

Assessment of blood metabolites, serum enzymes, and serum minerals in dietary probiotics fed captive Asian elephants

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Abstract: This study was organised to evaluate the effect of supplementation of dietary probiotics on blood biochemistry in 18 captive Asian elephants of 30-62 years age and 3495 ± 133.34 kg body weight (BW) for two months. To observe the results in different groups, i.e. control with no probiotic (T_1) and the other two with probiotics which contained 1×10^9 CFU/g concentrate of *Lactobacillus acidophilus* (T_2) and 1×10^9 CFU/g concentrate of *Saccharomyces cerevisiae* (T_3), they were given 50 kg BW/day (dose range 48-89 g per day/elephant). The elephants were randomly divided into three groups with six elephants each. They were fed on *ad libitum* green fodder. Blood biochemical constituents namely: (i) blood metabolites like glucose, total protein, albumin, cholesterol, creatinine, and blood urea nitrogen, (ii) serum enzymes like aspartate transaminase, alanine transaminase, and alkaline phosphatase, and (iii) serum minerals like calcium and phosphorus were analysed at the end of research to evaluate the physiological impacts of nutrients in the elephant's body. It didn't establish any health problem to the elephants since the parameters determined in the blood were observed well within the normal physiological range, and no significant differences were noticed due to the effect of treatment. It is concluded that blood biochemistry remained unaffected on the supplementation of probiotics in Asian elephants.

Key words: Asian elephant, blood biochemistry, physiological impact, probiotics

1. Introduction

The Asian elephant (*Elephas maximus*) is a monogastric herbivore, and its digestive system is comparable to that of equids [1], rabbits, and rodents. The elephant's large fermentative chambers harbour diverse anaerobic microbes consisting of bacteria, fungi, and protozoa for the fermentation and breakdown of fibrous feed materials [2]. The intestinal microorganisms have a vast impact on the gut health and performance of elephants and their vital aspects in wildlife health care, welfare management, and conservation programs.

Amongst of nutraceuticals, probiotics are the live microbial cultures that have been extensively used in animals because of their ability to modulate intestinal microorganism and immune system. The inclusions of probiotics furnish health advantages: improve growth performance and nutrient digestibility to the host when supplemented in adequate dosage [3]. It also lessens serum cholesterol and the incidence of diarrhea in livestock [4,5].

Appraisalment of the health and nutritional status of livestock is inestimable in present-day animal husbandry. Blood biochemical 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. Serum concentrations of metabolites such as total proteins, glucose, globulin, albumin, cholesterol, blood urea nitrogen, creatinine, and minerals indicate the degree of the metabolism of proteins, energy, and other nutrients in the livestock [6,7]. Variations in the concentration of circulating nutrient-sensitive metabolites are essential signals of the livestock's metabolic status and the activity of the organs [8,9]. Factors including the physiological condition of livestock, nutrition, health status, breed, age, and season may vary the concentration of these metabolites in the serum [10]. In this context, Olorode & Longe [11] have summarized that nutrition plays an important role and intervenes with the serum metabolites and other constituents' concentration levels. Therefore, a study was determined to assess the impact of administration of probiotics on the serum biochemical profile of captive Asian elephants.

2. Materials and methods

2.1. Study animals and design

The study was organised from August to October 2019 at Elephant Village, Jaipur (India) ($26^\circ 59'47''$ N and 75°

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52°35' E). Eighteen adult captive female Asian elephants of 30-62 years age, having similar body weight (3495 ± 133.34 kg), and of uniform conformation were randomly selected. They were divided into three similar groups with six elephants each. The elephants were stall-fed a consistent diet of green pearl millet fodder as basal diet throughout the research period, i.e. for a period of two months including ten days for adaptability with basal diet and fifty days for probiotics feeding. They were also allowed for a routine elephant safari. Fresh and clean *ad libitum* water was provided to all the elephants. A prophylaxis dose of broad-spectrum anthelmintic, i.e. Panacur Bolus (fenbendazole) as 5mg/kg body weight, was given to all the elephants prior to experimental feeding of probiotics.

