Occasionally vomits soon after eating	Weight loss. Decreased appetite.	NAF
19 Stomach, 2 full thickness: no significant findings. Small Intestine, 3 full thickness: the lamina propria is moderately diffusely expanded by plasma cells with fewer lymphocytes and eosinophils. There are scattered lymphocytes among the epithelial cells. In few villi there is an increased amount of pink stained material in the lamina propria among the inflammatory cells.	Moderate	7Y	Don't know	CONTROL WITH DIET	N	Y	N	NAF
20 Stomach, 2 pieces: no significant lesions. Small Intestine, 4 pieces: there is slight to moderate expansion of the lamina propria by plasma cells and fewer neutrophils. There is slight edema and rare dilation of lacteals.	Moderate	2.5Y	NUMEROUS	CONTROL WITH DIET	N	Cat biscuits, fresh meat, 3 times a week	Abdominal pain. Leukopenia and thick bowel	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
21 Stomach, 1 piece: no significant findings. Small Intestine, 5 full thickness: the lamina propria is moderately widened by edema and a cellular infiltrate composed mostly of plasma cells with few lymphocytes. Colon, 1 piece: the cellularity of the lamina propria is normal. There is a prominent lymphoid follicle (gut associated lymphoid tissue).	Mild	10Y	ONE	CONTROL WITH DIET	N	Food immediately after eating.	Decreased appetite	ND
22 Small Intestine, 2 pieces: there is moderate diffuse expansion of the lamina propria by plasma cells, lymphocytes, a fewer neutrophils. Lacteals are slightly to moderately dilated and contain pink, sparsely cellular material. There is regional extensive lymphocytic infiltration of the epithelium. Pancreas, 2 pieces: there is regionally extensive infiltration by lymphocytes and neutrophils. There is associated atrophy of acini.	Moderate	10Y	Don't know	CONTROL WITH DIET	N	Y	N	ND
23 Stomach, 1 piece: no significant findings. Small Intestine, 1 piece: there is slight expansion of the lamina propria by plasma cells and fewer lymphocytes with scattered neutrophils and rare eosinophils. Occasional lymphocytes are among the overlying epithelial cells.	Mild	5Y	NUMEROUS	CONTROL BY MEDICATION	N	Food or water 20 minutes after eating or drinking	N	ND

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
24 Stomach, 1 piece: no significant lesions. Small Intestine, 2 pieces: there is slight widening of the lamina propria due to an infiltrate composed mostly of plasma cells with fewer lymphocytes. There is slight edema. Colon, 1 piece: no significant lesions.	Mild	2Y	ONE	CONTROL BY MEDICATION	N	Food (20 minutes after eating), 2-3 per day	Weight loss. Decreased appetite. Dermatitis around the neck. Scratching.	ND
25 Small Intestine, 3 pieces: there is moderate expansion of the lamina propria by a mixture of plasma cells, lymphocytes, and eosinophils. There is slight oedema. Few lymphocytes are scattered among the overlying epithelial cells.	Moderate	12Y	ONE	CURED	N	White foam, bile, food (20 minutes after eating), 6 times a day	Weight loss.	ND
26 Small Intestine, 2 pieces: there is moderate expansion of the lamina propria by oedema and an infiltrate composed mostly of plasma cells and few lymphocytes. Rare lymphocytes are scattered among overlying epithelial cells.	Moderate	17Y	ONE	EUTHANIZED BECAUSE OF IBD	N	Small volume, food or fluid 30 minutes after eating, once a day. Progressed to 2 per week	Decreased appetite. Thickened intestines. Depressed.	ND
27 Stomach, 1 piece: no significant lesions. Small Intestine, 1 piece: the lamina propria is widened due to oedema and an infiltrate composed mostly of plasma cells with fewer lymphocytes and scattered eosinophils. Scattered lymphocytes are among the overlying epithelial cells.	Moderate	8M	ONE	CONTROL BY DIET AND MEDICATION	N	Small volume, food, grass, fluid at variable times after eating once a week. Progressed to 4 per day	Abdominal pain	ND

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>28</p> <p>Lymph Node, 1 piece: no significant lesions. Small Intestine, 1 piece: there is moderate widening of the lamina propria due to edema and a cellular infiltrate composed of plasma cells, lymphocytes, and rare neutrophils. Lymphocytes are common among the overlying epithelial cells. Colon, 1 piece: there is regionally extensive superficial necrosis. There is moderate to marked widening of the lamina propria due to an infiltrate composed mostly of neutrophils. Eosinophils are among them. There are regions in which plasma cells and lymphocytes are more common, but these tend to be over regions of gut associated lymphoid tissue. The gut associated lymphoid tissue is prominent and there is increased cellularity in the lamina propria between the follicles. This infiltrate is composed of monocuclear cells with many eosinophils and neutrophils. The intestinal epithelium is hyperplastic and there are scattered lymphocytes, eosinophils, and neutrophils among them.</p>	Severe	9Y	NUMEROUS	CURED	N	N	Weight loss. Straining. Blood in the faeces. Prolapse. Intusuception.	ND
<p>29</p> <p>Small Intestine, 3 pieces: there is slight, diffuse edema and an infiltrate of plasma cells with fewer lymphocytes and scattered eosinophils in the lamina propria</p>	Mild	14Y	CONTINUOUS	CONTROL BY DIET AND MEDICATION	N	Undigested food immediately after eating once a day. Progressed to 1 every 4 days	Weight loss. Depressed	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
30 Stomach, 2 pieces: no significant lesions. Small Intestine, 2 pieces: there is slight to moderate widening of the lamina propria by plasma cells with fewer lymphocytes. Lymphocytes are common among the overlying epithelial cells.	Moderate	9Y	10 PER YEAR	CONTROL WITH DIET	Pasty, yellow, small volume, 1 per day	N	Decreased appetite. Weight loss. Increased defecation frequency. Abdominal bloating. Depressed	NAF
31 Stomach, 1 piece: no significant lesions. Small Intestine, 1 piece: There is gut associated lymphoid tissue. The lamina propria is expanded by edema and a slight to moderate infiltrate of plasma cells, fewer lymphocytes, and scattered eosinophils.	Moderate	8Y	ONE	CONTROL BY MEDICATION	N	Food or fur, once every two weeks	Weight loss. Heart murmur	ND
32 Small Intestine, 3 pieces: There is moderate, diffuse expansion of the lamina propria by plasma cells, lymphocytes, and few eosinophils or neutrophils. Lymphocytes are common among the overlying epithelial cells.	Moderate	7Y	CONTINUOUS	CONTROL WITH DIET	N	Food immediately after eating, once a month. Progressed to 2-3 per week	Decreased appetite. Depressed.	ND
33 Small Intestine, full thickness, 4 pieces: There is expansion of the lamina propria due to edema and an infiltrate of lymphocytes, plasma cells, and eosinophils, with fewer neutrophils. There are few intra-epithelial lymphocytes.	Moderate	10Y	10	CONTROL WITH DIET	N	N	Decreased appetite. Depressed after eating. Ropy gut	ND

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
34 Small Intestine, full thickness, 2 pieces: There is moderate to severe expansion of the lamina propria due to an infiltrate of lymphocytes and plasma cells. There are few neutrophils and eosinophils. There is locally extensive infiltration of the overlying epithelium.	Severe	10Y	3 PER YEAR	EUTHANIZED BECAUSE OF IBD	Large volume, dark brown, slightly formed, twice a day four times a week	Food or grass, yellow colour, immediately after eating, 2-3 per day for 2 days	Borborygmus. Fluid contents in the small intestines	ND
35 Small Intestine, full thickness, 3 pieces: There is moderate, diffuse expansion of the lamina propria by edema and a mixture of lymphocytes and plasma cells. There is multifocal to regionally extensive infiltration of the overlying epithelium.	Moderate	16Y	2	CONTROL WITH DIET	N	Grass, food, once every two days when constipated	Decreased appetite. Straining.	ND
36 Stomach, full thickness, 1 piece: no significant lesions. Small Intestine, full thickness, 1 piece: There is moderate, diffuse expansion of the lamina propria due to edema and an infiltrate composed of plasma cells and lymphocytes with few, scattered eosinophils. Colon, full thickness, 1 piece: no significant lesions	Moderate	3Y7M	4	CONTROL BY DIET AND MEDICATION	Yellow, watery, constant	Food, small volume, yellow, immediately after eating, several per day	N	ND

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
37 Small Intestine, 1 piece: there is moderate to severe widening of villi due to an infiltrate composed mostly of lymphocytes with fewer plasma cells, scattered macrophages, and neutrophils. The cellular infiltrate is less dense at the base of the glands due to edema. Lymphocytes and neutrophils are common among epithelial cells, particularly at villous tips.	Moderate	13y6m	2	DIED/ UNRELATED CAUSE (CHRONIC CYSTITIS)	N	Food or fur, once every 10 days. Frequency increased with time	Decreased appetite. Weight loss. Enlarged mesenteric lymph nodes.	ND
38 Small Intestine, 7 endoscopic pieces: There is slight to moderate expansion of the lamina propria by plasma cells, fewer lymphocytes, and scattered eosinophils and neutrophils. There is mild edema.	Moderate	5y	2	CONTROL BY DIET AND MEDICATION	Watery, once a day	Clear fluid, grass or food, 3-4 per day. Progressed to 1-2 per day	Slightly thickened bowel loops	Duodenum inflamed, excessive bleeding. No normal collapse (fibrosis?)
39 Stomach, fragments. No significant lesions Small Intestine, 4 endoscopic pieces: The lamina propria is slightly oedematous. The cellularity appears within normal range, but small lymphocytes are fairly common among overlying epithelial cells. Colon, 7 endoscopic pieces: Lymphocytes extend from submucosal lymphoid follicle into the overlying lamina propria. There is mild edema, but the cellularity of the lamina propria appears to be within normal limits..	Mild	8y	4	CONTROL BY DIET AND MEDICATION	Soft or watery	Occasionally	Weight loss. Blood in faeces. Depressed. Facial and scapular muscle atrophy. Licks belly (bold) before getting sick	Stomach Normal. Duodenum friable. Colon normal

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
40 Stomach, 6 endoscopic pieces. Aggregates of lymphocytes are within the deep mucosa. Small Intestine, 2 endoscopic pieces. The lamina propria is slightly expanded due edema and an infiltrate composed mostly of plasma cells with fewer lymphocytes and scattered eosinophils and neutrophils.	Mild	4y	NUMEROUS	CONTROL BY DIET AND MEDICATION	N	Fur. food or fluid. 1-2 per week. Progressed to food with bile, 1-2 per day	Failure to grow	NAF
41 Stomach, 7 endoscopic pieces. No significant lesions. Small Intestine, 7 endoscopic pieces plus fragments: There is moderate diffuse expansion of the lamina propria by plasma cells and fewer eosinophils. Individual or small clusters of mast cells are widely scattered. There are fewer lymphocytes.	Moderate	5y	ONE	CONTROL WITH DIET	N	Clear fluid, small volume, 1-2 per day	Thickened bowel loops. Depressed	Stomach mild erythema and granularity. Scant mucus, few vessels visible. slow to insufflate. Duodenum Idem
42 Small Intestine, 8 endoscopic pieces plus fragments: A mixture of plasma cells and lymphocytes causes diffuse, moderate expansion of the lamina propria. Plasma cells predominate in some regions and lymphocytes in others. There are scattered eosinophils and neutrophils, either individually or in small aggregates. Intraepithelial lymphocytes are common in some sections.	Moderate	15Y	CONTINUOUS	DIED/D	N	Dry food and saliva, once a week	Decreased and increased appetite at different times. Weight loss. Depressed. Bowel distended. Thyroid nodules. Transient diabetic. Polyuria/polydipsia.	Duodenum granular and friable

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
43 Stomach, 7 endoscopic pieces: Large, coalescing aggregates of lymphocytes are within the lamina propria. In some regions the cells are ruptured and distorted. Small Intestine, 7 endoscopic pieces: There is moderate diffuse expansion of the lamina propria by plasma cells with fewer lymphocytes among them. Intraepithelial lymphocytes are common in some regions.	Moderate	13.5Y	NUMEROUS	NOT CONTROLLED	N	Food (20 minutes after eating), small volume,	N	Stomach mild erythema, friability and mucus. Vessels visible Duodenum mild to moderately friable and few vessels visible.
44 Stomach, 9 endoscopic pieces: There are small aggregates of lymphocytes in the deep mucosa. Small Intestine, 2 endoscopic pieces plus fragments: The lamina propria is expanded by mild edema and an infiltrate of plasma cells with few lymphocytes. Small lymphocytes are common among the overlying epithelial cells.	Mild	8Y	NUMEROUS	EUTHANIZED BECAUSE OF IBD	Watery	Food (5 minutes after eating), occasionally	weight loss. Flatulence. Blood in the faeces and increased defecation frequency. Urgency to defecate. Abdominal pain and abdominal bloating. Thickened bowel loops	Stomach mild to moderate granularity, mild erosions, mild friability. Duodenum mild to moderate granularity, mild erosions, mild friability
45 Stomach, 8 endoscopic pieces: There is a focal infiltrate of lymphocytes and plasma cells with few neutrophils in the superficial mucosa. The underlying glands are dilated with mucus. Small Intestine, fragments of villi: There is minimal expansion of the lamina propria by a mixture of small lymphocytes and plasma cells. Lymphocytes are scattered among the overlying epithelial cells.	Mild	10Y	5 PER YEAR (SUMMER)	CONTROL WITH DIET	N	Occasionally vomits medication	Obesity. Asthma. Urinary Tract Infection. Stomach hair ball/mass	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
46 Intestine, 1 endoscopic piece: There is moderate diffuse expansion of the lamina propria due to an infiltrate of plasma cells with widely scattered neutrophils and fewer eosinophils and lymphocytes.	Moderate	6Y	ONE	CONTROL WITH DIET	Soft faeces	Y	Blood and mucus in faeces. Obesity	Colon moderate granularity, mild erosions, mild friability, moderate mucus
47 Stomach, 7 endoscopic pieces: No significant lesion. Small Intestine, 1 endoscopic piece: There is moderate diffuse expansion of the lamina propria by plasma cells with few lymphocytes and scattered small aggregates of eosinophils and neutrophil. Occasional globule leukocytes are prominent.	Moderate	9y	CONTINUOUS	EUTHANIZED BECAUSE OF IBD	Yellow with blood, soft faeces, twice a day	Clear fluid, 2 per week	Decreased appetite and weight loss. Straining and blood in the faeces. Mildly thickened bowel loops	Gastric folds oedematous & thick Duodenum granular, difficult to inflate Colon slight erythema

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Voniting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>48 Small Intestine, 6 endoscopic pieces plus fragments: There is moderate diffuse expansion of the lamina propria by plasma cells with few lymphocytes and scattered eosinophils and neutrophils. Glands are occasionally dilated by mucus and two sections are surrounded by blood (this is unusual in endoscopic specimens).</p>	Moderate	8Y	FOUR	CONTROL WITH DIET	Large volume, watery	Food, fur, blood	Prominent bowel loops. Delayed gastric emptying.	<p>Two polyps in the pyloric antrum. Mild mucus, most vessels visible. Duodenum severe erythema, moderate erosions, moderate to severe granularity and friability. Mild/moderate mucus, mild-few to no vessels, mild poor insufflation. Colon poor preparation, moderate erythema and granularity, mild erosions, mild to moderate friability. Moderate to severe mucus. Few vessels visible, slow insufflation</p>

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>49 Stomach, 5 endoscopic pieces: Lymphoid aggregates are common within the deep mucosa. Stomach, 7 endoscopic pieces: No significant lesions. Small Intestine, 9 endoscopic pieces: There is moderate, diffuse expansion of the lamina propria by plasma cells, fewer lymphocytes, eosinophils, and occasional neutrophils. There is mild edema. Lymphocytes and, to a lesser extent, eosinophils and neutrophils are among the overlying epithelial cells. In some sections the proportion of goblet cells is decreased. Scattered glands are lined by flattened epithelial cells and are dilated by mucus.</p>	Moderate	6Y	ONE	CONTROL WITH DIET	N	Fluid or food once a day for 2 days	Heaving before vomiting	Stomach moderate to severe friability, mild mucus, few absent vessels, slow to insufflate. Corrugated antrum. Duodenum normal
<p>50 Stomach, 2 full thickness pieces (there are 2 pieces without mucosa). Aggregates of lymphocytes are within the deep mucosa. Small Intestine, 2 full thickness pieces: Plasma cells, lymphocytes and few scattered eosinophils and neutrophils cause slight to moderate expansion of the lamina propria.</p>	Moderate	11Y	CONTINUOUS	NOT CONTROLLED	Reddish, once	Food (minutes after eating) once a day	Oxalate stones-Chronic Renal Failure. Hyperdynamic cardiac function. PEG	ND

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>51 Stomach, 4 endoscopic pieces: No significant lesions. Small Intestine, 9 endoscopic pieces: There is moderate to severe expansion of the lamina propria due to an infiltrate of plasma cells, neutrophils, lymphocytes, and few scattered macrophages. There are occasional eosinophils. There is slight to moderate edema and occasional lacteals are dilated. Intraepithelial lymphocytes and neutrophils are common.</p>	Severe	10Y	CONTINUOUS	EUTHANIZED BECAUSE OF IBD	N	Food or fluid. 2-3 per day	Increased appetite and weight loss. Congenital heart disease. Thickened bowel loops. Enlarged mesenteric lymph nodes.	Duodenum oedematous mucosa
<p>52 Small Intestine, 1 endoscopic piece: There is moderate expansion of the lamina propria due to edema and an infiltrate composed of plasma cells with scattered lymphocytes, eosinophils, and neutrophils. Globule leukocytes are occasionally prominent. There is slight dilation of lacteals. Colon, 15 endoscopic pieces: There is moderate, diffuse expansion of the lamina propria by an infiltrate composed mostly of plasma cells with slightly fewer lymphocytes and scattered eosinophils and neutrophils. There are few, individual mast cells. Occasionally, globule leukocytes are prominent and scattered lymphocytes, neutrophils, and eosinophils are among the overlying epithelial cells. In some sections the proportion of goblet cells appears decreased. Scattered glands are dilated by mucus.</p>	Moderate		3	NOT CONTROLLED	N	N	Blood and mucus in faeces. Fever. Lymphocytosis. Another cat with FeLV in the same household	Colon normal

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
53 Small Intestine, 4 endoscopic pieces: There is widening of the lamina propria due to edema and a slight to moderate infiltrate composed of plasma cells with fewer lymphocytes among them. Lymphocytes are in low number among the overlying epithelial cells.	Mild	10Y	MULTIPLE	CONTROL BY DIET AND MEDICATION	N	N	Weight loss. Borborygmus. Hypertrophic Cardiomyopathy. Unilateral thyroid nodule. Enlarged lymph nodes. Eosinophilic plaque. Polycystic kidney. Hypocalcaemia	Duodenum pale, edematous, friable, cobblestoned
54 Stomach, 1 full thickness section: No significant lesions. Small Intestine, 1 full thickness section: There is moderate diffuse expansion of the lamina propria due to mild edema and an infiltrate composed mostly of plasma cells and fewer lymphocytes. Only rare lymphocytes are present among the overlying epithelial cells. There is slight dilation of lacteals. Small Intestine, 1 full thickness section. There is moderate diffuse expansion of the lamina propria due to an infiltrate of plasma cells and lymphocytes. Lymphocytes are common among the overlying epithelial cells. In the submucosa, small lymphocytes are mildly increased in number. They are diffusely distributed, with a slight increase in prominence around vessels.	Moderate	14Y	CONTINUOUS	CONTROL BY DIET AND MEDICATION	N	Food. 2-3 per day. PEG tube	Decreased appetite. Weight loss. Chronic renal failure. Microcytic anaemia. Systolic murmur.	NAF
55 Colon, 6 endoscopic pieces. An infiltrate of plasma cells causes very slight expansion of the lamina propria.	Mild		ONE	CONTROL WITH DIET	N	Fluid several hours after eating, once a week	Increased appetite. Blood and mucus in faeces. Obesity. Chronic renal failure.	Colon. Erythema with some erosions

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
56 Stomach, 3 endoscopic pieces: No significant lesions. Small Intestine, 3 endoscopic pieces: In two pieces there is moderate, diffuse expansion of the lamina propria by plasma cells with fewer lymphocytes and mild edema. Occasional lymphocytes are among the overlying epithelial cells. There are no significant lesions in the third piece of tissue.	Moderate	14Y	2-3 PER YEAR PROGRESSED TO CONTINUOUS	EUTHANIZED BECAUSE OF IBD	Large volume and watery	Fluid several hours after eating, once a day	Decreased appetite. Weight loss. Abdominal pain. Depressed. Bowel loops thickened. Fever. Severe leukopenia.	Stomach thick rugal folds, pylorus thickened, cardiac necrotic. Duodenum multiple erosions & plaques, fibrin appears to be present in debris, thickened, granular
57 Small Intestine, 8 endoscopic pieces: There is slight to moderate expansion of the lamina propria by plasma cells, fewer lymphocytes, and scattered neutrophils. There is a decrease in the proportion of goblet cells and epithelial cells near the surface appear slightly flattened. Mucus causes mild dilation of scattered glands.	Moderate	5Y	CONTINUOUS	DIED IBD	Y	N	Blood and mucus in faeces. Chronic impaction of anal glands. Inappropriate defecation and urination	Colon vessels easily visible, mild erythema, mild granularity, mild/moderate friability, mild/moderate mucus
58 Small Intestine, fragments. There is moderate expansion of the lamina propria by plasma cells, fewer lymphocytes, and neutrophils. Occasional lymphocytes and neutrophils are among the overlying epithelial cells. Globule leukocytes are occasionally prominent. The proportion of goblet cells appears decreased.	Moderate	3Y	CONTINUOUS	DIED IBD	N	Food once a day. Progressed to occasional vomit only	Decreased appetite and weight loss. Flea allergy and food allergy. Thickened bowel loops. Dehydration.	Stomach mild erythema, mild/moderate friability, slow to insufflate. Duodenum mild erythema, mild/moderate granularity, mild erosions, moderate friability, only partially insufflated

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>59 Small Intestine, 1 full thickness section: . There is marked diffuse expansion of the lamina propria by a heterogeneous infiltrate of inflammatory cells. Plasma cells predominate and there are slightly fewer lymphocytes. Eosinophils and neutrophils are present in much lower number, usually individually or in small aggregates. There is extensive infiltration of the epithelium mostly by lymphocytes and plasma cells and, to a lesser extent neutrophils and eosinophils. Villi appear blunted and there appears to be mild fibrosis. The epithelial cells are cuboidal to flattened. There are fewer than expected goblet cells.</p>	Severe	3Y	CONTINUOUS	DIED IBD	Constant, similar to fed gruel	Food 2-3 per day for two days of the week. Progressed to vomit after every meal	Decreased appetite. Weight loss. Depressed. Thickened gut. Mesenteric lymph nodes enlarged.	Stomach pale w/occasional mucosal folds, firm wall, difficult to insufflate
<p>60 Stomach, 3 endoscopic pieces: No significant lesions. Small Intestine, 10 endoscopic pieces: There is diffuse expansion of the lamina mostly by plasma cells and fewer lymphocytes. Lymphocytes are common in the lamina propria. There is a decreased proportion of goblet cells lining the villi.</p>	Severe	13Y	4	CONTROL BY DIET AND MEDICATION	Large volume, malodorous	Fur	Polyuria/polydipsia. Dehydration. Decreased appetite. Weight loss. Blood in faeces. Thickened bowel loops, enlarged lymph nodes	Stomach reddened, bleed excessively Duodenal dark purple-grey mucosa, bleed excessively

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Voniting	Other clinical signs and tests with abnormal results	Endoscopy findings
61 <p>Colon, 7 endoscopic pieces: There is slight to moderate expansion of the lamina propria by plasma cells and fewer lymphocytes. There is mild edema. One section includes a gut associated lymphoid nodule. Colon, 5 endoscopic pieces: Four of the 5 pieces are of normal cellularity. There is mild expansion of the lamina propria of one piece by plasma cells and few lymphocytes.</p>	Moderate	1Y	3	CONTROL WITH DIET	N	N	Blood in faeces	NAF
62 <p>Small Intestine, 3 full thickness pieces: There is slight to moderate widening of the lamina propria by plasma cells and fewer lymphocytes. Lymphocytes are occasionally among overlying epithelial cells. There is mild edema and slight dilation of lacteals. Small Intestine, 1 full thickness piece: similar to above. Colon, 1 full thickness piece: There is moderate diffuse expansion of the lamina propria by plasma cells and fewer lymphocytes. Globule lymphocytes appear increased. There are lymphocytes among overlying epithelial cells. The submucosa is slightly edematous and there is a slight to moderate infiltrate of plasma cells and lymphocytes.</p>	Moderate	7.5	ONE	EUTHANIZED BECAUSE OF IBD	Brown, large volume, fluid, 5 to 10 times a day	Food (1 hour after eating) once	Short ileum. No cecum. Short colon	ND

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
63 Small Intestine, 7 endoscopic pieces: There is slight, diffuse expansion of the lamina propria by plasma cells and fewer lymphocytes. Lymphocytes are occasionally present among the overlying epithelial cells.	Mild	5.5	ONE	NOT CONTROLLED	Bloody, stringy, once	Food or saliva (2 hours after eating) once every 2 days. Progressed to once every day	Decreased appetite. Weight loss. Blood in faeces. Depressed. Large liver. Icteric. Hepatic lipidosis	Stomach normal Duodenum granular, hyperaemic & very friable
64 Stomach, 5 endoscopic pieces: no significant lesions. Small Intestine, 1 endoscopic piece: There is very slight expansion of the lamina propria by plasma cells and few lymphocytes.	Mild	5.9	ONE	CURED	N	Food (1 hour after eating) once a day for a week	Decreased appetite and weight loss. Abdominal pain. Eosinophilia. Thickened bowel loops	Stomach pale, plaque lesions. Duodenum friable
65 Stomach, 1 full thickness piece: There are aggregates of lymphocytes in the mucosa. Small Intestine, 1 full thickness piece: There is slight to moderate expansion of the lamina propria by plasma cells, fewer lymphocytes, and eosinophils. The eosinophils are generally widely scattered, but sometimes form small aggregates.	Moderate	3	CONTINUOUS	CONTROL WITH MEDICATION	N	Foam or food frequently	Decreased appetite. Weight loss. Thickened Small Intestine NH4=82(N=11-35 uM/L)	ND
66 Stomach, 3 endoscopic pieces: There are few small aggregates of lymphocytes in the mucosa. Small Intestine, 7 endoscopic pieces: There is slight to moderate expansion of the lamina propria by plasma cells, with fewer lymphocytes, and widely scattered eosinophils.	Moderate	2	ONE	CONTROL WITH MEDICATION	N	Food (20 minutes after eating), 3 times a day	Weight loss. Thickened small intestine	Friable mucosa in S & D

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
67 Small Intestine, 2 full thickness pieces: There is moderate, diffuse expansion of the lamina propria by plasma cells and fewer lymphocytes. Colon, 1 full thickness piece: Gut associated lymphoid tissue is prominent, but the overall cellularity of the lamina propria is within normal limits.	Moderate	5	CONTINUOUS	CONTROL WITH MEDICATION	Soft to liquid, yellow, 4-5 times a day	Yellow, twice a day at the end of the disease	Increased or decreased appetite at different times. Weight loss. Flatulence. Straining. Blood and mucus in faeces. Increased defecation frequency. Depressed. Hepatomegaly and osteopenia. Increased liver enzymes. Oil covering faeces. Anaemia. Poor haircoat	ND
68 Stomach, multiple endoscopic pieces: There are few small aggregates of lymphocytes in the deep mucosa. Small Intestine, 3 endoscopic pieces: There is moderate, diffuse expansion of the lamina propria by plasma cells and fewer lymphocytes. There are rare eosinophils among these cells. Small lymphocytes are scattered among the overlying epithelial cells.	Moderate	11	CONTINUOUS	EUTHANIZED BECAUSE OF IBD	Very occasionally	Food or fluid occasionally	Increased appetite and weight loss. Colangiohepatitis	Duodenum pale and very friable
69 Stomach, 2 endoscopic pieces: no significant findings. Small Intestine, 2 endoscopic pieces: no significant findings. Colon, 2 endoscopic pieces: Few neutrophils are among a low number of lymphocytes and plasma cells within the lamina propria. Few neutrophils are among the overlying epithelial cells.	Mild	8M	NUMEROUS	CONTROL WITH DIET	Viscous-liquid, 10-12 times a day	N	Flatulence and straining. Blood and mucus in the faeces. Increased defecation frequency. Persistent leukocytosis-conjunctivitis. Cries during defecation and urination	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
70 Stomach, endoscopic fragments: non diagnostic. Small Intestine, 3 endoscopic pieces: There is slight to moderate expansion of the lamina propria by plasma cells with fewer lymphocytes. Lymphocytes multifocally infiltrate among the overlying epithelial cells.	Moderate	13	CONTINUOUS	NOT CONTROLLED	Grey, liquid, 3 times a day	Once a day for 3 days	Increased appetite. Weight loss. Depressed.	NAF. Huge hairball
71 Stomach, 2 endoscopic pieces: There are few small aggregates of lymphocytes in the deep mucosa. Stomach, 3 endoscopic pieces: no significant findings. Small Intestine, 2 pieces: There is minimal expansion of the lamina propria by few plasma cells and lymphocytes with few eosinophils among them.	Mild	11	CONTINUOUS	CONTROL BY DIET AND MEDICATION	Y	Fur, food or bile twice a day. Progressed to vomit fur once every 3 weeks	Decreased appetite. Heart murmur	Mild to moderate gastritis and mild duodenitis
72 Stomach, 2 endoscopic pieces plus fragments: no significant findings. Small Intestine, 6 endoscopic pieces: There is slight to moderate expansion of the lamina propria by plasma cells, lymphocytes, with scattered eosinophils. Lymphocytes are scattered among the overlying epithelial cells.	Moderate	9	NUMEROUS	NOT CONTROLLED- DIED OF UNRELATED CAUSE	Liquid, normal volume	Food or brown liquid (1 hour after eating), 2-3 per day	Decreased appetite. Depressed.	Duodenum friable & erythematous
73 Stomach, 5 endoscopic pieces: no significant findings. Small Intestine, endoscopic fragments: There is slight expansion of the lamina propria by plasma cells, lymphocytes, with scattered eosinophils. Lymphocytes are scattered among the overlying epithelial cells.	Mild	11	CONTINUOUS	NOT CONTROLLED- DIED OF NEUROLOGICAL DISEASE	N	Food (1 hour after eating), 4-5 per day	Weight loss. Dehydration	NAF

Sources: MASSEY=Veterinary Teaching Hospital at Massey University; AUK-NZ=Private Practices in NZ; Davis=Veterinary Teaching Hospital at the University of California at Davis; PENN=Veterinary Teaching Hospital at the University of Pennsylvania. **Endoscopy findings** : NAF=no abnormal finding; ND= not done. **Diarrhoea and Vomiting** : Y=presence; N=absence.



CHAPTER 4

TOLERANCE TO DIFFERENT STARCH SOURCES BY HEALTHY CATS AND CATS WITH INFLAMMATORY BOWEL DISEASE

By definition carbohydrate tolerance is the ability to endure dietary carbohydrates without effect or injury. Carbohydrate malassimilation and intolerance are common complications of gastrointestinal disease ¹ and can produce osmotic diarrhoea, bacterial overgrowth and ill thrift in cats ². Carbohydrate malabsorption has been shown in dogs and humans to delay gastric emptying and accelerate small intestinal transit leading to bloating and diarrhoea ³. In addition, carbohydrate intolerance in rats has been associated with the development of gastrointestinal lymphocytic infiltration and morphological changes consistent with tissue injury ⁴ that could further affect nutrient absorption. In this context, the adverse effects of carbohydrate malassimilation or intolerance can be compounded by maldigestion or malabsorption of other nutrients, especially protein ^{5,6} and by anorexia ².

The pathophysiology of carbohydrate malabsorption in feline gastrointestinal disease is poorly understood. Small intestinal disease can be accompanied by morphological changes or enterocyte injury that may induce carbohydrate malabsorption⁷. However, morphological gastrointestinal changes are not always apparent in animals with small intestinal disease⁸. Carbohydrate malabsorption may occur in Inflammatory Bowel Disease (IBD) because of the adverse effects of inflammation on digestive enzymes. Inflammatory mediators in man⁹ and gastrointestinal hypersensitivity reactions in rats¹⁰ have been found to adversely affect the level of intestinal saccharidases. The degree by which inflammatory infiltrates can act as a nutrient diffusion barrier in the small intestine is unknown, although in the colon it has been shown that the magnitude of cellular infiltration correlates negatively with absorptive function¹¹. A nutrient diffusion barrier is suspected to occur in feline alimentary lymphoma, which can produce similar gastrointestinal signs to IBD, including weight loss beyond that expected with the syndrome of cancer cachexia¹². The association of feline IBD with pancreatitis¹³ could also, in the long term, increase the possibility of malassimilation. Similarly, exocrine pancreatic insufficiency in man not only affects the availability of pancreatic enzymes but also intestinal transport systems¹⁴.

The retrospective epidemiological study conducted in cats with IBD (Chapter 3) investigated the possibility that dietary carbohydrates in starchy table foods could be associated with IBD. In that study, no differences were found between the healthy controls and the affected cats in the consumption of these carbohydrates. However, the widespread use of commercial diets, the large amount of carbohydrates they

contain and the frequent changes in these diets formulation suggest that handling of dietary carbohydrates by cats with gastrointestinal inflammation warrants further studies in the. Furthermore, the peculiarities of the digestion, absorption and metabolism of carbohydrates by cats ^{18 19 20} (see below or Chapter 1 for more details) may make cats with gastrointestinal disease more vulnerable to carbohydrate maldigestion and malabsorption.

Cats are classified as strict carnivores and show a series of adaptations to a diet rich in animal flesh. The carbohydrates usually contained in their natural diet are muscle glycogen and plant material found in their prey's gastrointestinal tract. Collectively, these sources of carbohydrates have been reported to represent only 1-2 % carbohydrate in the whole natural diet of the cat ¹⁵. No feline requirement for carbohydrates has been reported (NRC, 1985). Terminal studies in healthy cats indicated that different starches are digested to different extents in the feline small intestine and that prececal digestion is not complete ¹⁶. Nevertheless, it has been shown that healthy cats can thrive on a diet rich in carbohydrates ^{15,17}.

The above observations suggest that optimising the digestibility and utilisation of dietary carbohydrates might improve the outcome of dietary management of feline inflammatory bowel disease. This is likely to be true when one considers that the manufacturing of most commercial dry cat foods require a minimum of 40% carbohydrate ²¹. Furthermore, improved carbohydrate assimilation might improve the utilisation of other macronutrients. Morris, Trudell and Penkovic, (1977) ⁵, showed that apparent protein digestibility is decreased in cats in which diarrhoea has been induced with lactulose. Similarly, Kienzle (1993) ¹⁶, demonstrated decreased protein

digestibility in healthy cats consuming poorly digestible raw maize and raw potato starch when compared to those that consumed highly digestible cooked maize starch. Furthermore, dogs have been reported to show increased apparent digestibility of protein when consuming rice of high digestibility compared to rice of lower digestibility²².

Carbohydrate malabsorption in the small intestine has been difficult to measure in clinical patients because the malabsorbed carbohydrates are fermented in the colon^{23,24}. The breath hydrogen technique takes advantage of this process allowing not only identification but also quantification of carbohydrate malabsorption^{25,26}. A study in ileostomates has shown that the breath hydrogen method can accurately assess the loss of carbohydrate from the small intestine²⁷, although this has not been universally demonstrated or accepted^{28,29}.

Hydrogen gas excretion has been used in dogs³⁰ and cats³¹ for the purpose of evaluating carbohydrate malassimilation. Unfortunately, previous reported work with breath hydrogen collection in cats has not been very extensive and breath hydrogen production was compared with malabsorption tests³² that are now considered unreliable^{33,34}.

The objective of this study was to compare the absorption and tolerance to four different sources of starch - namely corn, rice, barley and tapioca – by healthy cats and cats diagnosed with IBD. Malassimilation was evaluated by the breath hydrogen method. In addition, faecal characteristics (grade, water content and faecal osmolar gap) have been studied to understand how well they indicate the presence of

carbohydrate malabsorption and how important the tested sources of dietary carbohydrates are in producing lower grade stools in cats.

Materials and methods

Diets

The test diets were prepared by Master Foods, Austria on behalf of the Waltham Centre for Pet Nutrition. All diets contained chicken as the principal source of protein and either tapioca, maize, barley or rice as the principal carbohydrate. Tapioca was added as ground meal, maize as meal and broken maize, and barley as flour and grits. The rice containing diet (Waltham Whiskas Selected Protein Chicken and Rice) had rice added as flour and whole rice. Gelatinisation of the starch in all diets occurred during canning. Dietary composition is presented in **Table 1**.

Table 1
Diet Composition

Diet	Protein	Fat	NFE	Sodium	Potassium
Chicken	64	36	0	-	-
Barley	25.3	48.9	25.8	0.201	0.253
Corn	27.9	48.9	23.2	0.195	0.245
Tapioca	25.6	48.6	25.8	0.206	0.316
Rice	28.7	52.9	18.4	0.170	0.215

Note: Protein, Fat and NFE (Nitrogen Free Extract) of the chicken diet are expressed as %ME (metabolizable energy) (Donoghue S. Kronfeld DS. Home-made diets. In: *The Waltham book of clinical nutrition of the dog and cat*. Ed. By J.M. Wils and K.W. Simpson. Elsevier, Oxford, England). Protein, Fat and NFE (Nitrogen Free Extract) of the carbohydrate containing diets are expressed as %ME (metabolizable energy) and have been provided by the manufacturer (Waltham Centre for Pet Nutrition). The content of sodium and potassium is expressed as g/100g and were performed at Hill Laboratories (NZ).

Animals

Two groups of cats were used for this study, healthy *controls* and *IBD cats*. The control group had 15 cats and the IBD group 7 cats. Control cats were provided by the Best Friend Feline Unit at Massey University and lived in a colony situation. The control cats were determined to be healthy by history and physical examination. Specifically, there was no history of vomiting and/or diarrhoea or weight loss amongst these cats and their physical examinations were unremarkable. These cats are monitored regularly with blood tests, free of gastrointestinal parasites, FIV and FeLV. They are observed daily. The control cats were divided into two groups to carry out the trial because of the number of metabolic cages available.

The cats in the IBD group (n=7) were referred to the Massey Veterinary Teaching Hospital for chronic diarrhoea, vomiting, weight loss or other signs of gastrointestinal disease. They were diagnosed with IBD by way of a standardized diagnostic protocol (see below) and selected into the dietary trial. For ethical reasons, animals with marked anorexia, weight loss, vomiting or those that were very choosy with their food were eliminated from this trial. Extremely excitable cats were handled and befriended before starting the trial until they felt comfortable with the handling and techniques required.

