

CHAPTER 4 DISCUSSION

4.1 THE INTESTINAL FLORA OF HEALTHY CHEETAHS

There were no aerobic bacteria isolated from the proximal duodenum of the two cheetahs and the number of anaerobic bacteria and *Enterococcus* spp. varied. Many of the Enterobacteriaceae and *Enterococcus* spp. are facultative anaerobes. They will grow in the presence or absence of oxygen.

Only two animals were available for duodenal sampling. One can therefore only deduce that there is a difference in bacterial numbers in the proximal duodenum between cheetahs. The difference might be associated with diet. The two animals were fed on a different diet, namely meat-based or adult cat food (IAMS). Vulfson *et al.* (2003) proposed a difference in bacterial counts associated with diet in mink. He also showed an effect on the different bacteria where beta-haemolytic staphylococci decreased after birth and *E. coli* increased gradually from birth to weaning.

High numbers of aerobic bacteria were cultured from the proximal intestine in cats (Johnston, *et al.*, 1993). The reason that no growth of aerobic bacteria occurred from the duodenal samples of the two cheetahs might be associated with the proximity of sampling to the stomach. Even so Johnston, *et al.* (1999), proposed no differences in bacterial counts in either qualitative or quantitative microbiological flora when duodenal juice is collected by endoscopy compared with direct needle aspiration during laparotomy.

Low numbers of anaerobes and high numbers of enterococci were found in the duodenum of the cheetahs in this study. An increase in fermentable fibre has been associated with a trend of lower total bacterial counts in the duodenum of cats (Johnston *et al.*, 1999). The IAMS diet contains more fermentable fibre than the meat-based diet. Therefore the difference in fibre or fundamental difference in the diet composition could be associated with the difference in bacterial counts found in the cheetahs.

High numbers of anaerobic bacteria and aerobic bacteria were isolated in the faeces of cheetahs in this study. The bacterial counts found by Howard *et al.* (1993) differed. The predominant bacterial isolates in cheetahs were Gram-negative (98 %) in comparison to 43 % of Gram-negative bacteria in cats.

There were higher numbers of bacteria, both aerobic and anaerobic, isolated from faeces of cheetahs fed a meat-based diet in this study. The higher bacterial counts could be related to their exclusive meat-based diet. Johnston *et al.*, (1993), proposed the higher number of bacteria in the gastrointestinal tract of cats to be associated with an exclusive meat-based diet. Higher numbers of bacteria should also be expected in cheetahs on a meat diet.

The most common bacterium found in cheetahs and cats in the study of Howard *et al.* (1993) was *E. coli*, but only 1.4 % of their isolates were *Enterococcus spp.* In contrast, 8 % of isolates were *Enterococcus spp.* in this study. Medium to high numbers of *E. coli* were found in this study but no colony counts were performed making it difficult to compare the two studies.

Howard *et al.* (1993) isolated the following Gram-negative bacteria from rectal swabs of cheetahs: *E. coli*, *Campylobacter*, *Klebsiella* and *Proteus*. *Enterococcus spp.* were the only Gram-positive bacteria present. This is in contrast with the results of this study where a variety of bacteria and yeasts were isolated (see Table 6). The variety of isolates was similar to the composition of isolates found in the domestic cat (Howard *et al.* 1993). All cheetahs in their study were housed in zoos and received a commercial carnivore diet with the addition of various meat supplements. The differences in housing and diet may also be responsible for the differences in bacterial isolates. The use of selective media in our study may also have enhanced the recovery of those bacteria only present in small numbers. No selective media were used by Howard *et al.* (1993). To get a true representation of the microflora of cheetahs, more animals would need to be studied to evaluate the effects of age, diet and housing. The influence of the diet on the susceptibility of cheetahs to intestinal upsets and diarrhoea also needs to be studied more.

