# The use of a probiotic in captive cheetahs (*Acinonyx jubatus*)

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## ABSTRACT

Juvenile captive cheetahs (*Acinonyx jubatus*) often present with diarrhoea that is commonly associated with bacterial infections. A species-specific probiotic containing *Lactobacillus* Group 2 and *Enterococcus faecium* was prepared from healthy adult cheetahs. Juvenile cheetahs ($n = 27$) between 8 and 13 months of age were included in the probiotic trial. The animals were observed prior to and after feeding of the probiotic which was made available for 28 days. Feeding of the probiotic resulted in a significantly increased body weight in the treatment group ($P = 0.026$), while there was no increase in the control group. A relative improvement in the faecal quality in the probiotic group during the treatment period compared with the pre-treatment ($P = 0.0363$) and post-treatment ($P = 0.004$) period was observed. This was accompanied by an absence of blood and mucus in the faeces during the treatment period in the probiotic group.

**Key words**: *Acinonyx jubatus*, cheetah, diarrhoea, *Enterococcus, Lactobacillus,* probiotic.

## INTRODUCTION

Probiotics have been used for centuries to treat specific intestinal problems in humans and animals.[5,6,11] They have been used to reduce mortality, suppress neonatal diarrhoea and improve the growth of young and stressed animals.[20,25] Probiotics are defined as live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance[5]. Probiotics usually consist of lactic acid-producing bacteria such as lactobacilli, enterococci, bifidobacteria and yeasts. Commercially available probiotics are usually not species-specific. Clinical trials have shown that probiotics work best in the species from which they have been isolated, as the receptors required for attachment to the epithelial layer of the host are host species-specific.[5] Lactic acid-producing bacteria lower the pH in the intestinal content and reduce the number of pathogenic *Escherichia coli* adhering to the microvilli of the lymphoepithelial cells, clinically prevent-ing severe diarrhoea and death.[25] Lactobacilli inhibit the growth of potential pathogens such as *E. coli, Salmonella, Shigella, Pseudomonas, Bacillus* and *Vibrio* species. They have also been shown to improve the intestinal immunity by stimulating immunoglobin A production and the local release of interferon $\alpha$ and $\beta$.[24] Bifidobacteria have also been shown to antagonise *Salmonella* by means of the production of acids as well as modification of the redox potential and production of bacteriocin-like inhibitory substances.[3]

Since free-ranging cheetahs do not usually return to a carcass and have never been observed to scavenge,[21] their intestinal tract is not adapted to deal with spoiled and contaminated food.[14] It has been found that cheetahs are highly susceptible to intestinal infections. Neonatal mortalities of up to 87 % and poor survival rates of cheetah cubs have been encountered in breeding facilities and zoos.[12,13,28] Enteritis was observed in 50 % of captive-reared cheetahs that died in South Africa between 1975 and 1995.[14] Three of 10 deaths in cheetahs younger than 7 months of age were associated with enteritis and infection by pathogenic bacteria such as *E. coli* and *Salmonella* species.[18] *Salmonella typhimurium* and *Salmonella muenchen* were the most frequently isolated *Salmonella* spp. from faeces of cheetahs in a reserve in South Africa.[27] The raw meat fed to the cheetahs was identified as the source of the *Salmonella* species.[27] *Salmonella* organisms were identified as a secondary factor in increasing mortality in cubs, particularly if they are not fed a nutritionally balanced diet.[14,27]