The protocol used to diagnose IBD included a full haemogram and serum biochemistry panel, FIV and FeLV serology, assay of serum thyroxine concentration (if older than 5 years old), three faecal flotations (if diarrhoea was the main complaint), faecal culture for *Salmonella*, *Campylobacter* and *Yersinia* (if considered to be indicated based on historical findings), breath hydrogen collection and

radiographs following administration of radiopaque markers (BIPS, MedID, Grand Rapids). Some cats had already undergone dietary trials to determine if the gastrointestinal problems were due to an adverse reaction to a food or if the problem was responsive to a 'therapeutic diet'. However, if there was any doubt about the thoroughness of the dietary trials, the cats were fed an elimination diet based on novel protein sources for a week and observed. If clinical signs continued it was considered that dietary hypersensitivity was unlikely to be the primary cause of the cat's clinical signs, in agreement with the reported view that response to novel protein diets occurs within a week in cats with gastrointestinal food sensitivity^{35,36}. Miscellaneous tests occasionally considered necessary to diagnose IBD included ultrasound imaging, triiodothyronine suppression test, bone marrow aspiration, and aspiration of mesenteric lymph nodes. If all of these tests were negative, an endoscopy and biopsy of the stomach duodenum and colon were performed. The diagnosis of IBD was confirmed if the biopsy findings were compatible with those previously described for IBD^{1,37-40} and the aforementioned diagnostic procedures had not revealed any other cause for the gastrointestinal signs (see details at the end of the Appendix).

The radiopaque marker procedure involved the administration of 10 large BIPS and 30 small BIPS mixed in a feline commercial diet of lamb and rice (Hill's feline d/d) recommended by the manufacturer. This diet was fed early in the morning and if it was not voluntarily consumed in less than 20 minutes the cats were manually fed. Radiographs were taken the following morning to confirm that the radiopaque markers had passed through the gastrointestinal tract unimpeded by physical bowel obstructions. The procedure to obtain the breath samples during the diagnostic stage was similar to the one used for the experimental stage (see below) but lasted only 8

was similar to the one used for the experimental stage (see below) but lasted only 8 hours and used the same diet as the radiopaque marker procedure.

The endoscopy was performed under general anaesthesia after a two day fast. The day before the endoscopy was scheduled the cats had two or three enemas. Endoscopic biopsies were obtained from the stomach (incisura, cardia and the junction of the fundus and pyloric antrum), duodenum (ascending duodenum and cranial flexure) and colon (descending, transverse and ascending colon). If any particular area of mucosa appeared abnormal, biopsies were also taken from these lesions. A minimum of 8 biopsy specimens per site were obtained and kept moist in a biopsy sponge wet with saline. Once the procedure was finished the biopsy specimens were submerged in 10% formalin and sent for histopathology.

Informed consent was obtained from the owners before starting the experimental stage and all procedures were approved by the Massey University Animal Ethics Committee.

Allocation of treatments

A crossover trial design was chosen to compare the carbohydrates. The experimental period was thus separated in 5 stages. On the first day of the trial all cats were fed a 'carbohydrate free' diet (boiled chicken flesh) to assess basal hydrogen gas production and the ambient concentration of hydrogen gas (stage 1). This was followed by a sequence of four carbohydrate sources. These next four stages involved the consecutive feeding of different carbohydrate sources according to the particular carbohydrate feeding sequence allocated to each cat. The carbohydrate feeding

Figure 1.- Feeding Schedule

Treatment Number	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
	Day 1	Day 2 & 3 + CrO	Day 4 & 5	Day 6 & 7 + CrO	Day 8 & 9
1	Chicken	Tapioca	Maize	Barley	Rice
2	Chicken	Tapioca	Barley	Rice	Maize
3	Chicken	Tapioca	Rice	Barley	Maize
4	Chicken	Tapioca	Maize	Barley	Rice
5	Chicken	Maize	Tapioca	Rice	Barley
6	Chicken	Maize	Barley	Tapioca	Rice
7	Chicken	Maize	Rice	Tapioca	Barley
8	Chicken	Maize	Barley	Tapioca	Rice
9	Chicken	Rice	Maize	Tapioca	Barley
10	Chicken	Rice	Tapioca	Barley	Maize
11	Chicken	Rice	Barley	Maize	Tapioca
12	Chicken	Barley	Rice	Maize	Tapioca
13	Chicken	Barley	Tapioca	Maize	Rice
14	Chicken	Barley	Maize	Rice	Tapioca
15	Chicken	Barley	Tapioca	Maize	Rice

Chromic Oxide (CrO) to be added to the diet in stage 2 and 4.

Totals for consecutive carbohydrate combinations are:

- Tapioca-Barley 4
- Tapioca-Maize 4
- Tapioca-Rice 4
- Maize-Tapioca 4
- Maize-Barley 4
- Maize-Rice 4
- Barley-Maize 4
- Barley-Tapioca 4
- Barley-Rice 4
- Rice-Maize 3
- Rice-Tapioca 3
- Rice-Barley 3

The carbohydrate sequences were distributed as follows:

❖ Stage 1	Chicken			
❖ Stage 2	Barley 4	Corn 4	Rice 3	Tapioca 4
❖ Stage 3	Barley 4	Corn 4	Rice 3	Tapioca 4
❖ Stage 4	Barley 4	Corn 4	Rice 3	Tapioca 4
❖ Stage 5	Barley 3	Corn 3	Rice 6	Tapioca 3

sequences were devised so that the carbohydrate sources would be approximately equally represented in each of the stages of the experiment besides stage 1 (chicken flesh). An effort was also made to ensure that any two consecutive carbohydrate combinations would be approximately equally represented (See Feeding Schedule in **Figure 1**). The allocation of the cats to each sequence of starches was randomised in both groups –controls and IBD cats. Each sequence ('treatment') was given a number and randomly matched with the name of each cat. All tests were performed in a blind fashion regarding diet consumed.

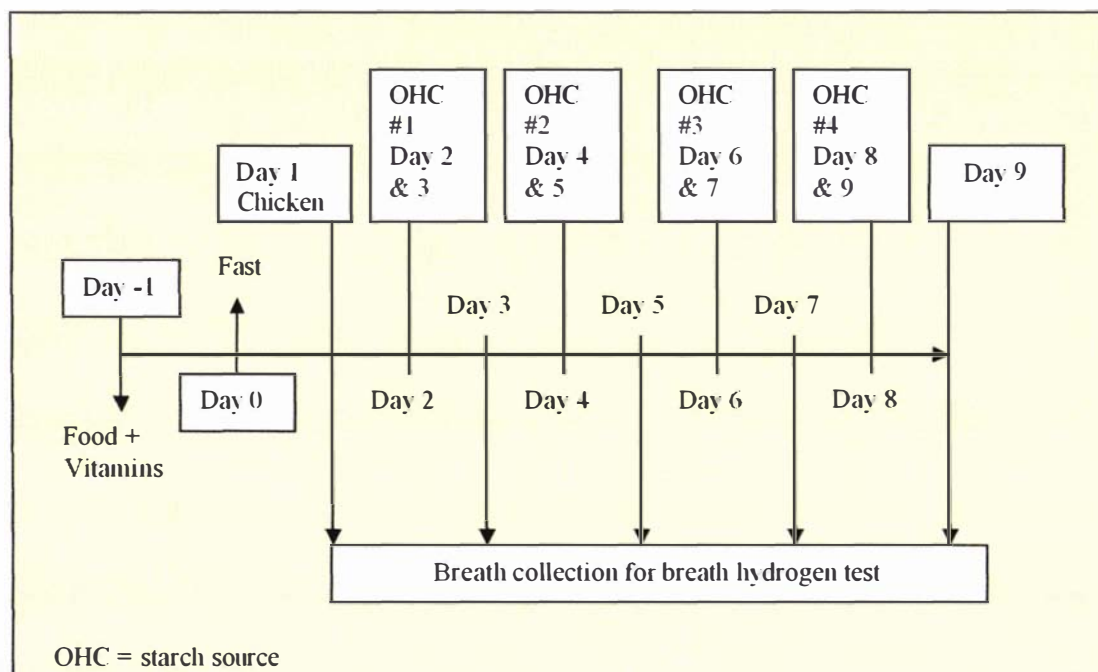
Experimental protocol

The control animals were moved to metabolic cages but otherwise remained in their habitual surroundings. The cats diagnosed with IBD were admitted to the Massey University Veterinary Teaching Hospital. The diagnostic work up of the IBD cats was conducted during their first week of hospitalisation. Once the diagnosis had been confirmed the experiment commenced.

All cats were fed *ad libitum* for a day with a complete balanced controlled diet (Whiskas Feline Selected Protein Chicken and Rice diet manufactured by Waltham) supplemented with multivitamins. The additional multivitamins were given to counteract the fact that these cats were going to be fed only 75% of their daily energy requirements during the trial. The reduced food intake could have affected the vitamin status of IBD cats, which may have already had pre-existing deficiencies. A 24 hours fast followed and the next day the trial proper started. Cats were fed only once a day in the early morning during the trial.

On day 1 of the trial all cats were weighed, and baseline breath samples were taken. The cats were then fed 75% of their energy requirements ($70 \times \text{Body Weight} = \text{kcal ME/day}$) as cooked chicken flesh. Cats that did not consume

Diagram 1
Experimental Protocol



their meal promptly were manually fed. The cats were fed only 75 % of their energy requirements in order to encourage voluntary consumption of the entire meal and decrease variability of food intake between cats which could have confounded the results. A breath collection followed. The cats were weighed again in the middle and at the end of the trial.

On day 2 of the trial, one of the carbohydrate containing diets was fed. Each of the different carbohydrate diets was fed for 2 days without washout period between consecutive diets (see **Diagram 1**). On the second day of each carbohydrate diet a breath hydrogen test was performed.

Chromic oxide was added (after being strained) and manually mixed with the diets (1/8 teaspoon per feeding bowl) in stages 2 and 4 (days 2, 3, 6 and 7 of the trial), independently of the dietary carbohydrate being tested. Chromic oxide was used with the sole purpose of recognising from which diet each stool was derived.

Food intake and faecal output

Food intake was measured daily. All food bowls and food given were carefully weighed each morning before feeding. The following morning the feeding bowl and all remnants of food in it and around the cage were weighed as well. Food intake was calculated as the difference between the food given the previous morning and the food recovered that morning.

Faeces were collected three times a day at least or when seen in the cages, graded according to a photographic faecal grading scale (see insert for scale description), immediately labelled and refrigerated prior to determination of osmolar gap and water content.

Faecal Grading Scale

GRADE 1: *Liquid faeces, no shape.*

GRADE 2: *Soft faeces. Paste-like. Collects into a mound but has no shape.*

GRADE 3: *Soft faeces but cylindrical shape is present.*

GRADE 4: *Stools with normal shape. Cylindrical but separated in pellets. Easily crushed out of shape.*

GRADE 5: *Separated in hard pellets difficult to crushed out of shape.*

Note: Intermediate grades were given if the faeces contained portions of different consistency, and hence grade.

Breath collection and breath hydrogen measurement

A semi-closed collection system was used to collect expired air from the cats. This system consisted of a face-mask connected to a 0.5 litre anaesthetic reservoir bag through a one-way non rebreathing valve. The cats breathed in room air and the expired air was collected in the reservoir bag until it was full. Paired samples of expiratory air were then taken with a 20 ml plastic syringe through a three-way valve. Some of the control cats were excessively difficult to handle and required training to accept the face-mask. This was done over a period of 5 days in which only the mask and rebreathing valve were used at first, and later the rebreathing bag was added. The training sessions increased in frequency until the cats tolerated the procedure. Training was undertaken to stop the cats getting excessively excited as excitation can change breath hydrogen content in the expired air ⁴¹. Some IBD cats were restrained by being wrapped in a towel during collection. This had the effect of calming them down and stopped them from clawing the reservoir bag. Breath-filled syringes were fitted with hypodermic needles embedded in a rubber stopper to avoid room air diluting the contents. Breath hydrogen content was measured within 24 hours with a hydrogen air analyser (GMI Exhaled Hydrogen Monitor, Renfrew, UK). The breath collection protocol included a baseline collection before feeding followed by collections every hour for 8 hours and then 3 collections two hours apart. The last breath sample was therefore collected 14 hours after the meal was fed.

Faecal dry matter and water content

Refrigerated stools were separated into two portions of equal grade (if the sample included faeces of different grade each portion contained equal amount of

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Faecal dry matter and water content

Refrigerated stools were separated into two portions of equal grade (if the sample included faeces of different grade each portion contained equal amount of

faeces of both grades). One portion was used to determine the dry matter and water content of the faeces by oven drying at 70 °C until constant weight was achieved. The faeces were weighed on tinfoil (tared), wrapped in the tinfoil and then placed into the oven. Pilot trials determined that the faeces required a minimum of 5 days in the oven before their weight began to stabilize. The faeces were then weighed twice more, two or three days apart (See Appendix to Chapter 4) until a constant weight was reached. The difference between the weight prior to and after oven drying was taken to represent the weight of evaporated water (+/- some minor quantities of volatile substances). The weight of the faeces after heating was considered to equal the dry matter content of the faeces.

$$\text{H}_2\text{O Content (\%)} = \frac{(\text{Weight IN} + \text{foil}) - (\text{Weight OUT} + \text{foil})}{\text{Weight IN}} \times 100$$

Ultracentrifugation of faeces

The second portion of the faeces was ultracentrifuged at 20,000 rpm (48,000g) in a Sorvall RC 5C Plus ultracentrifuge for 2 hours at 4°C to obtain faecal fluid^{42,43}. Pilot trials determined that this was the best method to obtain faecal fluid from cat faeces. However, failure to separate fluid from feline faeces was common when the faeces were firm and dry ('grade 5'). A method of faecal dilution to ensure faecal fluid could be obtained for analysis was investigated. Unfortunately the method had

to be abandoned because it produced inconsistent electrolyte measurements (see Appendix to Chapter 4).

The faecal supernatant produced after ultracentrifugation was commonly a thick dark fluid that was difficult to aspirate with a standard pipette. To overcome this problem, a tuberculin syringe attached to a long hypodermic needle was used to retrieve the faecal fluid from the centrifuge tube. The fluid was then filtered, if needed, using filter paper (Whatman 541, 12.5 cm, fast speed, for retention of coarse and gelatinous precipitates). The filtration step was necessary in some samples to remove small particles that could block the flame photometer. Unfortunately, the filtration reduced even further the amount of fluid left to work with. When insufficient faecal fluid remained to measure sodium and potassium the fluid was mixed with distilled water in the collection syringe and a dilution factor calculated. Dilution was required in only two samples.

Faecal Osmolar Gap

The faecal osmolar gap was calculated as follows:

$$\text{Faecal Osmolar Gap} = 314 - [2 \times (\text{Na}^+ + \text{K}^+)]$$

The value 314 represents the average osmolality of serum in the cat⁴⁴. The rationale for the use of this formula was reached after preliminary studies (presented in

the Appendix of this chapter) demonstrated that this was the most accurate method to obtain a faecal osmolar gap in the cat. The concentrations of sodium and potassium in the faecal fluid were measured by flame emission photometry (Corning 400 Flame Photometer)^{45,46}. A calibration curve was generated for every set of samples. Stock solutions were prepared for this purpose with distilled water containing 0, 10, 40, 100, 200 mmol/l of sodium chloride and 0, 1, 4, 10, 16 and 20 mmol/l of potassium chloride. The stock solutions were kept refrigerated at all times.

Statistical Analysis

Statistical analysis was carried out using SAS software, vs. 6.12. A probability of $p < 0.05$ was considered significant for all tests. Descriptive statistics including means and standard errors were used to summarise data. All data were analysed for normality. Data transformation or non-parametric methods were chosen for the analysis of data that were not normally distributed or had an ordinal or nominal scale

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Carbohydrate malabsorption was estimated by plotting the cumulative breath hydrogen production curve and calculating the area under the curve (AUC) for each carbohydrate in each cat using Microcal Origin v. 4.00, Microcal software, (Northampton, USA). An analysis of variance was performed using PROC MIXED for a repeated measures analysis using the model *parameter of interest = diet + group of cats (controls or IBD) + their interaction* including all diets (four carbohydrates and chicken). The data showed a deviation from normality that was corrected by using

a Log_{10} transformation of AUC. The analysis of variance of the Log_{10} AUC was performed using a compound symmetric type of covariance structure ⁴⁸. A large difference was found between healthy cats and cats diagnosed with IBD in the expiratory hydrogen curves AUC. Hence a term was included in the factorial model to take account of this heterogeneity of variance between sources ⁴⁸. Contrasts compared 'rice vs. other carbohydrates', 'tapioca vs. grains', 'rice vs. barley' and 'rice vs. corn'. In addition post-hoc comparisons using least squares means differences (with Tukey correction for multiple comparisons) were carried out between all diets, IBD and control cats, and their interactions.

The time at which the maximum expired breath hydrogen concentration was recorded (the 'peak time') for each carbohydrate source was compared by the non-parametric Friedman's test for repeated measurements based on ranks. Multiple comparisons of the peak time between diets within each group (IBD or control cats) were performed using the Student-Newman-Keuls test ⁴⁷. The peak time for each diet was compared between IBD and control group with a Wilcoxon rank sum test.

Both IBD and control cats were further compared by calculating a Breath Hydrogen Production Index for each test carbohydrate. In humans ⁴⁹, as well as dogs ⁵⁰ breath hydrogen has shown large individual variation. Indexes have been calculated in this study to eliminate part of this type of variation and obtain results that are more widely applicable. This process is similar to the categorisation of foods for diabetic patients according to a glycemic index ⁵¹. The Index of breath hydrogen production utilises one of the carbohydrates as a standard in accordance to the following formula:

$$\text{H}_2 \text{ Production Index} = \frac{\text{AUC (tested carbohydrate)}}{\text{AUC (standard)}}$$

This method incorporates the same variables affecting breath hydrogen production in the numerator and denominator, thus eliminating these variables as a source of variation in the data⁵². It is important to note, however, that because of the different stoichiometry of different carbohydrates in the production of hydrogen⁵³ this does not constitute a malabsorption index per se.

Rice was used as a standard carbohydrate to calculate the Hydrogen Production Index. Log10 transformation of the index was needed to restore normality. An analysis of variance using the same model as described above was used to compare indexes between diets, IBD and control cats and their interaction.

Food intake was analysed with an analysis of variance for repeated measures using diet, group of cats and their interaction as factors.

Faecal water content was calculated for each faecal sample and then correlated with faecal grade using the Spearman rank correlation coefficient. An analysis of variance of faecal water content was performed using PROC MIXED for repeated measures using the same model as described above. Friedman's test based on ranks for repeated measures was used to compare faecal grade between diets for each group

of cats⁴⁷. Faecal grade results were averaged when there were several stools produced per diet by a single cat so as to have one observation per cat for each different diet. Chicken was eliminated from the analysis because a large number of cats did not produce a stool after consuming chicken. Faecal grade was also correlated to breath hydrogen AUC using the Spearman correlation coefficient. The total number of stools produced in IBD and control cats by each diet was compared by a Chi-Square test. The number of stools per diet was measured as a cumulative number of stools produced by all cats in each group because the diets were fed only for two days. In this situation the range of possible stools per day equals 0-1. Chicken was eliminated from this analysis because it was fed only for one day.

The faecal osmolar gap, sodium and potassium content of faecal fluid were averaged for each cat on each different diet so as to have one observation for each of these parameters per diet and per cat. The faecal sodium/potassium ratio of each cat was also calculated. Normality of the data was confirmed and one IBD cat identified as an outlier was removed from the analysis. Chicken was eliminated from the analysis because approximately half of the cats did not produce enough faeces for analysis when consuming chicken. An analysis of variance was performed using PROC MIXED for a repeated measures analysis with a compound symmetric covariance type using the model *parameter of interest = diet + group of cats (controls or IBD) + their interaction*. Sequence order was included as a random factor to account for correlations between data collected at different times. This was possible because the elimination of chicken from the analysis (which was fed only in the first stage) meant that all diets were represented in each stage. Heterogeneity of variance between

sources was present for the faecal sodium content and the faecal osmolar gap. Hence for these variables a factor was added to the analysis to take care of the different variances between groups. The same pre-planned contrasts that were mentioned in the section describing the analysis of the breath hydrogen results were tested. In addition, post-hoc comparisons using least square means differences (with Tukey correction for multiple comparisons) were carried out between all diets, between controls and IBD cats and their interactions.

Results

The area under the breath hydrogen curve (AUC) was significantly lower for the chicken flesh diet than for any of the carbohydrate sources ($p=0.0001$). All carbohydrate diets behaved similarly and no differences were found between any two carbohydrates within each group of cats. However, IBD cats showed a significantly higher area under the curve ($p=0.0001$) than the control cats for all diets (**Figure 2**). The breath hydrogen production curves for each diet consumed by IBD and control cats are presented in **Figure 3**.

The breath hydrogen production peak time occurred significantly earlier when cats had consumed a tapioca based diet than the other diets in the control group ($p=0.05$). No significant differences were found between different diets in the IBD group. However, the breath hydrogen production peak while consuming a tapioca based diet occurred also significantly earlier in the control cats when compared with the IBD cats ($p=0.02$). See **Table 2**.

Figure 2.-Feline breath hydrogen AUC in healthy and IBD cats while consuming different sources of carbohydrates

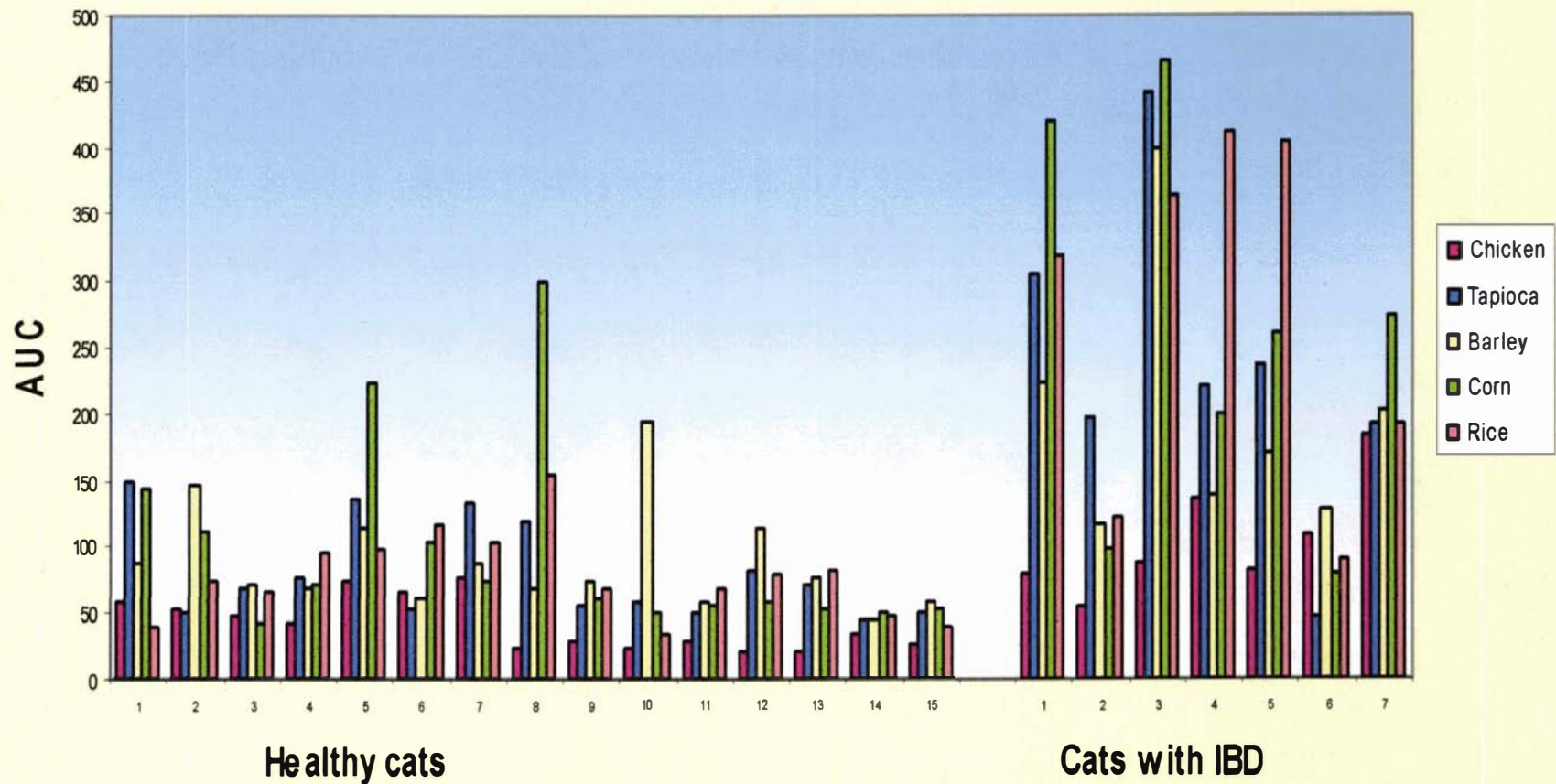


Figure 3
Breath hydrogen curves from healthy and IBD cats consuming diets with different sources of carbohydrates

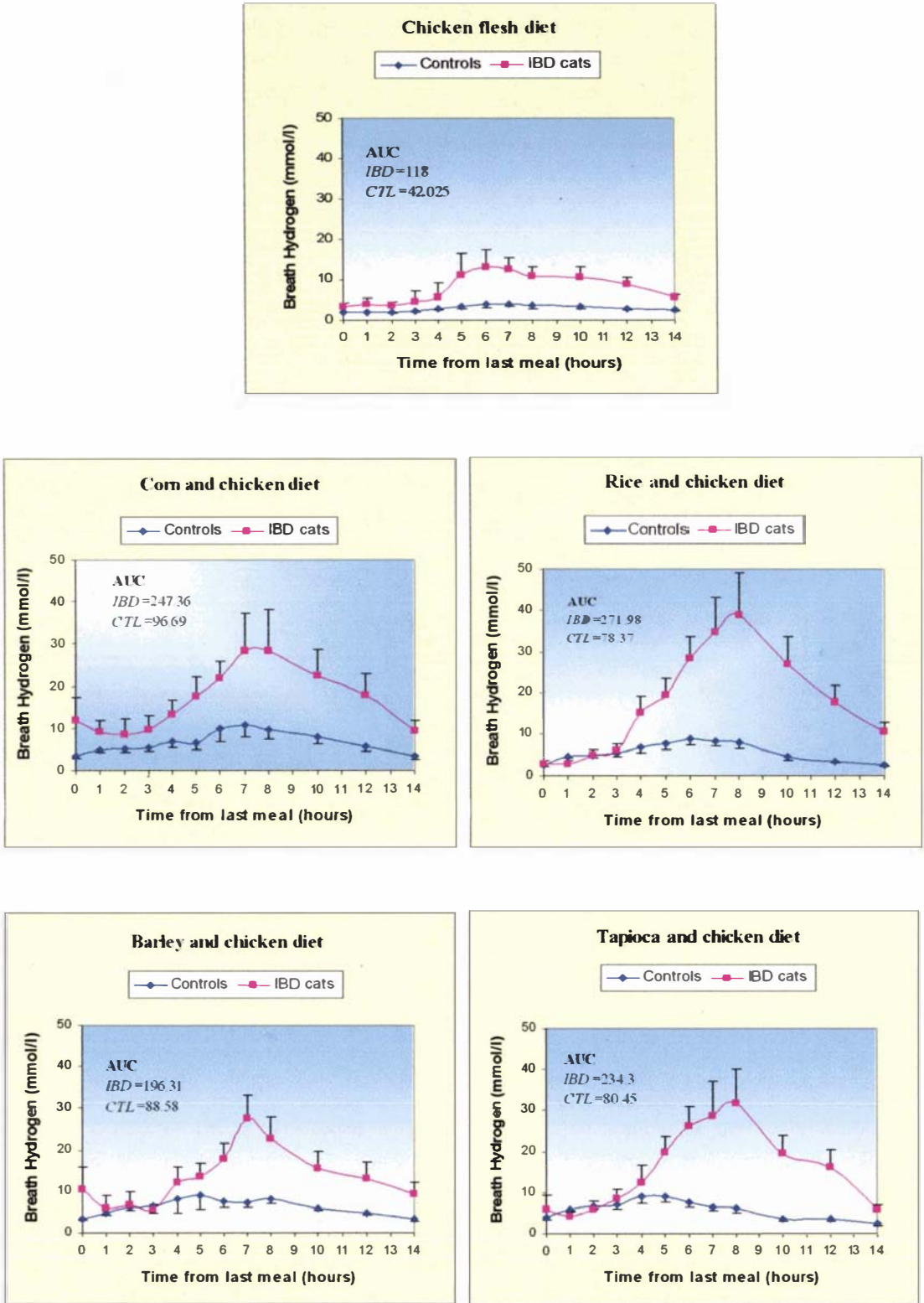


Table 2
Median Breath Hydrogen Peak Times (hours) in healthy and IBD cats consuming different sources of carbohydrates

Diet	Controls	IBD
Barley	6	7
Corn	7	8
Rice	6	7
Tapioca	5	7
Chicken	7	7

The rice based Breath Hydrogen Concentration Index was significantly lower for chicken flesh ($p=0.0001$) when compared to any of the carbohydrate diets (barley, tapioca and corn) in both IBD and control cats. No significant differences were present between carbohydrates (corn, barley or tapioca). Means of the rice based index for each diet within each group can be seen in **Table 3**.

Table 3
Mean +/- SEM indexes of hydrogen production in cats consuming different starch sources.

Diet	Control cats		IBD cats	
	Rice Based Index		Rice Based Index	
	<i>Mean</i>	<i>SEM</i>	<i>Mean</i>	<i>SEM</i>
Barley	1.42	0.33	0.85	0.15
Corn	1.28	0.21	0.98	0.14
Tapioca	1.17	0.21	0.92	0.15
Chicken	0.60	0.08	0.52	0.15

Note: Control cats $n=15$; IBD cats $n=7$

No significant differences were found in food intake between the different diets or between healthy and IBD cats.

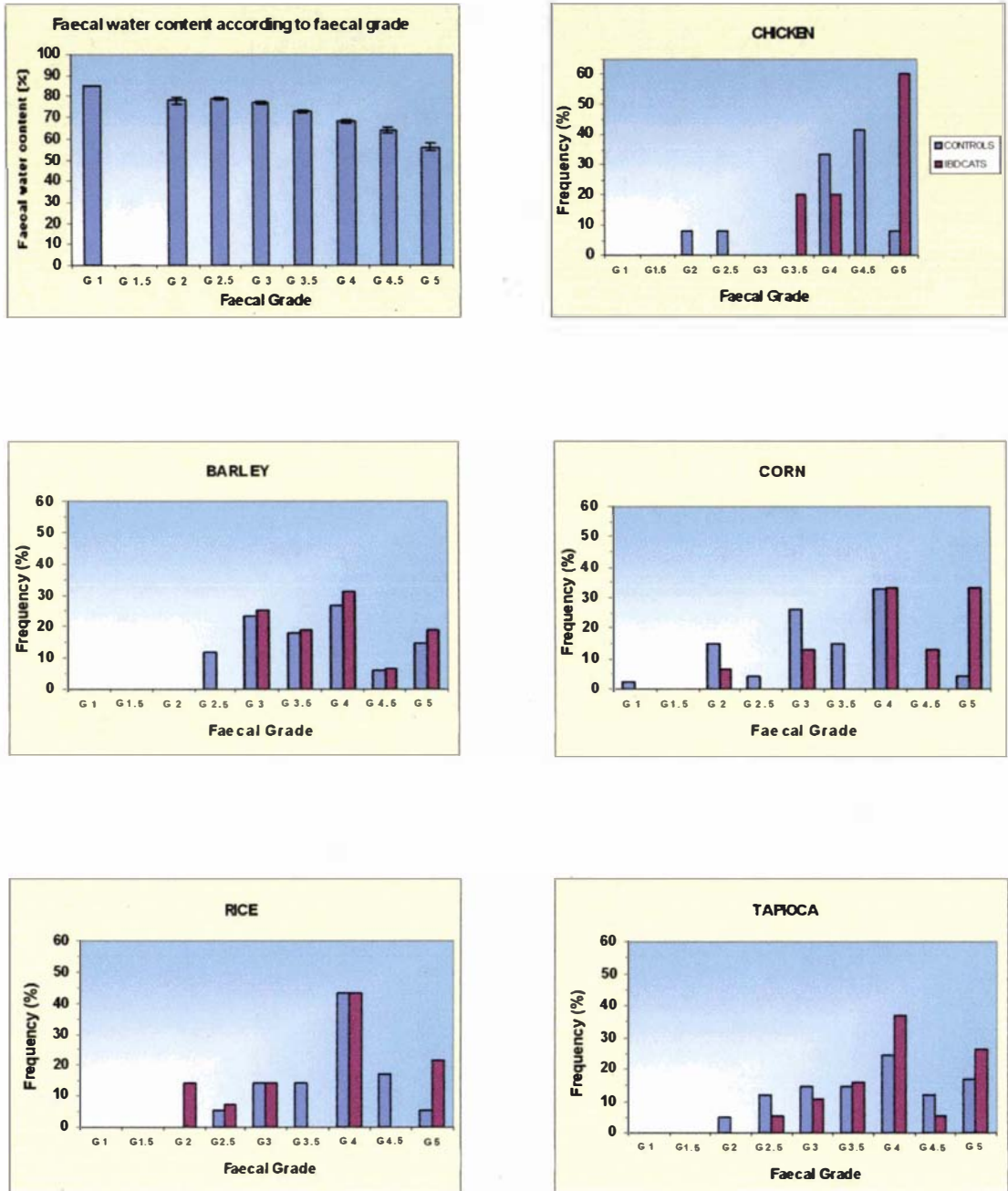
Faecal fluid content was inversely correlated to faecal grade ($r = -0.76$). The faecal grading system was not accurate to predict water faecal content for grade 2 to 3 faeces. However, very few samples were given these grades -grade 2=13/237; grade 2.5=16/237. See **Figure 4**.

No significant differences were found in faecal grade produced by the different carbohydrate sources in IBD or control cats or when all the cats were treated as a single group. Please refer to **Figure 4** for frequencies of different faecal grades with different diets. A weak correlation coefficient $r=0.28$ was found between breath hydrogen area under the curve (AUC) and faecal grade. Water content was significantly lower in the stools produced after the cats had consumed chicken flesh when compared to the stools produced following consumption of any of the carbohydrate diets ($p=0.0001$). Paradoxically, IBD cats also produced faeces with significantly less water content than the control cats ($p=0.016$). The number of stools per each carbohydrate diet was not different in IBD or control cats (**Figure 5**) as a percentage of the total number of stools for the group (IBD or controls).

There were no significant differences between the faecal potassium content or faecal osmolar gap of the cats fed the different carbohydrate diets or between the IBD and control cats. It should be noted that the data from cats fed chicken were not part of the statistical analysis although **Figure 6** shows the data for completeness. The differences of faecal sodium contents between diets tended towards significance ($p=0.056$) as barley produced faeces with less sodium content than rice. In addition, IBD cats that consumed the barley based diet showed less faecal sodium than the IBD cats that ate the rice based diet ($p=0.023$). Contrasts showed that overall, cats when

Figure 4

Faecal water content and faecal grade



Faecal water content and faecal grade observed in cats consuming different diets consisting of cooked chicken flesh or commercially prepared canned diets based on chicken and one source of carbohydrate (corn, rice, barley or tapioca).

eating the rice based diet had higher concentration of sodium in the faecal fluid than with any other carbohydrate ($p=0.046$) and that when eating the barley based diet cats

Table 4
Faecal Sodium, Potassium content and Faecal Osmolar Gap in IBD and healthy cats consuming different starch sources.

