CHAPTER 3 RESULTS

3.1 NORMAL INTESTINAL FLORA

Table 3 shows the aerobic and anaerobic bacteria and enterococci grown per ml of duodenal fluid, obtained by endoscopy. No aerobic bacteria were isolated. The anaerobic bacteria in the sample are obligate anaerobes. *Enterococcus* species was the only member of the facultative anaerobes isolated.

Table 3: Bacteria isolated from duodenal fluid (CFU)

	Cheetah ID			
	F 352	M 286	Mean	Std Dev
Diet	IAMS	Meat		
Strict Aerobes	0[a]	0[a]		
Anaerobic	60	110	85	35.36
Enterococci	300	100	200	141.42

[a] no growth occurred after 48 hours of incubation

Table 4 shows the number of aerobic and anaerobic bacteria and enterococci isolated from 1 g of faeces from 8 healthy adult cheetahs on different diets.

Table 4: Bacterial counts (CFU) of cheetah faecal samples

	Cheetah ID							
	F309	**F283**	**Q46**	**F282**	**F318**	**F331**	**F362**	**F327**
Aerobic	$4.67x10^6$	$1.56x10^9$	$6.40x10^5$	$3.50x10^7$	$1.86x10^6$	$2.34x10^8$	$4.77x10^8$	$1.44x10^6$
Median				$1.99x10^7$				
Mean				$2.89x10^8$				
Std Dev				$5.41x10^8$				
Min				$6.40x10^5$				
Max				$1.56x10^9$				
Anaerobic	$4.66x10^7$	$6.12x10^8$	$1.94x10^6$	0[a]	$2.54x10^6$	$1.32x10^6$	$3.06x10^9$	$1.30x10^6$
Median				$2.54x10^6$				
Mean				$5.32x10^8$				
Std Dev				$1.14x10^9$				
Min				$1.30x10^6$				
Max				$3.06x10^9$				
Enterococci	$1.00x10^5$	$5.00x10^8$	$1.40x10^5$	$6.60x10^3$	$1.88x10^4$	$2.20x10^3$	$4.60x10^5$	$7.00x10^3$
Median				$5.94x10^4$				
Mean				$6.26x10^7$				
Std Dev				$1.77x10^8$				
Min				$2.20Ex10^3$				
Max				$5.00x10^8$				

[a] no growth occurred after 48 hours of incubation

A high proportion of bacteria isolated were *Enterococcus* species. The average number of anaerobic bacteria from the faecal samples was higher than the aerobic isolate numbers. The mean number of aerobic and anaerobic bacteria, and enterococccci isolated from the eight samples was $2.89x10^8$ (SD $5.41x10^8$), $5.32x10^8$ ($1.14x10^9$) and $6.26x10^7$ ($1.77x10^8$), respectively.

The mean comparative number of bacteria isolated from the eight faecal samples is shown in Figure 4.

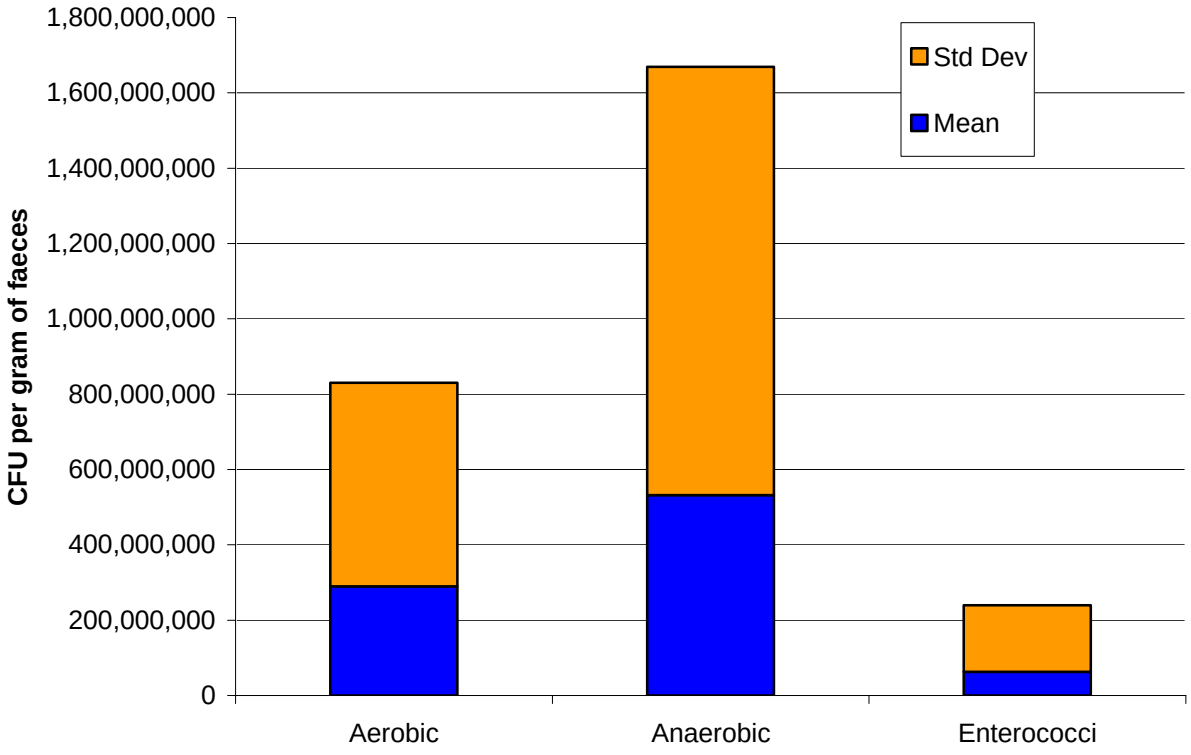


Figure 4: Bacterial numbers (CFU) isolated from cheetah faecal samples

Table 5 shows the effect of diet (IAMS adult cat or meat based) on the numbers of bacteria in the faeces of adult cheetahs.