## MATERIALS AND METHODS

### Probiotic development

All animals enrolled into the study were housed at the DeWildt Cheetah and Wildlife Centre, South Africa. Healthy, adult cheetahs were used to obtain the bacteria used in the probiotic. Half were fed a 3 kg mixed horsemeat and chicken carcass diet (ratio 70:30) and the other half IAMS adult cat pellets (IAMS Company, Dayton, OH, USA). Faeces were collected from 8 cheetahs and placed in sterile containers. Duodenal aspirates were collected under anaesthesia from 2 cheetahs, a male and female. Food was withheld from them for 24 hours prior to the procedure and they were anaesthetised with a combination of intramuscular medetomidine (Domitor, Novartis Animal Health, Isando, South Africa, 2 mg) and ketamine (Anaket-V, Centaur, Bayer (Pty) Ltd, Animal Health Division, Isando, South Africa, 100 mg)[17] in a squeeze-cage using a pole syringe. Endoscopies were performed in left lateral recumbency. A 7.9 mm diameter, 1.3 m length flexible fibreoptic scope (Olympus Optical, Tokyo, Japan) was introduced as far as possible into the proximal duodenum and a duodenal wash was obtained by lavage with 10 m$\ell$ of sterile saline solution and aspirating as much of this fluid as possible. Both specimen types were then placed in an anaerobic jar with a commercial anaerobic gas generator (GasPak, Oxoid, Basingstoke, UK) and reached the laboratory within 6 hours.

Kanamycin aesculin medium (Oxoid, Basingstoke, UK) was used for the selection of enterococci[26], SL medium[22] for the selection of lactobacilli and Beeherens medium for the selection of bifidobacteria. Aesculin-utilising, gram-positive cocci were identified to species level by Lancefield cell-wall antigen-grouping (Streptococcal Grouping Kit, Oxoid, Basingstoke, UK),

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their ability to ferment lactose, arabinose, sorbitol, mannitol and growth in 6.5 % salt broth.[19] Regular, gram-positive rods that grew on SL medium and were catalase-negative were identified by their ability to utilise bile-aesculin and to ferment lactose, galactose, maltose, mannitol, melibiose, salicin, sorbitol, sucrose, trehalose, and xylose.[10,15] Very few bifidobacteria were cultured. *Enterococcus faecium* and *Lactobacillus* group I were common in all the specimens and met the necessary criteria to be used in a probiotic. Both bacteria were stored in broth in 1.5 m$\ell$ cryotubes at –86 °C until required. *Enterococcus faecium* was stored in brain heart infusion broth (BHI, Merck, Germany) and *Lactobacillus* group I in MRS broth (Oxoid, Basingstoke, UK). Just before the trial the bacteria were thawed and 0.2 m$\ell$ of *E. faecium* and *Lactobacillus* group I was placed in BHI and MRS broth, respectively, and incubated at 37 °C for 48 hours. The optical density of each solution was determined with a spectrophotometer at 560 nm wavelength and adjusted to 1.0. This density had been determined by the use of standard plate counts for both bacteria to be between $10^9$ to $10^{10}$ colony forming units per millilitre (cfu/m$\ell$). The bacterial suspensions were then mixed in equal volumes and stored in a refrigerator.

### Probiotic trial in cheetah cubs

Juvenile cheetahs ($n = 27$) between the 8 and 13 months of age (median: 12 months) were included into the probiotic trial. The animals were randomly divided into 2 groups depending on their camps, since different feeding schedules of animals in 1 camp was not possible due to logistical reasons. Sixteen animals were placed in the probiotic group (PG) and 11 in the control group (CG). The trial was split into 3 monitoring periods, pretreatment (days 70 to 1), treatment (days 0 to 28) and post-treatment (days 29 to 42). One m$\ell$ ($10^9$–$10^{10}$ cfu) of the prepared bacterial suspension was mixed daily in the food of each cheetah in the PG. Each member of the CG received the same volume of BHI/MRS broth without the probiotic mixed in the food. The cheetahs were then observed to be assured that all the food was eaten.