	<i>Mean</i>	<i>SEM</i>	<i>Range</i>
Controls			
Sodium (mmol/l)			
Barley	34.91	3.14	17.57 - 64.75
Corn	35.11	3.10	12 - 55.84
Rice	31.20	2.59	18.5 - 53.5
Tapioca	43.47	3.02	21.25 - 58.5
Chicken	56.48	4.97	34 - 80
Potassium (mmol/l)			
Barley	13.11	1.24	4.65 - 25.2
Corn	11.58	0.91	3.77 - 17.45
Rice	12.19	0.8	7.4 - 18.7
Tapioca	13.07	0.96	8.8 - 24.2
Chicken	11.46	1.77	1.6 - 16.8
Osmolar Gap			
Barley	217.96	7.51	162.40 - 264.7
Corn	220.61	6.88	181.84 - 282.47
Rice	227.22	5.22	183.6 - 258.53
Tapioca	200.9	5.11	171.2 - 239.2
Chicken	178.11	12.29	126 - 242.8
IBD Cats			
Sodium (mmol/l)			
Barley	26.33	3.91	16.5 - 42.8
Corn	36.28	10.24	9 - 71.25
Rice	50.05	11.65	14.5 - 83.5
Tapioca	33.26	9.60	14.5 - 57
Chicken	-	-	43
Potassium (mmol/l)			
Barley	11.11	0.97	7.75 - 20.47
Corn	11.24	2.09	5.75 - 15.75
Rice	8.81	1.52	5.4 - 13.8
Tapioca	12.96	1.88	8.8 - 17.57
Chicken	-	-	15.4
Osmolar Gap			
Barley	239.12	8.34	203.3 - 258
Corn	218.96	19.86	145.6 - 264.5
Rice	196.28	23.45	183.6 - 258.53
Tapioca	221.57	22.95	171.2 - 239.2
Chicken	-	-	197.2

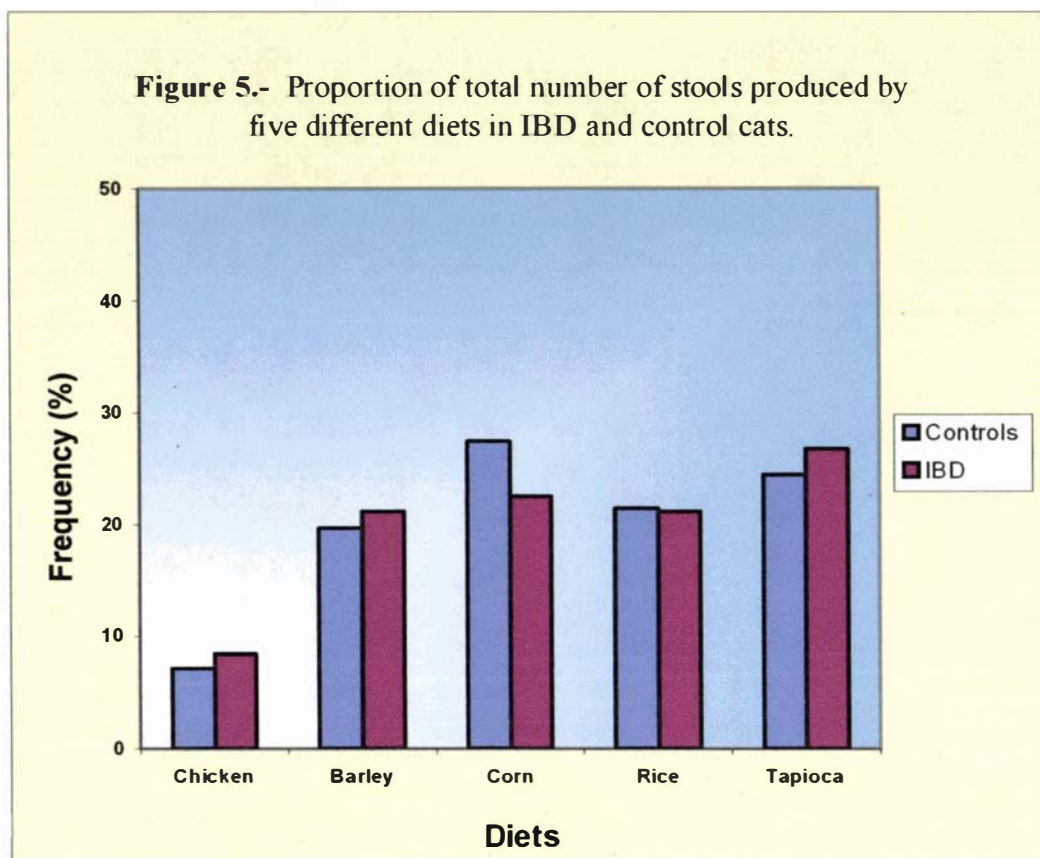


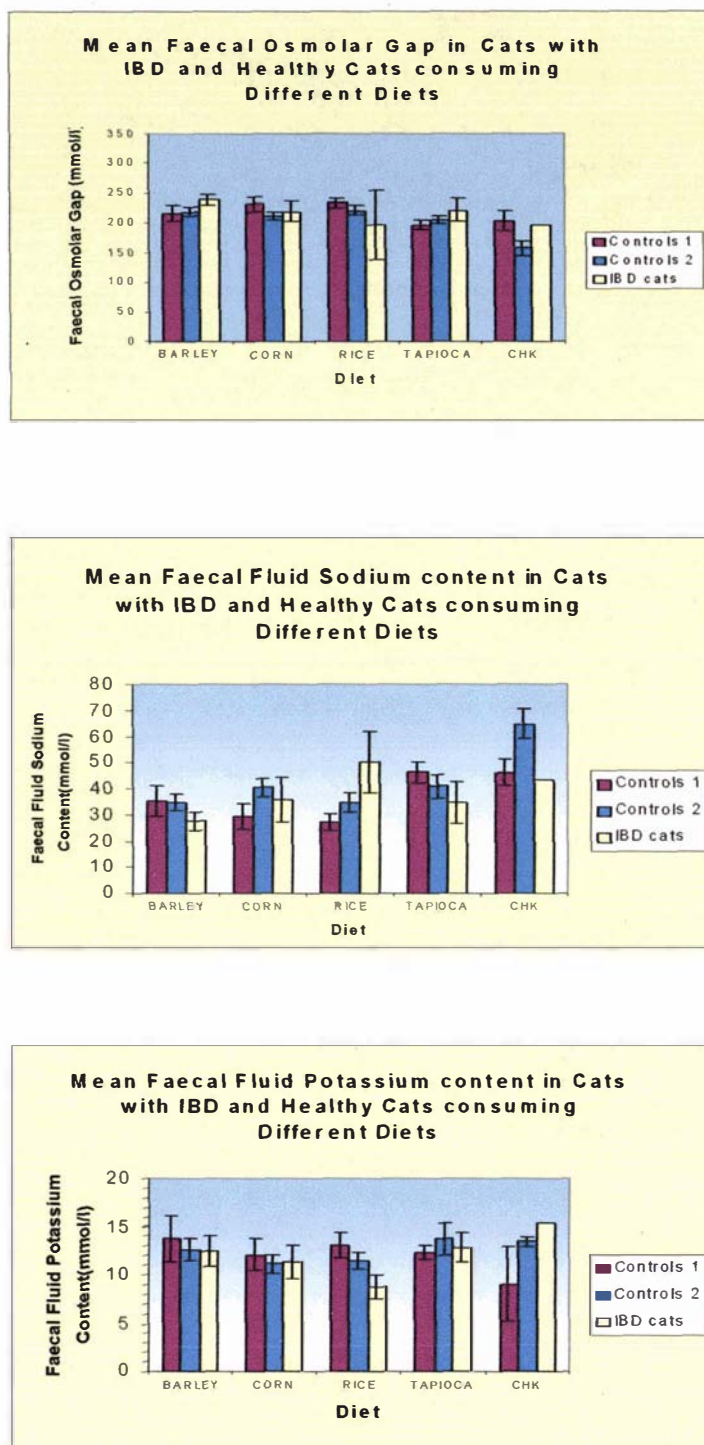
Table 5

Mean faecal sodium/potassium ratio in feline faeces produced while consuming four different carbohydrates

Diet	Control cats		IBD cats	
	Ratio	SEM	Ratio	SEM
Barley	2.90	0.30	2.44	0.36
Corn	3.21	0.33	3.98	1.23
Rice	2.75	0.31	6.17	1.76
Tapioca	3.63	0.36	2.35	0.37

Figure 6

Faecal osmolar gap, sodium and potassium



Mean faecal osmolar gap, sodium and potassium content of faecal fluid \pm SEM in cats consuming different diets consisting of cooked chicken flesh or commercially prepared canned food based on chicken and one of four carbohydrates (rice, corn, tapioca or barley).

had much less faecal sodium than when eating rice ($p=0.007$). These data were highly variable as it can be appreciated in **Table 4**.

Cats overall had a higher faecal sodium/potassium ratio when consuming rice than when eating barley ($p=0.002$) or tapioca ($p=0.0146$). More interestingly IBD cats consuming rice showed a higher ratio than when consuming tapioca ($p=0.0014$), barley ($p=0.0013$) but not corn ($p=0.42$). In addition the sodium/potassium faecal ratio from IBD cats consuming a rice diet was higher than control cats eating rice ($p=0.0025$), tapioca ($p=0.032$), corn ($p=0.0065$) and barley ($p=0.018$). Furthermore, when rice was contrasted with all other carbohydrates, it showed a significantly higher ($p=0.001$) faecal sodium/potassium ratio (barley ratio= 2.77 +/- 0.24; corn ratio= 3.40 +/- 0.38; rice ratio= 3.60 +/-0.58; tapioca ratio= 3.31 +/- 0.31). Contrasts also showed that overall when cats consumed the rice diet they had a significantly higher sodium/potassium faecal ratio than when consuming barley ($p=0.0004$) but not when consuming a corn based diet ($p=0.11$). **Table 5** shows the faecal sodium/potassium ratio in controls and IBD cats.

Discussion

A cross over design with repeated measurements in the same individual was chosen because it reduces experimental error and the number of animals needed. In addition, repeated measurements designs are particularly effective when the main source of random variation is the subject and the subject response over time is expected to be relatively uniform⁵⁴. Both, inter-individual variability and consistency of individual response through time have been shown to occur in man⁵⁵ and dogs^{50,56}

when using breath hydrogen to assess carbohydrate malabsorption. Another assumption of this experimental design is that there is no carry over effect of different treatments⁵⁴. Although, in dogs the fermentation of each starch, as measured by breath hydrogen concentration, can be affected by the order in which the starches have been given⁵⁷, enzymatic adaptation to carbohydrate diets has not been observed in cats^{18,58} to expect that the order of the diets will affect the results. However, the adjustment of the cat to hospitalization, human contact and expiratory breath collection, is expected to improve as the trial moves through different stages and could affect the results. For this reason different sequences of carbohydrate diets were included in the experimental design.

Fortunately, no differences in dietary intake were present between diets. Carbohydrate malabsorption in ileostomates has been shown to be directly related to starch intake⁵⁹ and could affect the results of the breath hydrogen collection⁵⁰.

Even a diet practically devoid of carbohydrates (chicken flesh) resulted in the production of small amounts of breath hydrogen in healthy cats. Glycoproteins present in mucus and cells have been demonstrated to be an adequate substrate for the production of hydrogen gas by the colonic flora^{49,60}. The breath hydrogen produced by IBD cats consuming only chicken flesh was significantly higher than that produced by healthy cats. This could be due to protein malabsorption⁵⁷ but, most probably, it is the result of gastrointestinal inflammation which increases cell desquamation and mucus production⁶¹.

Of most note, is the production by the IBD cats of high concentrations of breath hydrogen from all carbohydrate containing diets irrespective of starch source in comparison to that produced by healthy cats. Variability in the AUC resulting from different carbohydrates among healthy cats indicates that some individual cats may absorb one type of carbohydrate better than others. But, among IBD cats the presence of disease was the most important factor in the carbohydrate malabsorption since these cats malabsorbed all carbohydrates irrespective of source. The reasons for carbohydrate malabsorption have already been mentioned in the introduction, but which of these is more important in the cat is unknown.

High individual variability in the AUC produced by cats fed corn and barley were observed. The physical form of the carbohydrate sources may have affected some of the responses. Two of the diets, those containing barley and corn, contained larger particles which were from time to time found in the faeces of many cats. It has been shown in humans that particle size⁶², physical form⁶³, source⁶⁴ and processing⁶⁵ affect the hydrolysis of starchy foods. Consequently the degree of malabsorption and the breath hydrogen production would be affected also. These factors have also been shown to be important in the cat^{5,15}. Perhaps these variables are important in the significant difference found in breath hydrogen peak time between tapioca and all other diets. Tapioca was added as a meal only, while the other carbohydrates were added with larger particles (whole rice, barley grits and broken corn) included.

In addition to producing the lowest levels of breath hydrogen, chicken flesh also produced faeces with the lowest faecal water content. However, the consistency (grade) of faeces was good on average for all diets and the faecal grading system used

was shown to be consistent and accurate to assess faecal water content. All carbohydrates produced a higher amount of breath hydrogen in cats with IBD compared to healthy cats, but the higher fermentable load did not affect faecal characteristics in these cats. This discordance may be due to the very high efficiency by which the feline colon can absorb water and produce faeces with high osmolality (See appendix).

Nutritionally, fermentation is not as efficient in energy recovery as small intestine absorption⁶⁶ but the importance of colonic salvage of dietary calories has been reported in human patients with short bowel syndrome⁶⁷. Rice has often been recommended as a good carbohydrate source for cats with gastrointestinal problems because of presumed good digestibility⁶⁸. Rice antisecretory properties have been reported⁶⁹. However, all other carbohydrates tested in this study produced similar or less hydrogen than rice especially in the IBD group. The differences in hydrogen production cannot be directly equated to malabsorption because of possible differences in the amount of hydrogen produced per gram of carbohydrate in different sources⁵³. However, it does suggest further research in this field is warranted.

Despite the presence of normal grade faeces, the feeding of rice increased the concentration of sodium in the faecal fluid of all cats when compared with the other carbohydrates, but especially in cats with a diagnosis of IBD when compared to a barley containing diet. The increased content of sodium in the faecal fluid while cats were consuming rice was highlighted by the faecal sodium/potassium ratio, which was significantly higher than the ratio found with the other diets. These findings are interesting and question the anti-secretory effects of rice in the cat gastrointestinal

tract. However, it is uncertain how absorptive and secretory functions relate to electrolytes present in faecal fluid. Rice based oral rehydration solutions have been found to be beneficial in the treatment of cholera and non-cholera diarrhoea in humans, although other tested cereals (maize and wheat) have also been reported to be beneficial ⁷⁰. A secretory inhibitory factor has been isolated from boiled rice that blocks the secretory response to 3',5'-cyclic-adenosine monophosphate (cAMP) of some cryptal chloride channels ⁶⁹ and could underpin the beneficial effects of rice in cholera diarrhoea. It is uncertain if this rice factor would be as effective in other type of secretory diarrhoea or if it survives food processing and appears in normal commercial petfoods containing rice. However, the presence of sodium in faecal fluid at higher concentrations in stools produced while consuming a rice diet than in another stool of similar water content is paradoxical since sodium absorption drives water absorption in the colon of most mammals studied (see Chapter 6). However, colonic water absorption on a background of sodium secretion has already been reported in the cat consuming fermentable fibres ⁷¹, which in this instance is equivalent to the presence of malabsorbed fermentable carbohydrates in the colon. Our knowledge of absorptive processes in the colon of the cat is poor and requires further study before we can make sound conclusions on the effects of malabsorbed carbohydrates in feline gastrointestinal disease.

No significant differences in faecal osmolar gap were found between diets or among the IBD and healthy cats in this study. This is perhaps not surprising because there were very few faecal samples that could be classified as diarrhoea. Because of the way in which the faecal osmotic gap was calculated, the main determinant of the results was the electrolyte content of the faeces. Electrolyte content in faecal fluid is

stable⁴⁵ but the reference range is very wide in healthy humans^{45,72}. This was also true for the cats in the present study. The value of the faecal osmotic gap to discern between osmotic and secretory diarrhoea is controversial⁷². Most of the reference values in humans have been obtained by producing diarrhoea artificially^{73,74}. Besides many gastrointestinal complaints are multifactorial in origin and both, osmotic and secretory diarrhoea can be present at the same time. In this situation, both type of diarrhoea can balance each other out producing a normal faecal osmotic gap⁴⁵.

Serum osmolality is considered the limit of achievable osmolality in the colon⁴⁵. The original formula for calculating the faecal osmolar gap involves measuring faecal water osmolality⁴⁵. The use of serum osmolality rather than faecal osmolality in the formula used in this study was required for practical reasons (see Appendix to chapter 3). It was very difficult to obtain anything other than small samples of faecal fluid from cat faeces, which at times made the use of the freezing point osmometer impossible. However, osmolality increases in faeces from the moment they leave the colon, pass into the rectum and are voided as bacterial metabolic activity breaks down large molecules into small ones⁷⁵. For this reason, the use of serum osmolality in the formula to calculate faecal osmolar gap instead of a measurement of faecal osmolality has been found to be more reliable than the original method⁴⁶. This may be important in the cat since cats can store faeces in the large intestine for considerable time (36-48 h average time between ingestion of diet and faecal evacuation of that diet in this trial).

However, an anecdotal observation from the present study suggests further investigation of the diagnostic value of faecal sodium is worthwhile. The faecal

sodium of the cat eliminated from the trial because of presumed stress-induced diarrhoea (due to loud noise from a neighbouring building site) was well above the range of the other cats (225 and 200 mmol/l of faecal fluid). Consequently, this cat had a negative faecal osmolar gap in that instance. On the same diet she had a normal motion, grade 4, with half the content of sodium before the noise started. Collectively, these observations suggest a strong secretory response induced by stress was the most likely cause of this cat's diarrhoea. Shiau et al., (1985)⁷⁵, reports that a negative faecal osmotic gap in humans indicates secretory diarrhoea. Therefore it is possible than in diarrhoea of a single origin (secretory as opposed to secretory + osmotic), the use of the faecal osmolar gap may be useful. Unfortunately, in chronic feline IBD the value of the technique appears to be questionable.

Conclusions

Cats with gastrointestinal disease show broad spectrum carbohydrate malabsorption, as per breath hydrogen collection, but remarkable tolerance as cats seem to show no untoward effects in this situation. Further investigations into the responses to different carbohydrates by cats with gastrointestinal disease are needed. The use of rice as the preferred carbohydrate in the feline gastrointestinal tract needs to be further examined. Faecal grade does not seem to be a reliable measure of dietary carbohydrate malabsorption in cats.

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APPENDIX

to CHAPTER 4

Faecal Osmolar Gap

1.- Acquisition of faecal fluid

Most faecal samples produced only small amounts of faecal fluid following the ultracentrifugation step described in the Material and Methods section. The small amount of fluid collected frequently prevented the use of the freezing point osmometer (requires at least 0.4ml) and the use of the flame photometer on the same sample. The latter consumes the sample while measuring electrolytes and requires at least 1 ml for each electrolyte, sometimes more, to reach equilibration and accurate measurement. The volume of fluid acquired is listed in **Table A1**

Table A1
Faecal fluid obtained from feline faeces by ultracentrifugation

Faecal Sample	Faecal Grade	Faeces (g)	Faecal Fluid (ml)	Faecal Fluid ml/g
1	4	2	1.3	0.65
2	5	2	0.6	0.30
3	3 to 4	2	1	0.50
4	3 to 4	2	0.5	0.25
5	4	2	0.7	0.35
6	5	2	0.4	0.2
7	4	2	0.6	0.3
8	5	3.71	None	None
9	-	15	1.6	0.11
10	-	10	0.5	0.05
11	-	15	3.4	0.23
12	-	15	1.2	0.08
13	-	15	2.8	0.19
14	-	10	0.6	0.06
15	-	15	0.45	0.03
16	-	15	2	0.13

2.- Osmolality

Cats showed very high osmolality in their faecal fluid compared to the faecal osmolality reported by others for humans ¹{Shiau Y 1987 #9580}² even when the faeces were processed immediately after collection. Because of the small volume of faecal fluid obtained from most samples, several dilution methods were compared to assess if any would be suitable to gain an accurate measure of faecal fluid osmolality. Thus, osmolality was measured in undiluted faecal fluid, diluted faecal fluid (1 in 4) and a mixture of faeces and distilled water homogenized with a kitchen beater.

All samples were graded and separated in 2 groups. The first group of faecal samples was used to measure faecal fluid osmolality and diluted faecal fluid osmolality. **Table A2** shows the osmolality of the faecal fluid and the calculated osmolality of the diluted faecal fluid by applying the dilution factor (i.e. multiplied by 4).

Table A2
Osmolality (mOsm/l) of Faecal Fluid (FF) , diluted Faecal Fluid and conversion to true osmolality using the dilution factor

Sample No	Osmolality FF	Osmolality Diluted Faecal Fluid 1:4	Converted Osmolality Diluted FF
1	815.5	291	1164
2	631.5	236.5	946
3	440.5	117	468
4	405.5	109.5	438
5	417.5	146.5	586
6	467.5	161	644
7	781	291.5	1166
8	None	None	None

The second group of faecal samples was used to compare two different methods to calculate the dilution factor to be used to measure osmolality of diluted faeces. The faeces in this second group were separated in 3 portions, one for ultracentrifugation (19500 r.p.m. x 2 h at 4°C), one for dilution and the third for oven drying. For the dilution, 5 grams of faeces were mixed with 10 ml of 0.05N HCl. Then distilled water was added and the mixture was processed with a small kitchen beater for 1 1/2 minutes. The final volume was made up to 100 ml with distilled water in volumetric flasks. After thoroughly mixing the faecal suspension by agitation, approximately 10 ml of the mixture was centrifuged at 1500 r.p.m. for 10 minutes. The supernatant was kept and filtered. This final faecal solution was used to measure the cations and osmolality of the processed diluted faeces. The portion of the faeces that was oven-dried was placed in an oven at 68 °C for several days until at a constant weight. The amount of water per gram of faeces was then calculated.

The osmolality of the processed faecal solution (Osm PF; obtained by mixing faeces with distilled water and processing with a kitchen beater) was calculated by using two types of dilution factors. One was based on the faecal fluid produced by ultracentrifugation of that sample (PF(FF)DF) and the other based on the faecal

Dilution factor based on the amount of faecal fluid recovered

$$\text{PF(FF)DF} = 100 / (5 \text{ g} \times \text{Faecal Fluid ml/g})$$

Dilution factor based on the amount of faecal water

$$\text{PF(FW)DF} = 100 / (5 \text{ g} \times \text{Faecal Water ml/g})$$

water content calculated by oven drying of the same sample (PF(FW)DF).

None proved useful as can be seen in **Table A4**. **Table A3** shows the content of faecal water and faecal fluid used for the calculation of the dilution factors.

Table A3
Faecal fluid and Faecal water content

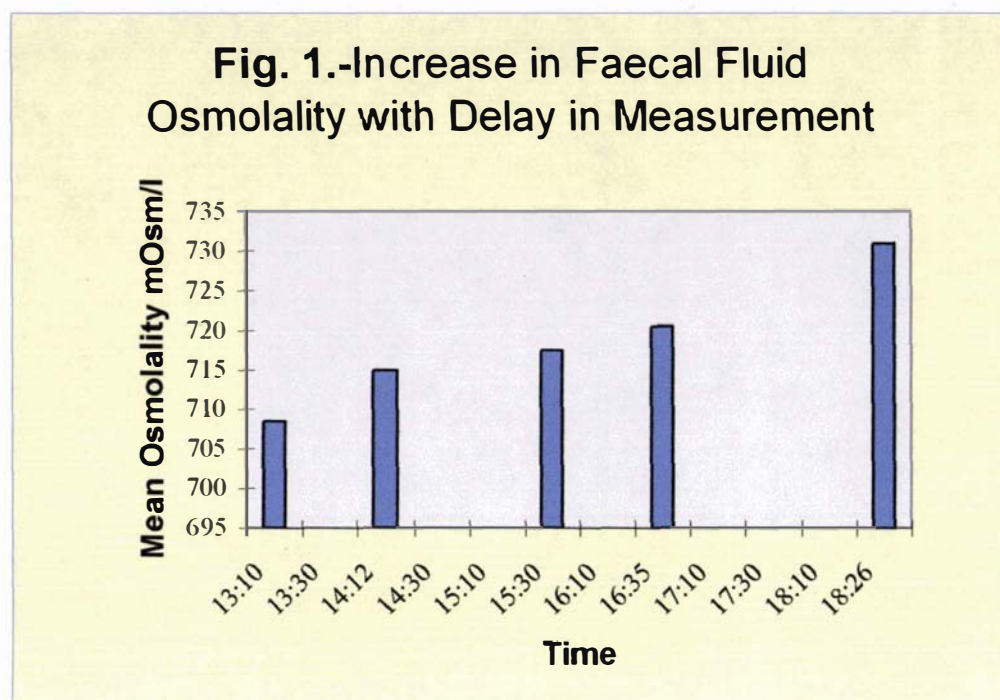
Sample No	Faecal g for Ultracent	Faecal Fluid Recovered	Faecal Fluid x g	Wgt Oven In (g)	Wgt Oven Out (g)	Faecal Water x g
1	15	1.6	0.11	3.8	2.249	0.41
2	10	0.5	0.05	1.209	1.199	0.01
3	15	3.4	0.23	5	2.038	0.59
4	15	1.2	0.08	5	2.472	0.51
5	15	2.8	0.19	5	2.182	0.56
6	10	0.6	0.06	5	2.517	0.50
7	15	0.45	0.03	5	2.224	0.56
8	15	2	0.13	5	2.195	0.56

Table A4
Osmolality of faecal fluid and of diluted faeces calculated by two dilution methods

Sample No	Osm FF	Osm PF	PF(FF)DiIF	PF(FW)DiIF	Osm PF FF	Osm PF FW
1	730	36	187.50	49.00	6750	1764.02
2	662.5	31.5	400.00	2418.00	12600	76167.00
3	818.5	38	88.24	33.76	3352.94	1282.92
4	656	35.5	250.00	39.56	8875	1404.27
5	807.5	42	107.14	35.49	4500	1490.42
6	730	41.5	333.33	40.27	13833.33	1671.37
7	555	34.5	666.67	36.02	23000	1242.80
8	751.5	35	150.00	35.65	5250	1247.77

Note: OsmFF= Osmolality of the faecal fluid. OsmPF= osmolality of the processed faeces. PF(FF)DiIF= dilution factor based on faecal fluid. PF(FW)DiIF= dilution factor based on faecal water. OsmPFFF= calculated osmolality based on faecal fluid dilution. OsmPFFW= calculated osmolality based on faecal water dilution

Osmolality of faecal fluid increases with time as reported by several investigators^{3 2}. The effects of delay in measurement of faecal fluid osmolality on the samples is shown in **Figure A1**.



The very high osmolality of the fluid recovered from the faeces of the cats in the present study was unexpected. It was possible that the high osmolality was an artifact resulting from fermentation of faecal substances in the time elapsed prior to collection or during storage. As a result of these concerns, a small study was undertaken as an adjunct to the principal study reported in this chapter. The aim of the adjunctive study was to measure the osmolality of fresh gastrointestinal contents of cats at different sites in the gastrointestinal tract.

The gastrointestinal tracts were harvested immediately after death from five cats euthanised at the Massey University Veterinary Teaching Hospital. The reasons for euthanasia of these cats and their signalment is shown in **Table A5**. Owners consent was granted.

Table A5

Details of cats that provided feline intestinal contents for measurement

Cat No.	Reason for euthanasia	Sex	Age	Breed
1	Feral Cat	F	Adult	DSH
2	Diabetes	MC	13y	DLH
3	Flea Allergy Dermatitis	F	Adult	DSH
4	Sacral Spinal Luxation	F	Adult	DMH
5	Uncontrolled Diarrhoea	MC	Adult	DSH

Immediately after death the abdominal cavity was opened and the gastrointestinal tract from stomach to rectum was removed. The stomach, duodenum, jejunum, ileum, cecum and colon were promptly separated from each other with scissors. A sample of the contents of each region of the gastrointestinal tract was collected and placed into an ultracentrifuge tube. In some regions of the bowel the contents of the tract were pasty and they needed to be gently scraped from the mucosa before placement in the ultracentrifuge tube for centrifugation at 20,000g and 4°C for 1 to 2 hours. The contents of a region of the bowel were sometimes present in insufficient quantities to collect an adequate sample for centrifugation. For this reason, it was occasionally necessary to pool the contents of adjacent bowel regions to obtain sufficient fluid. **Table A6** specifies the samples in which admixture was required and shows the osmolalities of the contents in the different bowel regions of the cats.

An analysis of these data shows that osmolalities of the intestinal contents from the duodenum to the rectum of cats are higher than 314 mOsm/l (serum osmolality in cats). Serum osmolality is considered as the limit of the attainable osmotic pressure in the colon in people ⁴. In the duodenum and jejunum, the digestion of food macromolecules into smaller molecules might be expected to increase osmolality of intestinal contents. However, the rapid absorption of the products of digestion and the freely permeable (to water) small intestinal mucosa would be expected to offset the increase in osmolality expected from digestion. The fermentation of non-absorbed carbohydrates to volatile fatty acids by bacteria in the small intestine (in particular the ileum) and colon might also contribute to the very high osmolalities recorded in the lower small intestine and large intestine in these cats. However, it is uncertain how the high osmolality is maintained in the face of what is presumed to be a gastrointestinal mucosa that is freely permeable to water.

Table A6

Osmolality (mOsm/l) of gut contents in cats immediately after euthanasia

Site	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5
Stomach	-	273.5	-	-	-
Duodenum	357.5	378.5	397.5	457.5	-
Jejunum	-	531.5	406.5	345	-
Ileum	422	470.5	354.5	322	-
Cecum	488	-	-	368.5	594
Cecum-Right Colon	-	766	-	-	-
Cecum-Total Colon			447.5	-	-
Total Colon	-		441	416	672.5
Left Colon- Rectum	-	804.5	-	-	-
Rectum	525	-	-	431	-

These observations seem to suggest the cat has a gastrointestinal mucosal mechanism that allows absorption of water against a high osmotic pressure in the bowel lumen.

3.- Electrolytes

Flame Emission Photometer: The small volume of faecal fluid (FF) obtained by ultracentrifugation of the faeces constrained the measurement of electrolytes in faecal fluid by flame emission photometry because the faecal fluid had to be diluted several fold to obtain enough fluid for the electrolyte measurements. The

differences incurred by dilution of faecal fluid can be seen in the **Table A7**.

Table A7

Content of sodium and potassium in diluted and non-diluted faecal fluid

Na mMol/l FF dil 1:2	Na mMol/l FF	% Diff	K mMol/l FF dil 1:4	K mMol/l FF	% Diff
110	104	-0.06			
95	90	-0.06	15.2	12	0.21
64	51	-0.25	11.2	8.7	0.22
48	42	-0.14	12.2	9.6	0.21
40	32	-0.25			

Equally, comparisons between the direct measurements of electrolyte concentrations in faecal fluid (FF) and the measurements obtained on fluid (PF) derived by mixing the faeces with distilled water (using the dilution factors – PF(FF)DF and PF(FW)DF described in the osmolality section – **Table A3**) were poor. This can be appreciated in **Table A8**.

The inaccuracies of both methods of dilution may originate in the fact that faecal fluid obtained by centrifugation is not a constant proportion of the total faecal water content (as measured by oven drying). Faeces are a complex system that can retain water in different phases and ultracentrifugation may not act to the same extent on all of these phases. Another potential source of inaccuracy would be the disruption of faecal matter by the processing perhaps allowing electrolytes to be released or bound in different ways than when disruption does not occur.

Table A8

Sodium and potassium content (mmol/l) of faecal fluid and diluted faeces using two different dilution factors

Potassium

Sample No	K Content Faecal Fluid	K Content Diluted Faeces	Converted K PF(FF)DF	Converted K PF(FW)DF
1	13.75	0.41	76.88	20.09
3	10	0.38	33.53	12.83
4	16.5	0.42	105	16.61
5	14	0.44	47.14	15.61
6	16.25	0.39	130	15.71
7	14.25	0.38	253.33	13.69
8	11.75	0.37	55.5	13.19

Sodium

Sample No	Na Content Faecal Fluid	Na Content Diluted Faeces	Converted Na PF(FF)DF	Converted Na PF(FW)DF
1	110	2	375	98.00
3	175	4	352.94	135.04
4	110	3	750	118.67
5	155	4.2	450	149.04
6	190	3.3	1100	132.90
7	200	3.2	2133.33	115.27
8	195	4.2	630	149.73

Another source of variation is the method used for the measurement of electrolytes. The flame photometer is designed to measure watery fluids in a very thin film. Feline faecal fluid was invariably a dense fluid that did not mix well with water and that contained impurities (which initially cause many problems with the operation of the flame photometer, hence the introduction of the filtration step). Thus, the measurement of a diluted solution may not be equivalent to the measurement of a thicker fluid. The flame photometer also measures electrolytes in a tight range determined by a calibration curve. Dilution may mean that the best calibration curve for the diluted fluid was not the one chosen for the undiluted faecal fluid. Because of the wide range of sodium and the small amount of faecal fluid to work with it was impractical to use different calibration curves to test these possibilities.

A regression analysis was performed between the electrolyte concentration measured in undiluted faecal fluid and that measured in diluted faeces (using faecal water to calculate a dilution factor). Faecal water was calculated by freeze-drying faeces in this instance. The equation representing the regression line for sodium was:

$$\text{Na Content in Faecal Fluid} = 1.96 \pm 0.33 \times \text{Na (in Processed Faeces)} + (-0.64.52 \pm 22.25)$$

The r^2 is 0.86 ($p < 0.005$) and the standard error of the estimate of Na concentration in faecal fluid is 10.85. See **Figure A2** and **Table A9**

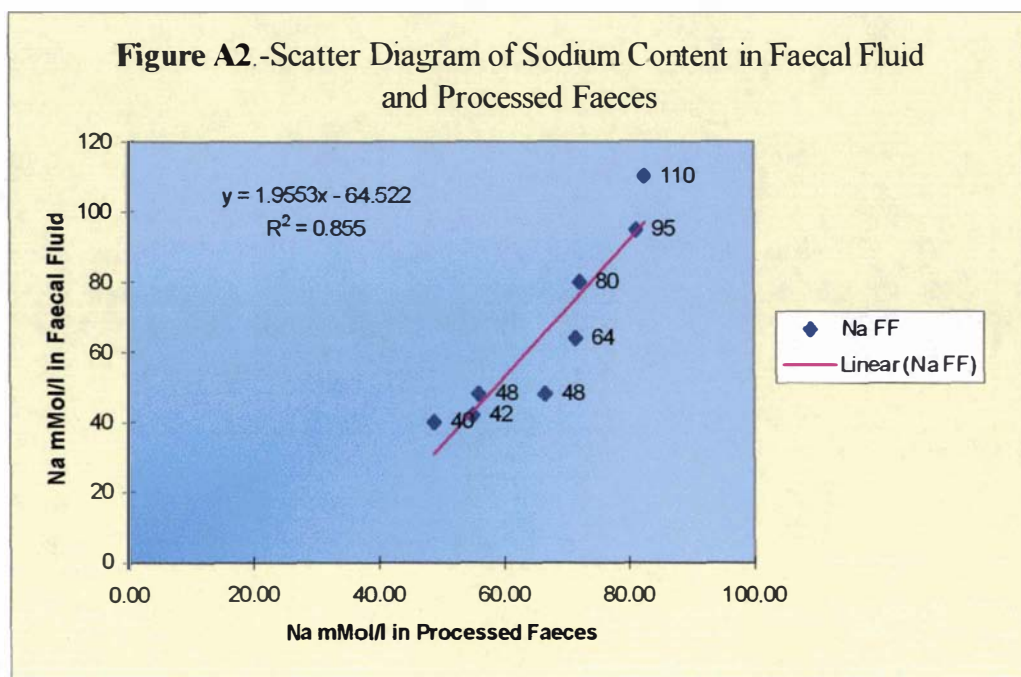


Table A9

Sodium Content in diluted faeces calculated according to a regression equation

Converted Na PF(FW)DF	Na Faecal Fluid	% Diff w/Faecal Fluid	Na by Regression Equation	% Diff w/Faecal Fluid
72.08	80	-0.11	76.76	-0.04
82.62	110	-0.33	97.41	-0.13
81.23	95	-0.17	94.70	0.00
55.00	42	0.24	43.28	0.03
55.86	48	0.14	44.96	-0.07
71.42	64	0.10	75.45	0.15
66.50	48	0.28	65.83	0.27
48.80	40	0.18	31.12	-0.29

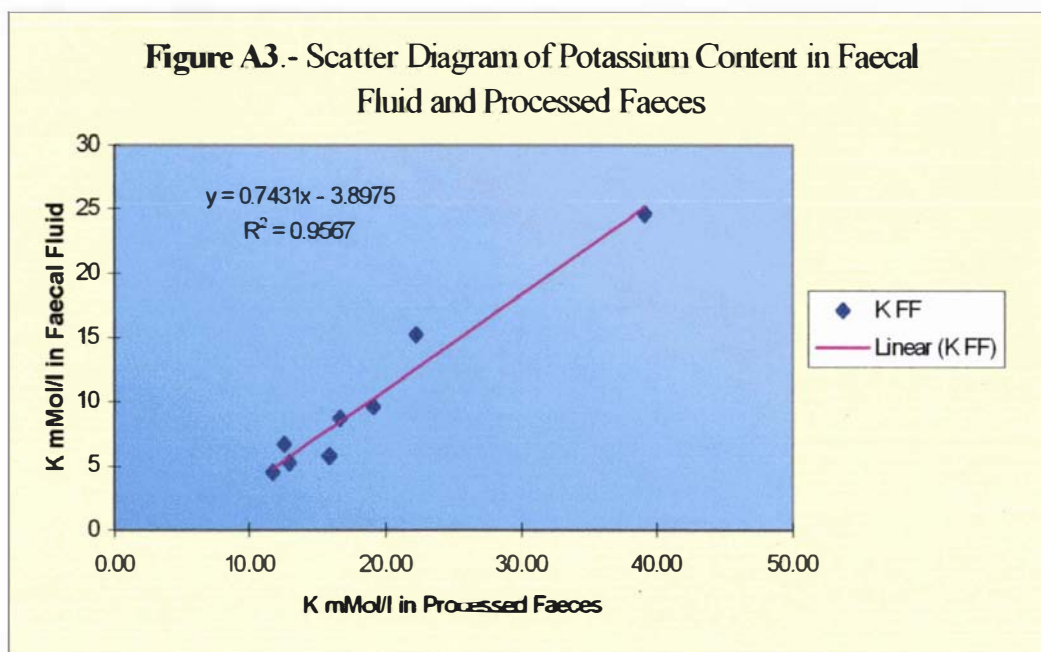
When the sodium content is calculated according to the regression equation the differences between the processed faeces and the undiluted faecal fluid decreases. However, when this formula was applied to the processed faeces electrolyte concentrations of some osmotic diarrhoea samples (produced by suddenly changing diet, see later) there was an overestimation of sodium content in

these samples and an underestimation in the non-diarrhoeic samples. These two errors in different directions eliminate the basis of the method on which osmotic diarrhoeas are identified when using the faecal osmolar gap.

The regression equation calculated for the potassium concentration of undiluted faecal fluid and diluted faeces was:

$$\text{K Content of Faecal Fluid} = 0.74 \pm 0.06 \times \text{K (in Processed Faeces)} + (-3.9) \pm 1.33$$

The $r^2 = 0.96$ ($p < 0.001$) and the standard error of the estimate is 1.53. See Figure A3 and Table A10.



Potassium had a much better agreement between the measurement in diluted faeces and undiluted faecal fluid but there were still some errors of high magnitude.

Table A10
Potassium content in diluted faeces calculated with a regression equation

Converted K PF(FW)DF	K Faecal Fluid	% Diff w/Faecal Fluid	K by Regression Equation	% Diff w/ Faecal Fluid
22.19	15.2	0.31	12.52	-0.214
16.65	8.7	0.48	8.42	-0.033
19.04	9.6	0.50	10.19	0.058
12.57	6.7	0.47	5.40	-0.240
39.10	24.6	0.37	25.03	0.017
15.90	5.8	0.64	7.87	0.263
11.74	4.55	0.61	4.78	0.049
12.97	5.25	0.60	5.70	0.079

Selective Electrode: Measurement of faecal electrolytes by an ion selective electrode was attempted and compared with the electrolyte concentration measured by flame photometry. The faecal fluid needed to be diluted to measure potassium by ion selective electrode. The range of measurement of this particular electrode for potassium was for concentrations between 1.5 to 10 mEq/l. The potassium concentration of feline faecal fluid had been previously measured to be 10 mEq/l or above by flame photometry. The range of the electrode for sodium was 80 to 180 mEq/l. The sodium concentration of faecal fluid samples had been previously measured to be inside, above and below this range by flame photometry.

Different dilutions of the faecal fluid were used to measure electrolytes with the ion selective electrode, as allowed by the total volume of faecal fluid available. This was done with the aim of learning if outside the known range of the electrode there was still a linear relationship for the electrolyte contents that could be exploited. Unfortunately the ion selective electrode was very inaccurate for the potassium measurement, even if faecal fluid was diluted to bring the concentration of potassium inside the accepted range. It is possible that the nature of faecal fluid affected the functioning of the electrode as the manufacturer includes correction factors in the calculation of electrolyte concentration to correct for the mass concentration of water of the fluid intended to be measured by the specific ion selective electrode. This is a reported source of differences between the flame photometer and ion selective electrode ⁵.