Table 5: Bacterial counts (CFU) of faeces and diets of adult cheetahs on two diets

Diet	Aerobic	Anaerobic	Enterococci
Mean IAMS	$2.70x10^{6}$	$2.43x10^{7}$	$1.20x10^{5}$
Median IAMS	$2.70x10^{6}$	$2.43x10^{7}$	$1.20x10^{5}$
Std Dev	$2.91x10^{6}$	$3.16x10^{7}$	$2.83x10^{4}$
Mean Meat	$3.85x10^{8}$	$7.35x10^{8}$	$8.34x10^{7}$
Median Meat	$1.35x10^{8}$	$2.54x10^{6}$	$1.29x10^{4}$
Std Dev	$6.05x10^{8}$	$1.33x10^{9}$	$2.04x10^{8}$

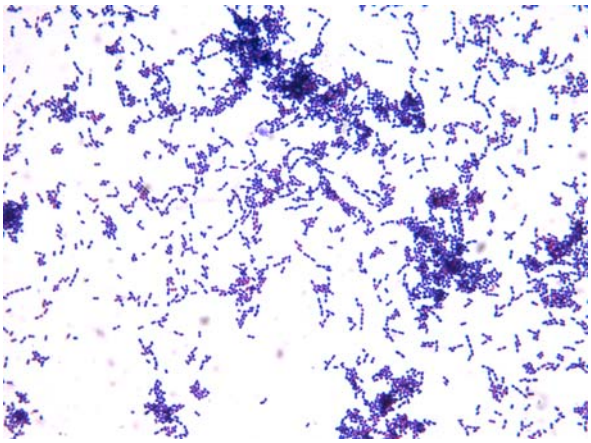
Table 6 shows the different bacteria and yeasts isolated from the faeces and duodenal samples of the cheetahs. Not all genera were identified to species level.

Table 6: Bacteria and yeasts isolated from cheetah faeces

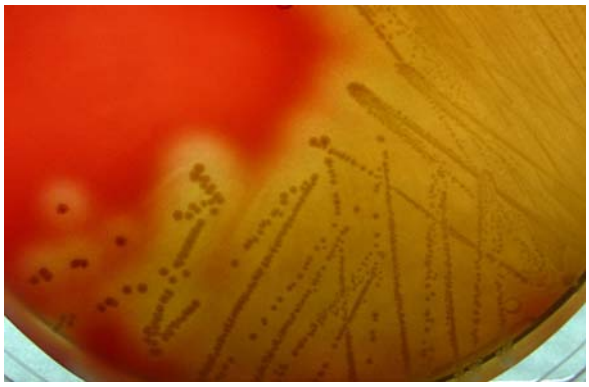
Bacteria isolated	
Genus	Species
Acinetobacter spp.	*A. wolfii, A. calcoaceticus*
Bacillus spp	
Clostridium spp.	*C. perfringens*
Corynebacterium spp.	
Edwardsiella spp.	*E. hoshinae*
Escherichia spp.	*E. coli*
Enterobacter spp.	*E. agglomerans*
Enterococcus spp.	*E. durans, E. agglomerans, E. faecium*
Lactobacillus spp.	Group 1, Group 2
Moraxella spp.	
Pasteurella spp.	
Proteus spp.	
Pseudmonas spp	
Staphylococcus spp.	*S. epidermalis*
Vibrio spp.	*V. alginolyticus, V. cholera*
Yeasts isolated	
Cryptococcus spp.	

3.2 BACTERIA FOR THE PROBIOTIC

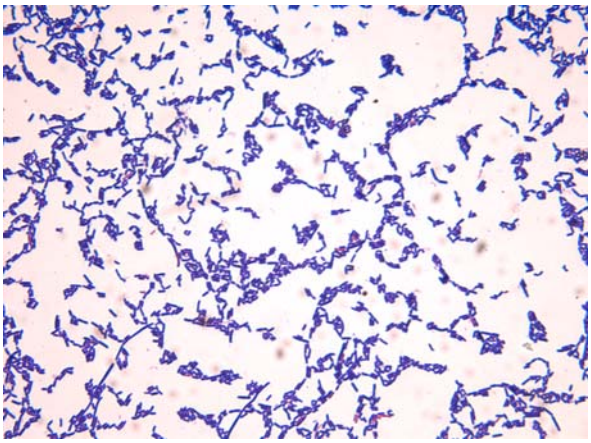
Enterococcus faecium and *Lactobacillus* group 1 were isolated from cheetah faeces to be included in the probiotic. The bifidobacterial isolates were only present in very low numbers. The isolates of *Bifidobacterium* ssp stored did not grow in the BHI or MRS broth and could therefore not be used in the trial.



Entercoccus faecium

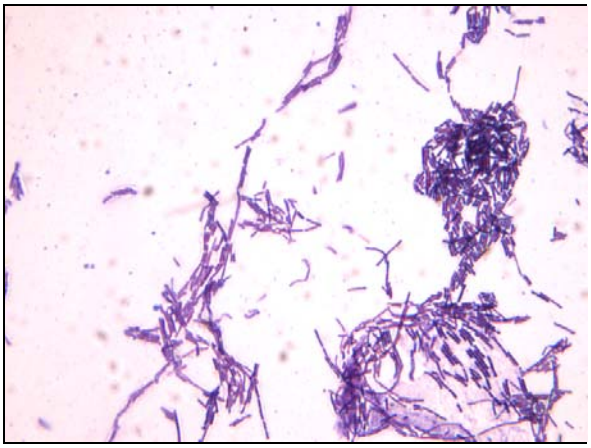


Enterococcus culture

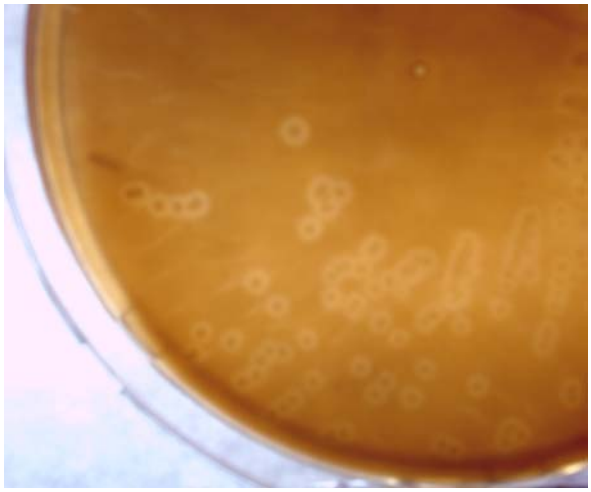


Lactobacillus species

Lactobacillus culture



Bifidobacterium species



Bifidobacterium culture

Figure 5: Smears and culture of bacteria used as probiotic

3.3 HEALTH OF JUVENILE CHEETAHS

All animals were healthy at the beginning of the trial on day –70. There was episodic diarrhoea present in all camps, rarely accompanied with a depressed or altered appetite. No animal showed signs of systemic disease before, during or after the treatment period (day 0 to day 28), except diarrhoea. F 457 was treated with 2 ml Synulox (140 mg amoxycillin and 35 mg clavulanic acid per ml; Pfitzer) and 2 ml Duplocillin (procaine penicillin 150 000 IU per ml; Intervet) on day –56 due to a bite wound on the shoulder. Synulox treatment was continued for 5 consecutive days.