2.2. Probiotics

The probiotics, i.e. *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were supplemented along with basal diet during the feeding trial of 50 days to all the elephants in T₂ and T₃ treatment groups, respectively. The probiotic supplemented in this study was a commercially available powder product by Meteoric Biopharmaceuticals Pvt. Ltd. (Gujarat, India). The products contained 1×10^{10} colony-forming units (CFU)/g concentrate of *Lactobacillus acidophilus* and 1×10^9 colony-forming units (CFU)/g concentrate of *Saccharomyces cerevisiae*. One gram probiotic was given per 50 kg body weight orally with jaggery, whereas the only jaggery was supplemented as a placebo along with the basal diet to the elephants of T₁ i.e. control group. The horse was considered as a model animal for calculating probiotic requirements and designing a diet for elephants [1].

2.3. Blood collection and analysis

For the analysis of blood metabolites, serum enzymes, and serum minerals, five mL of blood was collected after the end of the feeding trial between 07:00-09:00 AM from all elephants by puncturing ear vein. The blood was taken directly into a vacutainer tube and transported to the laboratory on ice, where serum was harvested and tested on the same day. From the serum samples, (i) blood metabolites like glucose, total protein, albumin, cholesterol, creatinine, and blood urea nitrogen (ii) serum enzymes like aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), and (iii) serum minerals like calcium and phosphorus were analysed using the commercial kits on fully automated biochemistry analyser "Turbochem100" (Awareness Inc., Connecticut, USA) as per manufacturer's protocol. Each sample was labelled with a unique identity (ID) number, date, and time of sampling.

2.4. Statistical analysis

All statistical analysis of data was performed using SPSS 16 version (IBM Corp., Armonk, NY, USA) for windows. The difference among groups was determined by one-way

ANOVA analysis. The significant effects of different means were compared by Duncan's new multiple range test [12]. Significance was defined at $P < 0.05$. All values represent mean \pm standard errors of the mean.

2.5. Ethical considerations

All applicable national, international, and institutional guidelines for the care and handling of animals were followed during blood sample collection. The study was conducted with the prior approval of the Additional Principal Chief Conservator of Forest and Chief Wildlife Warden, Rajasthan, Jaipur, India (Ref. no. 1159, dated May 28, 2019) and the Government of India, Ministry of Environment, Forest and Climate Change, Wildlife Division, New Delhi (Ref. no.1-22/2019 WL, dated October 16, 2019).

3. Results and discussion

The animals were in excellent condition throughout the study period without signs of abnormal health. Supplementation of probiotics, i.e. *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* for 50 days did not affect their health. Serum biochemical analysis is used to determine liver, heart, and kidney functioning and is mostly used as a signal of the physiological effects of nutrients in the body of animals.

The data that were enumerated during the investigation of serum biochemistry parameters namely: (i) blood metabolites like glucose, total protein, albumin, cholesterol, creatinine, and blood urea nitrogen, (ii) serum enzymes like aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), and (iii) serum minerals like calcium and phosphorus are shown in Table.

3.1. Blood metabolite

The mean values of serum glucose observed in this investigation were found well within the normal physiological range of 25.2-102.6 (mg/dL) as reported in Asian elephants [13,14,15]. The mean value of glucose was observed lowest in T₃ and highest in T₁ groups. However, the supplementation of probiotics did not have any effect on glucose among the groups. A similar nonsignificant effect, supporting the result of the present investigation, has also been noticed earlier in rabbits [16,17,18,19,20]. In another study, elevated ($P < 0.05$) serum glucose level was also reported in *Saccharomyces cerevisiae* fed pigs [21].

The mean values of total protein obtained in the present investigation for different groups were within the physiological range of 7.2-10.2 (g/dL) [13] and it attests to the results of earlier studies conducted in Asian elephants [22,14,23]. The statistical analysis of data of total protein revealed a nonsignificant effect among the treatments. The results are consistent with earlier findings [24,20], which indicate that serum level of total protein remained the same on the administration of probiotics in lamb and

Table. Different blood biochemical constituents in probiotics fed Asian elephants.