Ideally faeces of free-ranging cheetahs should also have been analysed. The normal flora of free-ranging cheetah may be different from the flora of captive cheetah fed on IAMS adult cat

pellets, horsemeat, or chicken. Different diets fed to animals in captivity may also change the normal flora. Unfortunately no faecal specimens from free-ranging cheetahs were available. One of the female cheetahs (F331) used for faecal collection was wild-caught (see Table 20), but not recently enough to qualify as free-ranging. The faecal cultures of F331 were not significantly different from those of captive-bred animals.

4.2 COMPOSITION OF THE CHEETAH PROBIOTIC

Lactobacillus Group 1 was selected as one of the bacteria to be included in a probiotic. *Lactobacillus* Group 1 contains several species of lactobacilli, namely: *L. delbrueckii*, *L. acidophilus*, *L. amylophilus*, *L. amylovorus*, *L. animalis*, *L. crispatus*, *L. farciminis*, *L. gasseri*, *L. helveticus*, *L. jensenii*, *L. ruminis*, *L. salivarius*, *L. sharpeae*, *L. vitulinus* and *L. omanashiensis* (Kandler and Weiss, 1986). The species of lactobacilli are closely linked when comparing rRNA sequences (de Waard *et al.*, 2002). This makes analysis of individual species difficult using biochemical tests. Several species of *Lactobacillus* Group I are currently used as probiotics (Fuller, 1998; Fox, 1988) and none of them are considered to be animal pathogens. Their use is thus considered to be safe. No further analyses of specific species were performed. Supplementing the diet with a probiotic containing lactobacilli increases the numbers of lactobacilli in the gut. Supplementing the diet of diarrhoeic cheetahs with a probiotic could increase survival rate as well as reduce the severity of clinical signs of diarrhoea caused by infectious diseases, stress and antibiotic use. The probiotic must be able to survive an acid and bile environment, adhere to the intestinal wall, colonize the intestinal tract and inhibit pathogens (Weese, 2002). A species-specific probiotic produced from healthy cheetahs was presumed to be more effective in supporting the intestinal flora of cheetahs than non-specific probiotics.

Enterococcus faecium was the most common *Enterococcus* spp. isolated from the cheetahs in this clinical trial. *Enterococcus faecium* has been used in commercial probiotics (Fox, 1988). It has been used to increase weight gain in pigs and feed conversion in calves (Fox, 1988). In calves the addition of *E. faecium* to the feed also lowered the requirement for medical treatment (Fox, 1988) and lowered the severity of *E. coli*-induced diarrhoea (Underdahl, 1982). *E. faecium* could reduce the severity of *Salmonella* infection in captive cheetahs. It has been found to reduce the severity and increase survival time of mice challenged with

Salmonella (Maia *et al.*, 2001). Lloyd *et al.*, (1977) also reduced the severity of *Salmonella* infection in chickens by feeding intestinal content of adult birds. *E. faecium* was thus selected as the second bacterium to be included in a probiotic for cheetahs.

Lactobacillus Group 1 and *E. faecium* have for the above-mentioned benefits on animal health, been selected to be used in a cheetah-specific probiotic in this trial.

4.3 EFFECT OF THE PROBIOTIC ON FAECAL QUALITY

If the difference in percentage diarrhoea is compared before and after the treatment period with the treatment period in the PG group, it can be concluded that there was a significant decrease in the percentage of diarrhoea in the PG during the feeding of the probiotic. The CG had a lower percentage of diarrhoea throughout the trial. This was incidental.

The reduction in diarrhoea during the feeding of the probiotic in the PG was accompanied by an absence of blood and mucus in the faeces, which had been present prior to the start of the 28-day administration of the probiotic (see Figure 9). In pigs treated with probiotic, Underdahl *et al.* (1982) were able to reduce the severity and duration of diarrhoea brought about by challenging them with different strains of *E. coli*. However, in this study the effects of the probiotic was only short term; mucoid/bloody faeces reappeared on day 42, 14 days post treatment (see Figure 9). Antibiotics were administered on two occasions to camp 57 and camp 55 on day -56 and day -35 respectively. These will also have affected the faecal qualities in these camps.