An outbreak of severe diarrhoea in camp 55 on day –35 was treated with Biosol (neomycin sulphate 200 mg, methscopolamine bromide 2 mg; Pharmacia and Upjohn). Each animal received 1.5 ml orally. Antibiotic therapy was continued for three days with Enteritis Tablets (sulphathiazole 75 mg, phthalylsulphathiazole 175 mg, neomycin sulphate 15 mg, streptomycin sulphate 10 mg, aminopentamide sulphate 0.025 mg, kaolin 200 mg, pectin 2 mg; Bayer Animal Health). The dosage was three tablets per animal twice daily.

There was a significant difference between the ages of the Probiotic and Control groups (two sampled t-test, $P<0.0069$).

3.4 FAECAL SCORING

The faeces were analysed from day –70 to day 42. Days –70 to –56 showed significant difference ($P=0.0137$) in the Probiotic Group in comparison to days –42 to –7. This is thought to be associated with subjective differences in scoring faeces, as a different person scored the first three weeks (days –70 to –56). For statistical analysis the first three weeks were excluded from the statistics in both groups.

Table 7: Comparison of diarrhoeic scores of Probiotic and Control groups in the pre-treatment period

Probiotic Group				
Camps	5	54	55	57
Diarrhoea Score	35.71%	26.32%	42.31%	39.39%
Control Group				
Camps	6	53	56	
Diarrhoea Score	27.54%	26.67%	11.11%	

Table 7 compares the differences in percentage diarrhoea between different camps in the PG and CG. The percentage diarrhoea was lower in camp 56 in the pre-treatment period but it was not significant. There were no significant differences in the percentage diarrhoea between camps in the PG and CG in the pre-treatment period (days –42 to –7).

Table 8: Percentage diarrhoea in Probiotic and Control groups during probiotic trial.

	Percentage diarrhoea		
Period	Probiotic Group	Control Group	Total
Pre-treatment	46.85%	24.68%	37.77%
Treatment	30.77%	31.37%	31.03%
Post-treatment	75.00%	36.00%	53.33%

Table 8 compares the percentage diarrhoea between PG and CG during the trial. There was a significant difference (P=0.0021) in the percentage diarrhoea between the PG and CG in the pre-treatment period. There was no statistical significant difference between PG and CG during the treatment period, but a significant difference was noted in the post-treatment period (P=0.0092).

Comparing the diarrhoeic scores between the pre-treatment, treatment and post-treatment periods in the control group, there was no statistical significant difference in percentage diarrhoea during different periods. However, there was a statistical significant difference between the pre-treatment and treatment period in the PG (P=0.0363) and the treatment and post-treatment period (P=0.0004) (see Table 8 and Figure 6).

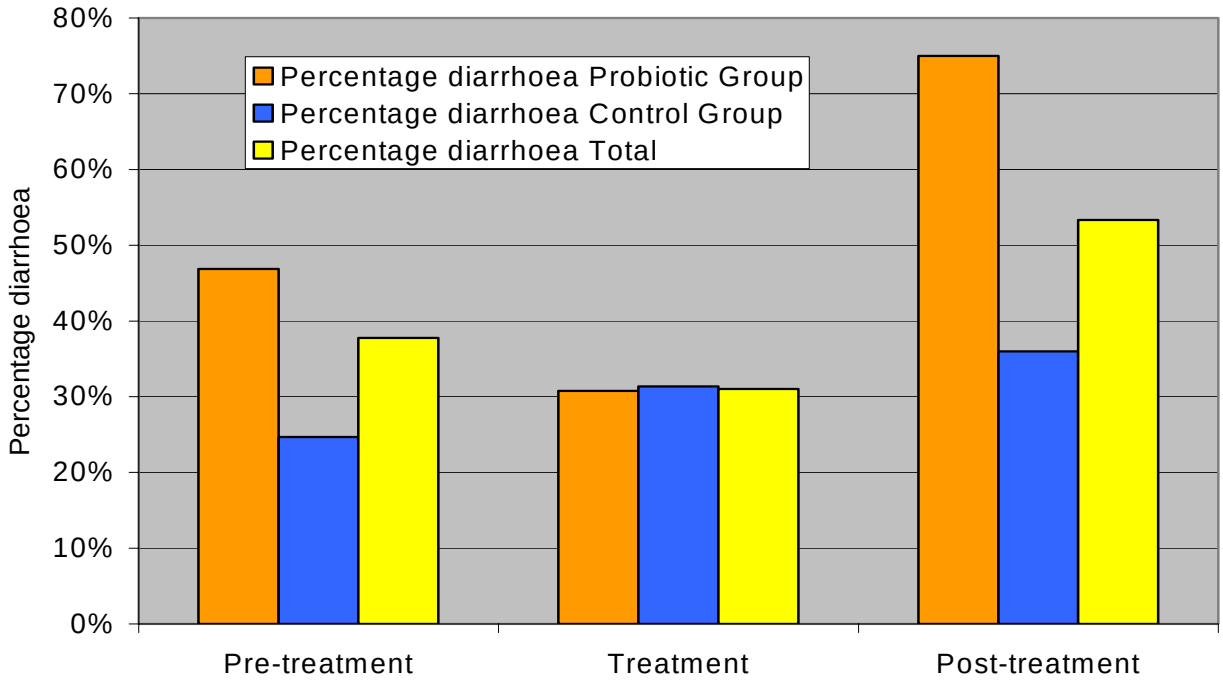


Figure 6: Comparison of percentage diarrhoea in Probiotic and Control groups during trial