Most faecal fluid had sodium concentrations below the validated range for the ion selective electrode. Therefore, nothing could be done to alter the concentration of these samples to bring them inside the accepted range. For the samples that had a sodium content inside the validated range of the ion selective electrode, there was good agreement between the sodium concentrations estimated by the flame photometer and the ion selective electrode. Agreement between the two methods was poor outside of the validated range. These results indicate that the ion selective electrode should not be recommended for measurement of faecal electrolytes. See **Table A11**

Table A11

Differences in sodium and potassium measurements in faecal fluid between the flame photometer and an ion selective electrode

Sample No.	Na Flame Photometer	Na Ion S Electrode	% Diff	K Flame Photometer	K Ion S Electrode	% Diff
1	80	84.5	0.053	15.2	32.76	0.54
2	110	109.7	-0.003	11.2	29.04	0.61
3	95	97.2	0.023	12.2	24.88	0.51
4	42	58	0.276	6.7		
5	48	56.6	0.152	24.6	38.08	0.35
6A	64	77.6	0.175	5.8		
6B	48	61.1	0.214	4.55		
7	40	54.3	0.263	5.25		

4.- Faecal Osmolar Gap

There are two principal methods for calculating faecal osmolar gap, one uses faecal osmolality and another serum osmolality.

$$\text{FAECAL OSMOLAR GAP} = \text{Faecal or Serum osmolality} - [2 \times (\text{Na}^+ + \text{K}^+)]$$

The faecal osmolar gap was calculated using different methods of measuring or calculating osmolality, sodium and potassium concentrations. The direct measurement of the faecal osmolar gap did not agree well with dilution methods or regression methods, even when the osmolality of faecal fluid was replaced by the mean feline serum osmolality (313 mOsm/l). The agreement was better when dealing with diarrhoea samples (Faecal Grade 2 to 3). The diarrhoea was assumed to be osmotic in nature because it was produced by changing the diet and it disappeared once the diet was returned to normal. The original technique

Table A12

Comparison of the faecal osmolar gap in normal consistency faeces and diarrhoea samples using the original (Osmolar Gap) and dilution methods -PF(FF) and PF(FW)

Normal Consistency Faeces			Diarrhoea Samples		
Osmolar Gap	Osm Gap PF(FF)	Osm Gap PF(FW)	Osmolar Gap	Osm Gap PF(FF)	Osm Gap PF(FW)
482.5	5846.3	1527.84	657	2598.82	1273.023
448.50	2580.0	987.17	557.3	2001.08	958.7266
403	7165.0	1133.70	494.4	1592.17	1061.421
469.5	3505.7	1161.11	418.7	1295.51	913.3986
317.5	11373.3	1374.14			
126.50	18226.7	984.87			
338	3879.0	921.93			
<i>Correlation with the true osmolar gap</i>					
	-0.88	0.40		0.995	0.84

(called 'true osmolar gap' in **Table A12**) using undiluted faecal fluid to measure osmolality, sodium and potassium concentrations tended to show a difference between faeces of normal consistency and the osmotic diarrhoea samples. The calculations based on dilution factors in accordance with faecal fluid content - PF(FF) or faecal water content - PF(FW), did not identify any difference between diarrhoea samples and normal faeces.

The osmolar gap was also calculated in faecal fluid from another set of faecal samples using the dilution factor based on faecal water and the regression analysis equations (see above) for electrolyte calculations (**Table A13**). The serum osmolality was used in this instance and assumed to be 313 mOsm/l.

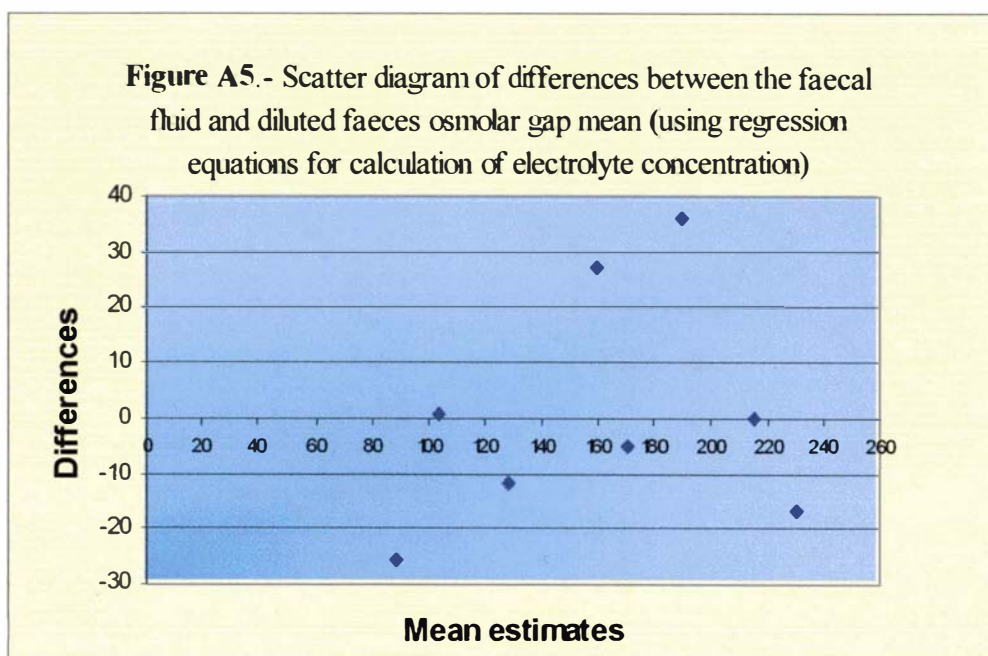
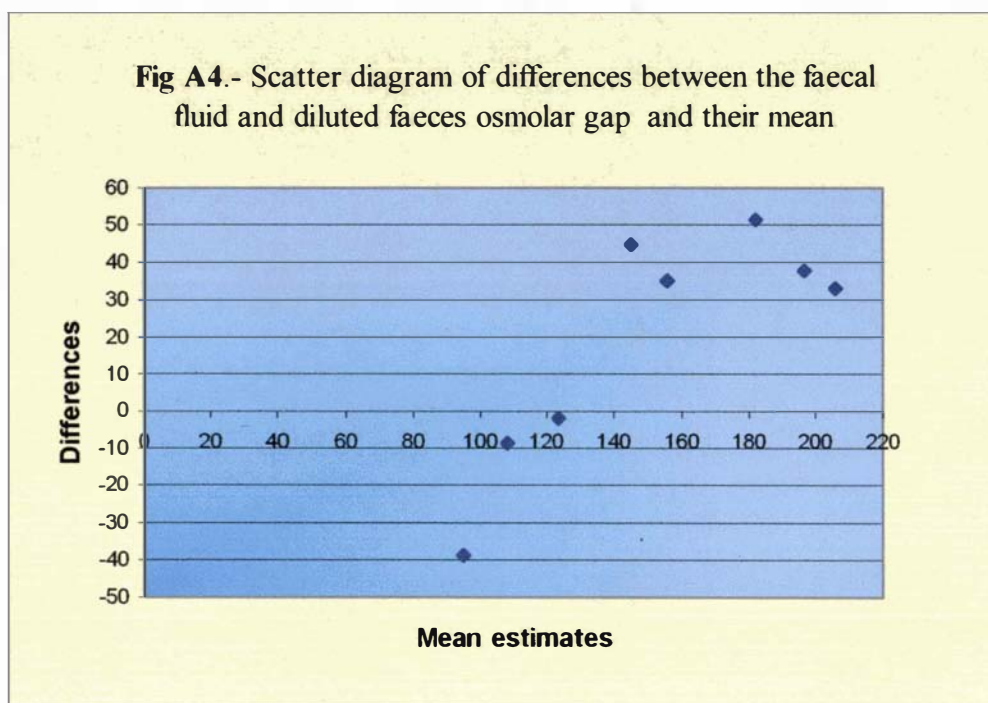
Table A13
 Faecal osmolar gap calculated by using serum osmolality in faecal fluid (Osm Gap), diluted faeces (OG PF) and using a regression equation (OG EQ)

Sample	Grade	OSM GAP	OG PF	OG EQ
1	5	122.6	124.46	134.44
2	4-5	75.6	114.46	101.34
3	4-5	103.8	112.46	103.23
4	3	215.6	177.86	215.63
5	2	167.8	123.08	173.01
6A	3	173.4	138.37	146.36
6B	2	207.9	156.52	171.78
7	2	222.5	189.46	239.35
<i>Correlation w/faecal fluid Osmolar Gap</i>			0.89	0.92

Once again the dilution method did not differentiate diarrhoea samples from normal samples. The method utilizing faecal fluid did separate the samples. The regression equation eliminated some of the variability and identified at least 4 out of 5 diarrhoea samples, if we use a reference range of faecal osmolar gap between - 115 to 167 (derived from the data already accumulated).

A better way of comparing two analytical methods is the calculation of the bias, the limits of agreement, and their confidence interval ⁶. These calculations demonstrated that the differences encountered between the dilution method and the original method for calculating faecal osmolar gap was substantial.

Figure A4 and **A5** show the degree of agreement between the dilution method and the original method for calculating faecal osmolar gap, and between the original method and the calculations using the regression equation. Obviously,



there is a bias present when higher differences are obtained in samples with higher osmotic gaps. This improved when using figures obtained by regression equations, yet the level of disagreement between the two techniques remained marked.

Table A14 indicates that the limits of agreement of the samples are too broad and not acceptable. The bias is the mean difference between the two estimates of faecal osmolar gap. The limits of agreement calculated with the use of the standard deviation of the differences indicate that the mean difference (bias) is 95% of the time between -43.2 to 81.3 when the direct measures are use, and between -41.1 and 42.1 when the regression equations are used. The confidence

Table A14

Calculation of bias, limits of agreement and their confidence intervals between the osmotic gap measured in faecal fluid (Osm Gap), diluted faeces (OG PF) and using regression equations (OG EQ).

OG	OG PF	OG EQ	OG-OGPF	Mean OG/OGPF	OG-OGEQ	Mean OG/OGEQ
122.60	124.46	134.44	-1.86	123.53	-11.84	128.52
75.60	114.46	101.34	-38.86	95.03	-25.74	88.47
103.80	112.46	103.23	-8.66	108.13	0.57	103.51
215.60	177.86	215.63	37.74	196.73	-0.03	215.62
167.80	123.08	173.01	44.72	145.44	-5.21	170.40
173.40	138.37	146.36	35.03	155.89	27.04	159.88
207.90	156.52	171.78	51.38	182.21	36.12	189.84
222.50	189.46	239.35	33.04	205.98	-16.85	230.93
		Bias	19.07		0.51	
		SD	31.76		21.21	
		SE	11.23		7.50	
		Limits	-43.19	81.32	-41.06	42.07
		t	2.36		2.36	
Confidence Interval Bias			-7.49	45.62	-17.22	18.24
SE Agreement			19.45		12.99	
Confidence Interval Lower Lim			-89.19	2.80	-71.77	-10.35
Confidence Interval Higher Lim			35.33	127.32	11.36	72.78

intervals for the upper and the lower estimates of the limits of agreement are found in the last two lines of the table and further indicate the lack of agreement of the two methods.

The reasons why dilution of faeces with water failed to provide fluid upon which accurate estimates of electrolyte concentrations and osmolality, as compared to faecal fluid measurements, could be made is not completely understood. Dilution affects osmolality in a non-linear manner ⁴ but the effects of dilution on electrolyte concentrations should be linear. However, faeces have a complex composition involving the presence of fluid, solids, electrolytes, bacteria and different chemical compounds. Because of the presence of colloidal substances like proteins and polysaccharides the distribution of electrolytes through the system may not be even and may be influenced by surface phenomena at the interface between solid and fluid ⁷. It is known that the size of the hydration crown of electrolytes, the pH of the system compared to the pK of ionizable substances, the presence of chemicals that affect surface tension (like bile acids), the swelling of colloids and other interactions can affect the distribution of particles in a colloidal system ⁸. Furthermore, dietary fibres and other macromolecules differ in their water holding capacity. Some of the water is unavailable if the molecular structure is not disturbed. The degree of disruption of the solid phase and the resultant availability of water held in this phase may differ between the ultracentrifugation and dilution

In conclusion, these results suggests that the measurement of the faecal osmolar gap using diluted faeces does not have the discriminatory power that the faecal osmolar gap measured directly in faecal fluid has to differentiate secretory from osmotic diarrhoeas. The method of measuring faecal osmolar gap using serum osmolality and faecal fluid electrolyte concentrations (**Figure A6**) identified the diarrhoea samples as different from the normal faeces but also indicated the nature of the diarrhoea (osmotic diarrhoea in this case). Even though it required direct measurement of electrolytes in the faecal fluid, this method was chosen for the dietary trials. It is noteworthy that the sodium concentration in the diarrhoea samples is consistently below the normal samples, which is expected in osmotic diarrhoeas. See **Table A15**.

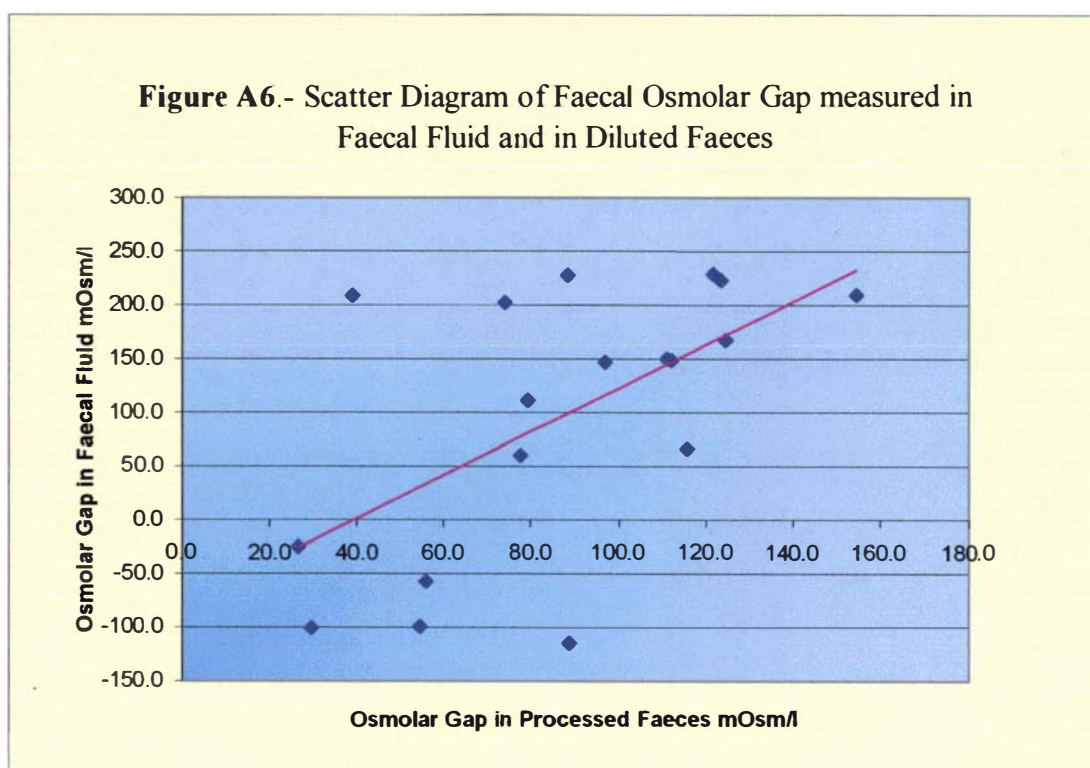


Table A15

Use of the faecal osmolar gap calculated with serum osmolality and faecal fluid electrolytes to differentiate diarrhoea from normal faeces

Faecal Grade	Osm Gap	Na	K
4	65.50	110	13.75
4	-49.86	160	21.43
4	-57.00	175	10
4	60.00	110	16.5
4	-25.00	155	14
4	-99.50	190	16.25
4	-115.50	200	14.25
4	-100.50	195	11.75
3	223	34.20	10.8
2	227.76	38.00	4.62
2-3	202.4	43.00	12.3
2	208.2	43.00	9.4
2-3	209.4	38.2	13.6
3-4	111.4	94	6.8
3-4	229	32	10
3-4	135	80	9
3-4/blood	147.2	77	5.9
3-4/blood	149.8	73	8.6
4	149	62	20
3-4	167.1	64	8.95

Faecal Water Content

Two methods to measure faecal water content were evaluated. Cat faeces are very compact and dense which slows down evaporation. It was noticed that normal faeces were still losing water at a very slow rate after 4 days in the oven. This would have affected the calculation of the dilution factor based on water content as well as our evaluation of dry matter content of the faeces. The results of oven drying the faeces at approximately 68-70°C can be seen in **Table A16**.

Freeze drying of faeces to measure faecal water content suggested there was a rather even amount of water in all samples, including diarrhoeal faeces (Table A17). The lower average water content of these faecal samples, than that of the faecal samples measured by the oven-drying method may indicate that there was a slight overestimation of water content with the oven drying method. Alternatively the freeze-dry method may have underestimated faecal water content. Certainly, there was poor discrimination of faecal consistency by the freeze drying compared to the oven drying. The oven drying technique was selected for use in the carbohydrate diet study reported in this chapter.

Table A16

Results of oven drying of feline faeces. Weight in grams after being in the oven a certain number of days

Grade	g Faeces Oven IN	Weight Foil	5 d	6 d	7 d	18 d	Difference 7 d-18 d	g Faecal Water
2-3	10	0.524	3.101	3.096	3.09	2.553	0.537	0.80
3-4	10	0.888	2.559	2.552	2.546	2.522	0.024	0.84
3-4	5	0.776	3.27	3.262	3.26	3.236	0.024	0.51
3-4	10	0.758	2.686	2.679	2.67	2.652	0.018	0.81
3-4	10	0.52	3.128	3.123	3.115	3.082	0.033	0.74
3-4	10	0.902	3.098	3.089	3.079	3.054	0.025	0.78
4	5	0.722	2.146	2.142	2.137	2.131	0.006	0.72
3-4	5	0.894	2.176	2.172	2.169	2.16	0.009	0.75

Table A 17

Results of freeze drying faeces. Weight in grams.

Grade	g Fr-Dr	g Foil	g OUT	g Dr Faec	g FW/g Faeces
5	18.84	1.39	6.9	5.51	0.71
4-5	14.29	1.94	5.16	3.22	0.77
4-5	14.33	1.72	4.76	3.04	0.79
3	9.35	1.02	3.23	2.21	0.76
2	18.56	1.12	5.06	3.94	0.79
3	25.13	1.03	7.51	6.48	0.74
2	31.83	1.09	8.51	7.42	0.77
2	27.25	1.09	7.12	6.03	0.78

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Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>Stomach, seven pieces: there is diffuse infiltration of the gastric lamina propria with low numbers of lymphocytes and plasma cells, and occasional mast cells and eosinophils. There are also several lymphoid follicles deeper in the mucosa which contain mild to moderate numbers of eosinophils and macrophages. Duodenum, ten pieces: there are mildly increased numbers of lymphocytes and plasma cells, as well as a few eosinophils. There are moderate numbers of intra-epithelial lymphocytes and lower numbers of intra-epithelial neutrophils. Two lymphoid follicles are present. There is mild oedema of the lamina propria. Colon, eight pieces: these contain dilated mucosal glands filled with mucus and sloughed cells. There are several lymphoid follicles, and one fragment shows mild fibrosis within the lamina propria. The total number of cells in the lamina propria is within normal limits. Rectum, one large piece: No abnormal findings</p>	Moderate	2.5y	CONTINUOUS (2 BOUTS PER YEAR)	CONTROL WITH DIET	Mustard-yellow. Liquid, normal volume. Two per day	N	Flatulence. Mucus in faeces. Increased defecation. Abdominal pain. Urgency to defecate	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>Stomach, ten pieces: There is multifocal moderate increase in cellularity of the lamina propria (mainly lymphocytes, plasma cells and eosinophils), moderately increased numbers of intra-epithelial inflammatory cells, and moderate numbers of globular leucocytes, especially deeper in the mucosa.</p> <p>Duodenum, four damaged fragments and one undamaged piece: the total numbers of cells within the lamina propria are within normal limits but there are moderately increased numbers of intra-epithelial lymphocytes. There is mild oedema of the lamina propria and increased mucin between villi.</p>	Mild	7y	NUMEROUS	CONTROL WITH DIET	One or two stools a day. Variable colour. Normal volume	Y	Decreased appetite. Lethargy. Weight loss. Abdominal pain. Borborygmus. Positive RAS to several antigens	Pyloric antrum pale with reddish irregular erosive areas.
<p>Stomach, six pieces: there are multiple discrete lymphoid follicles present in both the superficial; and deep submucosa. Very occasional globular leucocytes are present within glandular epithelium. There are loss numbers of lymphocytes and plasma cells scattered diffusely throughout the lamina propria. In one biopsy fragment there is fibrosis of the lamina propria. Duodenum, eleven pieces: Most of the duodenal biopsies suffer from squash artefact. Within the intact tissue there is mild focal increase in cellularity of the lamina propria (mainly plasma cells and lymphocytes, with occasional eosinophils), increased numbers of globular leucocytes and moderate numbers of intra-epithelial lymphocytes.</p>	Mild	3y	NUMEROUS	NOT CONTROLLED	N	Food (immediately after eating) fur or grass. Two vomits a day.	N	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>Stomach, six pieces: there are low numbers of lymphocytes and plasma cells in the lamina propria. Duodenum, seven pieces: There are moderate numbers of plasma cells, lymphocytes and eosinophils scattered throughout other lamina propria, as well as small focal aggregates of neutrophils. Many plasma cells contain Russell bodies. Colon, seven pieces: there are low numbers of lymphocytes, plasma cells (some with Russell bodies), eosinophils and mast cells within the lamina propria. Rectum, one piece: similar to colon</p>	Mild	2y	CONTINUOUS	CONTROL WITH DIET AND MEDICATION	N	Food. One vomit per day	Increased appetite. Flatulence. Abdominal bloating. Weight loss	NAF
<p>Stomach, seven pieces: Helicobacter present 5 + Duodenum, eight pieces: increased numbers of intra-epithelial lymphocytes, mild increase in lymphocytes and plasma cells in lamina propria with occasional neutrophils. Colon, six pieces: mild increase in lymphocytes and plasma cells in lamina propria. Globule leukocytes increased in number and prominent. Rectum, one piece: goblet cells reduced in number, globule leukocytes increased and neutrophils prominent in lamina propria and proprial capillaries.</p>	Mild	4y	NUMEROUS	CONTROL WITH DIET	Liquid to cream, 4-5 times a day.	N	Has been diagnosed with Giardia and treated effectively. Urgency to defecate. Flatulence. Anus and surrounding skin red. Mild leukopenia. Developed a FeLV + test	NAF

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>Stomach, nine pieces: prominent focal lymphoid aggregations were present in the middle and lower mucosa in five of these samples. Duodenum, four pieces: moderately excessive numbers of intraepithelial lymphocytes were present in all four samples. About 20-30 plasma cells were present in each villus cross section and 10 eosinophils. Larger clumps of plasma cells were present in the middle mucosal lamina propria. Colon: focal mixed lymphocyte and plasma cell infiltration of the mucosa in one of eight samples. Rectum: contains a focal lymphoid nodule with an active germinal centre in the submucosa.</p>	Moderate	10y	NUMEROUS	CONTROL WITH DIET	Liquid , 4-5 times a day. Long periods without problems	Small amounts of foam with fur	Weight loss.	Duodenum bled easily

Endoscopy findings : NAF=no abnormal finding; ND= not done. *Diarrhoea* and *Vomiting*: Y=presence; N=absence.

CHAPTER 5

THE EFFECTS OF INULIN ON THE GASTROINTESTINAL TRACT OF HEALTHY CATS AND ITS POSSIBLE APPLICATIONS TO FELINE INFLAMMATORY BOWEL DISEASE WITH PARTICULAR EMPHASIS ON THE LARGE BOWEL

Feline Inflammatory Bowel Disease (IBD) is an idiopathic condition characterized by non-specific gastrointestinal clinical signs ^{1,2}. It can affect any segment of the gastrointestinal tract i.e. stomach, small intestine and colon ³. Diagnosis is based mainly on the detection of inflammatory infiltrates in the epithelium and lamina propria of the gut and the elimination of other diseases that are known to cause bowel inflammation ¹⁻⁵. Several investigators have reported inflammatory infiltrates in colonic biopsy specimens from cats with presenting clinical signs suggestive only of small intestinal dysfunction ^{2,6,7}. These reports suggest that colonic structure and function should always be considered when evaluating animals with IBD, whether or not the presenting signs can be localised to the 'large bowel' or 'small bowel'.

The main functions of the colon and rectum are the absorption of water and electrolytes and the storage and elimination of faeces⁸. Although the mechanisms involved in these tasks^{9,10} are not fully understood, it is reasonable to expect that during colonic disease these functions would be adversely affected. The epithelial lining of the colonic lumen and crypts is involved in the processes of absorption and secretion¹⁰⁻¹² and thus, its anatomical and functional integrity is important for these functions. In addition, gastrointestinal motility in humans¹³ and dogs¹⁴ is thought to be influenced by the cells of the immune system in health and disease.

Thus, it is no surprise that a decrease in colonic absorption has been demonstrated in colonic perfusion studies of people with diarrhoea¹⁵. More interestingly, absorption rate was inversely correlated with the intensity of lamina propria inflammatory infiltration and directly correlated to the number of colonic epithelial cells¹⁵. Similarly, decreased and disordered colonic motility accompanies ulcerative colitis in humans¹⁶, acute chemically induced colitis in dogs¹⁷ and cats¹⁸ and idiopathic diarrhoea in cats¹⁹. In dogs and cats disturbances of colonic motility can still be present when clinical signs have disappeared^{17,18}. The physical basis for deranged motility during inflammatory bowel disease is unknown but localised ultrastructural changes in the interstitial cells of Cajal –putative gastrointestinal pacemakers- have been reported in ulcerative colitis in humans²⁰. The abrogation of motility abnormalities by anti-inflammatory therapy suggest that inflammatory mediators are involved in the motility abnormalities seen during gastrointestinal disease¹³.

Given that alterations in the absorptive functions and motility of the colon are present during gastrointestinal diseases such as colitis, effective therapy should logi-

cally focus on normalising these parameters. Dietary fibre has a number of beneficial effects on gastrointestinal structure and function, such as motility and absorption of fluid and electrolytes (See review in Chapter 1). Many of the effects of dietary fibre on colonic function and colonocyte integrity depend on the production of short chain fatty acids (SCFA) -especially butyrate- by the bacterial fermentation of dietary fibre²¹. Impaired butyrate metabolism has been shown in mice with experimental colitis²². Butyrate enemas have been successful in reducing the clinical signs of experimental colitis in mice, stimulating electrolyte absorption and decreasing macroscopic colonic damage²³. Butyrate enemas have also been shown to ameliorate histopathological abnormalities in people with distal ulcerative colitis²⁴. Furthermore, inhibition of SCFA oxidation induced disease in a rat model of ulcerative colitis²⁵.

Short chain fatty acids have been shown to increase proliferation of colonic epithelium and colonic crypt size in rats²⁶. In addition, the capacity of SCFA to reverse net secretion of colonic fluid to net absorption in human patients has been confirmed²⁷. The importance of SCFA for colonic fluid absorption in the dog has also been demonstrated²⁸.

The effects of dietary fibre on colonic motility are more difficult to interpret. Insoluble dietary fibres alter the pattern of colonic motility in dogs by increasing the amount of residue in the diet^{9,29}. More recent *in vitro* studies have shown that butyrate and propionate stimulate longitudinal smooth muscle contractions in the canine colon³⁰ and rat colon³¹. Treatment of dogs with idiopathic colitis with diets containing insoluble fibre has been reported to be poor³². In contrast, soluble dietary fibre has been used successfully to control large bowel signs in dogs³³.

No extensive work examining the gastrointestinal effects of fibre in a predominantly carnivorous species such as the cat has as yet been published. As a consequence, there is yet no convincing evidence that dietary fibre is beneficial in treating large bowel conditions in the cat. Dennis et al. (1993)³⁴, reported the successful use of high fibre diets as single therapy for cats with IBD. However, it is uncertain if the dietary fibre was the reason for the improved clinical results because the study lacked a control group and because the therapeutic diets used in the study were also highly digestible and may have contained proteins that were novel to the cats. Therefore, the value of dietary fibre in the management of feline large bowel disease remains uncertain. Of particular note, is the lack of information regarding the influence of fibre on large intestinal epithelial architecture and large intestinal motility in both healthy cats and those with IBD.

Inulin is a fermentable, non-digestible oligosaccharide that forms non-viscous solutions³⁵. Although some of the effects of inulin are similar to some dietary fibres, its full effects on gastrointestinal structure and function are as yet controversial³⁶⁻³⁸. Inulin is known to favour a bacterial flora that in humans and rats is considered beneficial to bowel function. In particular, one of the most recognised effects of inulin and other oligosaccharides in the gastrointestinal tract is its selective utilisation by *Bifidobacteria*. *Bifidobacteria* are considered beneficial for large bowel function³⁹. This is the area in which most of the recent research on the effects of oligosaccharides in the cat has focused⁴⁰⁻⁴².

The effects of inulin on gastrointestinal motility and its epitheliotropic attributes are still debated³⁶. However, inulin is completely fermented in humans³⁶ and if this is also true in the cat, inulin supplementation should provide ample quantities of butyrate and other SCFA to the feline large intestine. As a result, inulin supplementation may be beneficial to the colonic health of cats provided the feline colonic mucosa responds in a similar manner to SCFA as that of other species.

The original intention was to investigate the intake of dietary fibre as a risk factor for IBD as part of the epidemiological study (Chapter 3). Unfortunately, the quantity of dietary fibre in the diet of the cats studied could not be determined. Nevertheless, given the broad effects that fermentable dietary fibres and their equivalents have in the gastrointestinal tract (especially the large bowel) and the therapeutic claims ascribed to dietary fibres it was considered important to study the role of this nutrient in the gastrointestinal tract of healthy cats and cats with IBD. The principal objective of the study was to determine if inulin supplementation affected several parameters of gastrointestinal structure and function. The parameters studied included dietary acceptability, the amount and appearance of faeces, macronutrient digestibility, small and large intestinal morphology, and the movement of faeces through the colon. A secondary aim of the study was to compare the gastrointestinal morphology and function of colony cats with publicly-owned cats with and without dietary inulin supplementation.

Materials and methods

Diets

Three diets were formulated for this study by the sponsor. The diets had a chicken and rice base and were formulated to be complete and balanced. The 'petfood family' to which these diets belonged had been determined to be complete and balanced in clinical trials⁴³. Diet 'NF' had no inulin added (control diet). 'Diet F1' had

Table 1
Diet composition

Diet	Protein %	Fat %	NFE %	Ash %	Inulin %
NF	36	17	37	10	0
F1	35	18	37	10	0.1
F2	33	17	40	10	0.2

Note: Composition is expressed on a % Dry Matter basis for all nutrients. Inulin was added on an as fed basis. NFE = nitrogen free extract.

0.1% inulin added and diet 'F2' had 0.2% inulin added. These percentages of inclusion were determined after a preliminary trial at the sponsor facilities showed that inulin supplementation at these levels was not detrimental in any obvious way to healthy cats. The nutritional analysis of the three diets is shown in **Table 1**.

Feeding Regimen

The cats were randomly assigned to a diet group by a technician not involved in the trial (see "Experimental Design" for description of the method used to randomly assign the cats). Neither the technical staff feeding the cats nor the principal investigator was aware of which experimental diet (i.e. NF, F1 or F2) each cat was receiving. All cats were fed their daily caloric requirements (calculated as 70 kcal/Kg body weight) once a day in the morning.

Animals

All cats were managed in accordance with the policies and procedures of the Massey University Animal Ethics Committee. All cats were kept in single metabolic cages that allowed the separation of faeces from urine for the whole experimental period. They were observed a minimum of 6 times a day.

Thirty healthy cats were used for this experiment. Twenty of the cats belonged to the Massey University Feline Unit and lived in a colony situation. These cats remained in the colony during the conduct of the present study. The other ten cats belonged to the public. The owners of these cats signed a form consenting to the participation of their cats in the study. The publicly-owned cats were housed in cages in the 'Small Animal Production Unit' of Massey University for the duration of the study.

One of the publicly-owned cats was withdrawn from the trial after 5 days because it refused to eat the experimental diet and lost weight. Therefore data from 29 healthy cats were used for analysis.

Thirteen cats were female and sixteen cats were male. All colony cats were domestic short haired cats between 4 and 5 years old. The publicly-owned cats ranged in age from 18 months to 14 years old, however, they were distributed equally in the three dietary groups. They were all domestic short hair.

The staple diets of the publicly-owned and colony cats prior to the trial were different. The publicly-owned cats were fed a mixture of canned and dry cat food

along with table scraps. In addition some of these cats captured prey. The colony cats were fed a variety of canned cat food.

Four cats with a diagnosis of inflammatory bowel disease affecting the colon⁴⁴ were also included in the study. These cats were referred to the Massey Veterinary Teaching Hospital with signs of chronic gastrointestinal disease suggestive of large bowel dysfunction (chronic diarrhoea, presence of mucus and blood in the faeces, vomiting). The cats had not responded to dietary changes before being referred. Prior to their inclusion in the trial, the cats underwent a standard diagnostic work up. The protocol used to diagnose IBD included a full haemogram and serum biochemistry panel, FIV and FeLV serology, assay of serum thyroxine concentration (if older than 5 years old), three faecal flotations (if diarrhoea was the main complaint), faecal culture for *Salmonella*, *Campylobacter* and *Yersinia* (if considered to be indicated based on historical findings), breath hydrogen collection and radiographs following administration of radiopaque markers (BIPS, MedID, Grand Rapids). Some cats had already been on several dietary trials to determine if the gastrointestinal problems were due to an adverse reaction to a food or if the problem was responsive to a 'therapeutic diet'. However, if there was any doubt about the thoroughness of the dietary trials, the cats were fed an elimination diet based on novel protein sources for a week and observed. If clinical signs continued it was considered that dietary hypersensitivity was unlikely to be the primary cause of the cat's clinical signs, in agreement with the reported view that response to novel protein diets occurs within a week in cats with gastrointestinal food sensitivity^{35,36}. Miscellaneous tests occasionally considered necessary to diagnose IBD included ultrasound imaging, tri-iodothyronine suppression test, bone marrow aspiration, and aspiration of mesenteric lymph nodes. If all of these tests were

negative, an endoscopy and biopsy of the stomach, duodenum, and colon were performed. The diagnosis was confirmed if the biopsy findings were compatible with those previously described for IBD^{1,37-40} and the aforementioned diagnostic procedures had not revealed any other cause for the gastrointestinal signs.

The radiopaque procedure involved the administration of 10 large BIPS and 30 small BIPS mixed in a commercial feline diet of lamb and rice (Hill's feline d/d) recommended by the manufacturer. This diet was fed early in the morning and consumed in not more than 20 minutes; otherwise it would be manually fed. Radiographs were taken the following morning to confirm that the radiopaque markers have passed through the gastrointestinal tract unimpeded. The procedure to obtain the breath samples during the diagnostic stage was similar than the one described in chapter 4 but lasted only 8 hours and used the same diet than the radiopaque procedure.

The endoscopy was performed under general anaesthesia after a two day fast. The day before the endoscopy was scheduled the cats had two to three enemas. Endoscopic biopsy specimens were obtained from the stomach (incisura, cardias and pyloric antrum), duodenum (ascending duodenum and caudal flexure) and colon (descending, transverse and ascending colon). If any particular area appeared abnormal, biopsy samples were also taken from these lesions. A minimum of 8 biopsies per site were obtained and kept moist in a biopsy sponge wet with saline. Once the procedure was finished the biopsies were submerged in 10% formalin and sent for histopathological preparation and assessment. Rectal incisional biopsy samples were also obtained. Informed owner consent was obtained before starting the experimental stage and all procedures were approved by the Massey University Animal Ethic Committee.

The results of these tests confirmed that the most likely diagnosis was inflammatory bowel disease (details of each IBD cat is presented in the following two pages). This histopathological diagnosis was based primarily in qualitative criteria and performed by the pathologist on duty. However, published parameters were adhered to¹.

One of the cats had to be withdrawn from the trial because it developed marked diarrhoea and weight loss while being fed the experimental diet containing the 0.2% inulin. The cat's diet was changed to the control diet NF (no inulin added) to determine if the inulin in the diet was exacerbating the diarrhoea. Only partial improvement was observed. A succession of novel protein dietary trials followed. The faeces of the cat improved within a few hours of the diet being changed to a product based on venison and potato (Innovative Veterinary Diets Limited Ingredients Feline venison and potato). As a result, it was considered unlikely that the adverse reaction to the experimental diet was due to the inulin.

Experimental Design

The cats were randomly assigned to consume only one diet through the experimental period. Thus, there would be 10 cats consuming each of the experimental diets. Randomization of dietary treatments was achieved by pairing a treatment number with a diet. Then a randomly allocated treatment list was created for 30 cats. Each cat's name was randomly paired with a number from 1 to 30, which had already been paired with the test diets.

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>Stomach, seven pieces: Helicobacter present 5 + Duodenum, eight pieces: increased numbers of intra-epithelial lymphocytes, mild increase in lymphocytes and plasma cells in lamina propria with occasional neutrophils. Colon, six pieces: mild increase in lymphocytes and plasma cells in lamina propria. Globule leukocytes increased in number and prominent. Rectum, one piece: goblet cells reduced in number, globule leukocytes increased and neutrophils prominent in lamina propria and proprial capillaries.</p>	Mild	4y	NUMEROUS	CONTROL WITH DIET	Liquid to cream, 4-5 times a day.	N	Has been diagnosed with Giardia and treated effectively. Urgency to defecate. Flatulence. Anus and surrounding skin red. Mild leukopenia. Developed a FeLV + test	NAF
<p>Stomach, nine pieces: prominent focal lymphoid aggregations were present in the middle and lower mucosa in five of these samples. Duodenum, four pieces: moderately excessive numbers of intraepithelial lymphocytes were present in all four samples. About 20-30 plasma cells were present in each villus cross section and 10 eosinophils. Larger clumps of plasma cells were present in the middle mucosal lamina propria. Colon: focal mixed lymphocyte and plasma cell infiltration of the mucosa in one of eight samples. Rectum: contains a focal lymphoid nodule with an active germinal centre in the submucosa.</p>	Moderate	10y	NUMEROUS	CONTROL WITH DIET	Liquid , 4-5 times a day. Long periods without problems	Small amounts of foam with fur	Weight loss.	Duodenum bled easily

Clinical, pathological and endoscopic findings in cats with IBD

Lesions	Grade	Age	Bouts	Outcome	Diarrhoea	Vomiting	Other clinical signs and tests with abnormal results	Endoscopy findings
<p>Stomach, six pieces: there are moderately increased numbers of mast cells, plasma cells and lymphocytes scattered throughout the lamina propria, with occasional aggregates of eosinophils. There are moderate numbers of globular leukocytes in the glandular epithelium. Duodenum, six pieces: there are increased numbers of intra-epithelial leukocytes, and mildly increased numbers of plasma cells and lymphocytes within the lamina propria. Colon, eight pieces: there are occasional aggregates of lymphocytes, plasma cells and a few neutrophils, within the lamina propria. Several very large lymphoid nodules are present. Rectum: no abnormal findings</p>	Moderate	6y	NUMEROUS	CONTROL WITH MEDICATION	N	Food, bile, no hair. Large volume. 3-4 per day	Weight loss. Straining. Blood and mucus in the faeces. Black faeces since last year.	NAF
<p>Stomach, seven pieces: moderate numbers of Helicobacter sp were present in surface mucus and globule leukocytes were prominent in gastric crypt epithelium. Duodenum, three pieces: a mild increase in proprial plasma cells, macrophages, eosinophils and neutrophils was present. Goblet cells were also increased.. Colon, five pieces: a mild increase in proprial macrophages, fibroblasts, plasma cells and lymphocytes was present. Patches of the lamina propria had increased fibrous tissue. One specimen had prominent lymphoid nodules.</p>	Mild	15y	CONTINUOUS	NOT CONTROLLED	Red brown to pale yellow. Large volume, pasty. 2-3 per day	Fur, food, bile, foam. Once a month for 2-3 days	Increased appetite, weight loss, increased defecation frequency. Abdominal pain. Urge to defecate. Abdominal bloating. Had surgery for hyperthyroidism (T4 normal).	NAF

Endoscopy findings : NAF=no abnormal finding; ND= not done. **Diarrhoea and Vomiting:** Y=presence; N=absence.

