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Research Article

HAEMATOLOGICAL PROFILES OF ASIAN ELEPHANTS FED WITH PROBIOTIC FEED ADDITIVES

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ABSTRACT: Haematological profiles indicate the health status and ability to resist infections as it plays a significant role in the nutritional, physiological, and pathological state of animals. Supplementation of intestinal microflora with probiotic microbiota was proven to help to treat infections. So an experiment was conducted to determine the effect of probiotic feed additives on haematological profiles in 18 captive Asian elephants for two months. The experimental probiotics; *Lactobacillus acidophilus* and *Saccharomyces cerevisiae,* were supplemented @ 1 gm 1 × 10^9 CFU /gm for every 50 kg body weight per day to all the elephants of Lacto (T2) and Sac (T3) groups respectively, whereas no probiotic was given to the Control (T1) group. Haematological constituents, namely (i) red blood cell indices, white blood cell indices, and platelet cell indices were analyzed. The observed values of red blood cell indices revealed a non-significant effect among the groups. A highly significant ($p < 0.01$) effect was observed in the differential leukocyte count, which shows signs of an immune-stimulatory effect. Significant ($p < 0.05$) effect of treatment was recorded on mean platelet volume. The addition of probiotic feed additives did not affect the physiological health of the captive Asian elephants since the parameters studied were well within the normal physiological range of a healthy animal.

Key words: Captive Asian elephants, Haematological profiles, Physiological health, Probiotic feed additives.

INTRODUCTION

The Asian elephant is the largest existing terrestrial animal in Asia, with population estimations exhibiting a decline in population size by approximately 50 percent over three generations (Choudhury *et al*. 2008). At this rate, elephants face probable extinction in their innate habitat, with the Asian elephant population appearing in a more critical state than their African counterparts. Elephants are a challenging species to keep in captivity, although they are considered a species of flagship conservation and thus a favorite species to exhibit (Bowen-Jones and Entwistle 2002).

Generally, captive elephants are not fed factually according to their food preference in natural circumstances. In most captive facilities, elephants are fed empirically based on the experience of the mahout. Chronic nutritional imbalances weaken the animal's immune system and predispose it to many pathogens (Das 2018). The haematological profiles of elephants indicate their health status and ability to resist infections. Captive animals, including elephants, have exhibited a trend towards being stressed (Elzanowsky and Sergiel 2006) and chronic stress has been shown to reflect in the haematology of animals. Blood investigation is the right way of assessing the health status of livestock used in various feed trials as it plays a significant role in the nutritional, physiological, and pathological state of animals (Chharang *et al*. 2021). However, blood parameter ranges tend to be very broad in elephants (Salakij *et al*. 2005), making interpretation difficult. Furthermore, values may not be relevant across all populations because factors like sex, age, management, exercise, and geographical location can affect values (Pintavongs *et al*. 2014).

Gut morphology and host phylogeny have been stated to play a vital role in shaping animals' gut microbiotas (Fang *et al*. 2012). Phylogenetically, the elephant belongs to the family Elephantidae. The intestine harbors a complex, dynamic, and fermentative microbial ecosystem that has several major functions. Various strategies have been developed to inhibit microbial disturbances in the animal intestine, with growing concerns about using antibiotics and other growth stimulants (Chharang 2021).

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Supplementation of intestinal microflora with probiotic microbiota was proven to support and help to treat infections at that level (Corcionivoschi *et al*. 2010).

Microbiota is currently an essential issue in disease and, health and many studies have revealed it to play an indispensable role in 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). Thus, an attempt was made to evaluate the effect of probiotic feed additives on haematological profiles in captive Asian elephants to ascertain elephants' physiological health.

MATERIALS AND METHODS

Study animals

The present study was conducted at Elephant Village, Jaipur (India) (26°59'47"N 75°52'35" E). The study protocol was duly approved by the Institute Animal Ethics Committee (PGIVER/IAEC/I9-05) and performed following relevant guidelines and regulations. A total of 18 adult captive female Asian elephants with ages ranging from 30 to 62 years, having similar body weights (3495 ± 133.34 kg), and uniform conformation were divided randomly into three groups with six elephants each. The elephants were stall-fed a consistent diet of green pearl millet forage as basal feed throughout the research period, *i.e.,* for two months, including preliminary ten days for adaptability with basal feed and fifty days for the feeding trial of probiotic feed additives. Fresh and clean *ad libitum* water was provided to all the elephants. A prophylaxis dose of broad-spectrum anthelmintic at 5mg/ kg body weight was given before the experiment.