Parameters (Mean \pm SEM)	T ₁	T ₂	T ₃	P-value
Blood metabolites				
Glucose (mg/dL)	31.16 \pm 6.11	29.58 \pm 9.18	26.65 \pm 8.25	0.92
Total Protein (g/dL)	8.12 \pm 0.38	8.12 \pm 0.41	8.24 \pm 0.47	0.97
Albumin (g/dL)	3.05 \pm 0.15	3.18 \pm 0.15	3.08 \pm 0.19	0.83
Cholesterol (mg/dL)	33.25 \pm 3.22	31.08 \pm 3.01	30.60 \pm 5.73	0.89
Creatinine (mg/dL)	1.30 \pm 0.07	1.24 \pm 0.10	1.25 \pm 0.06	0.87
Blood Urea Nitrogen (mg/dL)	8.26 \pm 0.17	8.18 \pm 0.09	8.26 \pm 0.09	0.87
Serum enzymes				
AST (IU/L)	24.68 \pm 1.25	23.75 \pm 1.74	27.22 \pm 1.77	0.32
ALT (IU/L)	2.07 \pm 0.48	3.37 \pm 0.48	2.28 \pm 0.32	0.11
Alkaline Phosphatase (ALP)(IU/L)	54.13 \pm 6.77	59.67 \pm 7.90	58.74 \pm 3.60	0.81
Serum minerals				
Calcium (mg/dL)	11.53 \pm 0.29	11.10 \pm 0.62	11.42 \pm 0.36	0.78
Phosphorus (mg/dL)	4.95 \pm 0.48	6.47 \pm 0.62	6.26 \pm 0.46	0.12

T₁- Control, T₂- probiotic (*L. acidophilus*), and T₃- probiotic (*S. cerevisiae*).

rabbit, respectively. However, Onifade et al. [25] revealed that serum protein level was elevated with dietary yeast in rabbits and highly significant ($P < 0.001$) with *Lactobacillus* spp. in weaned piglets [26,27].

The statistical analysis of data of serum albumin revealed a nonsignificant effect among the three groups. The results are in accordance with the earlier observations [24,20] in lamb and rabbit, respectively. However, Onifade et al. [25] revealed that albumin level was increased with dietary yeast in rabbit, *Lactobacillus* spp. in piglets [26,27] and *Bacillus* spp. in lambs [28]. The albumin level obtained in the present investigation for different groups was found to be well within the physiological range of 2.6-3.6 (g/dL) reported in Asian elephants [13,14]. However, it was found towards a slightly higher side as compared to the results observed in earlier studies [22,29].

The statistical analysis of data of serum cholesterol revealed a nonsignificant effect among the three groups. The mean values of the present study are in corroboration with the earlier findings as observed in broiler chicken [30] and lamb [24], whereas it exhibits significant differences with the results obtained in other studies in rat [31] and in mice [32]. Similarly, earlier studies [25,33] reported that serum level of cholesterol was decreased with dietary inclusion of yeast and *Lactobacillus* spp. in rabbits, respectively. The mean cholesterol level obtained in the present investigation for different groups was found to be well within the normal reference range of 8.1-51.66 (mg/dL) [13]; however, it was found towards the lower side as compared to the data observed in earlier studies

[22,29,14]. It was found lowest in T₃ and highest in T₁ (control) groups.

The mean values of creatinine obtained in the present investigation for different groups were found to be well within the normal physiological range of 0.9-1.8 (mg/dL) [23] and it attests to the earlier observations in Asian elephants [22,29,14,15]. The statistical analysis of data of creatinine revealed a nonsignificant effect among the three groups. The study was in agreement with earlier findings in weaned piglets [26]. However, its concentration was decreased significantly in probiotic fed large felids [34] and rats [35].