For logistical reasons, the experiment had to be conducted in three separate stages. This was necessitated by the limited number of metabolic cages (10) available at one time and by the requirement for prompt processing of microdissection samples. Samples of this type should not be kept for more than 4 to 6 weeks to avoid deterioration of the tissues⁴⁵. Unfortunately the necessity to undertake the experiment in separate stages resulted in an uneven distribution of colony cats and publicly-owned cats amongst the different experimental stages due to the varying availability of the cats. Thus, all colony cats were studied together in the first two stages (Stage I and II) and all publicly-owned cats were studied together at the end of the experiment (Stage III) (See **Table 2**). This experimental design does not allow the separation between source of cats (colony or publicly-owned) and experimental stage⁴⁶ as independent factors.

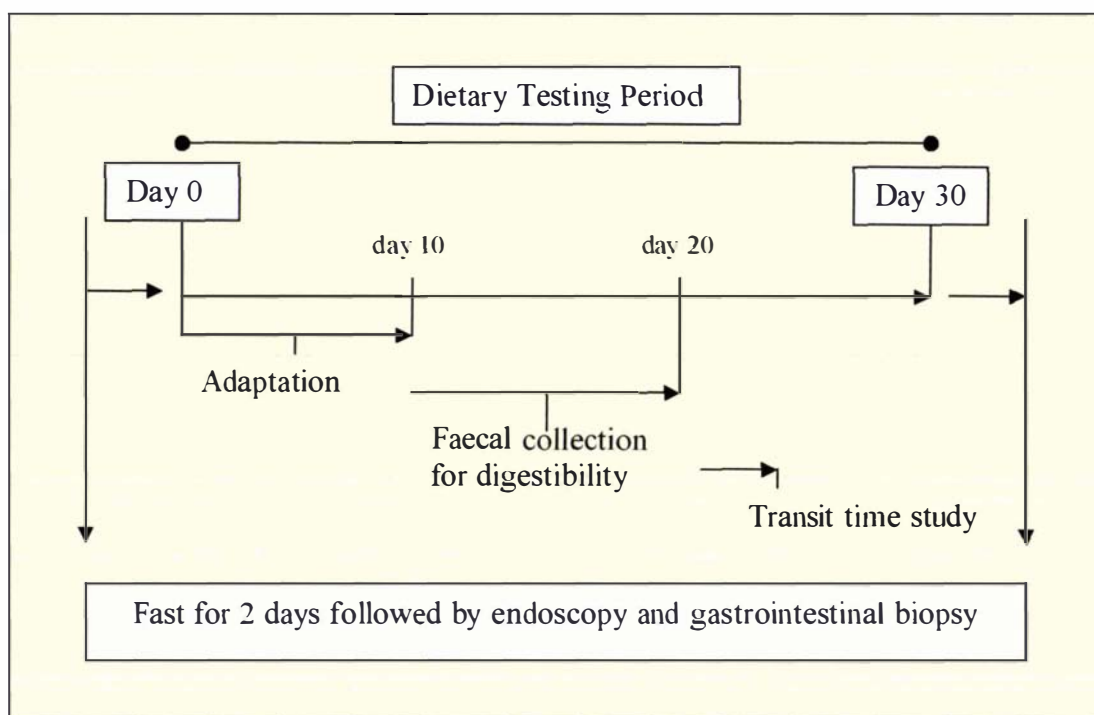
Table 2
Experimental Design

Diet	Stage	Source	<i>n</i> cats
NF (0% inulin)	I	Colony	3
	II	Colony	3
	III	Publicly-owned	3
F1 (0.1% inulin)	I	Colony	3
	II	Colony	4
	III	Publicly-owned	3
F2 (0.2% inulin)	I	Colony	4
	II	Colony	3
	III	Publicly-owned	3

The sex and age distributions were similar in each of the diet groups and at each experimental stage.

Each cat underwent an endoscopy and biopsy procedure similar to the one already explained for the cats with colitis at the start of the study. The assigned diet was then fed for 30 days. Faecal collection to assess dietary digestibility began on day 11 and continued until day 20, after a 10 day adaptation period to the diet. After 20 days on the assigned diet a radiopaque marker study was performed to assess colonic transit (see below). After the 30th day on the diet the cats were fasted for 2 days and a second endoscopy and biopsy procedure was performed. Please refer to **Diagram 1**

Diagram 1
Experimental design



Food intake and faecal output

Food intake was measured daily for each individual cat by subtracting the weight of the partially empty food bowls from the weight of the full food bowl. Any spilt food found in the cage was added to the food bowl prior to weighing.

Faeces were collected, weighed and graded every day. The faecal grade was assigned by comparing the faeces to a photographic chart showing faeces of five different consistencies ranging from liquid diarrhoea (grade 1) to firm dry faeces (grade 5). The Faecal Grading Scale is described in **Figure 1**.

Figure 1

<i>Faecal Grading Scale</i>
GRADE 1: <i>Liquid faeces, no shape.</i>
GRADE 2: <i>Soft paste-like faeces. "Cowpat" shape.</i>
GRADE 3: <i>Soft faeces but cylindrical shape is present.</i>
GRADE 4: <i>Stools with normal shape. Cylindrical but separated in "pellets". Easily crushed out of shape.</i>
GRADE 5: <i>Separated in hard "pellets" difficult to crush out of shape.</i>
Note: Intermediate grades were given if the faeces contained portions of different consistency, and hence grade.

Dietary digestibility

The digestibility of the experimental diets was assessed in accordance with a standard published technique⁴⁷.

Sufficient food was offered to meet the calculated daily energy requirement. Prior to feeding, 1-2% chromic oxide powder (CrO) was mixed in with each meal as an index substance. CrO is inert in the gastrointestinal tract and completely excreted. Therefore a measure of the digestibility of the diet or nutrient is given by the ratio of the concentration of the index material in the food to the concentration of the index material in the faeces⁴⁸.

Collection of food and faeces for digestibility analysis was started on day 11 of the testing period (once the cats had become accustomed to their diets) and continued for 10 days. Each day an excess of 20 g of food was added to the daily meal before mixing the CrO. Once the CrO was evenly distributed through the food a food sample weighing 20 grams was taken from the meal of each cat. The food sample was then labelled and immediately frozen.

Faeces were collected at least three times a day or whenever they were observed in the cages. As soon as the faeces were collected they were weighed, graded, labelled and frozen. Weighing of the faeces was done by difference since the weight of the toileting trays in the metabolic cages was known. On some occasions, the cats did not defecate on the trays. When this occurred, the faeces were collected as thor-

oughly as possible from the surface on which they had been deposited and placed onto a pre-weighed paper towel for weighing.

Once the period of collection was completed, the faeces and food samples of each cat were freeze-dried, ground in bulk, and sifted to remove excess hair (Hendricks, personal communication, 2000). The faeces were then analysed by the Massey University Nutrition Laboratory for chromic oxide, protein, ether extract, nitrogen free extract and dry matter content by standard methods (Dry Matter: AOAC 950.46 (B) (1995); ash: AOAC 923.03 (1995); fat: AOAC 392.05 (1995), Soxtec System HT 1043 Extraction Unit, Sweden; protein: Leco CNS Analyser FP-2000 Prot/N, Model 602, USA, conversion factor 6.25; NFE: calculated by difference).

Gastrointestinal biopsy procedures

Endoscopic biopsy samples were obtained before and after consumption of one of the test diets for 30 days. The preparation for endoscopy consisted of 2 days of fasting together with 2 or 3 enemas on the second day of the fasting. The enemas were performed with 60 ml of warm water placed in the rectum through a latex catheter.

The cats were sedated with acetylpromazine (0.05 mg/kg) or xylazine (2 mg/kg) depending on temperament. Anaesthesia was induced with ketamine (10 mg/kg) and diazepam (0.5 mg/kg) combined or with alfaxalone in cyclodextrin (3 mg/kg). The induction agents were given intravenously to effect. The cats were then intubated and anaesthesia was maintained with halothane and oxygen on a non-rebreathing circuit.

Once the cats were anaesthetised, an endoscope (Olympus XP10 Paediatric Gastroscope) was first passed into the stomach and duodenum and then into the large intestine. Eight to ten samples from each of the stomach, duodenum and colon were taken using an endoscopic biopsy instrument with a 2.2 or 2.8 mm biopsy cup. The samples were then placed in 10% formalin and underwent routine histopathological preparation and staining with Haematoxylin and Eosin. Four fresh samples of duodenum and colon were kept for microdissection and prepared accordingly (see 'Microdissection' below). The duodenal and colonic biopsies were taken at a consistent site by using the centimetre markings on the insertion tube of the endoscope (65-75 for duodenum and 15-20 for colon) to gauge the depth of the endoscope in the bowel.

Two rectal incisional biopsy samples were also taken. One rectal biopsy was processed for histopathology as above and the other for microdissection.

Histology

The Histology and Histopathology department of the Institute of veterinary, animal and biomedical sciences at Massey University processed and prepared by

Parameters assessed in Stomach, Small and Large Intestine Biopsy specimens in healthy cats

- Separation of crypts by cellular infiltrates
- Increased number of lymphocytes, plasma cells or eosinophils
- Presence of neutrophils anywhere in the mucosa
- Presence of granulomas or crypt abscesses
- Number of intraepithelial lymphocytes
- Presence of oedema or fibrosis
- Epithelial abnormalities
- Accumulation or depletion of mucus in goblet cells

Adapted from Guilford WG. Idiopathic inflammatory bowel disease. *In: Strombeck's Small Animal Gastroenterology. Ed. by Guilford, Center, Strombeck, Williams and Meyer. 3rd Ed. Saunders, USA. 1996a:451-486.*

standard methods all gastrointestinal biopsy sections. All tissue sections were then reviewed by one pathologist (Dr. M. Collett) using mainly qualitative assessments based on published parameters¹ (see insert in the previous page).

Microdissection

The microdissection technique has been used extensively for the study of cell division and morphometry of intestinal epithelial tissue^{49-52, 26,45,53-56}. Recently, it has been validated for the measurement of rate of cell division in intestinal tissues by comparison with standard techniques that involve the use of tritiated thymidine or bromodeoxyuridine⁴⁵, or by autoradiography in the dog⁵⁵. The principal advantage of the technique is that it can be used in clinical patients and requires only small amounts of tissue such as that which can be obtained during endoscopy^{49,54}.

Sample preparation for microdissection: the biopsy samples for microdissection were placed on a small square of foil for 1-2 minutes and allowed to dry sufficiently to attach to the foil. The samples were then refrigerated in Clarke's fixative for 8-12 hours. The samples were then transferred to 70% ethanol and kept refrigerated for a maximum of 6 weeks.

The samples were stained by the Schiff's technique to stain nuclei. A combination Schiff-Giemsa technique⁵⁰ was also tried but was found

Schiff's Bulk Stain

1.- Transfer to 50 % ethyl alcohol for 10 minutes

2.- Water for 10 minutes with 3 changes

3.- Molar hydrochloric acid at 60°C in a water bath for 6 minutes

4.- Water for 10 minutes with three changes

5.- Schiff reagent at room temperature for 20 minutes

6.- Water for 10 minutes with three changes

7.- Transfer to 40% glacial acetic acid

to offer no advantage to the single stain technique. The Schiff's technique is described in the insert.

It is noteworthy that rapid deterioration of the biopsy samples was observed when the staining and microdissection technique was being refined prior to the start of the present study. At the time, it was uncertain if the fixing or the preparation of the samples was to blame for the failure of the tissue to withstand microdissection and staining. Several trials were undertaken in an attempt to resolve the problem. These included modification of the acid step, dilution of the Schiff's reagent, alteration of the washing procedure and use of different mounting solutions. None of these steps improved the results. Modification of the microdissection tools and technique were not rewarding either. Eventually, it was discovered that refrigeration of the samples during fixing and storage, and the use of freshly prepared Schiff's stain (not older than 2 weeks) preserved the samples well and stained the cell nuclei strongly purple with excellent nuclear morphological detail. Refrigeration of the samples has been reported to be beneficial by other researchers also ⁴⁵.

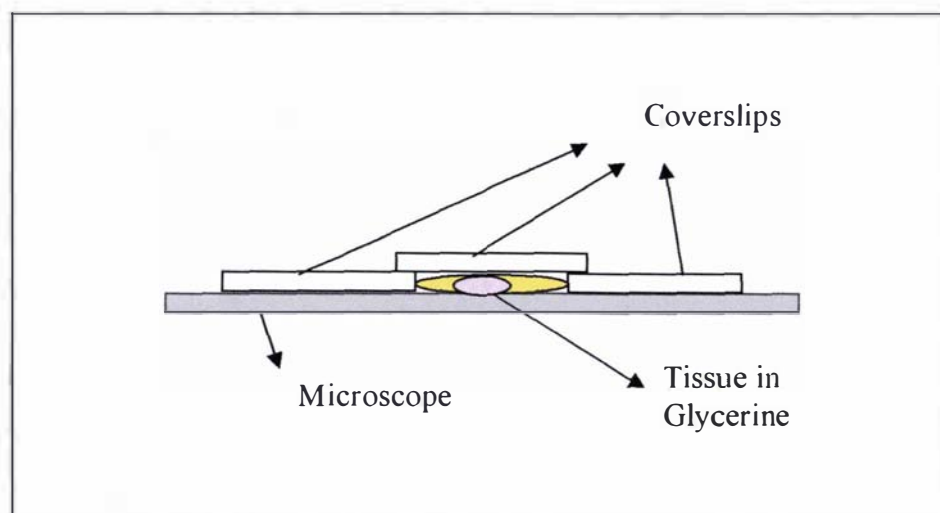
Microdissection measurements:

After the samples were Schiff-stained they were microdissected using a cataract scalpel and a 25G hypodermic needle attached to a 1 ml syringe under a stereomicroscope at a magnification of 15x (Nikon SMZ-1B). The bulk biopsy sample was carefully teased apart into single rows of crypts and villi whenever possible. The rows of crypts and villi were then mounted in different media according to their final use.

The separated crypts to be used for counting dividing cells were mounted in 40% acetic acid, covered with a coverslip and the borders of the coverslip sealed with acetate and nitrocellulose mixture dissolved in propyl-alcohol. This sealant avoided dehydration of the prepared sample and allowed the preparation of all samples prior to counting the dividing cells.

The crypts and villi to be used for morphometry were mounted on a drop of melted glycerine (40°C) on a microscope slide. To minimise deformation of the sample from the weight of a coverslip, two coverslips were positioned on the glass slide on each side of the drop of glycerine in which the tissue was to be mounted. After placing the microdissected tissue in the melted glycerine a coverslip was laid over the sample and the other two coverslips (Dr Greene, personal communication, 2000). This left a gap of approximately 50 microns under the upper coverslip in which the crypts and villi were mounted (see **Figure 2**). Thus, deformation was minimized, which allowed for more accurate measurements.

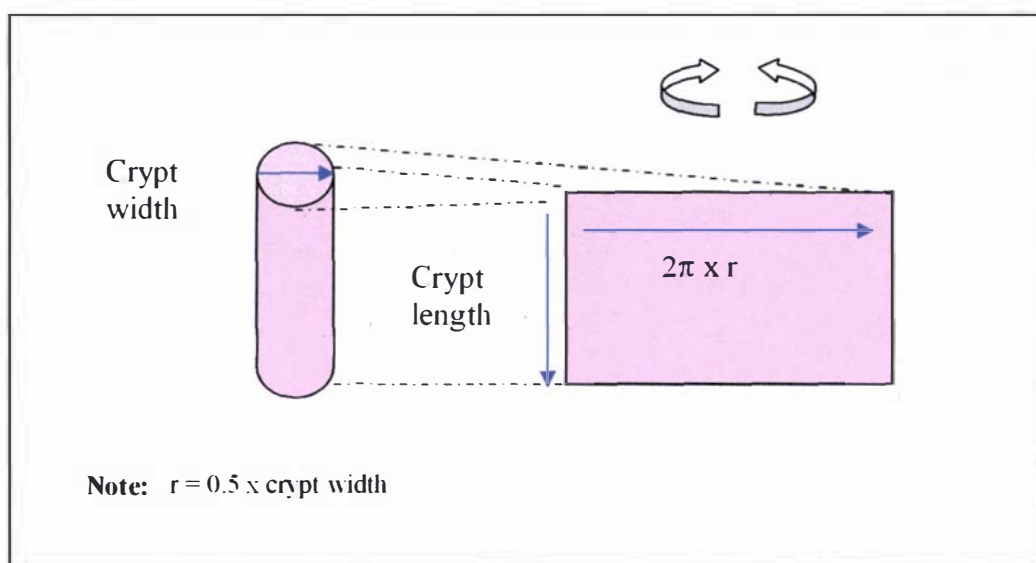
Figure 2
Mounting tissue for morphometry



SigmaScan™ Scientific Measurement System software (Jandel Scientific-San Rafael CA.) was used to measure the length and width of 10 villi or crypts in each tissue sample. Since crypts are mainly cylindrical (See **Figure 3**) with a slightly dilated end, their approximate surface area can be calculated using the formula by which the surface area of a hollow cylinder with only one cap can be measured ⁵⁷:

$$\text{Area} = 2\pi \times (0.5 \text{ width}) \times \text{length} + \pi \times (0.5 \text{ width})^2$$

Figure 3
Measurement of surface area in a crypt using a cylinder as a model



The same formula has been used previously to estimate the available physiological surface area of microvilli in the small intestine⁵⁸⁻⁶⁰. The length of the crypts was also measured by counting the epithelial cells (**Figure. 4**) in the outer column on one side of each crypt (from bulbous end to outlet)²⁶. Counting was done only by one person to maintain consistency.

Counting of the dividing cells in a crypt was done manually on a standard light microscope (Olympus CH-2) at 1000x magnification. Dividing cells were counted at the stages of prophase (if the cell looked enlarged and markedly different than the surrounding cells), anaphase and telophase (**Figure 5**) to obtain the total number of mitoses per crypt.

Colonic transit time

Colonic transit time was studied 3 to 5 days after the digestibility study was finished. Colonic transit time – specifically the colonic transit of indigestible particles - was measured using a simplified version of the radiopaque marker method described by Chandler et al (1997)⁶¹. Ten large and 30 small radiopaque markers (BIPS, MedID, Grand Rapids) were incorporated in a meal of the experimental diet. Cats that did not promptly eat the meal containing the radiopaque markers (approximately 50%

Figure 4
Crypt measurements

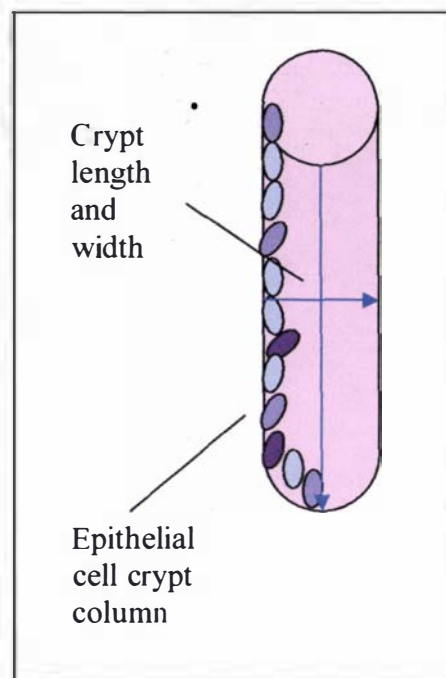
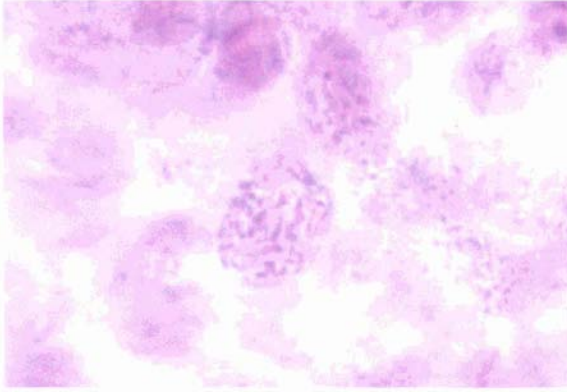
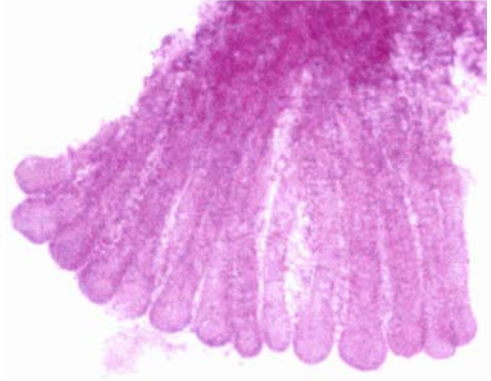


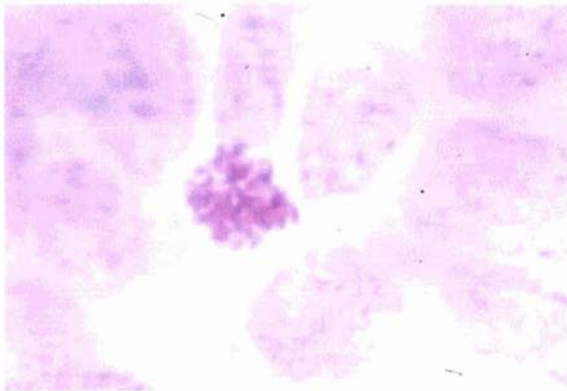
Figure 5
Mitotic figures and crypts from different segments of the gastrointestinal tract as seen when using the microdissection technique



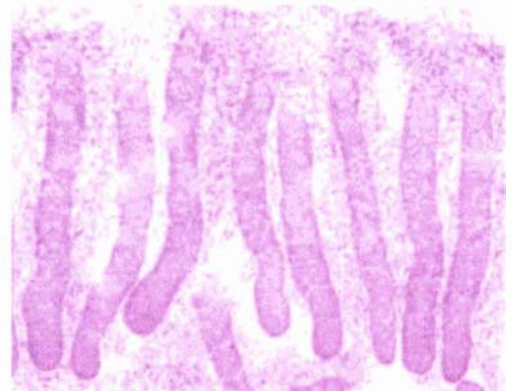
Prophase



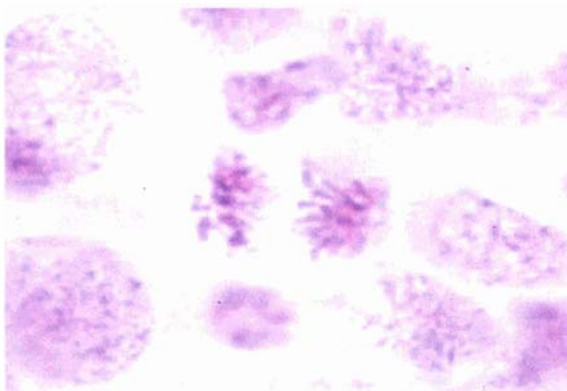
Duodenum



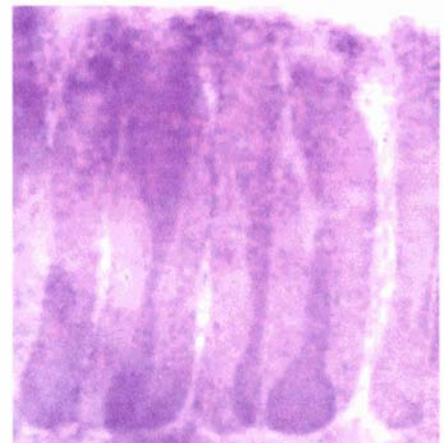
Anaphase



Colon



Telophase



Rectum

of the cats) were encouraged to do so by the administration of diazepam (0.1 ml IV) as an appetite stimulant. The administration of diazepam as an appetite stimulant does not affect gastric emptying or colonic transit time of radiopaque markers in cats⁶² and was considered preferable to force-feeding to ensure the test meal and markers were consumed completely and promptly. Force-feeding is stressful and stress has been shown to affect gastric emptying and colonic motility in rats⁶³.

The time at which the meal was completed by each cat was recorded. Right lateral and ventrodorsal abdominal radiographs were then taken at 12, 24 and 36 hours postprandially. Each radiograph then was studied and the markers in the stomach, small intestine, ascending colon, transverse colon, descending colon and rectum were counted.

A less rigorous radiographic schedule than the ones already published to measure colonic transit, was considered appropriate because the main interest was to gain an insight on the effect of different diets on the movement of faeces through the colon rather than fully measure colonic transit. This, however, limited the choices of analysis to the geometric centre method.

$$\text{Geometric Centre} = \sum \frac{\text{Intestinal Site Numeric Value} \times \text{Number of BIPS at site}}{\text{Total Number of BIPS}}$$

Note: Intestinal Sites Numeric value: Ascending Colon = 1; Transverse Colon = 2; Descending Colon = 3; Rectum and Faeces = 4.

The data was analysed by calculating the geometric centre for the small and large markers and comparing the percentage of markers at each site at different times. The geometric centre was calculated according to the following formula:

Statistical analysis

Descriptive statistics including means, standard errors and ranges were used to summarise data. All data were analysed for normality. Non-parametric methods were chosen for the analysis of data that was not normally distributed. All analysis were conducted with SAS vs. 6.12 software and values of $p \leq 0.05$ were considered significant for all comparisons.

Due to the unequal distribution of cats of different source per experimental stage an analysis of variance was performed to determine if there were any differences between the colony cats in stage I and II. If no differences were demonstrated between these two stages further statistical analysis considered stages I and II as a single group of animals i.e. 'colony cats' and the factor 'stage' was replaced by the factor 'source' (i.e. colony or publicly-owned cats) in the analysis. Otherwise when differences between stage I and II were significant any further analysis included the term 'stage' (I, II and III) as opposed to the term 'source'.

Once the prior analysis had determined which factor to use for each variable i.e. 'source' or 'experimental stage', an analysis of variance was used to analyse differences in food intake, faecal output, nutrient digestibility and microdissection measurements (before dietary intervention). The general model of this analysis was *pa-*

parameter of interest = diet + source or experimental stage + interaction between the two. Multiple comparisons between diets or stages were performed with a Tukey test.

In the case of the analysis of food intake the influence of chromic oxide on the food intake of the cats was assessed by a paired t-test.

An analysis of variance (using PROC MIXED) was performed to assess the differences in microdissection data between the first and second set of biopsy specimens. The model used was *difference between the means of the second and first biopsy = diet + source or experimental stage + interaction between the two.* An unstructured type of covariance was chosen. Differences between Least Square Means were used to determine the significant comparisons with Tukey adjustment for multiple comparisons. PROC CORR was used to find the Pearson correlation coefficient between crypt length and the number of epithelial cells per crypt column.

The numbers of radiopaque markers present at each time period (12, 24 or 36 hours) at each site were compared between experimental stages (I, II and III) and dietary groups (NF, F1 and F2) by the non-parametric Kruskal-Wallis test. Similarly, the geometric centres at each time period were compared between experimental stages (I, II and III) by the Kruskal-Wallis test. The geometric centres obtained when consuming different diets were compared by a non-parametric test to detect alternatives of ordered class differences – the Jonckheere-Terpstra test^{64,65} using PROC FREQ of SAS. This test is considered more powerful than the Kruskal-Wallis test when there is a possible order of treatment effect, resulting from the experimental design. The tested hypothesis is that the effects of treatment 1 (NF diet) < treatment 2 (F1 diet) < treat-

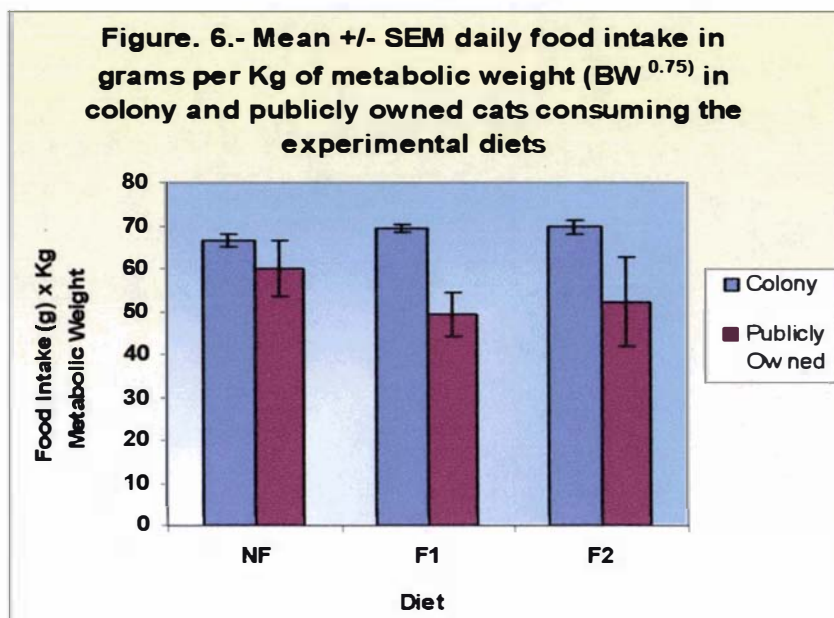
ment 3 (F2 diet) as opposed to the usual null hypothesis ($H_0 = \text{treatment 1} = \text{treatment 2} = \text{treatment 3}$)⁶⁵.

Results

Healthy cats – food intake

The mean daily food intakes of the colony and publicly-owned cats consuming the experimental diets are shown in **Figure 6**. All three diets were isocaloric therefore intakes in grams correlate directly with caloric intake when comparing the three diets. Colony cats consumed a significantly greater quantity of all diets than publicly-owned cats ($p=0.0002$). The colony cats consumed on average more than 96.5% of each meal including all experimental diets. In contrast, the publicly owned cats ate on average 72% of each meal of all of the diets. These differences in meal size were statistically significant ($p < 0.0001$).

There were no significant differences between the mean food intakes of each of the three experimental diets by the colony cats. Similarly, there were no significant differences between the mean food intakes of each of the experimental diets by the publicly-owned cats.

**Table 3**

Faecal production in colony and publicly owned cats consuming three different diets.

Diet	NF	SEM	F1	SEM	F2	SEM
Colony cats[#]						
Total faeces (g)	1183	144.5	1209	212.7	1663	302.1
<i>g.faeces</i> 100g food	52	5.21	53	4.16	63	8.76
Publicly owned cats[*]						
Total faeces (g)	883	166.5	608	87.7	673	209.9
<i>g.faeces</i> 100g food	34	1.23	35	2.75	32	1.99
Exp Stage I^a						
Total faeces (g)	910	37.16	1279	212.5	2065	433
<i>g.faeces</i> 100g food	51.66	0.99	58	7.36	78.2	9.61
Exp Stage II^a						
Total faeces (g)	1456	169	1157	365	1126	108
<i>g.faeces</i> 100g food	53.9	11.5	50.43	5.04	44.6	5.5
Exp Stage III^b						
Total faeces (g)	883.7	166.5	608	87.7	673	210
<i>g.faeces</i> 100g food	34.5	1.23	35.3	2.76	32.4	2.0

Note: Diets included a control diet (NF) or diets enriched with inulin (F1 0.1% and F2 0.2%). Different superscripts indicate significant differences $p=0.05$.

Food intake of all cats decreased slightly (mean reduction of 2%) when chromic oxide was added. This decrease in food intake while minor was statistically significant ($p < 0.048$).

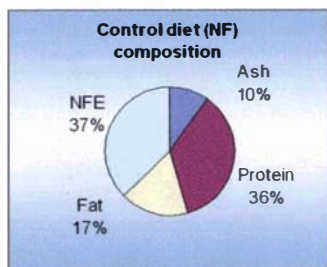
Healthy cats – faecal output

Five of the colony cats developed persistent diarrhoea during the study (two eating diet F2, two eating diet F1 and one eating diet NF). In spite of this, there were no significant differences in the number of motions passed throughout the testing period between the diet groups or the colony and publicly-owned cats.

Diet did not significantly influence either the total amount of faeces for the duration of the experiment or the grams of faeces produced per each 100g of food fed. Similarly, although the source of the cats did not significantly affect the total amount of faeces for the duration of the experiment, it tended towards significance ($p = 0.064$). In contrast, colony cats tested in stage I produced more faeces/100g food than the cats examined in stage II and stage III ($p < 0.05$) and cats tested in stage II produced more faeces/100g food than cats in stage III ($p < 0.05$). Please refer to **Table 3** for details.

Healthy cats – apparent digestibility of diets

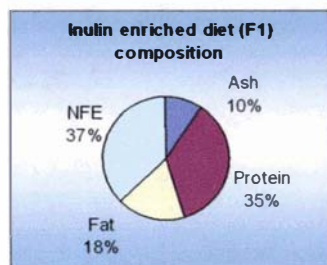
The apparent digestibility data for protein, fat and nitrogen free extract (NFE) are presented in **Figures 7 and 8**. No significant differences were found between the digestibility of the NF diet and the other experimental diets.



DIGESTIBILITY

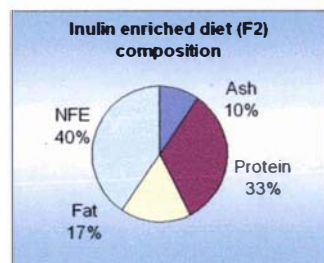
Diet NF (0.0% inulin)

Protein 78% +/- 0.01
 Fat 97% +/- 0.006
 NFE 84% +/- 0.009



Diet F1 (0.1% inulin)

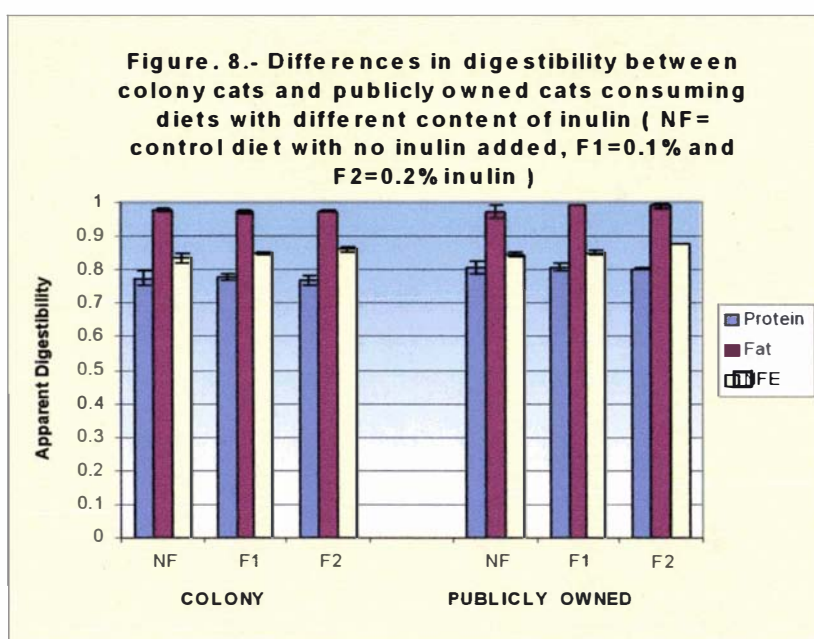
Protein 79% +/- 0.007
 Fat 97% +/- 0.004
 NFE 85% +/- 0.005



Diet F2 (0.2% inulin)

Protein 77% +/- 0.01
 Fat 98% +/- 0.003
 NFE 86% +/- 0.004

Figure 7.- Diet composition expressed on a dry matter basis. Digestibility expressed as mean +/- SEM.



Healthy cats – Histology

Before Dietary Intervention

Before the dietary intervention, all publicly-owned cats and 15 out of 20 colony cats showed a mild increase in intraepithelial and/or lamina propria leukocytes in the histological sections of the duodenum compared to the mucosal leukocyte content expected in the duodenums of healthy cats. Five colony cats, all of which were examined in experimental stage I, had normal numbers of leukocytes in the duodenal mucosa. The colonic histological sections prior to the dietary intervention showed occasional scattered neutrophils in nine colony cats and four publicly-owned cats. Another four publicly-owned cats had patchy neutrophilic infiltration (n=3) and crypt hyperplasia (n=1). The colonic histological sections of four of the colony cats examined in experimental stage I showed a mixed inflammatory infiltrate while the colonic sections of nine colony cats examined in experimental stage II had increased mononuclear infiltration in addition to occasional neutrophils. The baseline rectal histological sections of four publicly-owned cats had occasional neutrophils and those of another four publicly-owned cats showed crypt abscesses or patchy neutrophilic infiltration. In experimental stage I, the rectal biopsy specimens of four colony cats showed occasional neutrophils, those of another two colony cats had patchy neutrophilic infiltration and those of another three colony cats showed increased numbers of mononuclear leukocytes. In experimental stage II, the rectal biopsy specimens of six colony cats showed mildly increased mononuclear and neutrophilic leukocytic infiltration while one colony cat showed crypt abscesses.

It is noteworthy that *Helicobacter spp.* was present in large numbers in the stomachs of most of the healthy cats. The bacteria were sometimes associated with the

presence of globule leukocytes and lymphocyte foci, but not always. Only three cats (all colony cats) had no gastric *Helicobacter spp.* visible on the gastric mucosal biopsy specimens at the beginning of the trial.

After Dietary Intervention

Histological sections of the colony cats revealed some differences between the first and second set of biopsy specimens but did not reveal any consistent patterns of change in response to diet. The histological sections of three colony cats that had consumed the F2 diet showed an increase in lymphocyte foci in the colonic lamina propria in comparison to the baseline biopsy samples. In contrast, the biopsy specimens of another colony cat that had eaten the same diet showed the disappearance of mild lymphocytic plasmacytic infiltration from the duodenal lamina propria that had been present in the baseline specimens. The biopsy specimens of one colony cat that had eaten diet F1 had an increase in duodenal intraepithelial lymphocytes and colonic lamina propria lymphocytes in comparison to the baseline biopsy sections. On the contrary, a reduction in the number of inflammatory cells in the lamina propria of the colon and rectum was detected in the histological sections of another two colony cats that had eaten diet F1. One of the colony cats consuming the diet without inulin (NF) developed a patchy increase in the number of lymphocytes infiltrating the duodenum after consuming this diet. On the other hand, the feeding of the NF diet to another colony cat was associated with the disappearance of mixed inflammatory infiltrates from the rectal mucosa.

