

Impact of Oral Probiotics on Faecal Profile in Endangered Asian Elephant (*Elephas maximus*)

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ABSTRACT

A study was planned to determine the impact of oral probiotics on faecal profiles in 18 adult Asian elephants (*Elephas maximus*) for sixty days. The elephants were randomly divided into three groups, with six each. The experimental probiotics; *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*, were supplemented @ 1 gm (1×10^9 cfu /gm) / 50 kg body weight per day to all the elephants of T₂ and T₃ groups, respectively, whereas no probiotic was given to the control (T₁) group. The faecal samples were taken on 0 and 60th day of the experiment for the analysis of faecal profiles viz. pH, lactic acid, coliforms, and probiotic microbial counts, i.e., lactobacillus and mould. Oral feeding of probiotics did not exhibit any significant impact on faecal profiles; however, it seems to induce some shift in faecal microflora. A declining trend in lactobacillus count was observed in probiotic groups as compared to the control group, which could be implicative of positive health status in Asian elephants.

Key words: Asian elephants, Faecal profiles, Lactobacillus, Mould, Probiotics.

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INTRODUCTION

India is a place of vast diversity in flora and fauna. We must have knowledge of the welfare, and nutrition of wildlife like Asian elephants. Investigation of faecal profiles in animals has led to a better understanding of gut environment and faecal microbial communities, and they have underlined the significance of these communities for host's fitness and survival, physiology and utilization of nutrients (Jacobs and Braun, 2014). Elephants are simple stomached animals with hindgut fermentation. Caecum and colon are inhabited by anaerobic bacteria and protozoa, which are reported to be similar to those found in ruminants and horses (Stevens and Hume, 1998; Bunesova *et al.*, 2013). Senthilkumar *et al.* (2018), found that genus *Lactobacillus* seems to be common species occurring in the GI tract of Asian elephants. The enzymatic degradation of fibrous feeds, partly chewed plant materials, and lactic acid formed in the upper digestive tract results in the production of VFA. These VFAs are readily absorbed in the body and are the primary source of energy to the elephant (Chharang, 2021). Presently, the microflora in GI tract of the elephant has not fully been investigated and needs to be explained.

Various strategies have been developed to inhibit microbial disturbances in the animal intestine, with the growing concerns about using antibiotics and other growth stimulants (Chharang, 2021). Supplementation of intestinal microflora with probiotic microbiota was proven to support and help to treat infections (Corcionivoschi *et al.*, 2010) because of their ability to modulate intestinal microorganism and immune system (Chharang *et al.*, 2021). Many studies have revealed to play an indispensable role in

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physiological homeostasis and health promotion (Alayande *et al.*, 2020). The mode of action of probiotics is through beneficial changes in the gut flora with depletions in the population of pathogens, production of antibiotic-type substances, lactate production with subsequent changes in intestinal pH, competition for adhesion receptors in the intestine, production of enzymes, competition for nutrients and decline of toxin release and immune stimulation (Taklimi *et al.*, 2012). Therefore, this study was planned to determine the impact of oral probiotics on faecal profiles like pH, lactic acid and microbiota in Asian elephants.

MATERIALS AND METHODS

The present study was conducted at Elephant Village, Jaipur (India). The study protocol was duly approved by the Institute Animal Ethics Committee (PGIVER/IAEC/19-05) and performed in accordance with relevant guidelines and regulations.

Animals and Housing

Total of 18 adult captive female Asian elephants with age ranged from 30 to 62 years were divided randomly into three groups with six elephants in each. All the elephants were housed in a hygienic and well ventilated individual enclosure, having a concrete floor with a feeding arrangement so that each animal's faeces could be collected separately.

Experimental Feeding Trial

The elephants were stall-fed a consistent diet of green pearl millet forage as basal feed throughout the research period of 60 days. An adaptation period of 10 days was observed prior to start of experimental feeding trial. The elephants were then placed on three dietary experimental feeds for 50 days of experimental feeding trial. During the feeding trial of 50 days, experimental probiotics *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were administered @ 1 gm (1×10^9 cfu) / 50 kg body weight per day orally along with basal feed to all the experimental elephants of T₂ and T₃ groups, respectively. The experimental group T₁ served as control, and received no probiotic.