The percentage of faecal water increases when any intestinal problem causes an increase in secretions or a decrease in the absorption of fluids. A water content of 60 to 80 % is considered normal in dogs and cats. Water content of 70 to 90 % are found in unformed to watery faeces in domestic cats (Guilford and Strombeck, 1996). Small changes in faecal water content are normally responsible for the transformation of semi-solid faeces to liquid faeces.

The faecal water content did not change over time and there was no difference between the Probiotic and Control groups. The faecal water is highly dependent on the food provided. Water content increased as the proportion of IAMS adult cat diet increased. The quality of

food during the entire trial varied on a day-to-day basis and depended on the availability of horsemeat. In camp 55, the diet was changed to IAMS intestinal diet on Day 7 for approximately 14 days in an attempt to reduce the loose faeces in the group. In addition the group was moved to a new camp on Day 19 as a result of a high helminth burden found in faecal samples (see Figure 3). Animals in Camps 5, 6, 55 and 57 had to be moved to different camps or groups for managemental reasons during the trial. Possible stress associated with movement may also have affected faecal water content. All these factors and the two outbreaks of *Toxocara* spp. infestation probably contributed to the variation in diarrhoeic score and faecal water before and during the probiotic treatment period.

Faecal water may also be affected by the environment. The water content of the soil, drainage and weather conditions influence the water content of the faeces collected. Even though only fresh faeces were collected, some water would have already drained into the surrounding soil. To improve the reliability of the faecal water content animals should be housed on a concrete floor. Due to practical considerations this was not possible in this study.

4.4 EFFECT OF PROBIOTIC TREATMENT ON WEIGHT GAIN

The weight gains of each animal over the 28-day treatment period are shown in Figure 12. The Probiotic Group had a mean increase of 7.70 % and the Control Group 5.91 %. Cheetah M444 was excluded because his weight had decreased by 13.54 %. This would have been a substantial reduction in weight and would thus have been noticeable. Because it was in perfect condition the difference was put down to error and the reading excluded later when the data was evaluated (see Table 11). The animals were of different ages and sexes at the start of treatment. There was a difference between the ages of the Probiotic and Control groups. This was a constraint of the trial, which was determined by management at the De Wildt Centre. The animals were only available in those groups as this was determined by litters of siblings staying together, some of which were mixed with others long before the trial started. Later mixing to match or stratify animals in groups according to ages, weight and sex was not possible, as the animals would have fought causing severe stress.

Sex does not influence weight increase in neonatal cubs (Wack *et al.*, 1991), therefore it is unlikely that the sex of the juvenile cheetahs influenced their individual weight gains. Growth rate in neonatal cubs up to 40 days of life is linear (Beekman *et al.*, 1999). Birth weight of cubs influences individual growth rate rather than sex (Beekman *et al.*, 1999). Therefore the weight gain as a percentage of individual weight at the start of the probiotic feeding (day 0) was used as a measure of increase in weight. It is unlikely that the difference in ages between the Probiotic and Control groups influenced the weight gain of the animals in this study.

The difference in percentage weight gain between the Probiotic and Control groups of 1.79 % is significant particularly considering the short duration of the trial. Dilworth *et al.* (1978) reported increased growth rates in chickens fed *Lactobacillus* spp. over an eight-week period. The finding was similar to that of Bernardeau *et al.* (2002) who reported a weight increase of up to 31.7 % in mice fed different *Lactobacillus* spp. in comparison with controls over a 17-day period. The greater response seen in the mice compared to the juvenile cheetahs in this experiment was probably due to the shorter generation interval and smaller body mass of mice.

4.5 ROLE OF INFECTIOUS AGENTS AND PARASITES ON WEIGHT GAIN AND DIARRHOEA

Reduced weight gain and diarrhoea have also been associated with coronavirus infection and feline infectious peritonitis (FIP). The mortality due to FIP in cheetahs and reported in the literature is low but coronavirus can cause significant morbidity in a population (Munson *et al.*, 1998). It is thus important to evaluate diarrhoeic samples of the juvenile cheetahs for the presence of coronavirus, particularly if they are showing depression and reduced appetite alongside diarrhoea. Coronavirus could not be isolated from faeces and therefore was unlikely to have influenced results.