Histological sections of the publicly-owned cats showed changes after dietary intervention in only one cat. The duodenal biopsy specimens of this cat had mild infil-

trates of lymphocytes, plasma cells and globule leukocytes after eating the F1 diet that were not present in the baseline biopsy sections.

Healthy cats –microdissection measurements of the duodenum

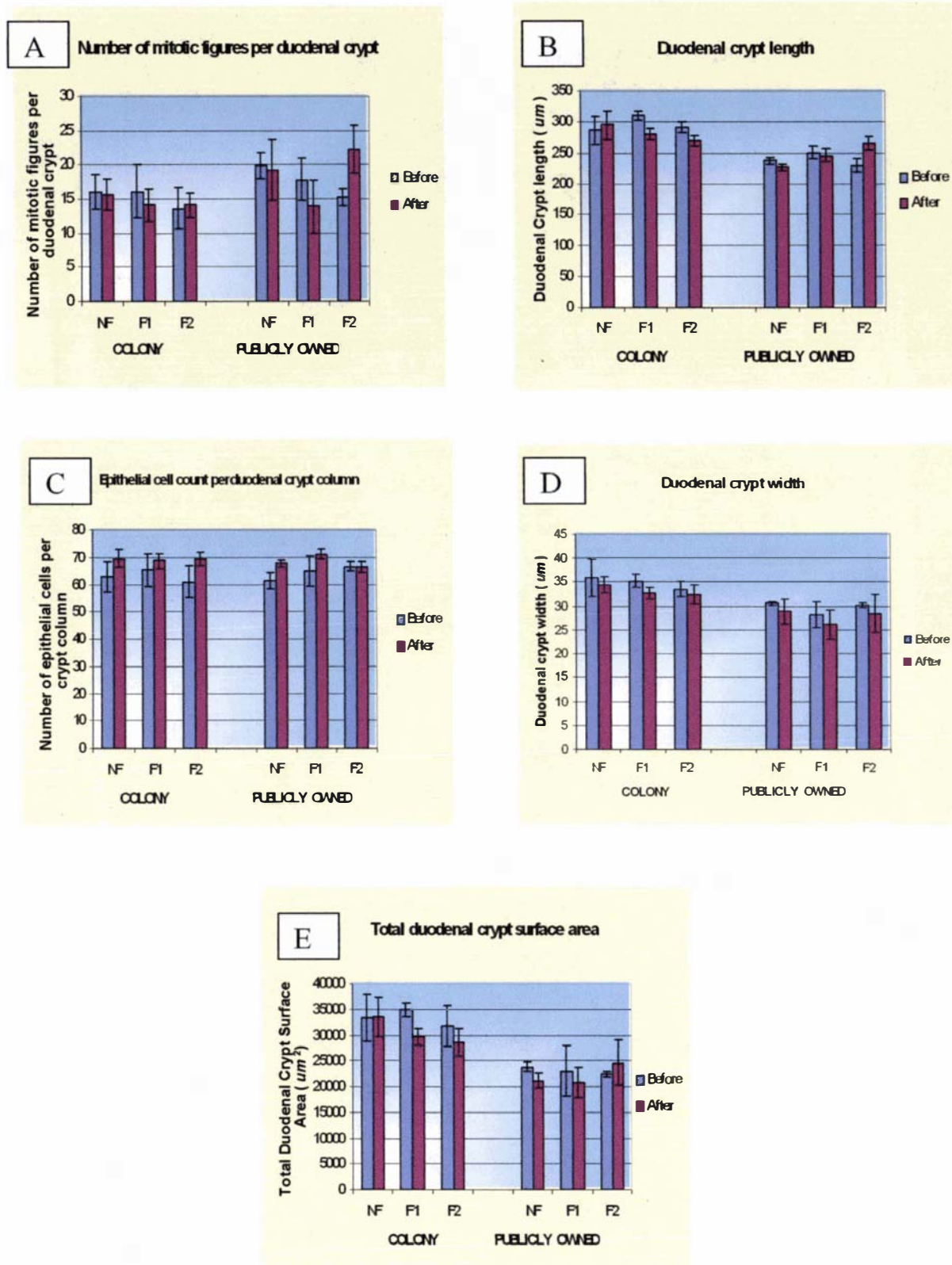
The microdissection data from the duodenum was incomplete because a number of the endoscopic biopsy specimens were of insufficient depth to perform microdissection of the gastrointestinal crypts. Most of the shallow biopsy specimens were from cats in experimental stage I. Only 4 sets of duodenal biopsy specimens from the 10 cats in this group were adequate. As a result, data from experimental stages I and II were combined. The pooling of these data preserved the ability to statistically compare the effect of the source (colony vs. publicly owned) of the cats on the duodenal data but prevented comparisons of the effects of experimental stage in the parameters of interest. Please refer to **Figure 9** and **10** and **Tables 4** and **5** for the results of the measurements carried out in duodenal biopsy specimens.

Duodenal crypt mitotic count

Before dietary intervention

No differences in the number of duodenal crypt mitotic figures between diet groups (NF, F1 and F2) or between cat sources were found before the testing period started. However, and considering the low number of samples from cats tested in stage I (see above), cats examined during experimental stage I showed a large significantly ($p \leq 0.05$) reduced number of mitotic figures compared to cats examined

Figure 9



Duodenal morphometric parameters (mean \pm SEM) in colony and publicly owned cats consuming three different diets (NF=control diet, F1= 0.1% inulin added, F2= 0.2% inulin added).

DUODENAL CRYPTS		Control diet NF				Diet F1(0.1% inulin)				Diet F2 (0.2% inulin)			
	Source	Before	SEM	After	SEM	Before	SEM	After	SEM	Before	SEM	After	SEM
<i>Mitotic figures per crypt</i>	Colony	16.07	2.59	15.6	2.25	16.12	3.94	14.11	2.45	13.58	3.12	14.06	1.81
	Public	19.9	1.85	19.23	4.47	17.83	3.08	13.9	3.92	15.26	1.30	22.27	3.54
<i>Epithelial Cells per crypt column</i>	Colony	62.7	5.54	69.61	3.28	65.32	5.85	68.61	2.61	61	5.72	69.48	2.54
	Public	61.43	3.02	67.8	1.31	64.76	5.63	71.1	1.61	66.56	1.96	66.3	2.0
<i>Crypt length (um)</i>	Colony	285.86	9.47	294.95	23.15	309.64	15.83	280.27	8.4	290.27	29.35	268.3	8.71
	Public	237.84	5.89	226.45	5.36	250.06	30.07	245.88	10.4	229.25	4.08	264.25	10.57
<i>Crypt width(um)</i>	Colony	35.82	3.96	34.38	1.63	35.13	1.34	32.65	1.32	33.48	1.56	32.47	2.01
	Public	30.53	0.46	28.84	2.56	28.13	2.69	26.12	3.12	30.16	0.51	28.48	4.0
<i>Crypt Surface Area(um)²</i>	Colony	33337	4459.8	33480.1	3785.1	34952.4	1396.4	29588.1	1487	31762	3929	28522	2687
	Public	23792	904	21179	1569	23041.4	4915.95	20870.1	2960.6	22447	525	24559	4379

Table 4.- Cell counts and morphometric mean + SEM measurements of duodenal crypts in colony cats and cats owned by the public.

during stages II and III. Stage I had a mean of 7.9 \pm 1.0 mitotic figures per duodenal crypt, whereas Stage II had 19.25 \pm 1.54 and Stage III had 17.66 \pm 1.3.

After dietary intervention

No significant differences occurred in the mean change in number of duodenal crypt mitoses between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet. It is noteworthy, however that the influence of diet on this parameter tended toward significance ($p=0.07$) as did the interaction between diet and cat source ($p=0.07$). These trends are seen most clearly in the publicly-owned cats fed the diet with the high inulin concentration (see **Figure 9A**). Duodenal crypt length

Before dietary intervention

No significant differences were found in duodenal crypt length between the diet groups (NF, F1 and F2) at the start of the trial (**Figure 9B**). However, publicly-owned cats had significantly ($p<0.0024$) shorter crypts than colony cats at the beginning of the trial (mean \pm SEM of the colony cats was 296 \pm 11 μm and of the publicly-owned cats was 239 \pm 9 μm).

After dietary intervention

No significant differences occurred in the mean change in duodenal crypt length between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet. However, the inclusion of inulin decreased the variation in crypt length between the publicly owned and colony cats. The standard deviation before the trial period was $\sigma=50$ for the colony cats and $\sigma=35$ for the publicly-

owned cats that were to consume diets F1 and F2 i.e. diets enriched with inulin. However, after consuming the trial diets $\sigma=21$ for the colony cats and $\sigma=19$ for the publicly-owned cats.

Number of epithelial cells per duodenal crypt cell column

Before dietary intervention

No significant differences were present in the mean number of epithelial cells per duodenal crypt cell column between dietary groups (NF, F1 and F2) or between cat sources (colony and publicly-owned cats) at the beginning of the trial (**Figure 9C**). However, cats examined in experimental Stage I had significantly ($p\leq 0.05$) fewer cells per duodenal crypt column than the other two experimental stages. Stage I had 50.8 ± 4 cells per crypt column whereas Stage II had 69.8 ± 2 and Stage III had 64.25 ± 2 cells per crypt column (mean \pm SEM).

After dietary intervention

Most cats showed a small increase in the number of cells per duodenal crypt column from baseline to the end of the period during which the experimental diets were fed regardless of the diet consumed (see **Figure 9C**). However, this change was not significant and there was no influence of diet or cat source on the magnitude of this change.

Interestingly, the correlation between the number of cells in a duodenal crypt column and the measured length of the crypt (in microns) was poor. The correlation between the number of epithelial cells per column of a duodenal crypt and the crypt

length in the first biopsy was $r = -0.15$ and for the second biopsy (after dietary intervention) $r = 0.34$.

Duodenal crypt width

Before dietary intervention

No significant differences were present in the mean duodenal crypt width between dietary groups (NF, F1 and F2) at the beginning of the trial (**Figure 9D**). However, publicly-owned cats had significantly ($p < 0.007$) narrower duodenal crypts than colony cats. The crypt width of the publicly-owned cats was $29.6 \pm 2.6 \mu\text{m}$ and that of the colony cats was $34.7 \pm 4.7 \mu\text{m}$ (mean \pm SEM) before the feeding of the experimental diets.

After dietary intervention

All cats, irrespective of the diet consumed, showed a small reduction in their duodenal crypt width between the first and second biopsy samples (**Figure 9D**). However, there were no significant differences in the mean duodenal crypt width between the first and second biopsy specimens of any of the dietary groups comprised of either the colony or publicly-owned cats.

Total duodenal crypt surface area

Before dietary intervention

No significant differences were present in the mean total duodenal crypt surface area between dietary groups (NF, F1 and F2) at the beginning of the trial (**Figure 9E**). However, publicly-owned cats had significantly ($p = 0.0007$) smaller mean total crypt surface area than colony cats at the start of the trial. In the publicly-owned cats

the total duodenal crypt surface area was $23093 \pm 1463 \mu\text{m}^2$ whereas in the colony cats it was $33351 \pm 1834 \mu\text{m}^2$.

After dietary intervention

The publicly-owned cats consuming the high fibre diet (diet F2) showed an increase of the duodenal crypt surface area from baseline to the end of the experiment. In contrast, the duodenal crypt surface area of all the other groups stayed the same or showed a reduction of surface area. However, these changes were not significant.

Duodenal villi length

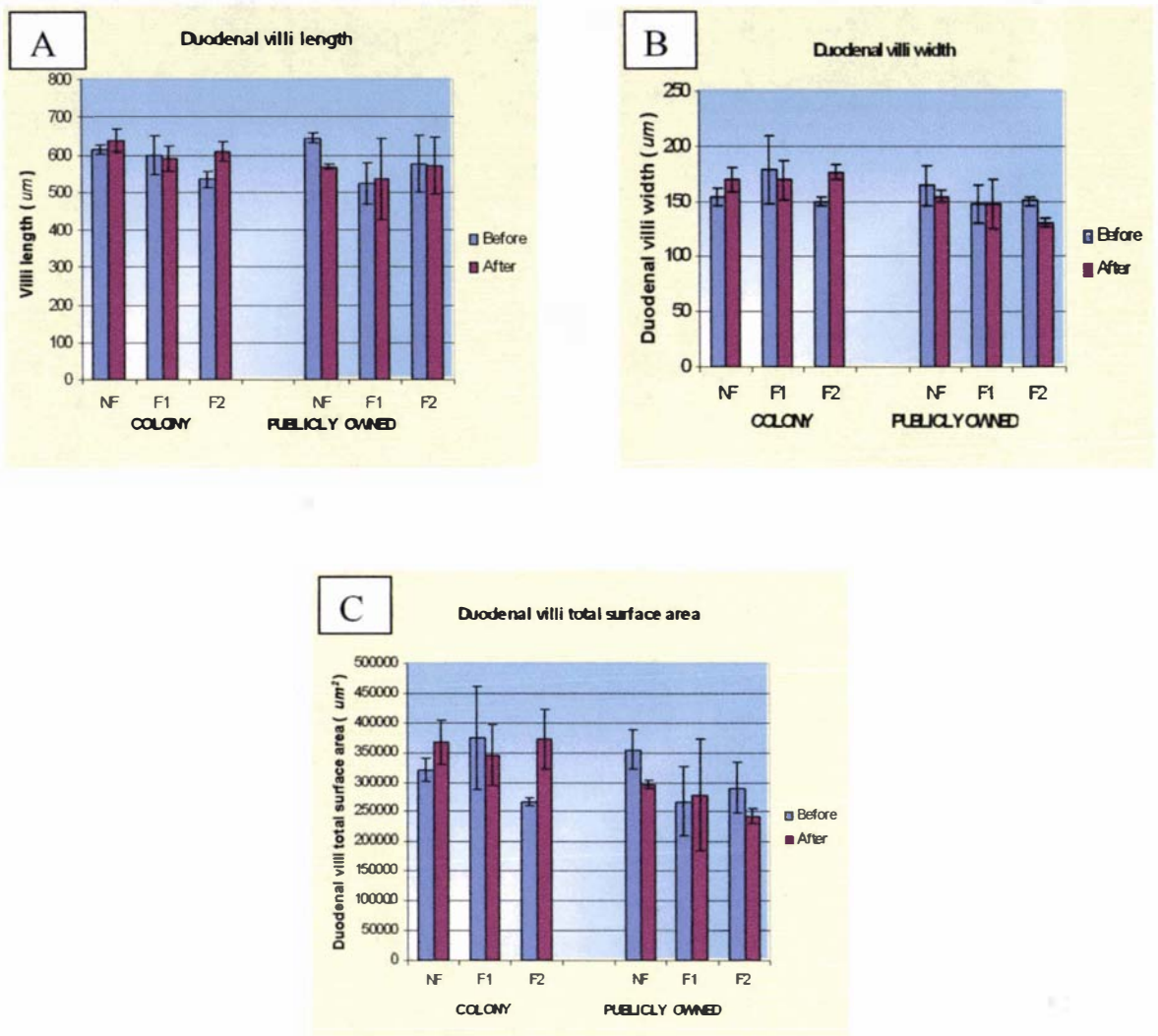
Before dietary intervention

No significant differences were present in the mean duodenal villi length between dietary groups or between publicly-owned and colony cats at the start of the trial (**Figure 10A**).

After dietary intervention

The colony cats, irrespective of diet consumed, showed a significant increase in the mean villi length (**Figure 10A**) from baseline to the end of the experiment when compared to the difference in mean villi length shown by the publicly-owned cats ($p=0.05$).

Figure 10



Duodenal villi morphometric parameters (mean \pm SEM) in colony and publicly owned cats consuming three different diets (NF=control diet, F1= 0.1% inulin added, F2= 0.2% inulin added).

DUODENAL VILLI		Control Diet NF				Diet F1 (0.1% inulin)				Diet F2 (0.2% inulin)			
	Source	Before	SEM	After	SEM	Before	SEM	After	SEM	Before	SEM	After	SEM
Villi Length (μm)	Colony	614.65	11.43	636.73	33.09	597.28	50.41	588.84	32.10	533.24	22.27	607.89	26.85
	Public	643.21	15.26	567.69	107.25	522.3	56.62	535.8	6.23	574.59	74.56	570.06	75.82
Villi width (μm)	Colony	154.63	7.67	169.39	18.35	178.62	30.66	169.23	11.06	149.81	9.61	176.68	16.1
	Public	164.56	18.04	154.83	21.88	148.34	17.29	147.76	4.93	150.29	5.6	130.82	9.95
Villi Surface Area(μm^2)	Colony	320236	19759	366542	52177	374430	87646	345851	36705	267357	7337	371806	49859
	Public	54194	33403	296649	93502	267998	59002	279512	6062	291087	41420	242592	12059

Table 5.- Morphometric mean +/- SEM measurements of duodenal villi in colony cats and publicly owned cats.

Duodenal villi width

Before dietary intervention

No significant differences were present in the mean duodenal villi width between dietary groups or between the two sources of cats at the beginning of the trial (**Figure 10B**).

After dietary intervention

No significant differences occurred in the mean change in duodenal villi width between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet.

Duodenal villi total surface area

Before dietary intervention

No significant differences were present in the mean surface area of the duodenal villi between dietary groups or between the two sources of cats at the beginning of the trial.

After dietary intervention

No significant differences occurred in the mean change of surface area of the duodenal villi between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet. However, the response of the publicly-owned and colony cats was significantly ($p=0.023$) different. Colony cats, showed an increase in the mean surface area of the duodenal villi between the first and second biopsy specimens irrespective of diet whereas the opposite was true for the publicly-owned cats. The mean increase in the duodenal villi surface area of the colony cats

was $30133\mu\text{m}^2$ whereas the mean decrease in this parameter of the publicly-owned cats was $31508\mu\text{m}^2$ (see **Figure 10C**). Although the interaction between diet and source of cats was statistically significant ($p=0.016$) multiple comparisons did not identify any significant specific interaction.

Healthy cats –microdissection measurements of the colon

Variation in the shape and density of crypts in the biopsy specimens of the colon was observed. The variation was seen between biopsy specimens from different cats but also between biopsy specimens from the same cat suggesting localised differences in mucosal morphology.

Many of the colony and publicly-owned cats had occasional colonic mucosal crypts with bilobulated ends. Most of the colonic crypts in the biopsy specimens from the youngest publicly-owned cat (11 months old) had a double end (**Figure 11**). This feature was present in both the first and second biopsy specimens of this cat. Please refer to **Figure 13** and **Table 6** for the results of the measurements carried out in colonic biopsy specimens.

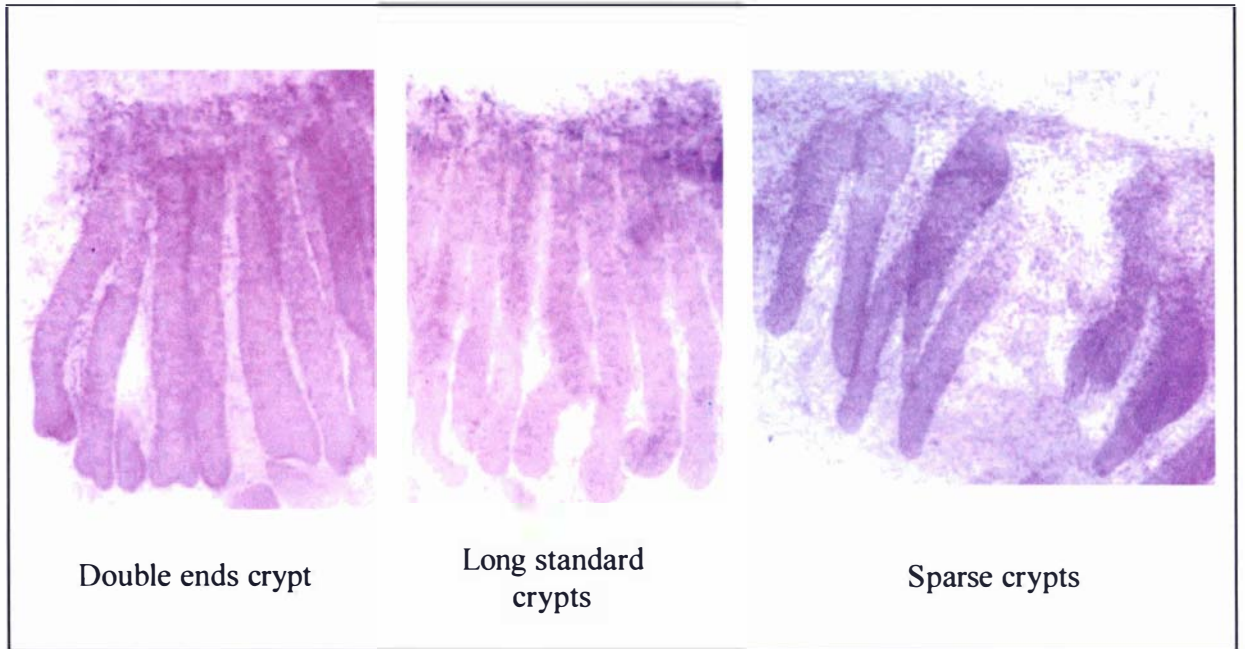
Colonic crypt mitotic count

Before dietary intervention

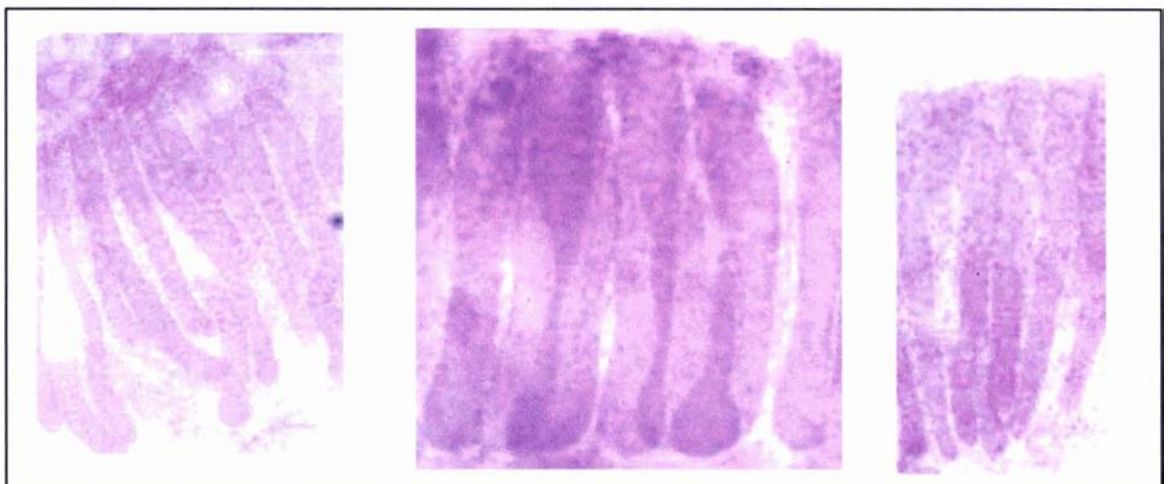
No significant differences were present in the mean colonic crypt mitotic count between the dietary groups (NF, F1 and F2) at the beginning of the trial. However, colony cats tended ($p<0.06$) to have lower numbers of mitotic figures in their colonic crypts than publicly-owned cats. The colonic crypt mitotic count of the colony cats at

Figure 11

Colon mucosal crypts from three different healthy cats as seen with the microdissection technique. Note variability in crypt length, width, shape and density.

**Figure 12**

Rectal mucosal crypts from three different healthy cats as seen with the microdissection technique. Note variability in crypt length, width and shape.



baseline was 9.4 +/- 3.3 and that of the publicly-owned cats was 15.2 +/-7.8 (mean +/- SEM).

After dietary intervention

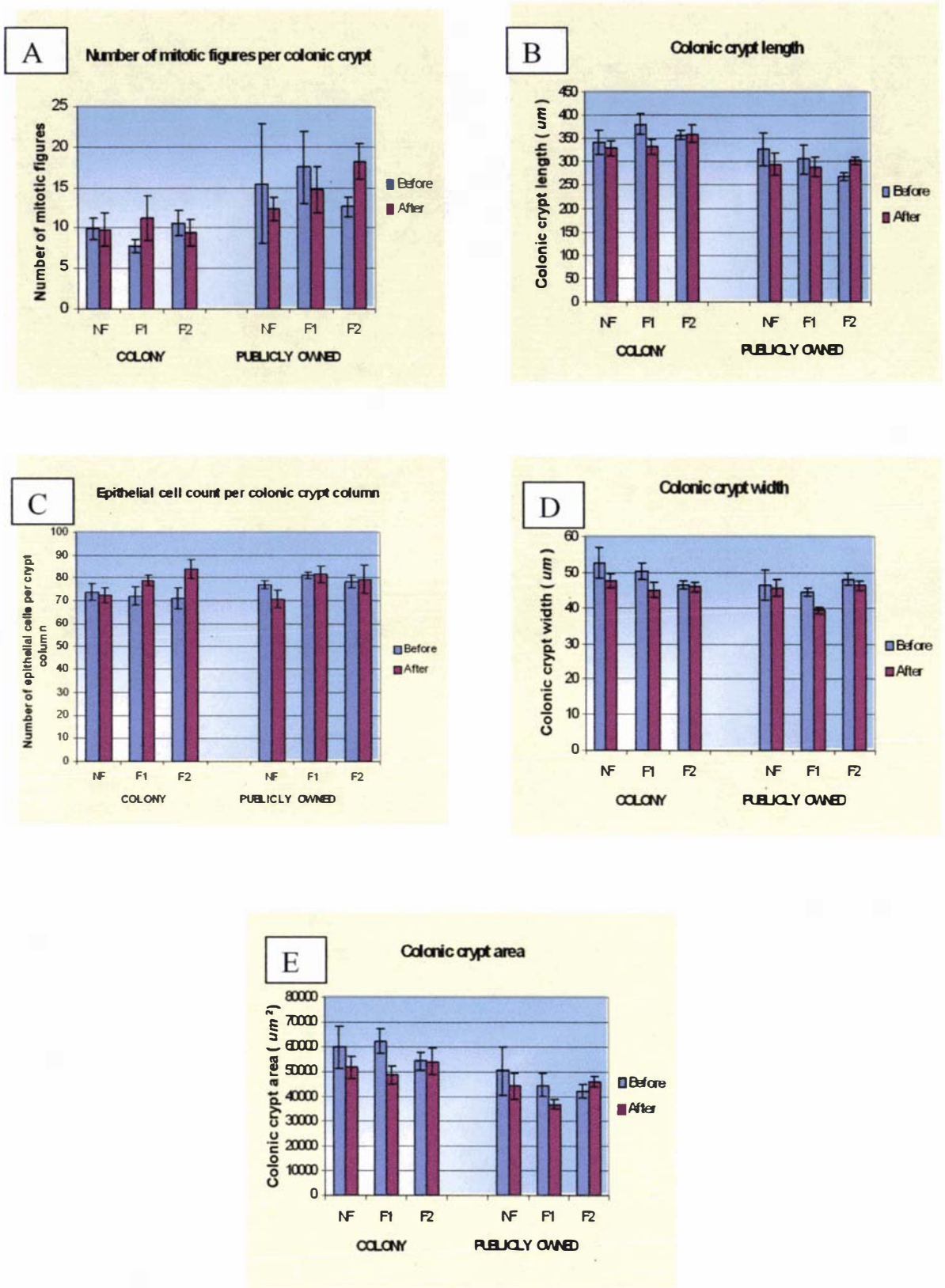
No significant differences occurred in the mean change in colonic crypt mitotic count between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet. However, the interaction between diet and cat source tended toward significance ($p < 0.069$). As seen in **Figure 13A**, the publicly-owned cats responded to the high inulin diet (F2) with an increase in colonic mitotic figures. This change, while substantive (the mean colonic crypt mitotic count in publicly owned cats that consumed diet F2 before the test was 12.6 and afterwards was 18.2) did not reach statistical significance perhaps because of the wide variation found in this parameter within the other dietary groups.

Colonic crypt length

Before dietary intervention

No significant differences were present in the mean colonic crypt lengths between the dietary groups (NF, F1 and F2) at the beginning of the trial. However, colony cats had significantly ($p = 0.006$) longer crypts than publicly-owned cats. The mean colonic crypt length of the colony cats was 361 +/- 11 μm and that of the publicly-owned cats was 300 +/- 16 μm (mean +/- SEM). Significant differences ($p \leq 0.05$) in the baseline colonic crypt lengths also existed between colony cats examined in experimental stages I and II. The difference in colonic crypt length between these two groups of colony cats was substantial (391.34 +/- 16.4 μm and 330.83 +/- 9.13 μm for stages I and II, respectively).

Figure 13



Colonic morphometric parameters (mean +/- SEM) in colony and publicly owned cats consuming three different diets (NF=control diet, F1= 0.1% inulin added, F2= 0.2% inulin added).

COLONIC CRYPTS		Control Diet NF				Diet F1 (0.1% inulin)				Diet F2 (0.2% inulin)			
	Source	Before	SEM	After	SEM	Before	SEM	After	SEM	Before	SEM	After	SEM
<i>Mitotic figures per crypt</i>	Colony	9.91	1.31	9.81	2.04	7.75	0.82	11.23	2.79	10.62	1.51	9.38	1.64
	Public	15.46	7.37	12.33	1.49	17.46	4.49	14.7	2.81	12.6	1.15	18.23	2.18
<i>Epithelial Cells per crypt column</i>	Colony	73.71	3.77	72.31	3.18	72.07	3.85	78.74	2.44	70.84	4.65	83.68	4.15
	Public	76.83	1.76	70.6	3.87	81.06	1.38	81.67	3.56	78.2	2.75	79.46	6.12
<i>Crypt length(um)</i>	Colony	341.81	26.23	329.17	15.74	381.13	21.27	331.78	15.11	357.56	11.24	359.92	19.44
	Public	326.78	35.43	295.84	23.25	306.36	30.29	289.59	19.87	268.82	9.42	303.31	7.11
<i>Crypt width(um)</i>	Colony	52.75	4.2	47.63	1.85	50.18	2.32	44.95	2.09	46.51	2.18	45.96	2.39
	Public	46.53	4.18	45.62	2.4	44.69	1.17	39.32	0.85	48.01	4.06	46.28	1.25
<i>Crypt Surface Area(um)²</i>	Colony	59760	8616	51602	4261	62367	5085	48752	3679	54203	3802	54092	5074
	Public	50297	9753	44188	5295	44665	4875	36874	1850	42346	2909	45879	2218

Table 6.- Cell counts and morphometric mean + SEM measurements of colonic crypts in colony cats and publicly owned cats.

After dietary intervention

Diet ($p=0.025$) modified the differences encountered in colonic crypt length between the first and second biopsy specimens. The mean colonic crypt length in the cats consuming diet F2 increased significantly when compared to that of the cats consuming diet F1 ($p=0.02$) as seen in **Figure 13B**. The mean colonic crypt length at baseline of the cats consuming diet F2 was $331\pm 16\mu\text{m}$ and after dietary intervention was $343\pm 16\mu\text{m}$, whereas the corresponding measurements in the cats fed diet F1 were $359\pm 20\mu\text{m}$ and $319\pm 13\mu\text{m}$, respectively. There were no significant differences in this parameter between diet F2 and diet NF or between diet NF and F1.

The change in the mean colonic crypt length between the first and second biopsy specimens was also significantly different between the cats examined in the different experimental stages ($p=0.0031$). Experimental stage I was significantly different from group II ($p=0.0029$) and group III ($p=0.034$). The cats examined in stage I showed a reduction in mean colonic crypt length across all diets from $391\pm 16\mu\text{m}$ at baseline to $338\pm 17\mu\text{m}$ at the end of the trial. The cats examined in stage II showed an increase of colonic crypt length from $331\pm 9\mu\text{m}$ at baseline to $343\pm 10\mu\text{m}$ at the end of the trial. The cats examined at experimental stage III (only publicly-owned cats) showed very little change from $301\pm 16\mu\text{m}$ at baseline to $296\pm 9\mu\text{m}$ at the end of the trial.

Epithelial cell count per colonic crypt cell column

Before dietary intervention

No significant differences were present in the mean epithelial cell count per colonic crypt cell column between dietary groups (NF, F1 and F2) at the beginning of the trial. However, publicly-owned cats had significantly ($p=0.017$) more cells per colonic crypt column than colony cats (**Figure 13C**). The mean epithelial cell count per colonic crypt cell column of the colony cats at baseline was 72.1 ± 2.3 and that of the publicly-owned cats was 78.7 ± 1.2 cells (mean \pm SEM). However, the colony cats examined in stage I and II of the trial were also not homogeneous. The cats examined in stage I had a significantly ($p\leq 0.05$) lower mean number of epithelial cells per colonic crypt column (64.7 ± 1.9) than those examined in stage II (79.6 ± 2.5).

After dietary intervention

The change in the mean number of epithelial cells per colonic crypt column between the first and second biopsy specimens was significantly affected by diet ($p=0.006$) and experimental stage ($p=0.011$). The differences between the cats consuming diet NF and those consuming diet F1 ($p=0.05$) and diet F2 ($p=0.005$) were significant. The feeding of diet NF was associated with a minor reduction in cell number per colonic crypt column from 74.75 ± 2 cells per crypt column at baseline to 71.74 ± 2 at the end of the trial (mean \pm SEM). In contrast, the feeding of diet F1 was associated with an increase of cells per colonic crypt column from 74.7 ± 3 to 79.6 ± 2 at the end of the trial. Similarly, the feeding of diet F2 was associated with an increase in cells per colonic crypt column from 73 ± 3 to 82 ± 3 after dietary intervention.

The change in the mean number of epithelial cells per colonic crypt column between the first and second biopsy specimens (**Figure 13C**) was significantly different between the colony cats examined in experimental stage I and the publicly-owned cats examined in experimental stage III ($p=0.009$). The cats examined in experimental stage I showed an increase of epithelial cells per colonic crypt cell column from 64.7 ± 1.9 at baseline to 74.7 ± 3.0 at the end of the trial whereas those examined in experimental stage III showed little change from 78.7 ± 1.2 at baseline to 77.2 ± 2.89 after dietary intervention.

Correlation coefficients were calculated between the number of cells per colonic crypt column and the length of the crypt. At the start of the trial the number of cells and the crypt length were negatively correlated ($r = -0.40$) while at the end of the trial these parameters were positively correlated ($r=0.51$).

Colonic crypt width

Before dietary intervention

No significant differences were present in the mean colonic crypt width between dietary groups (NF, F1 and F2) and cat source (colony or publicly-owned cats) at the beginning of the trial. Please refer to **Figure 13D**.

After dietary intervention

No significant differences occurred in the mean change of colonic crypt width between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet.

Colonic crypt surface area

Before dietary intervention

No significant differences were present in the mean colonic crypt surface area between the dietary groups (NF, F1 and F2) at the start of the trial. However, colony cats had a significantly ($p=0.025$) larger colonic crypt surface area than the publicly-owned cats at the beginning of the trial. The mean colonic crypt surface area of the colony cats was $58728\mu\text{m}^2 \pm 14802$ whereas that of the publicly-owned cats was $45770\mu\text{m}^2 \pm 10395$ (mean \pm SEM).

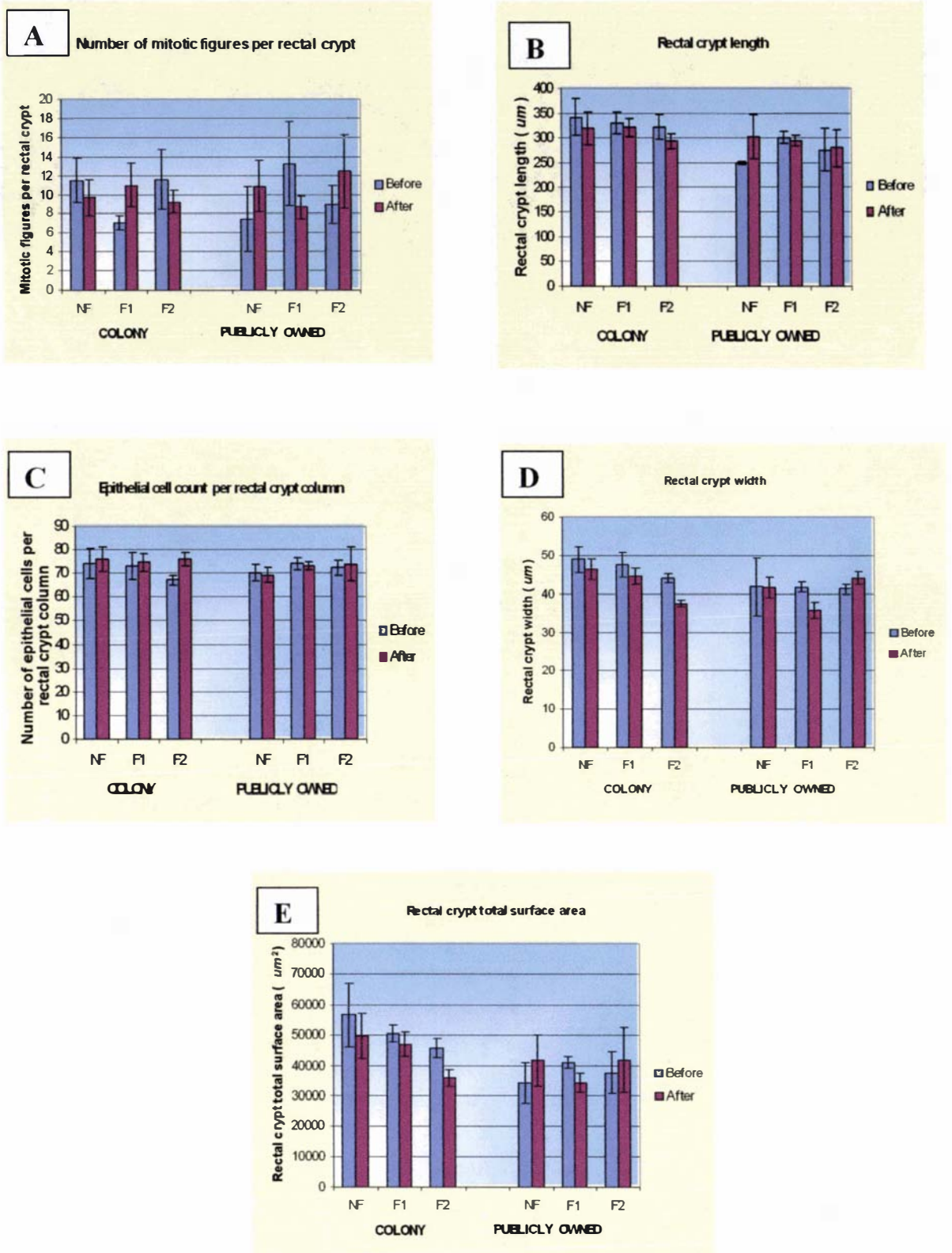
After dietary intervention

No significant differences occurred in the mean change of colonic crypt surface area between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet. Please refer to **Figure 13E**.