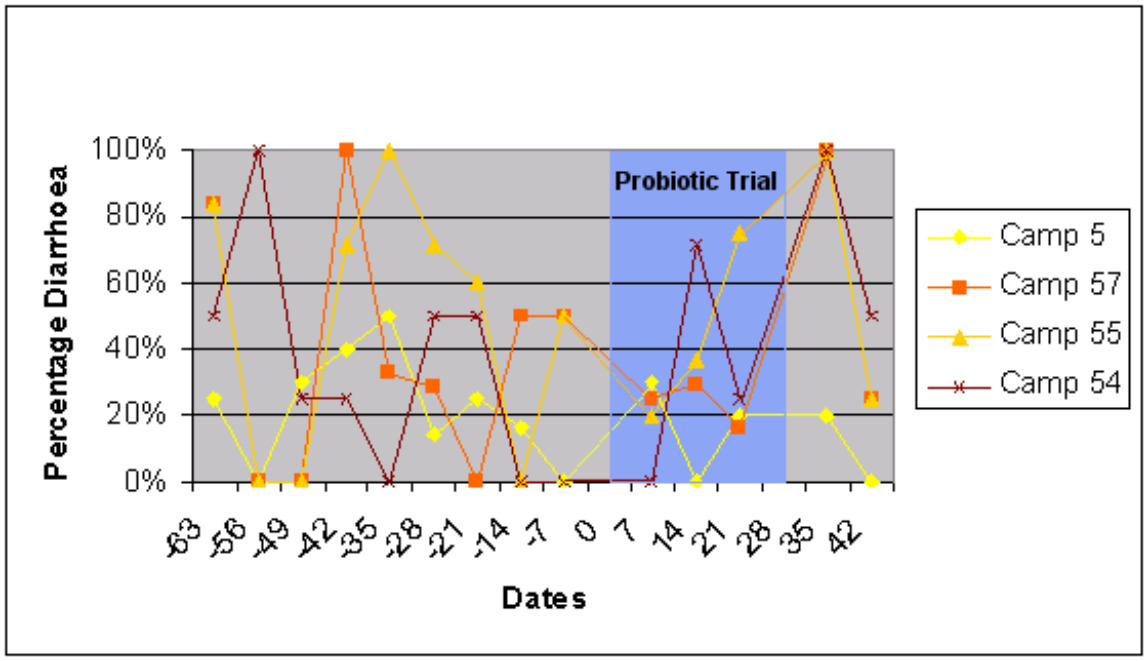


Figure 7: Percentage diarrhoea in the Probiotic Group during the trial

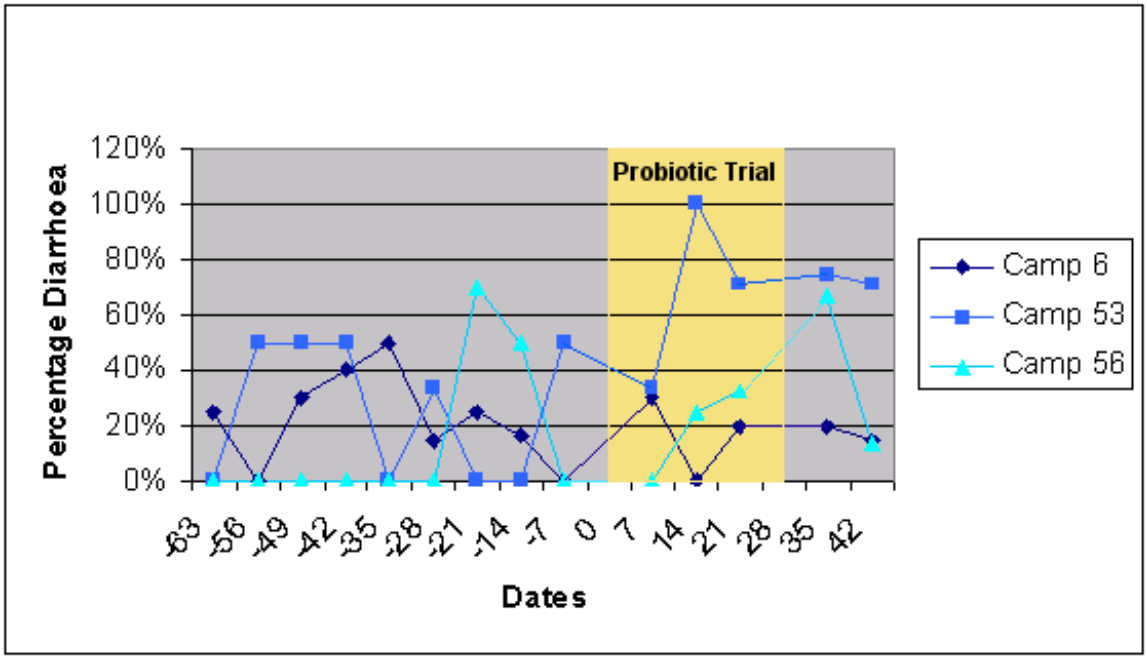


Figure 8: Percentage diarrhoea of the Control Group during the trial

Figure 7 and Figure 8 show the percentage diarrhoea of the PG and CG over time from day –63 to day 42, on weekly intervals. The probiotic was fed from the Day 0 until Day 28.

The prevalence of bloody or mucoid faecal samples in the PG decreased to nil during the treatment period (Figure 9and Table 24)

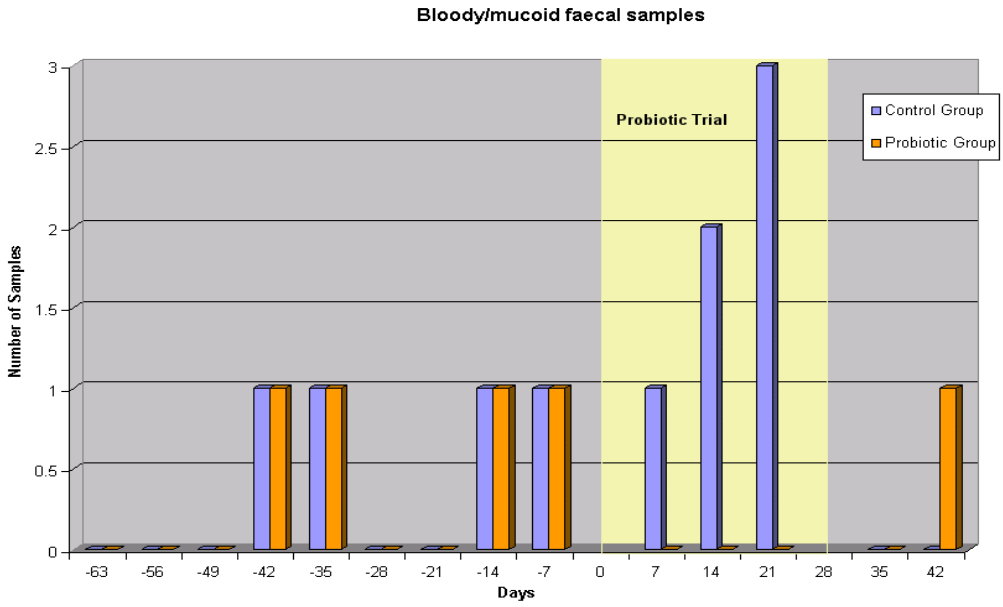


Figure 9: Prevalence of bloody/mucoid faecal samples in Probiotic Group and Control Group during trial

The lightly shaded area in Figure 9 shows the time during which the probiotic bacteria were fed to the Probiotic Group. The orange markers represent the PG and the CG is represented by the blue colour.