### Faecal scoring and percentage water

Faeces were evaluated in all camps once a week by the principal investigator. Only faeces that were watery with or without solids were considered as diarrhoea and were used to calculate the percentage of diarrhoeic faeces in each camp. The chi-square test for non-continuous variables was used to analyse this set of data. The
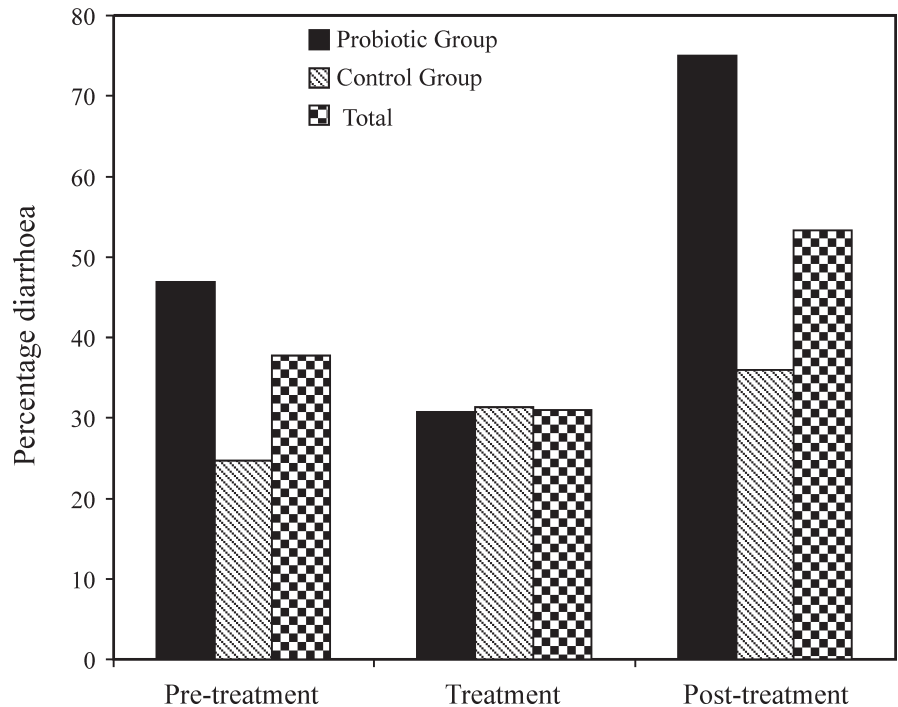
faeces were also evaluated for the presence of blood or mucus. The percentage water in the faeces was recorded by collecting 2 fresh faecal specimens randomly from each camp once a week. The faeces were weighed before and after drying in an oven at 100 °C for at least 5 days, until there was no further loss in weight, and the percentage water in the sample was calculated and recorded.

### Weight gain of cheetahs

All animals were weighed at the beginning (day 0) and end (day 28) of the probiotic treatment period. The animals were caught in the crush and transferred to a transportation crate and weighed on a flatbed scale accurate to 0.1 kg. The weight of the crate was subtracted to obtain the weight of the individual cheetahs. The increase in weight over the 4 week period was expressed as a percentage weight gain to account for the variation in weights and ages between the animals at the start of the trial. This data set was analysed using the ANOVA test.[8]

### Intestinal permeability

Intestinal permeability was tested at the beginning (day 0) and the end (day 28) of the 4-week treatment period using isomolar solutions of lactulose (L) and rhamnose (R). Food and water was withheld from the cheetahs for 24 hours and 12 hours, respectively, prior to the 2 sugars being given. An isomolar solution of the 2 sugars R and L was given orally. The cheetahs were placed into the crate to insure individual intake. The solution

was offered to the cheetahs in the crate with a bit of raw minced meat to facilitate voluntary intake. Each animal received 20 m$\ell$ of a solution containing 102.67 g L/$\ell$ and 61.55 g of R/$\ell$. This resulted in a solution with an osmolality of 300 mmol/$\ell$. The serum concentration of R and L was measured before and 60–90 min after ingestion of these sugars. The exact time of the blood sample collection was recorded.

To collect the blood sample, cheetahs were gently restrained in the crate and blood taken from the femoral vein. Serum was separated by centrifuging and frozen at –86 °C until sent frozen in dry ice to the Gastrointestinal Laboratory, Texas A & M University, College Station, USA, where they were analysed by HPLC method[23] for L and R. Analysis of variance was used to compare L and R values and L/R ratios between CG and PG. Repeated measures ANOVA were used to compare the effects of the timing of the blood collection around 60 min to those taken at around 90 m after the administration of the sugars.