Healthy cats –microdissection measurements of the rectum

The rectal samples were larger than the endoscopic pinch biopsy specimens from the duodenum and colon and their degree of fixation and staining was more variable. Marked morphological variability of the rectal mucosal crypts was observed. Some cats seemed to have very sparse crypts intercalated with considerable amounts of connective tissue. Some cats had short and wide crypts whereas others had long and slim crypts (**Figure 12**). It was not unusual to observe variation in crypt length and width in different areas of a biopsy samples. The cats that had sparse crypts showed crypts of different shape with a sharper tip, rather than a bulbous end. Leukocytes were easily identified in some preparations. Please refer to **Figure 14** and **Table 7** for the results of the measurements carried out in rectal specimens.

Figure 14



Rectal morphometric parameters (mean +/- SEM) in colony and publicly owned cats consuming three different diets (NF=control diet, F1= 0.1% inulin added, F2= 0.2% inulin added).

RECTAL CRYPTS		Control Diet NF				Diet F1 (0.1% inulin)				Diet F2 (0.2% inulin)			
	Source	Before	SEM	After	SEM	Before	SEM	After	SEM	Before	SEM	After	SEM
<i>Mitotic figures per crypt</i>	Colony	11.5	2.38	9.7	1.88	7.07	0.76	10.95	2.33	11.56	3.18	9.21	1.96
	Public	7.4	3.4	10.87	2.65	13.2	4.4	8.67	1.19	8.93	1.99	12.4	3.8
<i>Epithelial Cells per crypt column</i>	Colony	74.43	6.46	76	5.27	73.01	5.89	74.75	3.83	67.03	2.15	75.87	2.75
	Public	70.1	3.37	69.23	3.09	73.96	2.74	72.9	1.61	72.2	3.38	73.73	7.28
<i>Crypt length(um)</i>	Colony	342.64	36.86	319	32.65	330.40	21.89	321.27	17.91	322.37	25.29	293.47	14.32
	Public	249.1	3.26	303.2	43.79	300.89	12.47	295.49	11.34	276.18	43.51	279.97	37.17
<i>Crypt width(um)</i>	Colony	48.98	3.33	46.41	2.75	47.65	3.31	44.62	2.02	43.93	1.77	37.65	2.01
	Public	41.85	7.59	41.74	2.67	41.77	1.34	35.73	2.14	41.31	1.22	44.18	5.41
<i>Crypt Surface Area (um)²</i>	Colony	56715	10442	49529	7478	50522	2841	46832	3835	45849	3201	35922	2687
	Public	34324	6682	41749	8472	40881	1960	34230	3216	37625	6824	41771	10535

Table 7.- Cell counts and morphometric mean + SEM measurements of rectal crypts of colony cats and cats owned by the public (home).

Rectal crypt mitotic count

Before dietary intervention

No significant differences were present in the mean rectal crypt mitotic count between dietary groups (NF, F1 and F2) or cat source (colony or publicly-owned cats) at the start of the trial.

After dietary intervention

No significant differences occurred in the mean change in number of mitotic figures per rectal crypt between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet (**Figure 14A**).

Rectal crypt length

Before dietary intervention

No significant differences were present in the mean rectal crypt length between dietary groups (NF, F1 and F2) at the start of the trial. However, the colony cats had significantly ($p=0.0317$) longer rectal crypts than the publicly-owned cats at the beginning of the trial (**Figure 14B**). The rectal crypt length of the colony cats was $331\mu\text{m} \pm 15$ at baseline and that of the publicly-owned cats was $275\mu\text{m} \pm 15$ (mean \pm SEM).

After dietary intervention

No significant differences occurred in the mean change of rectal crypt length between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet.

Number of epithelial cells per rectal crypt column

Before dietary intervention

No significant differences were present in the mean number of epithelial cells per rectal crypt column between dietary groups (NF, F1 and F2) at the start of the trial. However, significant difference ($p \leq 0.05$) in this parameter was present between the colony cats examined in stage I and II. Colony cats examined in stage I of the experiment had 61.2 ± 3 cells per rectal crypt column whereas colony cats in experimental stage II had 80.8 ± 3 cells. The publicly-owned cats examined in stage III were also significantly different ($p \leq 0.05$) from the other two groups of cats and had 72 ± 2 cells per rectal crypt column.

After dietary intervention

Diet was not associated with significant differences in the mean change in number of cells per rectal crypt column between the first and second biopsy specimens (**Figure 14C**).

However, the colony cats examined in stage I of the experiment showed a significant increase in the number of cells per rectal crypt column when compared with the cats examined in stages II and III of the experiment ($p=0.025$ and $p=0.024$, respectively). Cats tested in stage I showed 61 ± 3 cells per rectal crypt column at baseline and 70.5 ± 3 at the end of the trial. In contrast, in the cats examined in stage II the number of cells per rectal crypt column changed from 80.8 ± 3 cells in the first biopsy to 80.5 ± 3 in the second biopsy. Similarly, in the cats examined in stage III this parameter changed from 72.1 ± 2 to 71.9 ± 2.4 cells.

The correlation between the number of cells per crypt column and the crypt length was better in the rectum than other parts of the intestine. ($r=0.45$ and $r=0.63$ at the start and end of the trial, respectively).

Rectal crypt width

Before dietary intervention

No significant differences were present in the mean rectal crypt width between dietary groups (NF, F1 and F2) or between cat sources (**Figure 14D**).

After dietary intervention

No significant differences occurred in the mean change of rectal crypt width between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet.

Rectal crypt surface area

Before dietary intervention

No significant differences were present in mean rectal crypt surface area between dietary groups (NF, F1 or F2) at the beginning of the trial. However, colony cats had a significantly ($p=0.023$) larger mean rectal crypt surface area ($51002 \pm 3535 \mu\text{m}^2$) than the publicly-owned cats ($37610 \pm 2969 \mu\text{m}^2$) at the start of the trial (**Figure 14E**)

After dietary intervention

No significant differences occurred in the mean change in rectal crypt surface area between the first and second biopsy specimens in either the colony or publicly-owned cats in response to diet.

Summary of microdissection results

There were a large number of significant differences in the microdissection measurements present between colony and publicly-owned cats at the start of the trial before any dietary intervention. The source of the cats was not only a very important factor associated with these differences but also appeared to have a significant influence on the response of the cats to the different diets (see below). There was also a high level of individual variation in the microdissection parameters – particularly amongst the colony cats as can be seen by the number of times significant differences were found between colony cats examined in stage I and II of the experiment. Variation in baseline microdissection measurements was observed between biopsy specimens from different cats but also between biopsy specimens from the same cat suggesting localised differences in mucosal morphology. Lastly, diet was associated with only a small number of statistically significant effects on the microdissection measurements.

Publicly-owned cats had shorter and narrower duodenal crypts with a reduced duodenal crypt surface area in comparison to colony cats. In addition, publicly-owned cats had a higher number of mitotic figures per colonic crypt, shorter colonic crypts with a higher number of epithelial cells per colonic crypt column and a smaller colonic

crypt surface area. In the rectum, publicly-owned cats had shorter crypts with a smaller surface area than colony cats.

The colony cats examined in stage I of the experiment showed a reduced number of mitotic figures in the duodenum, a reduced number of epithelial cells per duodenal crypt column, longer colonic crypts, fewer epithelial cells per colonic crypt cell column and fewer epithelial cells per rectal crypt cell column in comparison to the colony cats examined in stage II of the experiment.

The main differences associated with the consumption of the diet enriched with 0.2% inulin (Diet F2) were a trend, more pronounced for the publicly-owned cats, to show an increased number of duodenal crypt mitotic figures and increased number of colonic crypt mitotic figures after dietary intervention. The mean colonic crypt length increased significantly in the cats consuming Diet F2 compared to those consuming Diet F1 although the change in crypt length in response to diet varied significantly between the cats examined in the different experimental stages. The number of cells per colonic crypt column significantly increased in cats fed Diets F1 and F2 when compared to Diet NF.

Colonic transit time

No significant differences were found between diets (NF, F1 and F2) in the colonic transit time of the large and small radiopaque markers (as measured by calculation of the geometric centre) or their distribution in the different regions of the colon at different times (12, 24 and 36 hours postprandially).

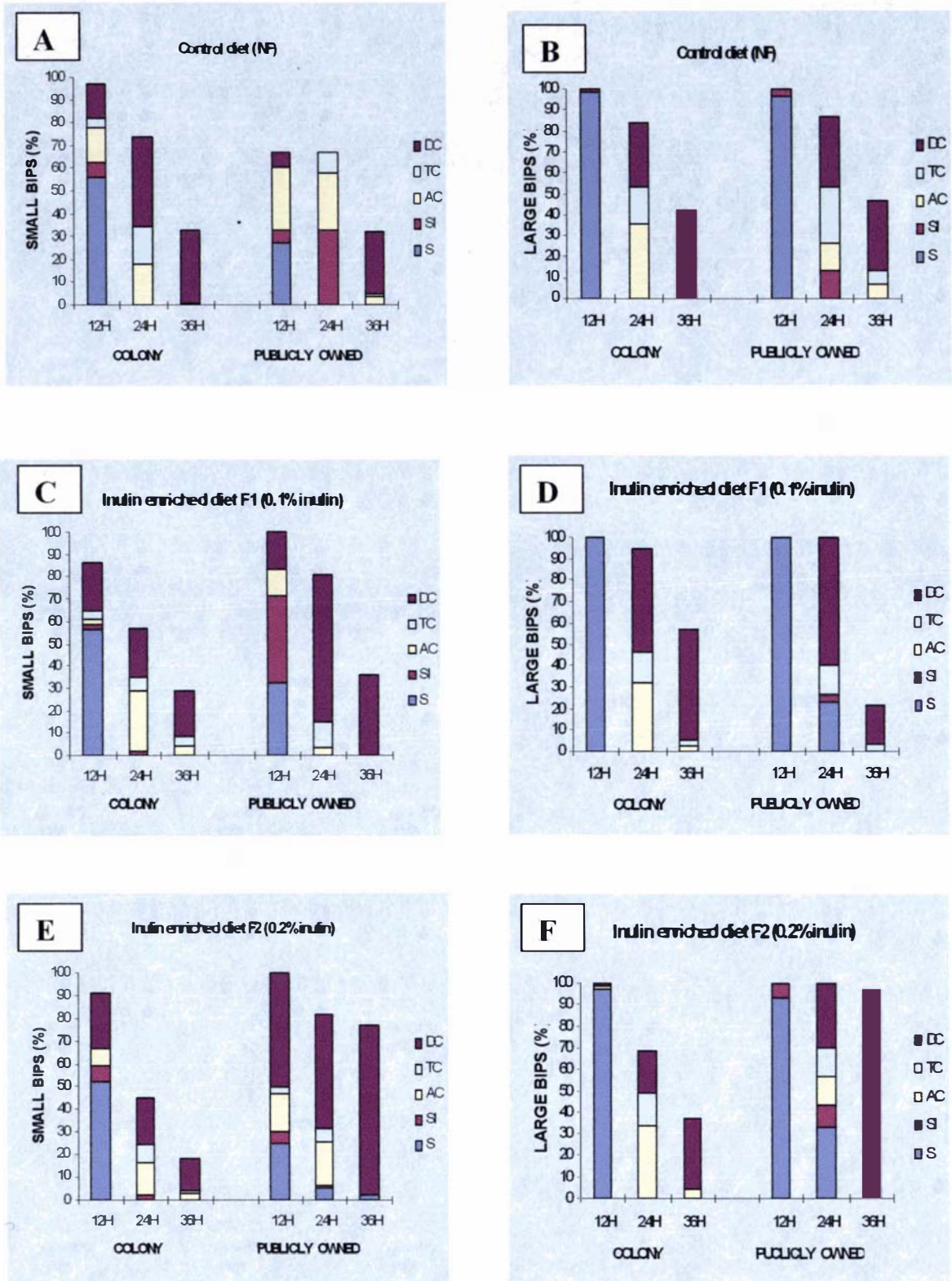
In general, the large 5mm markers were retained in the pylorus for more than 12 hrs, but by 24 hrs they were all mostly in the colon and by 36 hrs half of them had been eliminated in the faeces. The small markers moved faster than the large ones. Approximately half of the small markers were still in the stomach by 12 hrs, while most of the other half had reached the colon. By 36 hours only one third of the small markers had not been defecated.

The percentages (at the various time intervals) of the small and large radiopaque markers in the different regions of the gastrointestinal tract of the colony and publicly-owned cats fed the different diets are shown in **Figure 15**. The geometric centre data is shown in **Figure 16** and **17**.

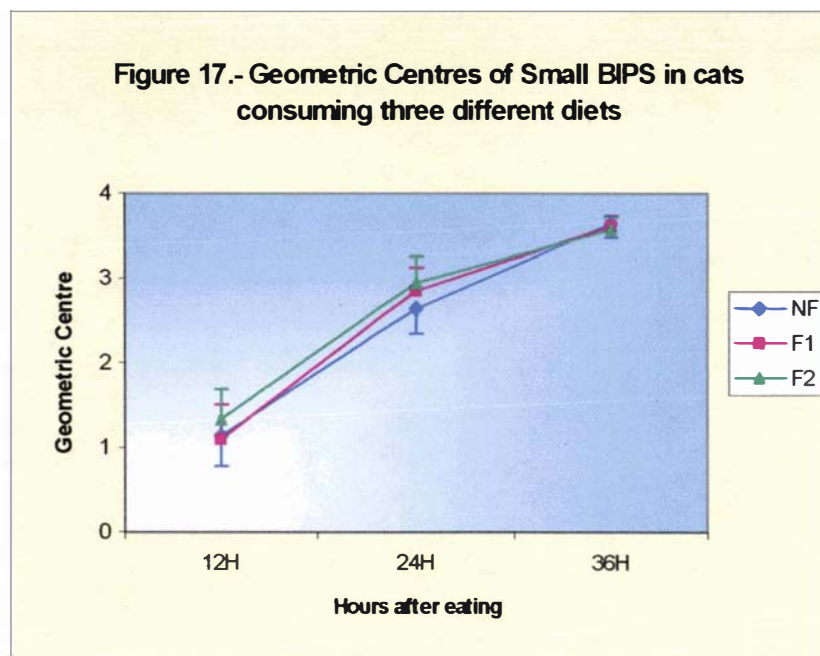
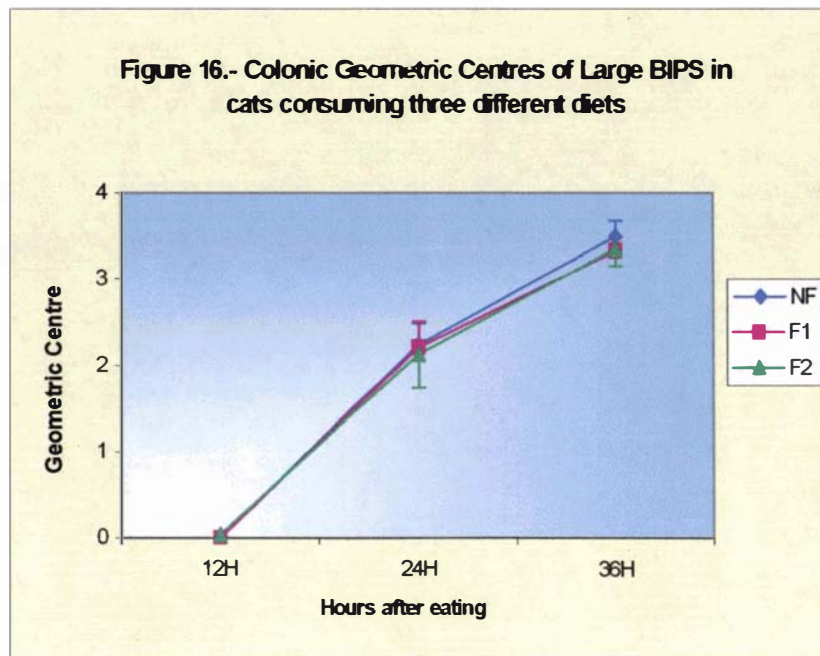
Colony cats showed a significantly ($p=0.042$) greater proportion of large markers in the ascending colon (33.6%) than the publicly-owned cats (8.9%) 24 hours after ingestion of the markers. It can be appreciated from **Figure 15**, that irrespective of diet, by 24 hours the colony cats did not have any large markers in the stomach or small intestine whereas the publicly-owned cats had small numbers of markers remaining in these regions. However, no difference in marker retention was present at 36 hours between colony and public owned cats (**Table 8**).

A significant difference ($p=0.042$) was found between the cats investigated in stage I and II of the experiment (both colony cats) regarding the number of large markers remaining in the descending colon at 36 hours. Cats examined in stage I had 19.2% +/-10.7 whereas those examined in stage II had 64% +/-10.4 of markers remaining.

Figure 15
 Distribution of BIPS (%) along the gastrointestinal tract of colony and publicly owned cats fed three different experimental diets.



Note: S= stomach, SI= small intestine, AC= ascending colon, TC= transverse colon, DC= descending colon



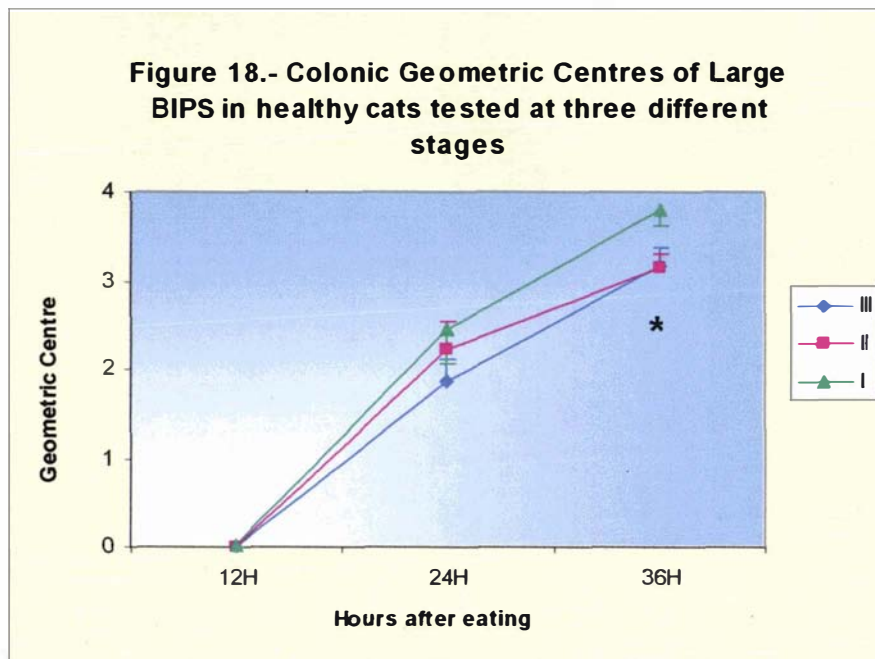
Note: NF=control diet with no inulin added. F1=diet with 0.1% of inulin added and F2=diet with 0.2% inulin added.

Geometric Centre = $\sum \text{intestinal site} \times (\text{Number of BIPS at site} / \text{Total Number of BIPS})$
 1=Ascending colon: 2=Transverse colon: 3=Descending colon: 4=Rectum and Faeces.

Table 8
Mean percentage of BIPS not eliminated at 36 hrs post ingestion.

DIET	NF	SEM	F1	SEM	F2	SEM
Large BIPS						
<i>Colony</i>	41.85	17.90	57.30	16.35	37.14	18.22
<i>Home</i>	46.66	29.05	66.66	17.63	96.66	3.33
Small BIPS						
<i>Colony</i>	32.78	17.93	28.55	10.15	18.29	11.86
<i>Home</i>	32.05	32.05	35.89	28.80	77.07	15.74

Note: Diets included a control diet (NF) and diets enriched with inulin (F2 0.2% and F1 0.1%)



Note: NF=control diet with no inulin added, F1=diet with 0.1% of inulin added and F2=diet with 0.2% inulin added. (*) $p < 0.01$

Geometric Centre = $\sum \text{intestinal site} \times (\text{Number of BIPS at site} / \text{Total Number of BIPS})$
 1=Ascending colon; 2=Transverse colon; 3=Descending colon; 4=Rectum and Faeces.

The geometric centre method also showed a significant difference between experimental stages at 36 hours. Cats examined in stage I of the experiment showed a significantly ($p=0.0087$) higher geometric centre of the large markers (i.e. more aboral distribution of the markers) than the cats examined in the other two experimental stages. The mean geometric centre of the large markers 36 hours after ingestion was 3.8 ± 0.15 for the cats examined in stage I of the experiment, 3.15 ± 0.16 for the cats examined in stage II and 3.17 ± 0.17 for those examined in stage III. See **Figure 18**.

Cats with idiopathic colitis

Unfortunately, only four cases of confirmed colonic inflammatory bowel disease (idiopathic colitis) were available for participation in the trial. Two cats (Pebbles and Zoe) consumed diet F2 and the other two cats (Puss and Raisin) had the control diet (NF). Pebbles had to be withdrawn from the trial so that the only results available from this cat are from the first biopsy samples before dietary intervention. Some samples from Puss were of insufficient depth for the microdissection measurements.

Cats with idiopathic colitis – food intake

The food intake of the cats was good for all diets although the cat being fed the F2 diet consumed only 80% of the food offered, while the cats on the NF diet ate all of their meals.

Cats with idiopathic colitis – faecal output & clinical signs

Mucus and blood in the faeces had been intermittently present before the trial but disappeared a few days after admitting the cats for the trial. During the trial period the faeces of the cats were in all cases firm (grade 5) with no mucus or blood.

Production of faeces per each 100g of food was 24.71g (Puss on NF diet), 12.93g (Raisin on NF diet) and 13.09 (Zoe on diet F2).

Cats with idiopathic colitis – apparent digestibility of diets

Data from two cats were available; Raisin which consumed the control diet and Zoe which consumed diet F2 (0.2% inulin). The digestibility of protein was 82 % for both diets, fat was 96 and 99% and Nitrogen Free Extract was 81 and 85%, respectively.

Cats with idiopathic colitis – histology

Puss was diagnosed histopathologically with a mild colitis and duodenitis, both of which resolved by the end of the trial after consuming the NF diet. Raisin had a moderate colitis and gastritis at the start of the trial. These partially improved by the end of the trial in association with the feeding of the NF diet but a concurrent enteritis became severe. Zoe (0.2% inulin diet-F2) developed chronic gastritis and duodenitis, with very little change in the mild colitis that was present at baseline but a resolution in a moderate proctitis.

IBD cats		Puss (NF)				Raisin (NF)				Zoe (F2)			
		Before	SEM	After	SEM	Before	SEM	After	SEM	Before	SEM	After	SEM
D I S E A S E D U M	Mitosis per crypt	-	-	17.7	0.86	6.1	0.54	5.1	0.43	15.8	0.91	12.9	0.99
	Epit. cells per crypt	-	-	71.1	0.83	64.9	1.97	54.4	1.0	59.6	1.78	59.2	1.37
	Crypt length (μm)	243.28	7.68	-	-	331.64	31.57	192.0	6.85	237.46	7.24	231.26	2.49
	Crypt width (μm)	29.52	1.55	-	-	38.24	5.05	31.46	1.08	27.86	1.57	24.54	0.58
	Crypt Area (μm^2)	23280	1503	-	-	45181	10562	19839	1195	21259	1110	18298	484
	Villi length (μm)	-	-	-	-	615.03	24.36	609.04	23.6	677.89	26.58	781.74	37.47
	Villi width (μm)	-	-	-	-	144.59	4.49	99.43	5.13	165.07	5.88	173.4	3.42
	Villi area (μm^2)	-	-	-	-	293897	8707	199260	14854	375844	25661	450678	25922
C O L O N	Mitosis per crypt	7	0.94	12.9	1.32	6.4	0.56	3.1	0.34	7.8	0.51	18.7	1.08
	Epit. cells per crypt	67.1	0.79	70.6	1.01	68.7	0.74	60	1.14	80.5	2.18	69.5	1.27
	Crypt length (μm)	354.58	6.9	268.32	6.6	282.26	7.54	235.15	8.41	368.76	4.55	308.55	10.64
	Crypt width (μm)	40.77	1.09	40.50	1.62	44.7	2.17	39.59	2.16	58.45	2.3	60.51	2.26
	Crypt Area (μm^2)	46738	1625	35537	1919	41385	2653	30451	1915	70364	2920	61623	3357
R E C T U M	Mitosis per crypt	-	-	-	-	6.2	0.89	3.5	0.83	12.2	0.71	14.9	0.78
	Epit. cells per crypt	-	-	-	-	66.7	0.88	62	2.01	84.4	2.49	75.4	1.80
	Crypt length (μm)	-	-	-	-	244.54	14.56	250.53	9.22	328.38	10.56	317.78	7.07
	Crypt width (μm)	-	-	-	-	30.86	3.37	39.14	2.25	41.88	1.92	39.15	1.71
	Crypt Area (μm^2)	-	-	-	-	25708	3957	31736	1669	44867	3059	40306	2068

Table 9.- Cell counts and morphometric mean +SEM measurements in cats with colitis.

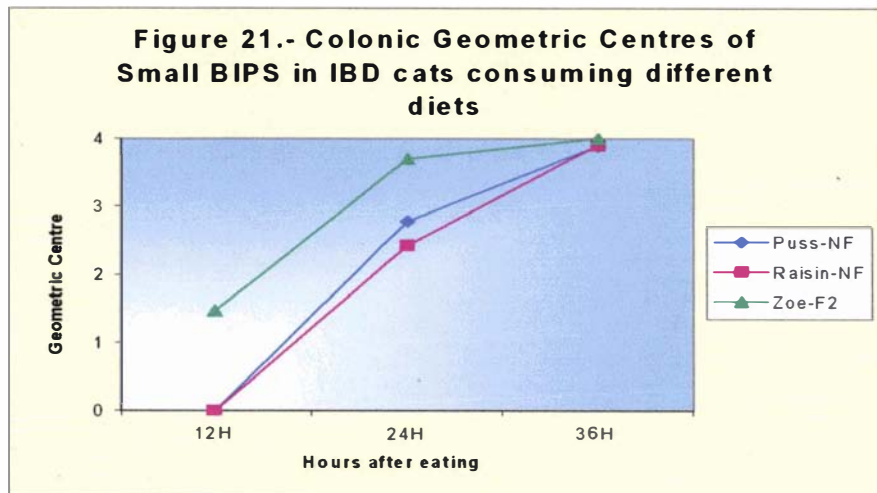
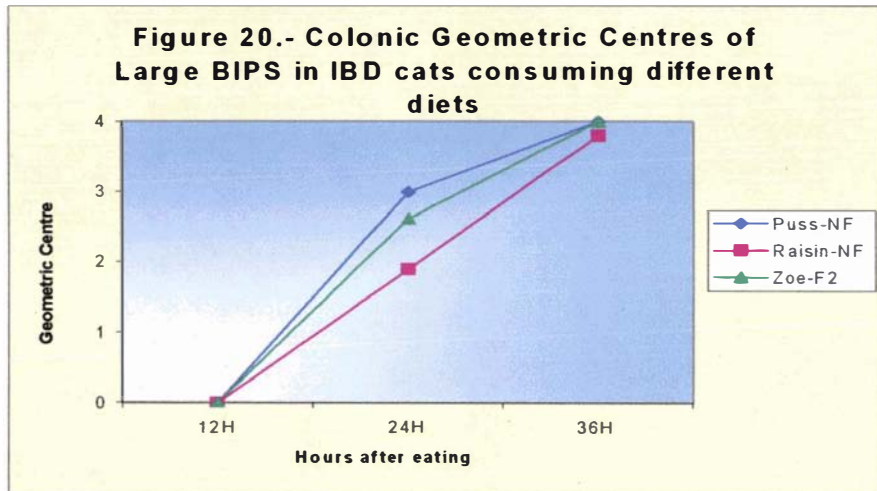
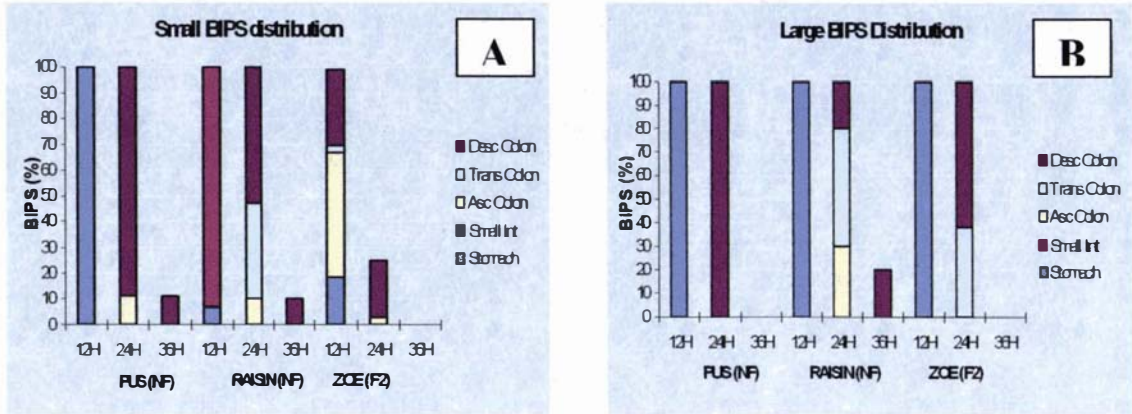
Cats with idiopathic colitis – microdissection measurements

The cat that was withdrawn from the trial, Pebbles, did show in the first biopsy marked differences with the other cats in regards to the duodenal parameters. Although outside of the main purposes of this research the results are disclosed because they add an extra dimension to the microdissection technique in its possible application to other gastrointestinal diseases rather than IBD (refer to Chapter 1). This cat had a large number of dividing cells in duodenal crypts (28 ± 1.16), longer duodenal crypts (333 ± 8.7) with a higher number of epithelial cells per column (81 ± 1.72) and slightly wider duodenal crypts (42.82 ± 1.93). Colonic and rectal parameters were not very different from the other cats. Ultimately this cat was diagnosed with food allergy.

The microdissection measurements of the cats with idiopathic colitis are presented in **Table 9**. All three cats had a lower mean number of mitotic figures per colonic crypt before dietary intervention than healthy cats (7.06 ± 0.4 compared to 11.2 ± 1.05 from all healthy cats).

All cats (regardless of diet) showed a decrease in colonic crypt length and width after dietary intervention. The number of mitotic figures in the colonic crypts increased in two of the cats – one of which had consumed the control diet (NF) and one the diet enriched with inulin (F2). The third cat (Raisin), which consumed the NF diet, showed a reduction of mitotic figures in all tissues, but this effect was most marked in the colon and rectum.

Figure 19
Gastrointestinal transit time of small (A) and large (B) BIPS in three cats with colitis



Note: Diets and Geometric Centre details as described in Figures 16 -18.

Cats with idiopathic colitis – colonic transit time

All three cats eliminated small and large radiopaque markers faster than their healthy counterparts (See **Figures 19, 20 and 21**). The geometric centre data of the cats consuming the NF diet suggest that these cats had a slower transit of small radiopaque markers into the colon than the healthy cats, but once there these markers moved faster through colon and rectum. The cat with colitis that consumed diet F2 had a faster transit of small radiopaque markers than the other two cats with colitis and the healthy cats. The geometric centres for the large markers suggested a slower transit into the colon in all cats with colitis irrespective of diet. Transit of the large markers through the colon was faster for two of the cats (Puss and Zoe that consumed diet NF and F2 respectively). The third cat (Raisin consuming diet NF) had a slower transit through the ascending and transverse colon and a faster transit through the distal colon.

Discussion

The acceptability of the experimental diets by the healthy cats and the cats with colitis was good and was not affected by the inulin content of the diets. However, the food intake of the colony cats was significantly greater than that of the publicly-owned cats. The reason for this difference was unclear. Cage confinement in unfamiliar surroundings could have adversely affected the intake of the public owned cats as cats are reported to refuse novel food in stressful situations⁶⁷. Furthermore, cats refuse foods with different texture to what they are accustomed to⁶⁷. Publicly-owned cats had a more varied diet than colony cats which mostly ate canned food of similar characteris-

tics to the diets being tested. It is possible that the different food intake between the colony and publicly-owned cats may have contributed in part to the differences noted in the present study between the cats from different sources. For instance, the higher faecal output of the colony cats when compared to the publicly-owned cats may have been partly explained by the difference in food intake between these groups.

The small quantities of inulin added to two of the experimental diets in the present study did not increase the total number of stools, total faecal output or amount of faeces/100g of food consumed. Inulin did not adversely affect digestibility of protein, fat or carbohydrate either. The mucus and blood that had been intermittently present in the faeces of the cats with colitis prior to the study did not reoccur while the cats were eating the experimental diets (with or without inulin). In contrast, diarrhoea occurred in one of the cats with colitis and a few colony cats eating an inulin-containing diet. The cause of the diarrhoea in the cat with colitis was determined to be unrelated to inulin. It is uncertain what caused the diarrhoea in the colony cats but no other signs of malaise were present. In addition, all diets including the control diet without inulin were associated with the presence of diarrhoea in very few animals which makes inulin an unlikely culprit.

Histological assessment before dietary intervention of the gastrointestinal tract of healthy cats showed interesting findings, especially because baseline data on gastrointestinal histology of healthy cats is rarely obtained by endoscopic biopsy. The presence of neutrophils in gastrointestinal biopsies of cats with IBD has been reported⁶⁸ and is considered an important hallmark of colitis in man⁶⁹. Increased lamina propria cellularity has been suggested to be an important discriminator in all types of colitis in

man⁷⁰ and veterinary patients^{1,3,68,71}. However, small numbers of neutrophils and mild increases in the number of lymphocytes and plasma cells were common in the mucosa of the healthy cats (colony and publicly-owned) examined in the present study. These findings could be interpreted as evidence that low-grade IBD (including colitis) was widespread amongst the healthy cat population examined in the present study. Alternatively, and perhaps more likely, these data might be taken to suggest that the veterinary profession is yet to fully understand the wide 'normal range' of leukocyte infiltration in the gastrointestinal lamina propria and how much weight should be placed on this assessment when attempting to diagnose IBD/colitis. The caveats of subjective histopathological assessment have been discussed in detail in Chapter 1 and have long been a concern even in the diagnosis of human inflammatory bowel disease^{69,70,72,73}. The Guidelines published by the British Society of Gastroenterology identified several problems in the diagnosis of suspected chronic idiopathic inflammatory bowel disease that are applicable to the situation in veterinary science. These problems are a.- lack of awareness of the range of normal, b.- lack of awareness of accuracy and reproducibility of changes used in diagnosis and c.- absence of standard terminology for pathological description and diagnosis⁷². Without doubt more knowledge is required on the factors that determine and modify lamina propria infiltration in the gut of domestic animals. Until then histopathological diagnosis of gastrointestinal chronic inflammation will be plagued with uncertainty and equivocal results.

There were no consistent patterns of change in the histological appearance of the gastrointestinal mucosa of the healthy cats or the cats with colitis in response to the addition of inulin to the base chicken and rice diet. This was not too surprising because the histological appearance of the mucosa – particularly the presence of chronic

inflammatory infiltrates such as lymphocytes and plasma cells – would be unlikely to change significantly in the short time frame of the current trial.⁷⁴ In addition, more animals with colitis need to be tested with the inulin containing diets to reach any conclusion.

There was a high level of individual variation in the microdissection parameters at the start of trial. Variable quality of endoscopic pinch biopsy specimens used for histopathology has been reported⁷⁵ and may partly explain the variation in the microdissection parameters seen in the present trial. However, the parameters measured by microdissection are less affected by endoscopic biopsy technique (than is histopathology) because sectioning (i.e. microdissection) is performed under microscopic guidance, which minimises inadvertent damage to the tissue and maximises the use of the small amounts of undamaged tissue of adequate depth. In addition, when compared with standard histopathological techniques, microdissection offers a more accurate method to assess morphology⁴⁹ and the number of dividing cells⁵⁵. Greater accuracy is achieved because the orientation of the sample and the plane and point of section have no bearing on the measurements. Furthermore, complete single crypts or villi (not sections) can be assessed one at a time^{49,55}.

The high level of variation present between the colony cats examined in stages I and II of the experiment was unexpected. The only difference in their experimental protocol was the time of the year at which the testing took place. A circadian rhythm is known to occur in the proliferation cycle, but no yearly rhythm has been reported⁷⁶. It is likely, therefore that the differences observed between the colony cats examined in the different stages were due either to chance or other unidentified factor.

The large number of significant differences in the microdissection measurements present between colony and publicly-owned cats at the start of the trial was an interesting observation. Diet has been previously shown to have a powerful influence on mucosal morphology (See Chapter 1). The diet of the colony cats was less varied than that of the publicly-owned cats and contained less dry food, table foods and prey. It is possible, but unlikely, that the colony cats may have had a higher prevalence of mild colitis than the publicly-owned cats that was not recognized by the histopathology. Longer crypts and crypt hyperplasia have been described as typical colitis changes¹ although crypt hyperplasia was not evident in the colony cats in the present study. One can also speculate that the consumption by the colony cats of higher amounts of carrageenan and other gums in canned foods may have caused some of the differences. Dietary carrageenans have been used to produce a model of colitis in guinea pigs⁷⁷.

Diet has important trophic effects on the gastrointestinal mucosa^{78,79}. In this study the addition of inulin was associated with a small number of statistically significant changes in intestinal microstructure of the feline colon. Some of these changes have been reported in other species in response to the inclusion of dietary fibre or its equivalents in the diet^{36,52,56,80}. Although these findings suggest that inulin can safely be added to feline diets and stimulate cell proliferation, its therapeutic significance remains to be discovered. However, the beneficial effects of dietary fibre and fructooligosaccharides in gastrointestinal disease are thought to be related to the many effects that the products of fermentation of dietary fibre have on the functions and structure of

the gastrointestinal tract. In particular, colonocyte energetic metabolism²¹, colonocyte health^{81,82} and repair^{23,24}; and colonocyte absorptive function^{23,28} (see Chapter 1 for details). The number of cells per colonic crypt column significantly increased in cats fed Diets F1 and F2 when compared to diet NF. Two reasons could support an increase in epithelial cells, one is an increase in proliferation, the other is an increase in cell life span^{83,84}. A significant increase in number of mitotic figures was not a feature of this study. However, large variation in number of mitotic figures per colonic crypt was present especially in the publicly-owned cats. Publicly-owned cats showed a trend to a higher number of mitotic figures per colonic crypt after consumption of diet F2 but high level of variation and low number of observations may have made statistical significance unattainable. On the other hand it is possible that a decrease in cell exfoliation and in cell turnover rate underlying an increase in cell life-span could have been the reason for the results, especially in colony cats that showed no tendency to a higher number of dividing cells per colonic crypt.