3.5 FAECAL WATER CONTENT

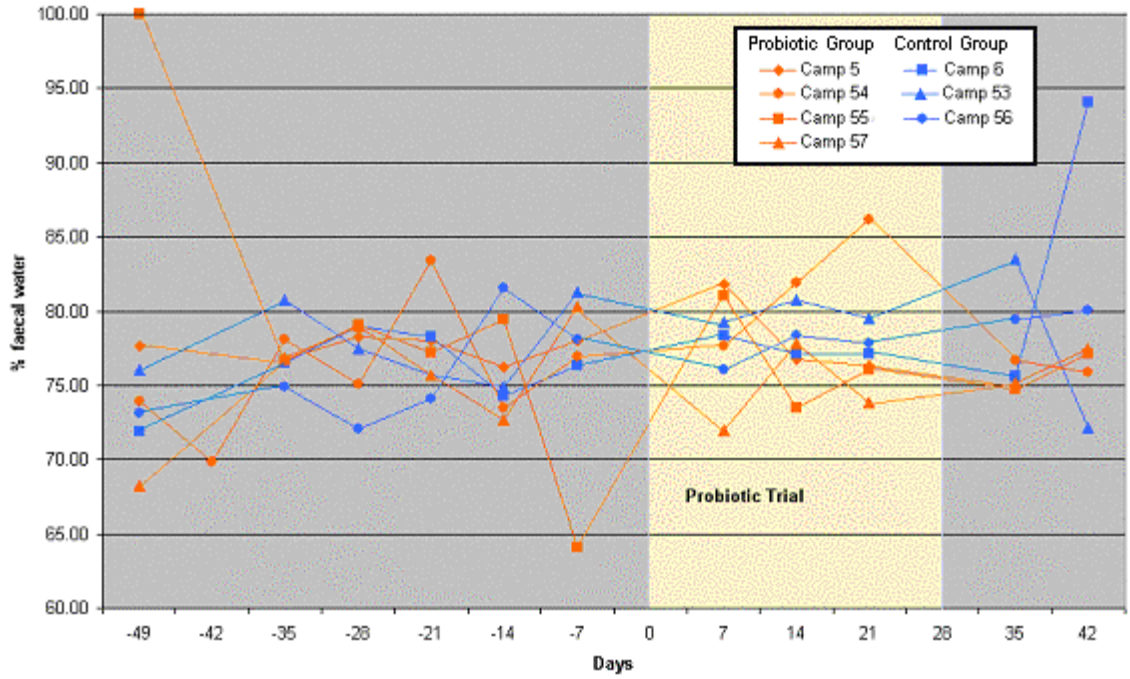


Figure 10: Percentage of faecal water in Probiotic Group and Control Group during trial

Figure 10 shows the maximal percentage of water in faeces in weekly samples collected in different camps during the trial.

There was no statistical difference in faecal water between different camps or different dates. The maximal faecal water was not affected by the administration of the probiotic bacteria.

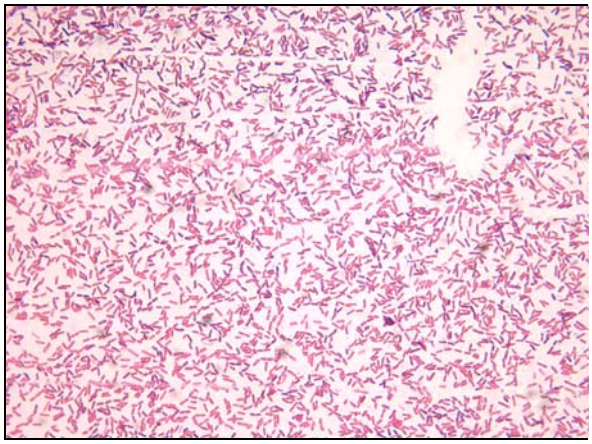
3.6 PATHOGENIC BACTERIA IN FAECES

Four diarrhoeic samples, collected in different camps, were cultured for pathogenic bacteria. No *Salmonella* spp. and no smooth *E. coli* were isolated (see Table 9).

Table 9: Bacteria isolated from diarrhoeic faeces

Day sampled	Camp	Bacteria isolated
Day -7	57	Rough *E. coli*
Day 14	53	Rough *E. coli*
Day 42	57	Rough *E. coli* and *Proteus* species
Day 42	55	Rough *E. coli*

In summary, no smooth *E.coli*, *Salmonella* or *Yersinia* were isolated from the diarrhoeic faeces during the entire trial.



Escherichia coli

Figure 11: *E. coli* smear- Gram's stain

Figure 11 shows Gram-negative slender bacilli typical for *E.coli.*

3.7 BODY MASS

Table 10 and Table 11 show the weight of each cheetah at Day 0 and Day 28, after the four-week treatment period. Only one weight was available for F460 as the cheetah was sold before day 28. F459 was also sold earlier, however, it was weighed on day 13. The percentage weight increase of each individual cheetah is also provided. Animals F460, F459 and F444 were excluded from the statically analysis. F444 was excluded as a result of an incorrect reading (see Table 11).