The blood urea nitrogen concentration obtained in the present investigation for different groups was found to be well within the normal range of 4.2-19.7 (mg/dL) [23] and the mean values are in corroboration with the earlier findings in Asian elephants [22,29,14,15]. Supplementation of probiotics did not have any effect ($P > 0.05$) on blood urea nitrogen among the groups. Similar results were reported in ewes [36], large felids [34] and weaned piglets [26]. However, a significantly decreased concentration of blood urea nitrogen was observed in probiotic fed rats [35] and lambs [28].

The liver enzymes, i.e., serum AST, ALT, and ALP activities, were used to estimate liver functions. The increased concentration of these enzymes was associated with the degeneration of hepatic cells [37,38] and muscle tissues [39,40].

The statistical analysis of data of AST revealed a nonsignificant effect among the groups. The values are

in corroboration with the findings reported earlier [41]. The serum level of AST was found to be reduced with the administration of dietary yeast in rabbits [25], multistrain probiotics in rats [31], and *Bacillus* spp. in lambs [28], whereas all the treatments (Kefir rich probiotic levels 3%, 6%, 9%, and 12%) significantly increased the AST enzyme level as compared to the control group ($P < 0.05$) in Japanese quails [42]. The AST level obtained in the present investigation for different groups was found to be well within the normal reference range of 10.1-39.6 U/L [23] and the mean values are consistent with earlier findings in Asian elephants [22,14]. The mean AST value of the present study was found to be highest in the T_3 group.

The statistical analysis of data of ALT enzyme in both treatment groups was more than the control group, but the variations were insignificant, and the mean values are in accordance with the earlier studies in broiler chicken [41] and Japanese quails [42]. On the other hand, serum level of ALT was decreased with dietary yeast in rabbits [25], multistrain probiotics in rats [31], and *Bacillus* spp. in lambs [28]. However, the ALT level observed in the present investigation for different groups was found to be well within the average range of 0-5.6 U/L, as reported in Asian elephants [23].

The ALP level observed in the present study for the different groups was found to be well within the average reference range of 0-281.5 U/L [23] and it attests to the results of earlier studies conducted in elephants [22,14]. The serum concentrations of ALP of the present study were not affected by dietary treatments and are in agreement with earlier reports in ewes [36] and weaned piglets [26]. However, serum levels of ALP were decreased in rabbits [25] and Japanese quails [42] treated with the low levels of probiotics.

3.2. Serum minerals

Limited information is available on the serum minerals in response to dietary probiotic administration. Macro and micro minerals are advantageous to livestock and act as structural and functional cofactors in metal-containing enzymes. These minerals play a vital role in livestock's physiology [43].

The mean serum calcium level obtained in the present investigation for the different groups was found

to be slightly lower than the normal reference range of 31.14-56.34 mg/dL [44] and 18.10 ± 1.78 mg/dL [45]. The statistical analysis of data of serum calcium revealed a nonsignificant effect among the groups. The present results are in agreement with the findings reported in pigs [46], broiler chicks [47] and rats [48]. However, increased and significantly decreased ($P \leq 0.001$) serum calcium levels were reported in rabbits [49,50] and lambs [28], respectively.

The statistical analysis of data of serum phosphorus revealed an insignificant increase in T_2 and T_3 groups as compared to the control group. These results are in agreement with earlier findings in cows [51]. However, a significant increase in serum phosphorus level was seen in rabbits [50] and lambs [28]. The serum phosphorus level recorded in the present investigation for different groups was observed to be in corroboration with the earlier studies [45] but found to be a little lower than the data cited in Asian elephants [44,13]. The mean phosphorus value was found to be lowest in the diet of the T_1 group. However, the Ca/P ratio was found to be well within the normal reference range [13].

4. Conclusion

It is concluded that the administration of dietary probiotics *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* to Asian elephant did no significant changes in blood metabolites, serum enzymes, and serum minerals. It didn't establish any health problem to the elephants since the parameters determined in the blood under the study conditions were observed well within the normal physiological range of a healthy animal.

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