Sample Collection and Preservation

Freshly voided faecal samples were collected prior to administration of probiotic (0 day), and at the end of the experiment (60th day) and sent immediately to the laboratory on ice for the analysis of faecal profiles viz. pH, lactic acid, coliforms, and probiotic microbial counts. Faecal samples were collected with care after removing the outer layer of dung to avoid any soil contamination.

Estimation of Faecal Profiles

Faecal pH: The faecal pH was determined directly using a pH meter, specially designed for direct pH measurement of semi-solid samples.

Faecal lactic acid: The lactic acid of the faecal sample (gm/kg) was estimated as described by Dowarah (2015).

Faecal coliforms and probiotic microbiotas: For faecal coliform, lactobacillus and mould counts in faecal samples were enumerated by using the pour plate method. About 10 gm fresh faeces were immediately mixed with normal saline (1:10 w/v) and vortexed for 3-4 min, and the supernatant was used for microbial counting. The filtrate was serially diluted to 10^{10} with normal saline. For coliform, lactobacillus and mould MacConkey Agar (HiMedia), MRS Agar and PDA Agar were used and, the relevant agar plates were incubated anaerobically at 37 °C for 48 h, 24 h and 48 h, respectively. After incubation, the colonies of coliform, lactobacillus and mould were counted as colony forming units (cfu)/gm faeces and expressed as log 10 cfu/gm of faeces.

Statistical Analysis

The data were subjected to statistical analysis (SPSS version 24) using one-way analysis of variance described by Snedecor and Cochran (2004). Significance was defined at $p < 0.05$. All the values represent mean \pm standard errors of the mean.

RESULTS AND DISCUSSION

The nutritional impact of supplementation of different oral probiotics on faecal parameters determined on day 0 and 60th day of the study is depicted in Table 1. Statistically, non-significant effects of probiotics supplementation were found on faecal pH, lactic acid, coliform, lactobacillus, and mould counts. Though the differences were non-significant but, on comparing the data at different period, the declined trend of average pH, coliforms, and mould counts was found to be associated with increased lactic acid and lactobacillus count on the 60th day as compared to the 0 day within all the respective groups. In contrast; pH, coliform, and mould counts were recorded to be higher, whereas lactic acid and lactobacillus counts were lower on day 60th in the T₂ group followed by the T₃ group than the T₁ group. Thus, the present study suggests that the trend of decline in lactobacillus count obtained from probiotics fed elephants as compared to those fed the control diet could be implicative of positive health status.

Table 1: Mean \pm SEM values of faecal parameters in the probiotics supplemented and control groups of elephants

Groups	Faecal pH		Lactic acid (gm/kg)		Coliform counts (cfu/gm)		Lactobacillus		Mould	
	0 Day	60 th Day	0 Day	60 th Day	0 Day	60 th Day	0 Day	60 th Day	0 Day	60 th Day
T ₁	5.59 \pm 0.10	4.79 \pm 0.13	19.60 \pm 0.50	61.65 \pm 3.52	7.15 \pm 0.27	6.17 \pm 0.33	2.67 \pm 0.38	8.02 \pm 0.57	7.43 \pm 0.91	3.78 \pm 0.24
T ₂	5.63 \pm 0.09	5.00 \pm 0.21	19.82 \pm 0.50	54.03 \pm 5.43	7.65 \pm 0.49	6.47 \pm 0.41	2.22 \pm 0.48	7.20 \pm 0.82	7.25 \pm 0.16	4.23 \pm 0.26
T ₃	5.61 \pm 0.14	4.80 \pm 0.18	19.47 \pm 0.31	60.35 \pm 4.55	7.38 \pm 0.44	6.32 \pm 0.27	2.38 \pm 0.37	7.32 \pm 0.76	7.40 \pm 0.14	4.03 \pm 0.20
p-value	0.961	0.649	0.856	0.469	0.699	0.829	0.730	0.697	0.604	0.423