Table 10: Body mass changes of cheetah in Probiotic Group

Camp	Animal	D.o.B.	Age months	Weight 23.04	2nd Weight	Date	% increase	Comment
5	460	01.06.2002	11	20.7				Sold
	465	Unknown	11 [a]	17.7	19.7	21.05.04	10.15 %	
54	50	17.04.2002	13	25.5	27.7	21.05.05	7.94 %	
	433	17.04.2002	13	29.1	30.2	21.05.06	3.64 %	
	434	17.04.2002	13	27.8	31.3	21.05.07	11.18 %	
	440	28.04.2002	13	23.7	25.9	21.05.08	8.49 %	
	441	28.04.2002	13	22.3	24.4	21.05.09	8.61 %	
55	438	28.04.2002	13	19.8	21.1	21.05.10	6.16 %	
	450	30.05.2002	12	20	22.1	21.05.11	9.50 %	
	455	05.06.2002	11	23.2	24.9	21.05.12	6.83 %	
	437	21.04.2002	13	17.2	17.8	21.05.13	3.37 %	
	446	18.05.2002	12	16.7	18.6	21.05.14	10.22 %	
	459	02.06.2002	11	19.1	20.8	06.05.03	8.17 %	
	461	01.06.2002	11	17	17.9	21.05.03	5.03 %	
57	458	01.08.2002	9	16.8	17.8	21.05.04	5.62 %	
	457	01.08.2002	9	13.9	15.54	21.05.05	10.55 %	

[a] approximated age of cheetah

Table 11: Body mass changes of cheetah in Control Group

Camp	Animal	D.o.B.	Age month	Weight 23.04	2nd Weight	Date	% increase	
6	430	16.04.2002	13	22.1	23.2	21.05.03	4.74 %	
	431	16.04.2002	13	18.6	19.8	21.05.04	6.06 %	
	432	16.04.2002	13	21.5	23.1	21.05.05	6.93 %	
	435	20.04.2002	13	27.4	28.2	21.05.06	2.84 %	
	436	20.04.2002	13	25.7	28.3	21.05.07	9.19 %	
	447	20.04.2002	13	21	22.7	21.05.08	7.49 %	
53	426	22.03.2002	14	30.6	32.6	21.05.09	6.13 %	
	427	22.03.2002	14	33.2	36.3	21.05.10	8.54 %	
	428	22.03.2002	14	30.6	32.2	21.05.11	4.97 %	
56	443	12.05.2002	12	26.5	27.1	21.05.12	2.21 %	
	444	12.05.2002	12	26	22.9	21.05.13	-13.54 %	Incorrect reading

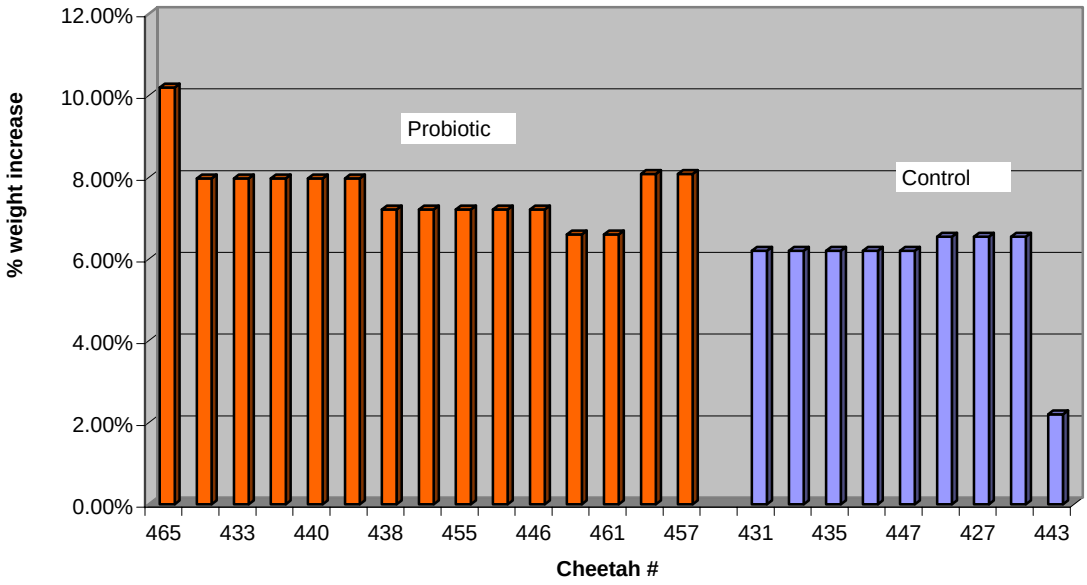


Figure 12: Comparison of percentage body mass increase in Probiotic Group and Control Group

There was no difference in weight increase between the PG and CG if actual weight change was considered, however when considered as a percentage, the PG gained considerably more weight than the CG ($p = 0.026$, ANOVA, $p < 0.05$).

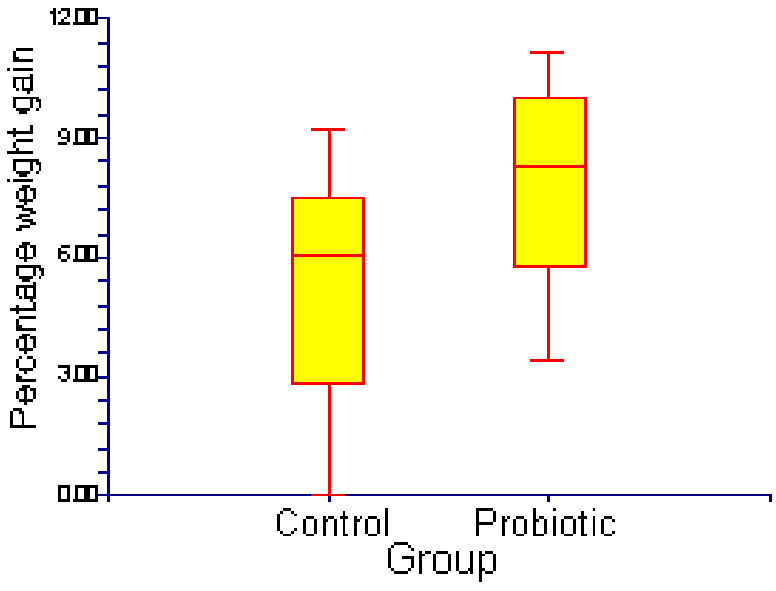


Figure 13: Box plot comparison of percentage weight gain of Probiotic Group and Control Group

The mean increase in weight of the PG was 7.754 % (SE=0.645) and of the CG 5.372 % (SE=0.778) (Tukey-Kramer test, $p < 0.05$). The mean and standard deviation are presented in Figure 13.

3.8 SERUM BIOCHEMISTRY AND HAEMATOLOGY

Except for the eosinophil count of cheetah F446, the leukocyte values of the cheetahs were all within the reference range, published by the International Species Information System (1999).