Diarrhoea may occur with *Babesia felis* infection in cats (Taboada, 1998). Three animals, M427, M440 and F446, were found to be positive for *Babesia* spp. in two consecutive blood smears. None of the animals however, showed severe diarrhoea, lethargy, anaemia, anorexia or icterus that are associated with *B. felis* infection in cats under the age of 2 years (Taboada, 1998; Schoeman *et al.*, 2001). Macrocytic, hypochromic, regenerative anaemia was present in

57 % of the young cats infected with *B. felis* (Schoeman *et al.*, 2001). No other haematological abnormalities were detected in the three cheetahs (Table 25, Table 26, Table 27 and Table 28).

Theileria-like piroplasms have been reported in lions (Averbeck *et al.*, 1990) in Serengeti National Park and Ngorongoro Crater, Tanzania and the Kruger National Park (KNP) South Africa. The piroplasms in lions in the KNP have been described as a distinct species: *Babesia leo*, based on the phylogenetic analysis of 18S rRNA gene (Penzhorn *et al.*, 2001). *Theileria*-like piroplasms have been described in the blood of all the cheetahs tested in the Serengeti National Park and Ngorongoro Crater, Tanzania (Averbeck *et al.*, 1990). None of these cheetahs showed clinical signs of disease. A non-pathogenic *Theileria*-like piroplasm has been previously identified at the De Wildt Center (D. Meltzer, University of Pretoria, DGA, personal communication, 2003). The *Theileria*-like piroplasm identified from the cheetah in this study were analysed by PCR and reverse line blot but did not match any known *Theileria*-like piroplasm. It is likely that the piroplasm identified in the cheetah is a new, non-pathogenic species specific to cheetah but further research is needed to evaluate this.

4.6 SERUM BIOCHEMISTRY AND HAEMATOLOGY

Serum biochemistry and haematology were monitored in this trial as an indicator of systemic disease. The results showed that none of the cheetahs suffered from systemic illnesses. Only results that deviated from the expected values were therefore considered.

The high eosinophil count found in F446 could have been associated with the *Theileria*-like piroplasm (Ettinger and Feldman, 2000b). F446 was one of the three cheetahs infected with the *Theileria*-like piroplasm. The two other cheetahs, M440 and M427, found to have positive smears, did not show increased eosinophil counts. It is unlikely therefore that the eosinophilia was associated with the *Theileria*-like piroplasm unless there were two different species with different pathogenicities present. In addition, Schoeman *et al.* (2001) did not report an eosinophilia in cats affected with *B. felis*.

4.7 PATHOGEN ISOLATION

No pathogenic bacteria (smooth *E.coli* or *Salmonella* spp.) were isolated from the cheetahs during the study. The pathogenicity of *E. coli* is *inter alia* associated with the morphological appearance of the colonies. Smooth colonies are round, shiny and domed and rough colonies are irregular, flat and have a pitted surface. This effect is best noted after two to three days of incubation. Rough colonies are usually non-pathogenic. The disturbances of the intestinal tract were more associated with a disturbance of the normal flora rather than growth of pathogenic bacteria or osmotic diarrhoea as a result of the diet.

Faecal samples from all camps had evidence of helminth infestations, but no individual faecal egg counts were carried out. Ettinger and Feldman (2000b) reported eosinophilia in association with parasitism, particularly where nematodes such as *Toxocara* were undergoing tissue migration. Faecal egg counts represents only the presence of adult worms in the intestinal tract. The higher eosinophil counts could have been associated with a higher helminth burden in that particular animal (F446). *Toxocara* spp. have been reported as a problem in various zoos around the world (Penzhorn *et al.*, 1998). This is probably due too high concentrations of animals in a confined area.