The digestive system in case of elephants is similar to horses. Hence, extrapolations were carried out with regard to the dosing regimen of the probiotic preparations from the resources used in the case of horses. In the present study, the probiotics, which contained 1×10^9 cfu /gm *Lactobacillus acidophilus* and 1×10^9 cfu /gm *Saccharomyces cerevisiae*, were given for every 50 kg body weight per day. Weese *et al.* (2003) used 1×10^{10} cfu per 50 kg body weight per day in a group of horses, pertaining to the *Lactobacillus* organisms and achieved a consistent intestinal colonization only with a high dose.

In this study, the faecal pH of the probiotics fed elephants showed a slight decreasing trend. Maintaining of a lower faecal pH and the elevation in faecal lactic acid concentrations suggest increased enteric populations of lactic acid-producing bacteria. An acidic pH in the intestine of elephants is thought to be detrimental due to the acid sensitivity of fibre-degrading bacteria; however, the fecal pH in all the elephants was within a physiological range during the experiment (Hydock *et al.*, 2014). Some *in vitro* studies have demonstrated that probiotic bacteria release carboxylic acids such as lactic acid and acetic acid, and have inhibitory effects on the growth and invasive function of *E. coli* in low pH conditions (Miyazaki *et al.*, 2010). Although, supplementation of *S. cerevisiae* has been reported to prevent acidification of faeces and improve microbial fibrolytic activities induced by a high-fibre or high-starch diet in horses (Jouany *et al.*, 2009).

Swanson (2002), Jounay *et al.* (2009) and Palagi *et al.* (2018) observed no differences in the numbers of lactic acid bacteria as well as on faecal pH on supplementation of *S. cerevisiae* in horses. However, a highly significant increase in the concentration of *Saccharomyces cerevisiae* strain was seen in the cecum and colon of live yeast supplemented fistulated horses by Jounay *et al.* (2009). Stercova *et al.* (2016) observed non-significant difference in pH, lactic acid bacteria, and total coliforms of freshly voided faeces between the live yeast (LY) supplemented and the control Beagle dogs. However, faecal lactic acid contents showed a significant effect of treatment and sex interactions ($p < 0.10$). The log cfu/gm values of the *S. cerevisiae* were also significantly higher in the faeces of the LY animals ($p < 0.01$).

On the contrary, the *L. acidophilus*'s inclusion in the diet of pre and post weaned foals increased faecal lactic acid (Swanson, 2002). Vase-Khavari *et al.* (2019) showed lower caecal coliforms in rabbits and colon pathogens in poultry, administered with probiotics, respectively. A prophylactic dose of *L. acidophilus* improved faecal conditions and increased lactic acid bacterial populations in the hindgut of adult horses (Ishizaka *et al.*, 2014). Similarly, decreased incidence of diarrhoea was observed when foals were supplemented with multiple strains of *Lactobacillus* and *Bifidobacterium* spp. (Tanabe *et al.*, 2014). Dowarah *et al.* (2017) reported that weaned piglets fed with *L. acidophilus* supplements in their basal diet exhibited significant improvements in terms of growth performance, digestion

rate, faecal microbial count, intestinal morphology, diarrhoea control and maintenance of pH in the GI tract. In addition, with increasing the inclusion level of a probiotics mixture in the diets, a linearly increased ($p < 0.05$) faecal *Lactobacillus* counts and decreased *Escherichia coli* counts in weaning pigs was recorded (Nguyen *et al.*, 2019), whereas Julliard *et al.* (2018) reported that the inclusion of *S. cerevisiae* (10×10^9 and 10×10^{10} cfu /animal per day) in horses' diet had no impact on gastric pH but reduced the lactic acid utilizing bacteria.

CONCLUSIONS

Thus, it can be concluded from the observations of the present study that probiotics *L. acidophilus* and *S. cerevisiae* can be safely incorporated up to 10^9 cfu /gm for every 50 kg body weight /day in the diet of elephants without affecting the faecal profiles.

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