The mean haemoglobin (Hb) at the beginning of the treatment period was higher in the PG than the CG and lower at the end, however, the difference was not statistically significant. The red cell count (RCC) showed a significant decrease in the CG over time (P=0.023), but none of the animals fell outside the published reference range for RCC. There was no significant difference in mean haematocrit (Ht) in both groups over time. There was a significant difference in the mean cell volume (MCV) over time in both groups (P=0.002). The initial MCV in the CG was higher. There was a significant increase in mean cell haemoglobin concentration (MCHC) in both groups over time (P=0.000002). The MCHC was higher in the PG at the start. There was a significant decrease in white blood cell count (WBCC) in both groups over time (P=0.0014). There was a decreasing trend in segmented neutrophils but this was not significant. The mean number of banded neutrophils did not change significantly over time but the PG had lower counts at all times. There was a lot of individual variation in neutrophil count over time. The mean number of lymphocytes in both groups decreased insignificantly over time. The number of monocytes at the end of the treatment period decreased non-significantly in the PG. There was little variation in eosinophil count between groups and over time. F446 had the highest eosinophil count on Day 0 (4.23 $x10^3/\mu l$) and on Day 28 it had increased to 7.92 x $10^3/\mu l$. The highest physiological reference recorded is 5.84 $x10^3/\mu l$ (International Species Information System, 1999).

Table 12: Biochemistry and haematology values of Probiotic and Control groups at the start (day 0) and end of treatment (day 28).

		Control Group day 0		Control Group day 28		Probiotic Group day 0		Probiotic Group day 28		Reference Range	
Variables	Units	Mean	St dev.	Mean	St dev.	Mean	St Dev.	Mean	St Dev.	Min	Max
TSP	g/l	62.35	4.87	62.05	2.26	63.24	2.18	62.52	2.69	51.00	88.00
Alb	g/l	33.85	2.58	34.37	1.51	35.18	1.70	35.03	1.37	23.00	51.00
Globulin	g/l	28.51	2.69	27.65	1.37	28.04	1.61	27.49	1.98	17.00	55.00
A/G		1.19	0.08	1.25	0.07	1.26	0.10	1.28	0.08	0.60	1.20
Bil-T	ymol/l	2.45	0.46	2.68	0.49	3.08	0.76	2.67	0.50	0.00	29.00
Cholesterol	mmol/l	3.24	0.72	3.68	0.50	3.05	0.46	3.49	0.38	2.07	13.68
Urea	mmol/l	12.93	1.30	10.55	0.68	12.18	2.05	11.65	1.85	5.36	29.63
Creatine	ymol/l	207.73	15.75	210.82	27.91	195.50	31.89	198.93	24.81	53.04	716.04
Haemoglobin	g/l	138.27	6.33	134.18	13.39	133.50	5.18	137.21	5.48	6.90	20.20
RCC	1000/yl	8.18	0.40	7.77	0.69	7.89	0.42	7.93	0.38	4.18	11.10
Haematocrit	l/l	0.47	0.02	0.43	0.04	25.92	0.02	0.45	0.03	0.20	0.58
MCV	fL	57.62	1.87	56.13	1.96	56.86	2.25	55.95	1.53	34.20	86.10
MCHC	g/dl	29.36	0.36	30.87	0.92	29.80	0.98	30.99	0.40	20.50	48.80
WBCC	1000/yl	11.55	2.41	9.81	1.09	11.76	2.88	10.50	2.56	3.70	25.20
Seg. neutrophils	1000/yl	6.88	1.57	6.16	0.96	6.81	2.09	6.02	1.23	1.34	20.90
Neutrophils (bands)	1000/yl	0.05	0.11	0.05	0.08	0.01	0.05	0.01	0.03	0.00	6.30
Lymphocytes	1000/yl	3.35	1.58	2.51	0.49	3.64	1.36	3.03	0.78	0.14	8.26
Monocytes	1000/yl	0.61	0.38	0.54	0.33	0.60	0.25	0.45	0.21	0.00	2.59
Eosinophils	1000/yl	0.66	0.42	0.50	0.28	0.64	0.98	0.96	2.02	0.00	5.80
Basophils	1000/yl	0.02	0.05	0.04	0.07	0.03	0.06	0.03	0.04	0.00	0.26
ThrC	1000000/yl	364.09	185.04	379.36	146.32	358.78	187.70	403.57	181.39	96.00	842.00

There was no significant difference in the mean number of basophils. There was no significance difference in the level of thrombocytes (ThrC), total serum protein (TSP), albumin or globulin. There was, however, a significant increase in albumin/globulin ratios (A/G) in the CG from Day 0 to Day 28 (P = 0.044). Although, total bilirubin (BilT) decreased in the PG, the difference was not significant. Cholesterol increased significantly in both groups over time (Epsilon probability level = 0.000014). Urea decreased significantly in the CG in relation to the PG and time (P = 0.0054), but there was no significant change in creatinine over time or between groups. The mean values of the PG and CG at the start (day0) and end (day 28) of the feeding of the probiotic are represented inTable 12.

3.9 PATHOGEN IDENTIFICATION

PCR tests for presence of feline coronavirus in diarrhoeic faecal samples were negative, as no nucleic acid could be detected. Blood smears from M427, M440 and F446 were positive for *Babesia* species on repeated blood sampling (seeTable 25, Table 26, Table 27 and Table 28). Blood samples were further analysed by PCR and reverse line blot to identify the species of *Babesia*, as described by Penzhorn *et al.* (2001). The parasite did not match any known isolates for *Theileria/Babesia* species (Anna-Marie Bosman, University of Pretoria, personal communication, 2003).

Table 13: Faecal flotation of faeces (no of eggs/gram of faeces)

Group	Camp	Dates	
		14.05.03 Day 21	28.05.03 Day 35
Probiotic	5	63	0
	54	226	0
	55	3	0
	57	35	0
Control	6	0	0
	53	110	0
	56	33	0

Results of the faecal flotations from faeces collected on day 21 and day 35 are presented in Table 13. Eggs showing the characteristics of *Toxocara leonina* and other *Toxocara* species were identified in the faeces. Adult helminths belonging to *Toxocara* spp. were identified in the faeces.

3.10 INTESTINAL PERMEABILITY

The intestinal permeability of the Probiotic and Control groups was measured at the start (day 0) and the end (day 28) of the treatment period. The timing between the administration of the sugars and the collection of blood is shown in Table 29 and Table 30. The median for day 0 for all animals was 82 minutes (range 40 to 170 minutes) and for day 28 was 70 minutes

(range 44 to 112 minutes). The delayed blood collection after administration of the sugars lactulose (L) and rhamnose (R) occurred mainly in the CG. Comparing L and R-values and L/R ratios, there was no significant difference between sugar concentration in the blood before and after one hour and one and-a-half hour.