### RESULTS

The percentage of diarrhoeic faeces was significantly higher in the PG (46.95 %) than in the CG (24.78 %, $P = 0.0021$) during the pre-treatment period (Fig. 1 ). No significant difference of diarrhoeic faeces was observed between the PG (30.77 %) and CG (31.37 %; $P = 0.0092$) during the treatment period, but there was a significant difference in percentage diarrhoeic faeces ($P = 0.0092$) between the PG (75.00 %) and the CG (36.00 %) in
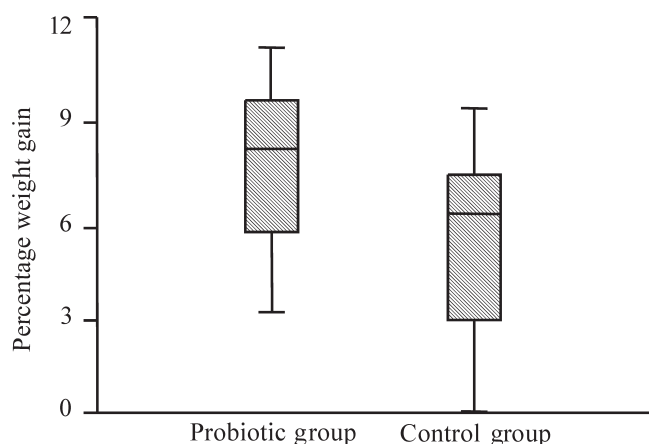


Fig. 1: **Comparison of percentage diarrhoea of faeces in probiotic and control groups during the trial.**

**Fig. 2: Box plot comparison of percentage weight gain in the probiotic group and the control group over the duration of the study.**

the post-treatment period. Comparing the diarrhoeic scores between the pre-treatment, treatment and post-treatment periods in the control group, there was no difference between periods. In the PG, however, significant differences between the pre-treatment and treatment ($P = 0.0363$) and the treatment and post-treatment periods ($P = 0.0004$) were identified. Marked subjective improvement in faecal quality in the PG during the feeding of the probiotic and a decrease in the number of faecal samples containing mucus and blood was noted.

No statistical difference in the mean faecal water between different camps, different sample collection dates, or between groups or periods was noted. No significant difference in weight gain between the PG ($1.73 \pm 0.71$ kg) and the CG ($1.63 \pm 0.78$ kg) was found when actual weight was measured. However, when weight changes were considered as a percentage of body weight, the PG gained considerably more weight than the CG ($P = 0.026$, ANOVA, $P < 0.05$) (Fig. 2). This was due to the large variations of individual weights of cheetahs at the start of the trial. The mean percentage weight increase of the PG and the CG over the entire period of the study were

7.75% (SE = 0.645) and 5.37 % (SE = 0.778), respectively ($P < 0.05$).

The mean time point for blood collection to evaluate the absorption of the 2 sugars from the intestinal tract was 86.78 minutes (SD = 31.04) on day 0 and 80.35 minutes (SD = 16.37) on day 28. No significant difference between the sugar concentrations in the blood of cheetahs sampled around 60 min to those sampled around 90 min after administration of the sugars was documented.

No significant differences were recorded in serum R concentrations between the PG and CG on day 0 ($P = 0.073$) and day 28 ($P = 0.065$) and the serum L concentrations on day 0 ($P = 0.865$) and day 28 ($P = 0.052$). No significant differences in the R:L ratios between the groups on day 0 ($P = 0.59$) and day 28 ($P = 0.134$) were calculated. The mean values for the PG and the CG are shown in Table 1. A significant increase ($P = 0.336$) in the R concentrations in the CG at day 28 was observed, if values were compared between groups and time. Both groups had an insignificant decrease ($P = 0.947$) in the mean serum L concentration on day 28. A significant difference in the mean L/R was observed between the probiotic and control group at the end of

the treatment period, with the CG showing a lower L/R ratio ($P = 0.044$).