It is noteworthy that a similar trend for the changes observed in the colonic crypts of the publicly-owned cats in response to the inulin also occurred in the duodenum. The trend towards a higher duodenal crypt mitotic rate in the diet enriched with the 0.2% inulin may have been a chance occurrence or it may have been due to fermentation of the fibre at this site. Epithelial cells from the small intestine have been shown to be able to oxidize short chain fatty acids⁸⁵. Cats are known to harbour larger numbers of small intestinal bacteria than other species with bacterial species that more closely resemble colonic flora⁸⁶. Inulin on the other hand has been found to be totally indigestible in the small intestine of man and not to be viscous³⁶. As described in Chapter 1, indigestible and viscous dietary fibres have been found to increase the

crypt cell production rate but only in the distal parts of the small intestine⁵². The reason for the difference between colony cats, which did not show a trend to a higher number of dividing cells, and publicly-owned cats, that did, is uncertain.

It is difficult to ascertain if the changes observed in the healthy cats would occur and benefit cats with IBD. Only one cat with colitis consumed a diet containing inulin (F2). This cat showed longer colonic crypts than any of the publicly-owned and colony cats before the trial, which is one of the morphometric features of colitis. No large changes were observed in the duodenum or rectum after dietary intervention. However, the consumption of diet F2 was associated with an increase in mitotic figures in colonic crypts, as the healthy publicly-owned control cats had shown. Despite this similarity, it also showed contrasting responses to the publicly-owned cats i.e. reduction in the number of epithelial cells per crypt column, shorter colonic crypts and a smaller total crypt surface area at the end of the trial. These changes might be speculated to suggest a good therapeutic response by increasing the number of healthy new colonocytes, reverting colonic crypt increased length characteristic of colitis and reducing secretory crypt surface area. However, it would be unwise to reach such conclusion without further research including larger numbers of cats with colitis.

We attempted to measure colonic transit by mixing radiopaque markers (BIPS) with the test diets. The use of radiopaque markers to study gut transit times was first reported by Hinton, Lennard-Jones and Young in 1969⁸⁷ and has recently been adapted for its use in cats by Chandler et al. (1997)⁶¹. Radiopaque markers have been used in the cat in several reports^{61,62,88-90} but validation of this technique for measuring food rate of passage in the cat has not been attempted by terminal studies. In addi-

tion no diet has been validated in the cat for measuring gastrointestinal transit time using BIPS, but it needs to be noted that the diet usually recommended by the manufacturer of the radiopaque markers is different from the one used in this study. In addition particulate matter like the radiopaque markers may not be strictly physiological in terms of the nature of the chyme normally delivered to the colon⁹¹.

Several methods –colonic filling times, the mean residence time (MRT) and the geometric centre- have been used in previous studies to measure colonic transit^{62,92} in cats and dogs. Only the geometric centre method could be applied to the data in the present study because the time of entry of the markers into the colon was not measured. It is known from previous studies that radiopaque markers can enter the colon well before the first radiograph taken in this study (12 hours after food consumption)^{61,62}. The geometric centre method has been postulated as being the most accurate means of measuring intestinal transit in rats because of its sensitivity to changes in intestinal transit produced by pharmacological intervention⁹³. The geometric centre method is also used for data analysis in colonic transit scintigraphy⁹¹ and has been previously applied to colonic transit in cats⁶².

Scintigraphy is considered the most physiological method for measuring gastrointestinal transit⁹¹, and radiopaque markers have been favourable compared to it when measuring colonic transit in man⁹⁴. In this study radiopaque markers were found to be slightly ahead of the radiolabeled food, but the difference was small and it was considered that probably it would not affect the clinical decisions made on basis of the results obtained with the radiopaque markers. Most importantly their study consisted only of 2 radiographs which would indicate a very practical type of study for

the clinician. The main concern was individual variability, which was larger with the radiopaque markers than with the radioisotope food.

Chandler et al. (1997)⁶¹ has suggested that gastric emptying is the main determinant of orocolic transit time in cats evaluated with BIPS, and then a delay in gastric emptying would affect the final results of the present study. Comparisons between scintigraphy and BIPS in the dog⁹⁵ and in the cat when studying gastric emptying⁹⁰ have been published. The results of the study in cats indicated a poor correlation between the two techniques but a diet different than the one recommended for use with BIPS was used. BIPS separated out and remained in the stomach longer as indigestible particles. An important observation was that gastric emptying was delayed even when measured by scintigraphy in the same day of BIPS evaluation. It was considered that the stress of repeated radiographs the cause of this difference. Interestingly, although the present study included only 3 radiographs, it did show longer transit times than others published^{61,62,88-90} with retention of BIPS in the stomach.

Both large and small BIPS showed delayed transit at 12 hours, most possibly because of retention in the stomach. The small BIPS looked well distributed through the intestine, but some seemed to be retained around the large BIPS in the pylorus. It is possible that this occurred because the small BIPS separated out from the diet and perhaps were sieved by the large ones. If they would had been separated out in a fluid phase they would have travelled ahead of the rest. The delay in transit of BIPS occurred with all diets including the control one and therefore cannot be ascribed to the fibre supplement. In addition, inulin does not form gels or viscous solutions so the sequestration of the food fluid phase to delay liquid emptying⁹⁶ is highly improbable.

The small quantities of inulin (0.1% or 0.2% of the diet) included in the experimental diets in this study did not appear to affect the transit of the radiopaque markers in healthy cats. These results are compatible with the observations on faecal output – specifically that the total number of stools passed by the cats during the experiment was not influenced by the diet. However, one must be cautious in drawing such inferences. Studies in man have shown that colonic transit is not necessarily well correlated to defecation frequency⁹¹ and it is probable that cats might show a similarly poor correlation between colonic transit and defecation given their reclusive toileting habits.

The finding of high variability in the results obtained with any technique, always questions how sensitive the technique in question is to detect departures from normality⁹⁷. The present study did show marked variability between individuals on the same diet even though some cats did receive IV diazepam to encourage food consumption which has been shown to reduce variability between cats when using BIPS⁶². Variability of individual results obtained with radiopaque markers has been noticed in man⁹⁴, dogs^{92,95} and cats^{61,88}. It is noteworthy that the high variability seen in the present study in the percentage of radiopaque markers in the different regions of the bowel at each time point was reduced when the geometric centre method was applied. Considering all the above points - i.e. technique, diet, species, variability- our results must be taken with caution.

In the present study, the large markers appeared to have a faster gastrointestinal transit in the colony cats than in the publicly-owned cats. This was particularly true of the colony cats examined in Stage 1 of the experiment. The reason for this difference (if any) is unclear although only the results at 36hours were significantly dif-

ferent, and hence the movement of the markers in the distal colon was accelerated. Differences in motility between colony research cats and publicly-owned cats have been explored in the past using imaging techniques of the gastrointestinal tract⁹⁸. Differences were not found but the radiographic technique used by Morgan et al (1981) is not considered very sensitive. In addition, the difference observed in the present study did not occur between publicly-owned cats and the colony cats tested in the second stage of the experiment. Colony cats examined in stage I of this study also showed other differences with the other colony cats i.e. longer colonic crypts and reduced number of epithelial cells per colonic crypt column. These differences were reversed by dietary intervention irrespective of diet. The increase in distal colon transit time may be the result of increased giant migrating contractions, which in dogs with colitis¹⁷ and people with ulcerative colitis¹⁶ increases in number especially in the distal colon. Cats with colitis have shown that over a background of decreased slow wave activity, contractions of greater amplitude and duration than control animals occur¹⁸. Therefore, combining the results of microdissection and colonic transit time, it might be reasonable to say that the cats tested in stage I may have had an incipient colitis. However, this group of cats had the least histological lesions.

All cats with colitis appeared to have a faster colonic transit of radiopaque markers than the healthy cats, especially of the cranial colon. The accelerated colonic transit time was more marked in the cat consuming diet F2. Despite this, the number of stools was lower in cats with colitis than in the healthy cats. The two cats on the control diet NF also showed a slower entry into the colon of small radiopaque markers, which it is most probably due to slower gastric emptying.

Interestingly, this pattern of accelerated cranial colonic transit is different to the pattern observed in the colony cats tested in stage I, which showed some signs of colitis but with accelerated caudal colonic transit. Since the presence of low grade colitis in cats tested in stage I is speculative, it is uncertain why there is a different pattern of accelerated colonic transit time but severity of disease may play a part.

Conclusions

In conclusion, this study has shown that the addition of inulin up to 0.2%DM was associated with minor changes in gastrointestinal structural parameters in healthy cats. In addition inulin did not appear to be detrimental to gastrointestinal functions such as colonic transit and nutrient digestibility. However, more research is needed on the effects of inulin and other dietary fibres in clinical feline colitis to evaluate therapeutic benefit in the control of clinical signs, in the progression of disease and in the severity of relapses.

This study has also shown marked differences in gastrointestinal morphometric and morphological parameters between publicly-owned (source of veterinary patients) and some research colony cats. At the same time it hinted at a difference in response to inulin and differences in colonic function (colonic transit time, amount of faeces produced) between these two groups.

Further research concentrating on identification of useful parameters of gastrointestinal health and determination of the range of normal for these parameters is required. The results of the histology assessment of healthy cats in the present study and the difference between publicly-owned and colony cats attest to the vacuum of knowledge that veterinary scientists are faced with when attempting to separate a healthy from an ill gut.

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CHAPTER 6

REVIEW ON THE USES AND COLLECTION METHODS OF FAECAL AND COLO-RECTAL MUCOSAL FLUID IN GASTROINTESTINAL DISEASE DIAGNOSIS AND DEVELOPMENT OF A NOVEL DEVICE FOR RECOVERY OF COLO-RECTAL FLUID IN THE CAT

The main functions of the colon are to conserve water and electrolytes and to serve as a storage site for faecal material ¹. More importantly there is evidence that disturbance of these functions may play a role in the clinical signs attributed to Inflammatory Bowel Disease ^{2,3}. Although the importance of colonic fluid and electrolyte absorption has long been recognized, the physiological ^{1,4,5} and pathological ⁶⁻⁸ processes that underpin them have been difficult to study and as yet remain incompletely understood.

In particular, our knowledge of epithelial electrolyte and fluid transport is not complete ⁴. As discussed in Chapter 3, the movement of water across the colonic cellular membrane is thought to follow an osmotic gradient generated by solute influx ⁴. In this context, electrolytes such as sodium and short chain fatty acids (SCFA) are thought to be the main determinants of water absorption in the colon ⁵. The absorption of sodium can occur in accordance with an electrochemical

gradient or by electro-neutral exchange ⁸. The latter mechanism entails the absorption of SCFA, which is associated in humans ⁵ and in the dog ⁹ with equivalent sodium absorption. It is not completely understood how SCFA are absorbed ⁵. Nevertheless, solute induced intracellular acidification is considered an important trigger for a sodium/hydrogen exchange transporter in the apical membrane ^{1,10}. The activity of these transporters builds up an osmotic gradient by causing the accumulation of intracellular solutes those results in water absorption. Thus, residual osmolality in the lumen of the bowel is an important determinant of the amount of water absorbed from the bowel. Species that form solid faeces, such as the rat and sheep, maintain a high sodium gradient in the interstitial space around the crypts favouring the absorption of water from the lumen. The opposite is true in species, such as cattle, that produce softer faeces. These species do not have a high sodium gradient around the colonic crypts because of trans-epithelial leakage of sodium ¹¹.

It is unknown if the same mechanisms are important in the colon of cats. The osmolality of fluid obtained from feline intestinal contents is extremely high (see the Appendix to Chapter 3). Furthermore, we have seen (Chapter 3) that feline faecal fluid contains more sodium on average than human faeces. This observation is difficult to reconcile with the very firm consistency of faeces from healthy cats.

From a teleological standpoint, it is also worth considering how important a role SCFA are likely to play in fluid and electrolyte transport in the colon of a carnivorous species when cats have evolved consuming low quantities of carbohydrates and fibre. However, dogs consuming meat only diets have shown

large concentrations of SCFA in the large intestine ¹² and cats consuming commercial diets have shown similarly high concentrations ¹³. Unfortunately, the true magnitude of the impact of SCFA on electrolyte and fluid absorption in the colon cannot be determined simply by measuring the concentration of SCFA in the faeces because over 95% of SCFA are absorbed in the colon ¹⁴. Furthermore, in a colonic perfusion study, Bueno et al. (2000) ¹⁵, demonstrated that sodium was secreted in cats at the same time that SCFA were being absorbed. This observation suggests SCFA may not be involved in electro-neutral sodium absorption in cats.

Given that absorption and secretion are important phenomena in the colon, the collection of colorectal fluid and the study of its composition have been considered the first steps necessary to gain a greater understanding of colonic fluid and electrolyte physiology and pathophysiology. Although faeces have a solid appearance, faecal material consists mostly of water ¹⁶. This is true even in cats that usually produce small hard stools (See Chapter 3). The recovery of this faecal water as representative of colo-rectal fluid was the core of earlier attempts to investigate colonic physiology ¹⁶⁻²². Some of the solutes present in faeces are dissolved in the free faecal water, while others are insoluble or trapped inside fibre or cells. The principle behind using faecal water to study colo-rectal absorptive and secretory mechanisms is that the free faecal water and its electrolytes are considered to be a reflection of the bowel absorptive and secretory activity ²³.

Several methods have been used by previous investigators to separate faecal fluid from the faecal mass including centrifugation, ultrafiltration, and *in vivo* and *in vitro* dialysis ²⁴. Analytes measured in the faecal fluid obtained by these

methods have included sodium, potassium, chloride, ammonia, short chain fatty acids (SCFA), amino-acids and inflammatory mediators.

Electrolyte content and osmolality of faecal fluid have been used to differentiate the pathophysiology of different types of diarrhoea^{25,26}. They have also been used to differentiate between diseases like Crohn's disease, ulcerative colitis and ulcerative proctitis²⁰, and to determine the best composition of oral rehydrating solutions in different types of diarrhoea²⁷. However, methodological differences can be important when appraising data originating in faecal fluid measurements.

Methods used to collect faecal fluid

a.- Dilution based methods of faecal fluid collection

Goiffon in 1961²³ presented data on the composition of faecal fluid obtained by homogenizing faeces, precipitating the mixture and filtering the supernatant. Other researchers used ultrafiltration in the final step instead^{18,28} for the same purpose. However, homogenization of the faeces required mixing the faeces with water prior to the concentration and purification steps. The shortcomings of dilution-based methods have been noted by several researchers as dilution affects, in a non-linear manner, osmolality, water volume of distribution and electrolyte activity^{23,24}. Data are presented in Chapter 3 of this thesis that also show dilution of faeces followed by centrifugation is not suitable to obtain reliable information from cat faeces. However, despite mixing the faeces with water the results from Goiffon (1961) agreed well with the average electrolyte content of

faecal fluid obtained by other researchers using *in vivo* dialysis²³. Similarly, ultrafiltration of diluted faeces has also been reported to yield similar sodium and potassium concentrations in faecal fluid to those concentrations found by *in vivo*¹⁸ and *in vitro*²⁸ dialysis, methods that do not include faecal dilution.

b.- Non-dilution methods of faecal fluid collection

Methods to obtain faecal fluid directly (i.e. that do not involve dilution) include centrifugation, ultracentrifugation and dialysis. Direct centrifugation and ultracentrifugation of homogenized human faeces (without mixing with another fluid) do not suffer from dilution artefacts but success in collecting faecal fluid depends on faecal consistency^{24,29,30}. Feline faeces, by nature, are of a harder consistency than other species and the harder the consistency the less faecal fluid is obtained by these techniques (See Chapter 3).

Wrong, Morrison and Hurst (1961)¹⁶ published the first report of *in vivo* dialysis. Dialysis involves the separation of solutes from proteins or other macromolecules by placing the mixture of solutes and macromolecules inside a dialysis bag made of a thin semi-permeable membrane and immersing the bag in water. The macromolecules are retained in the dialysis bags while a proportion of simple salts present in the mixture diffuse out through the pores in the membrane³¹. Usually cellulose tubing bags, filled with a solution that will not escape from the bag, are swallowed and collected from faeces. Alternatively, the dialysis bags are placed into the rectum for a period of time. *In vitro* dialysis involves collecting faeces and then placing a dialysis bag inside the faecal mass¹⁶. Equilibrium is

expected to occur between faecal fluid and the fluid inside the dialysis bag over a period of time in both *in vivo* and *in vitro* dialysis.

Tarlow and Thom, (1974) ²⁹, albeit with a very small group of human subjects, compared *in vivo* dialysis with direct ultracentrifugation. Their first aim was to detect artefacts produced by the processing of faeces that occurs prior to or during ultracentrifugation. This processing does not take place during *in vivo*, or for that matter, *in vitro* dialysis. Hence they ultracentrifuged and compared the results of faeces that underwent refrigeration, freezing and homogenization and some that did not. They found that refrigeration, freezing and homogenization caused only very slight differences in the faecal fluid composition obtained by ultracentrifugation. Furthermore, *in vitro* faecal dialysis produced a similar fluid composition to centrifugation (40,000g for 30 minutes) of the same faeces. These findings show that the processing (i.e. refrigeration, freezing and homogenization) that takes place before and during centrifugation and ultracentrifugation do not cause significant differences in faecal fluid composition. However, when comparing the composition of faecal fluid obtained by centrifugation and *in vivo* dialysis they reported a significant increase of sodium in centrifuged samples plus changes in osmolality, organic anions and pH. These observations challenge the view that equilibrium with faecal fluid is reached in dialysis bags, principle that is paramount to the belief that faecal fluid collected in dialysis bags truly represents the free fluid present in faeces.

Nonetheless, Owens and Padovan, (1976) ³⁰, found that the electrolyte content of faecal fluid obtained by centrifugation at 24,000g for 2 hours was very

similar to that obtained by *in vivo* dialysis in the same subjects. Even at centrifugation speeds of 40,000g there was little change in the composition of faecal fluid and no evidence that high centrifugal forces could introduce artefacts by causing cell rupture and release of intracellular fluid. Even sonication of these faecal samples did not change the concentration of potassium in the faecal fluid when compared to that obtained after ultracentrifugation. This was true even though the sonicated samples showed clear evidence of cell rupture (release of intracellular amino acids). This finding agrees with the previous group of researchers and confirms that processing by centrifugation does not cause significant changes in electrolyte measurements in faecal fluid samples.

Wrong and co-workers (1961)¹⁶, when first reporting *in vivo* dialysis, described the use of cellulose tubing containing polyvinyl pyrrolidone in solution. These bags were swallowed or inserted into the rectum. Both methods compared well with *in vitro* dialysis using the same faeces. The same authors later published more detailed information on the use of dialysis bags but this time containing dextran²³. This work indicated that *in vivo* dialysis bags were not effective in collecting dialysate from hard faeces. In addition, the dialysate obtained from *in vivo* dialysis bags varied markedly according to the faecal material immediately surrounding the bag. Tarlow (1974)²⁹, reported similar findings. These observations suggest that several dialysis bags are needed to obtain a representative sample of faecal dialysate from the faeces of a single patient and that a wide distribution of several dialysis bags through the faeces is desirable. Wrong et al. (1965)²³ reported also a large variation in electrolyte content in dialysate from *in vivo* dialysis of different individuals. The faecal sodium concentration showed the

highest variability between individuals. Faecal osmolality was only slightly above normal serum osmolality even though some subjects had the bags in their colon for up to 4 days. They also reported the presence of precipitated solids in the dialysate.

Dialysis bags have been used also for directly measuring *in vivo* colorectal absorption and secretion^{8,19,32,33} rather than for collecting faecal fluid as described prior. A dialysis bag filled with an electrolyte solution is placed in the rectum and after a period of time recovered so that net changes in the weight and fluid composition of the bag can be measured. Edmonds (1971)³² attempted to validate this system in rats by comparing the ionic movement of free fluid in the colon with the same fluid in a dialysis bag. They concluded that it was difficult to compare flux rates obtained by whole colon perfusion and dialysis bags. Direct contact with the mucosa was important, electrolyte flux varied with the electrolyte content in the fluid of the dialysis bag and faecal contamination was undesirable. The area of colonic mucosa involved in the movement of electrolytes from the dialysis bags was studied by using radioisotopic potassium. Absorbed potassium concentrated only in the mucosa in contact with the dialysis bag indicating that only the activity of this sector of the mucosa was being assessed. It is therefore reasonable to question how representative this technique is of total large bowel net absorption/secretion. This would be particularly of concern when the technique is used in diseases that may have a localized or patchy distribution.

To the author's knowledge other comparative studies between this use of dialysis bags, perfusion studies and Ussing chambers to measure colonic secretion and absorption are not available. However, these methods might not be directly

comparable either, as unlike *in vivo* techniques, motility will not play a part in the absorptive/secretory function in Ussing chambers. The link between absorption, secretion and motility has not been elucidated but it is believed to be important^{8,34} (Washabau, 2002 personal communication). For instance, it is possible that the cyclical changes in intraluminal pressure of the intestine resulting from the activity of the muscular layers of the intestine will influence water and electrolyte absorption. Colonic manometry has shown a reduction of intracolonic pressure in humans during diarrhoea³⁵. In contrast, cats with spinal lesions that become constipated maintain normal intracolonic pressures even though they cannot eliminate faeces⁷. Hence in this context the use of *in vivo* dialysis bags for the purpose of studying net absorption/secretion in the colon has the advantage of including the effects of colonic motility on water and electrolyte movement across the colonic epithelium.

Miscellaneous methods used to collect intestinal or mucosal fluid

Collection of intestinal fluid for bacteriological studies, or mucosal fluid for disease diagnosis has been achieved by the use of capsules, microcapsules and filter paper.

Plastic capsules have been used to sample intestinal contents for diagnosis of giardiasis (a peroral nylon string test)³⁶ and for bacteriological studies (Hiryzmann, 1962 as cited by Simon and Gorbach, 1984)³⁷. The first uses a string

as a wick to collect fluid, the second used an electronic signal to open the capsule and allow intestinal content/fluid to go in.

Semipermeable polymer microcapsules have also been used to trap intestinal compounds such as carcinogens³⁸. These microcapsules are used most commonly in food technology applications to deliver enzymes³⁹, as drug delivery devices⁴⁰ and in biomedical science to encapsulate mammalian cells⁴¹. Several types are available⁴²⁻⁴⁴ and they can be adapted for different uses. Their permeability characteristics and biodegradation have been intensely studied^{40,45,46} making them very versatile.

Another novel diagnostic method using filter paper to collect colonic fluid has been reported. The technique was first used to recover cytokines from the nasal mucosa to prove allergic responses after challenge⁴⁷. Recently direct application of filter paper to the colonic mucosa during rigid sigmoidoscopy or rectoscopy has been successfully used to measure cytokine and eicosanoid production in ulcerative colitis^{48,49}. The substances dissolved in the fluid were collected by incubation of the filter paper at 4°C for 24 or 72 hours in a buffer solution and then stored at -70 to -80°C^{48,49}. This was a rapid, safe, low cost method to study pathogenesis and response to treatment but may also be applicable to the diagnosis of colonic disease if appropriate biochemical markers for specific colonic diseases can be found.

Applications of and constraints on the use of faecal fluid and dialysis bags in gastrointestinal disease diagnosis

Historically, analysis of faecal fluid has been used to assist differentiation of Crohn's disease and ulcerative colitis in people ²⁰. Although differences were found between the diseases the methodology used in the study included dilution of faeces and so the results are open to question. Undiluted faecal fluid has been used to differentiate osmotic from secretory diarrhoeas in people ^{25.26.50} but with controversial results. Faecal fluid obtained from dialysis bags has been used for measuring production of histamine ⁴⁹, electrolyte movement ¹⁷ and to study therapeutic responses in the colon by measuring different cytokines and eicosanoids ^{21,51}.

In contrast, the analysis of faecal fluid as a mean of diagnosis has not been used extensively in veterinary science. Many of the observations noted previously in this Chapter concerning the different methods of colorectal fluid collection are important when considering the best methods to obtain colorectal fluid from cats. Cats have smaller amount of faeces of harder consistency in their colons than other species. It is possible that cats may have developed highly efficient mechanisms to absorb water and electrolyte from their intestine as has been suggested to be present in the small intestine of small mammals from desert environments ⁵². Like desert animals cats showed longer villi ⁵³ than other mammals-, longer microvilli, increased surface area and increased sodium, chloride and glucose absorption ⁵². Certainly, as described in Chapter 3, the acquisition of faecal fluid from cat faeces

by any method proved to be difficult. Please refer to Chapter 3 and its Appendix for a detailed report on the difficulties experienced in the collection of faecal fluid in healthy cats and cats diagnosed with IBD. A brief summary based on the constraints (previously described in Chapter 3) of the different methods for collection of faecal fluid in cats follows.

Mixing of cat faeces with distilled water and the use of several dilution factors proved unsuitable to obtain faecal fluid for analysis. In addition, although faecal fluid obtained by centrifugation or ultracentrifugation of human faeces has been shown to be very similar in composition to the fluid obtained by dialysis bags, direct ultracentrifugation of cat faeces proved to be ineffective to obtain faecal fluid from very firm cat faeces. Furthermore, when feline faecal fluid was obtained by ultracentrifugation it showed a large range of normal electrolyte values (as human dialysates have shown). The content of potassium in feline faecal fluid was substantially lower than those reported in the literature for humans^{18,23}. Precipitates were found in feline faecal fluid after ultracentrifugation. Filtration of samples with precipitates was required to avoid obstructing the flame photometer. Some precipitates were hard, dark concretions others were soft and white. Although not analysed, these white, round, greasy precipitates, had the characteristics of soap and could have affected the amount of free electrolytes in the faecal fluid by altering water activity in two ways. In non-filtered faecal fluid, the precipitates may have decreased the total electrolyte measurement⁵⁴. In filtered faecal fluid, the elimination of the precipitates may have increased the total electrolyte content²³. Both contributing to the large variation of electrolyte content

encountered in feline faecal fluid. Feline faecal fluid did not identify any colonic physiological alteration in feline IBD.

Likewise, the *in vivo* and *in vitro* dialysis bags used in humans are likely to be poorly suited for use in cats. They have shown to be ineffective with hard faeces, several bags are required per subject and distribution through faeces should be even ^{23,29}. Practical considerations regarding the size of the bags to be swallowed by an average size cat and the likelihood that only very small amounts of fluid would be collected -if any- discouraged me from trying them. Rectal insertion of the bags was possible but results would have only reflected the faeces the bags were in contact with (if any fluid collection took place) and the small size of the cat colon would have made any manipulations of the bags difficult.

Interestingly, the use of dialysis bags to study colonic secretion and absorption in dogs has been reported by Rolfe (1999 and 2001) ^{8,55}. Few details were given about the preparation of the colon before insertion of the dialysis bags. As a result it is difficult to assess if the bag was in contact with faeces or directly against the colonic wall. The dialysis bags filled with an electrolyte solution were left for one hour in the colon and changes to the electrolyte solution studied. Comparisons of changes in the electrolyte solution between healthy dogs and dogs with dietary sensitivity showed reduced absorption of water, sodium and chloride in the food-sensitive dogs. The same author has published more recently a more detailed study of the dietary effects on colonic structure and function in hypersensitive dogs reaching similar conclusions to those of the original study ³³. In the more recent study dialysis bags were inserted into the descending colon for

30 minutes after endoscopy and biopsy. Factors that require consideration in future studies using this method are 1.- whether the presence of blood or faeces or fluid in the colon from previous enemas affected the electrolyte changes inside the dialysis bag and 2.- whether the physical presence of the dialysis bags irritated the colon and altered (only in the disease affected animals) the production of prostaglandin E₂⁵⁶ and the absorption/secretion in the epithelium in contact with the dialysis bag. Notwithstanding these considerations, the use of dialysis bags in dogs in this study offered very interesting observations that now await confirmation.

Microcapsules may be more applicable to the cat than dialysis bags. Microcapsules have the advantage that they offer good distribution through the ingesta increasing the surface area available for transport, survive gastrointestinal transit intact^{45,46}, are non-invasive and some microcapsule designs allow easy magnetic recovery from faeces³⁸. Unfortunately, the production of microcapsules requires specialized, trained personnel and expensive technology. In addition, the high osmolality found along the large intestine of the cat may have affected their performance.

The objective of this study was to develop a simple and inexpensive system for collection of rectal mucosal fluid from cats to be used in future studies of feline gastrointestinal diseases.

Materials and Methods

Animals

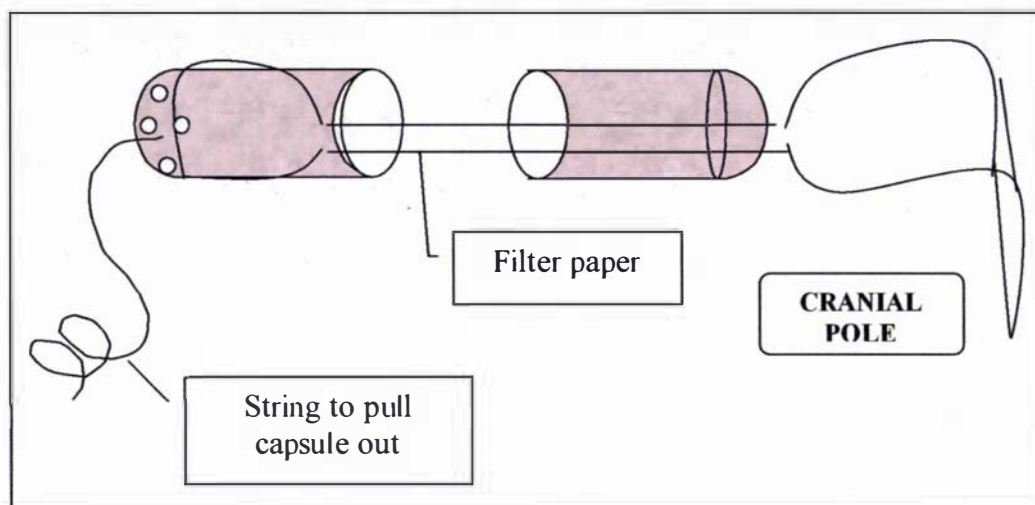
Ten cats owned by the Animal Health Services Centre or members of the public were used in the study. A consent form was signed by the cat owners. The protocol was approved by the Massey University Ethics Committee. Nine of the cats were determined to be healthy by history and physical examination. One of the cats had been diagnosed with IBD and had a history of intermittent diarrhoea although at the time of this trial no clinical signs were evident.

Dialysis Capsules

The outer casings of the capsules (**Figure. 1**) were manufactured by Stallion Plastics Ltd (Palmerston North). The casings were made of sturdy but flexible plastic and had multiple fenestrations on both poles. One pole of the casing was cut off with a scalpel blade to create a small opening through which filter paper (Whatman 1-medium fast speed) was threaded. This pole was the pole of the capsule that was inserted first into the rectum and is hereafter referred to as the “cranial pole”. The filter paper was cut into a 5mm x 145mm strip with two wider ends shaped as a spatula (300mm x 150mm). One spatulate end of the filter paper was wrapped around the inside of the caudal pole of the capsule and the middle strip was folded inside the rest of the capsule. The second spatulate end of the filter paper was threaded through the opening in the cranial pole of the capsule and folded over the exterior surface of the casing of the capsule. After insertion of the capsule, this exteriorized piece of filter paper was assumed to be in direct contact

with the rectal mucosa. A nylon string was tied to the fenestrations of the caudal pole of the capsule to facilitate recovery of the device.

Figure 1
Capsule for faecal fluid collection



Protocol Overview

The cats were housed in a ward of the Massey Veterinary Teaching Hospital. Food was withheld for 12 hours prior to the start of the trial and during the study period. Water was freely available.

On the day of the trial a capsule was manually inserted into the rectum of each cat. Prior to insertion, the anus was lightly lubricated with paraffin jelly to facilitate insertion. The capsules were left in the rectum for approximately 10 hours. They were manually removed after 10 hours if they had not been defecated prior.

The cats were observed continuously throughout the study period. The observer reported every 15 minutes on the behaviour of each cat after the insertion of the capsule.

The cats were returned to their owners once the capsule had been recovered.

Results

No sedation was required to insert the capsules into the rectums of the cats. Some cats showed minor discomfort during the procedure and attempted to move away from the person inserting the capsule. Nevertheless, tolerance of the capsules was very good with most cats not paying any attention to their anal or perineal area following insertion of the capsule. Most animals slept, groomed or rested quietly throughout the 10 hours of the study period.

One cat defecated the capsule twice immediately after insertion but it stayed in place after the third insertion. Another cat passed the capsule unnoticed sometime early in the study period. The filter paper of this capsule was dry when found and the capsule was discarded.

The capsules were manually removed from the rectum of eight cats at the conclusion of the 10-hour study period. One cat objected strongly to the attempt to remove the capsule from its rectum resulting in the retrieval string breaking. This capsule was passed with faeces the following morning and it was discarded. The other cats showed little or no discomfort during the manual recovery of the capsule.

Once the capsules were removed from the rectum the wetting of the filter paper could be clearly seen. In most cases it was only the cranial portion of the filter paper wick – the only portion of the filter paper in direct contact with the rectal mucosa – that was wet (see Table 1). The fluid wetting the filter paper was clear in most cats. No blood on the capsule or irritation of the anal area was observed.

Table 1

Rectal fluid collection in cats using a capsule with a filter paper wick inserted in the rectum

Filter paper area wet	Frequency	Observations
None	1	Faecal staining of spatula
Small area of spatula	1	Healthy cat
All spatula	4	Healthy cats
Spatula and small area of middle strip	1	Healthy cat
Spatula and middle strip	1	Healthy cat
All filter paper	1	Cat with history of IBD

Discussion

The procedure of inserting and recovering the capsules was well tolerated by most cats. This method of bowel fluid recovery had the advantages of being non-invasive, not requiring sedation or anaesthesia, and being easy to perform in a private practice situation. The procedure was successful in most cats although there were instances in which the volume of fluid recovered was low or absent.

Interestingly, the cat with the history of IBD was the only cat in which the entire filter paper was wetted. There did not appear to be a large amount of rectal fluid available to be soaked up by the filter paper in most of the other cats. It is

uncertain if the larger amount of fluid recovered from the cat with the history of IBD is a matter of chance or is a reflection of increased secretion in this cat due to the presence of gastrointestinal disease. Alternatively, there are precedents with dialysis bags in humans²² and rabbits⁵⁶ in which the presence of disease seems to sensitize the mucosa to the presence of the dialysis bag. It is reasonable to think that this sensitisation could also occur in cats with gastrointestinal disease in response to the capsule used in the present study.

In conclusion, a simple method to acquire small quantities of fluid from the rectum of cats has been successfully developed. Further studies are required to determine if the method provides representative samples of rectal fluid in sufficient quantities for the measurement of substances of diagnostic value in large bowel disorders of cats and other species.

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APPENDIX

to CHAPTER 6

Preliminary development of capsules

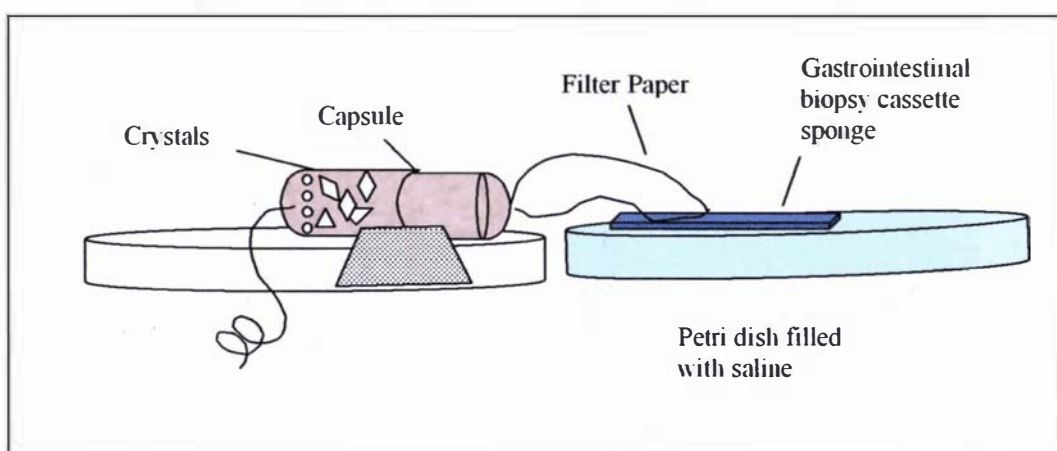
The objectives of these preliminary trials were to determine the ability of a filter paper strip (enclosed in a capsule) to act like a wick when in contact with a thin film of water and to determine if the action of the wick could be enhanced by placing the filter paper in contact with water absorbing crystals. The trials were conducted in two phases, one *in vitro* and one *in vivo* study.

A. Laboratory pilot study of a capsule containing filter paper and absorbent crystals.

Two capsules were prepared as described above in the Materials and Methods section but with the addition of 12-15 proprietary water absorbing crystals (AquaCrystalsTM manufactured by Aqua and marketed by Horticom, Auckland) used routinely in the gardening industry to retain water in the soil of pot plants. The composition of these crystals was not disclosed by the manufacturer but they were chosen for this experiment because, according to the manufacturer, they form gels which sponge water (up to 400 times their own weight in water) but do not trap solutes. The fluid collected in such crystals could then be recovered by compression of the water filled crystals against filter paper as described for measuring gel water-binding capacity¹. The capsule was placed on a small raised platform inside an empty Petri dish (**Figure 2**). The edge of the cranial spatula of filter paper was placed in contact with a small piece of sponge floating in saline in

an adjacent Petri dish lid (**Figure 2**). The filter paper was left in place for 20 hours and inspected every hour (for the first 6-7 hours) for swelling of the crystals. Swelling was not obvious until 4 hours had elapsed. Over night the crystal collected so much water that one capsule burst open and another capsule lost swollen crystals through the fenestrations in the capsule.

Figure 2
Capsule trial in the laboratory to demonstrate fluid absorption by the filter paper from a thin fluid film



This pilot experiment demonstrated that the filter paper in the capsule could act as an effective wick and that the crystals could collect fluid from this wick.

B. Use of capsules containing filter paper & water-absorbing crystals in a small number of cats

Capsules containing filter paper and AquaCrystals were inserted in the rectum of three cats (supplied by the Best Friend Feline Unit at Massey University)

and left in place for 24 hours until defecated the next morning. The cats were fed twice a day and were housed in individual metabolic cages.

Collection of fluid was poor. In all cases there was faecal staining of the filter paper (cranial and caudal spatula). Slight swelling and softening of the crystals occurred but the water did not appear to have reached the crystals via the filter paper wick because the strip in between the two spatulas was dry and clean. The crystals were stained brown and were mainly located in the caudal pole of the capsule around the caudal fenestrations. Faeces were found attached to the capsule, especially around the fenestrations in its caudal pole. It is considered that these faeces and/or water vapour present in the gas in the colon may have been the source(s) of the water that swelled the crystals.

This pilot study suggested that 1.- the addition of water-absorbing crystals to the capsule did not provide an advantage for the collection of fluid *in vivo* and the presence of crystals may have increased the likelihood of faecal contamination of the fluid collected. 2.- that it is preferable to avoid contact of the capsule with faeces to avoid faecal contamination. Thus, fasting the cats before the introduction of the capsule and during the testing period was considered desirable. 3.- that faecal contamination could be avoided further by reducing the length of time the capsule was left in the rectum and by recovering the capsule rather than waiting for defaecation.

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