The concentration of L and R was measured in the serum and the ratios of the two sugars were calculated for each animal. Table 14 and Table 16 show the sugar concentrations for the CG and PG at the start of the trial. Table 15 and Table 17 show the sugar concentrations of the PG and CG at the end of the trial.

Table 14: Sugar concentration of Control Group at the start of treatment period

Rhamnose (R) conc.		Lactulose (L) conc.		L/R ratio	
Min	0.00	Min	0.00	Min	0.00
Max	6.20	Max	1.10	Max	0.79
Mean	2.84	Mean	0.56	Mean	0.20
Median	2.20	Median	0.64	Median	0.19
SD	2.07	SD	0.47	SD	0.21

Table 15: Sugar concentration of Control Group at the end of treatment period

Rhamnose (R) conc.		Lactulose (L) conc.		L/R ratio	
Min	1.40	Min	0.02	Min	0.00
Max	5.20	Max	2.50	Max	0.48
Mean	3.22	Mean	0.40	Mean	0.09
Median	3.20	Median	0.20	Median	0.05
SD	1.33	SD	0.71	SD	0.14

Table 16: Sugar concentration of Probiotic Group at the start of treatment period

Rhamnose (R) conc.		Lactulose (L) conc.		L/R ratio	
Min	0.30	Min	0.00	Min	0.00
Max	5.40	Max	2.50	Max	0.92
Mean	1.89	Mean	0.55	Mean	0.27
Median	1.60	Median	0.40	Median	0.14
SD	1.30	SD	0.67	SD	0.29

Table 17: Sugar concentration Probiotic group at the end of treatment period

Rhamnose (R) conc.		Lactulose (L) conc.		L/R ratio	
Min	0.200	Min	0.010	Min	0.003
Max	7.200	Max	2.100	Max	0.913
Mean	1.993	Mean	0.382	Mean	0.264
Median	1.450	Median	0.130	Median	0.100
SD	1.927	SD	0.565	SD	0.308

Table 19 shows the changes in the L/R ratios of the individual animals in the Control and Probiotic groups, respectively. Figure 14 shows a comparison of the mean lactulose/rhamnose ratios of the CG and PG at the beginning and end of the probiotic trial.

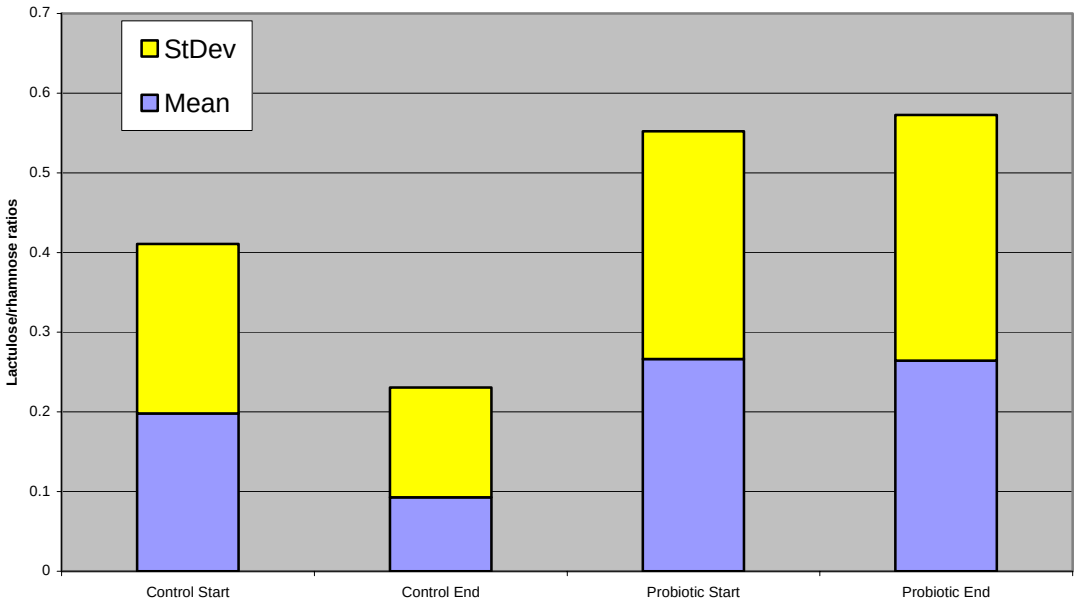


Figure 14: Comparison of lactulose/rhamnose ratios in PG and CG at the start and end of treatment period

Table 18: Differences in rhamnose (R) and lactulose (L) ratios in Control Group

Cheetah ID	Start L/R	End L/R	Difference
430	0.214	0.045	-0.169
431	0.045	0.010	-0.036
432	0.006	0.014	0.008
435	0.140	0.061	-0.080
436	0.190	0.128	-0.062
447	0.224	0.008	-0.217
426	0.188	0.154	-0.034
427	0.000	0.000	0.000
428	0.150	0.073	-0.077
443	0.786	0.481	-0.305
444	0.233	0.045	-0.187

Table 19: Differences in rhamnose (R) and lactulose (L) ratios in Probiotic Group

Cheetah ID	Start L/R	End L/R	Difference
465	0.500	0.733	0.233
438	0.000	0.100	0.100
450	0.076	0.132	0.056
455	0.917	0.338	-0.578
437	0.353	0.056	-0.297
446	0.100	0.003	-0.097
461	0.067	0.017	-0.050
50	0.045	0.008	-0.037
433	0.029	0.050	0.021
434	0.040	0.088	0.048
440	0.714	0.600	-0.114
441	0.338	0.913	0.575
458	0.072	0.10	0.03
457	0.653	0.56	-0.09
459	0.173	[a]	
460	0.181	[a]	

[a] no second reading available since cheetah was sold

There was a significant difference in rhamnose concentration between the CG and PG on Day 0 of the treatment period (P=0.034, ANOVA, significance α=0.05). The mean rhamnose

concentration of CG on Day 0 was 2.836 (SE 0.466) and the PG was 1.894 (SE 0.386). The mean concentration of rhamnose at the end in CG was 3.218 (SE: 0.466) and in PG was 1.993 (SE 0.413). There was no significant difference between lactulose concentrations between the two groups on Day 0. Both groups had a non-significant decrease in the mean lactulose concentration on Day 28. There was a significant difference in the lactulose to rhamnose ratio (L/R) between the Probiotic and the Control groups at the end of the treatment period, with the Control Group showing a decrease in the L/R ratio ($P=0.044$, ANOVA, significance $\alpha=0.05$).