Experimental probiotic feed additives

During the feeding trial of 50 days, experimental probiotic feed additives *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were administered @ 1 gm 1 × 10^9 CFU /gm for every 50 kg body weight per day orally along with basal feed to all the experimental elephants of Lacto (T2) and Sac (T3) groups respectively. The experimental group T1 was the Control group that received no probiotics. The horse was considered a model animal for calculating probiotic requirements and designing a diet for elephants (Clauss *et al*. 2003).

Blood collection and analysis

Blood samples were collected after the end of the feeding trial between 07:00- 09:00 AM from the elephants by puncturing ear veins. For the analysis of haematological profiles, 2 mL of blood was collected aseptically in a vacutainer tube containing di-potassium salt of EDTA (@ 1mg/ml of blood) as an anticoagulant. The vacutainer tubes were transported to the laboratory on ice and tested on the same day. From the blood samples, (i) red blood cell indices like red blood cell (RBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC); (ii) white blood cell indices like total leukocyte count (WBC) and differential leukocyte count; and (iii) platelet cell indices like platelet count, mean platelet volume (MPV), and platelet crit (PCT) were analyzed using the Automated Hematology Analyzer, *i.e*., Spincell 5 compact Veterinary Mode as per manufacturer's protocol.

Statistical analysis

All the statistical analysis of data was performed using SPSS version 24. The difference among groups was determined by one-way ANOVA analysis (Snedecor and Cochran 2004). The levels of significance and high significance were defined at $p < 0.05$ and $p < 0.01$, respectively. All the values represent mean ± standard errors of the mean.

RESULTS AND DISCUSSION

A haematological study was used to ascertain the impacts of probiotic feed additives on the physiological health of the elephants. Administration of probiotic feed additives, *i.e., Lactobacillus acidophilus* and *Saccharomyces cerevisiae,* did not affect their health. The elephants were in excellent condition throughout the research period without signs of abnormal health.

The data that were enumerated during the investigation of haematological profiles namely: (i) red blood cell indices like a red blood cell, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration; (ii) white blood cell indices like total leukocyte count and differential leukocyte count; and (iii) platelet cell indices like platelet count, mean platelet volume, and plateletcrit (Table 1-3).

Red blood cell indices

The inclusion of probiotic feed additives, *L. acidophilus* and *S. cerevisiae* on red blood cell indices *viz*. RBC, Hb, hematocrit, MCV, MCH, and MCHC in the Asian elephants revealed a non-significant effect among the groups (Table 1). Though the differences were non-significant the mean values of RBC, Hb, hematocrit, and MCHC were recorded to be higher in the Sac (T3) group, followed by the Lacto (T2) group and the Control (T1) group. In contrast, the mean values of MCV and MCH were recorded to be higher in the T2 group, followed by the T3 group and the T1 group. Higher values were observed in probiotics-fed groups as compared to the control group.

The physiological values of Hb and MCV were observed to be similar to Niemuller *et al*. (1990), Janyamethakul *et al*. (2017), and Gupta *et al*. (2020), whereas the values of RBC and hematocrit were found to be higher, as well as MCH and MCHC, were found to be lower in this study than Niemuller *et al*. (1990) and Gupta *et al*. (2020) in Asian elephants.

White blood cell indices

A highly significant ($p < 0.01$) effect of treatment was observed on the percent lymphocyte and neutrophil counts. However, it could not reveal any effect on other indices, *viz.* WBC, monocytes, eosinophils, and basophils in Asian elephants (Table 2). It is noteworthy to mention that the results of this study regarding mean white blood cell indices in different groups are following the reference ranges of Janyamethakul *et al*. (2017) and Gupta *et al*. (2020) in the Asian elephants and they were not adversely affected by the experimental treatment.

There was no difference in WBC count between the groups. WBC is important in defending the body against infections (Schalm *et al*. 1975). The WBC count, however, cannot give specific information since this requires differential leukocyte counts. In the differential leukocyte count, the neutrophil count was observed to be higher in the control group. The difference might be attributed that neutrophils execute phagocytosis of pathogenic microbiotas during the firstly hours after they enter into tissues (Bovera *et al*. 2012). The lymphocyte counts were recorded to be higher for the Lacto (T2) group, followed by the Sac (T3) group, and then in the Control (T1) group. Aattouri *et al*. (2001) found that oral administration of LAB in the rat increases lymphocyte proliferation and interferon-A production. In essence, the improvement in the lymphocyte counts observed in the T2 group shows signs of an immune-stimulatory effect.