4.8 EFFECT OF PROBIOTIC TREATMENT ON INTESTINAL PERMEABILITY AND TRANSIT TIME

Intestinal permeability varies greatly between different species and different breeds (Garden *et al.*, 1997). The level of exercise also has an effect on intestinal permeability, a lower permeability has been found in racing greyhounds (Randell *et al.*, 2001). The recovery of rhamnose and lactulose also varies between species (Delahunty and Hollander, 1987). Higher ratios of lactulose to rhamnose have been recorded in clinically healthy cats (Randell *et al.*, 2001). An increase in lactulose uptake has been associated with an increase in intestinal permeability, an increase in the perviousness of tight junctions and increased accessibility of molecules to the crypts. A reduction in the rhamnose blood concentration indicates a decrease in gut transit time or a reduced surface area. Normal cats have a shorter intestine and therefore a decreased surface area for absorption resulting in a higher intestinal permeability, when comparing sugar absorption, in relation to dogs (Randell *et al.*, 2001). The higher bacterial

counts in cat intestines has also been associated with an increased permeability (Johnston, 1999; Johnston *et al.*, 2001). Higher inherent permeability has also been recorded in juvenile animals (Garden *et al.*, 1997). All cheetahs in this study were juvenile therefore a higher intestinal permeability would have been expected.

The reduction in the L/R ratios, reflecting a lower permeability in the Control Group could have been caused by several factors. There might be a variation in permeability between different animals. There are different publications of mean values in healthy dogs. L/R ratios of 0.19 (\pm 0.07) has been reported by Randell *et al.*, (2001), but lower mean values of intestinal permeability in urine (0.08) and in plasma (0.09) have been reported by Sørensen *et al.* (1993, 1997). High L/R ratios of up to 0.40 \pm 0.20 have been recorded in clinically healthy cats (Johnston *et al.*, 2001). Randell *et al.*, (2001) reported even higher values (0.52 \pm 0.19 with a range of 0.3-0.98) as normal in clinically healthy cats. The L/R ratios in this study in cheetahs varied from 0.00 to 0.79, which is within the range of ratios reported by Randell *et al.* (2001) for domestic cats.

Another factor affecting the recovery of the sugars and thus intestinal permeability is the timing of the blood collection after the administration of the sugars. The timing will affect the recovery of the sugars in plasma. The percentage recovery of the sugars in plasma of cats is quite consistent between 60 and 90 minutes after oral administration of the sugars (Hawkins *et al.*, 1986). A sharp decline of the sugar concentration in plasma was noted at 120 minutes (Hawkins *et al.*, 1986). No reference for the ideal timing of collection of plasma after the administration of the sugar was available and therefore plasma was collected as close to 60 minutes after administration as possible. However there was a variation in the collection time of the plasma between cheetahs from 40 min to 170 min (median 74 min). There was no significant difference in the amount of R and L recovered after one hour and after one and-a-half hour between the CG and PG. The differences in timing between administration and blood collection were therefore unlikely to affect the results.

Johnston *et al.*, (2001) looked at the effect of diet and antibiotic therapy (metronidazole) on the intestinal permeability in healthy cats. Higher intestinal permeability has been associated with antibiotic therapy. Higher permeability has also been associated with canned cat food in relation to dry cat food (Johnston *et al.*, 2001). This could be associated with differences in bacterial numbers. More animals in the PG were treated with antibiotics than the CP during

the probiotic treatment period, which could have resulted in higher intestinal permeability in the PG. Food was also not kept as a constant during the trial and varied from day to day, and between camps, which would have had an effect on the intestinal permeability.

There was high variability in the collection time of the plasma for the analysis of sugar concentration as well as other factors affecting the intestinal permeability as discussed above. This in conjunction with no reference range for intestinal permeability in healthy cheetahs makes it difficult to analyse the results. Therefore it is difficult to discuss the significance of the different L/R ratios obtained in this study. The establishment of a reference range in cheetahs would be necessary to evaluate the results of this study further.