## DISCUSSION

Feeding of *Lactobacillus* group 1 and *Enterococcus faecium* resulted in a significant reduction in the frequency of diarrhoea in the PG during the treatment period in comparison to the pre- and post-treatment period. A higher percentage of diarrhoea (an increase of 44.23% in the PG versus 4.63 % in the CG) was observed post-treatment. Several factors contributed to variation between the 2 groups. There was a concurrent outbreak of *Toxocara* spp. in 2 camps of the PG and several animals of the PG were moved in and out of their familiar groups for management reasons in the post-treatment period. The stress associated with this is the most likely explanation for the increase of diarrhea, while a lasting effect of the probiotic may have contributed to a reduced severity of the diarrhea. No blood or mucus were noted in the PG post-treatment in comparison to the CG. A relative improvement in faecal quality between the PG and CG was observed if the higher prevalence of diarrhoea in the PG during the pre- and post-treatment period is considered. Underdahl *et al.*[25] have shown a reduction in the severity of diarrhoea in association with feeding a probiotic.

A significant increase in individual weight in the PG in comparison to the CG over the 28-day treatment period was noted but the 2 groups differed in age. The growth rate of cheetah cubs is linear up to 40 days of age and individual birth weight influences the individual growth rate rather than sex.[1] Therefore it is unlikely that the difference in age between groups had any significant effect on weight gain. Mice receiving a probiotic over a 17-day period had an increase in weight of 31.7 %.[2] There was not such a marked difference between the PG and CG (1.79 %) over the 28-day period, but considering the slower growth rate and larger body mass of the cheetah, such a difference should be considered as significant.

Intestinal permeability varies greatly between species and breeds.[7] The level of exercise also has an effect on intestinal permeability, with a lower permeability being found in racing greyhounds.[16] The urinary recovery of rhamnose and lactulose also varies between species.[4] Higher ratios of lactulose to rhamnose have been recorded in clinically healthy domestic cats than in domestic dogs.[16] An increase in urinary or serum lactulose recovery has been associated with an increase in intestinal permeability, an increase in the

Table 1: **Mean concentration of sugars in probiotic and control groups on days 0 and 28.**

| Sugars | Time | | Probiotic group | Control group |
|---|---|---|---|---|
| Rhamnose (R) | Day 0 | Mean | 1.84 | 3.12 |
| | | SD | 1.64 | 1.64 |
| | Day 28 | Mean | 1.99 | 3.36 |
| | | SD | 1.70 | 1.70 |
| Lactulose (L) | Day 0 | Mean | 0.58 | 0.62 |
| | | SD | 0.62 | 0.62 |
| | Day 28 | Mean | 0.38 | 0.42 |
| | | SD | 0.64 | 0.64 |
| R:L ratio | Day 0 | Mean | 0.28 | 0.22 |
| | | SD | 0.27 | 0.27 |
| | Day 28 | Mean | 0.26 | 0.10 |
| | | SD | 0.25 | 0.25 |

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 functional surface area. Felines have a shorter intestine and therefore a decreased surface area for absorption, which may also alter intestinal permeability.[16] The higher bacterial counts in cat intestines have also been associated with an increased permeability.[7,9] Higher inherent permeability has also been recorded in juvenile animals.[7] All cheetahs in this study were juveniles; therefore a higher intestinal permeability would be expected. Higher values of L/R recovery ratios (0.52 ± 0.19 with a range of 0.30–0.98) have been reported in clinically healthy cats.[16] The L/R ratios in this study in cheetahs varied from 0.00 to 0.79, which falls within the range of ratios reported for healthy domestic cats.[16] The time from administration to collection of blood samples varied considerably between animals. This in conjunction with no available reference range for intestinal permeability in healthy cheetahs made it difficult to interpret the results and makes it difficult to discuss the significance of the different L/R ratios obtained in this study. Essentially, a reference range for healthy cheetahs needs to be established.

The isolation of selected bacteria from healthy adult cheetahs facilitated the production of a probiotic that showed promising results in this study. The juvenile cheetahs treated with the probiotic over a 28-day period gained relatively more weight than the control animals. There was a significant reduction in diarrhoeic faeces, and blood and mucus in the PG during the feeding of the probiotic compared to the faeces from the CG. As only juveniles were tested, the study should be repeated on a wider range of cheetahs of different ages, because the effects of the probiotic will differ in different age groups.

### ACKNOWLEDGEMENTS

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