Platelet cell indices

Significant ($p < 0.05$) effect of treatment was recorded on mean platelet volume (MPV); however, it could not reveal any effect on platelet counts and plateletcrit (Table

Table 1. Effect of probiotic feed additives on red blood cell indices in different groups of captive Asian elephants.

Red blood cell indices	T_1	T_2	T_3	Overall	p-value
RBC (10^6 cell/µL)	5.62 ± 0.35	5.70 ± 0.44	5.80 ± 0.18	5.70 ± 0.19	0.929
Haemoglobin (gm/dL)	10.92 ± 0.57	11.32 ± 0.77	11.44 ± 0.31	11.22 ± 0.32	0.807
Haematocrit (%)	69.32 ± 3.63	71.52 ± 4.87	71.59 ± 2.11	70.81 ± 2.03	0.887
MCV (fL)	123.82 ± 1.92	126.50 ± 2.63	124.51 ± 1.23	124.94 ± 1.12	0.627
MCH (pg)	19.45 ± 0.32	19.90 ± 0.34	19.65 ± 0.23	19.67 ± 0.17	0.580
MCHC (gm/dL)	15.72 ± 0.09	15.78 ± 0.10	15.87 ± 0.08	15.79 ± 0.05	0.539

Table 2. Effect of probiotic feed additives on white blood cell indices in different groups of captive Asian elephants.

White blood cell indices	T_1	T_2	T_3	Overall	p-value
WBC (10^3 cell/µL)	14.31 ± 0.78	16.24 ± 1.49	16.21 ± 0.57	15.59 ± 0.60	0.339
Differential Leukocyte Count					
Lymphocytes (%)	33.52^a ± 6.27	54.83^b ± 2.43	45.53^a ± 2.16	44.63 ± 3.06	0.008^{**}
Monocytes (%)	16.25 ± 8.80	5.46 ± 0.91	6.99 ± 0.84	9.56 ± 3.01	0.303
Neutrophils (%)	45.96^b ± 3.73	33.31^a ± 2.06	42.01^b ± 1.26	40.42 ± 1.89	0.010^{**}
Eosinophils (%)	4.09 ± 1.22	6.15 ± 1.24	5.18 ± 0 .23	5.14 ± 0 .59	0.378
Basophils (%)	0.19 ± 0.07	0.26 ± 0.06	0.29 ± 0.04	0.25 ± 0.03	0.516

Means superscripted with a different letter within a column differ significantly from each other.
**Highly significant ($p < 0.01$).

Table 3. Effect of probiotic feed additives on platelet cell indices in different groups of captive Asian elephants.

Platelet cell indices	T1	T2	T3	Overall	p-value
Platelets (103 cell/µL)	483.17 ± 30.05	496.50 ± 56.10	433.17 ± 31.29	470.94 ± 23.17	0.529
MPV (fL)	2.40a ± 0.18	2.93a ± 0.23	3.27b ± 0.22	2.87 ± 0.14	0.034*
Plateletcrit (%)	0.11 ±0.02	0.15 ± 0.02	0.14 ±0.02	0.13 ± 0.01	0.511

Means superscripted with a different letter within a column differ significantly from each other. *Significant ($p < 0.05$).

3). It is noteworthy to mention that the results of this study regarding platelet indices in different groups are following the normal physiological values of Niemuller *et al*. (1990), Janyamethakul *et al*. (2017), and Gupta *et al*. (2020) in the Asian elephants and they were not adversely affected by the experimental treatment.

CONCLUSION

It is concluded that the addition of probiotic feed additives; *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* @ 1 gm 1 × 10^9 CFU /gm for every 50 kg body weight per day did not affect the physiological health of the captive Asian elephants since the parameters studied were well within the normal physiological range of a healthy animal. The observed values of red blood cell indices revealed a non-significant effect among the groups. However, the differences were non-significant but improved red blood cell indices in probiotics-fed elephants. A highly significant effect was observed in the differential leukocyte count, which shows signs of an immune-modulatory effect. A significant effect of treatment was recorded on mean platelet volume.

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