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RESEARCH ARTICLE

Nutrient utilization and energy balance profile in probiotic supplemented Asian elephants

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Probiyotik takviyeli Asya fillerinde besin kullanımı ve enerji dengesi profili

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Öz

Amaç: Bu çalışma probiyotik takviyesinin Asya fillerinde besin ve enerji kullanımını etkileyip etkilemeyeceğini değerlendirmek için yapılmıştır.

Gereç ve Yöntem: Beş günlük sindirilebilirlik denemesini içeren 60 günlük deney için 18 fil rastgele seçilerek her biri altı filden oluşan üç gruba ayrıldı. T1 grubundaki fillere probiyotik uygulaması yapılmadı. T2 ve T3 grubundaki fillere sırasıyla her 50 kg vücut ağırlığı için 1x109 CFU/gm konsantrasyonunda Lactobacillus acidophilus ve Saccharomyces cerevisiae probiyotikleri oral olarak verildi.

Bulgular: Eter özütü ve toplam kül alımları önemli, toplam kül ve asitte çözünmeyen külün sindirilebilirlik katsayıları ise tedavinin oldukça önemli bir etkisini ortaya koydu. Ancak, alımların diğer değerleri ve yaklaşık prensiplerin ve lif fraksiyonlarının sindirilebilirlik katsayıları herhangi bir önemli etki ortaya koyamadı. PPratik besin değeri, beslenme düzeyi ve enerji dengesi profili gruplar arasında anlamlı bulunmamıştır. Enerji alımı ve sindirilebilirliğindeki artış eğilimi ile DM (kuru madde) alımı ve NDF (nötral deterjan lif) sindirilebilirliği arasındaki göreceli fark artışla ilişkilendirilmiştir. TDN %, NR, sindirilebilir DM, OM ve TDN alımı için ortalama değerler, probiyotik takviyeli gruplarda kontrol grubuna göre daha yüksek bulunmuştur.

Öneri: Probiyotik takviyesinin fillerde besin ve enerji kullanımı üzerinde önemli bir etki gösteremediği sonucuna varılmıştır. Ancak, probiyotiklerin yüksek dozlarının, suşunun ve canlılığının etkilerini incelemek için daha fazla araştırmaya ihtiyaç vardır.

Anahtar kelimeler: Asya fili, besin kullanımı, enerji, probiyotikler, sindirilebilirlik

Abstract

Aim: A study was methodized to evaluate whether supplementation of probiotics could impact the utilization of nutrients and energy in Asian elephants.

Materials and Methods: Eighteen elephants were randomly selected for 60 days experiment including five days of the digestibility trial and divided into three groups with six elephants each i.e. control with no probiotic (T1) and the other two with probiotics *Lactobacillus acidophilus* in T2 and *Saccharomyces cerevisiae* in T3 groups at 1 × 109 CFU/gm concentrate per 50 kg body weight/ day orally.

Results: : The intakes of ether extract and total ash revealed significant whereas the digestibility coefficients of total ash and acid-insoluble ash exhibited a highly significant effect of treatment. However, other values of intakes and the digestibility coefficients of proximate principles and fibre fractions could not reveal any significant effect. Practical nutritional worth, plane of nutrition and energy balance profile were also found to be non-significant among the groups. Increasing trend of intake and the digestibility of energy; and the relative difference was associated with increased DM intake and NDF digestibility. The mean values for TDN%, NR, digestible DM and OM intakes and TDN intake were obtained to be higher for probiotic supplemented groups than the control group.

Conclusion: It is concluded that probiotic supplementation could not exhibit any significant effect on nutrients and energy utilization in elephants. However, further research is needed to examine the effects of high doses, strain and viability of the probiotics.

Keywords: Asian elephant, digestibility, energy, nutrient utilization, probiotics

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Introduction

Elephants are the long-ranging migratory, endangered, intelligent, gregarious and largest terrestrial herbivores. For the conservation of this species globally, the Association of Zoo and Aquariums Elephant Taxon Advisory Group has accredited research to better understand the causes of deprived health, nutritional and welfare concerns of elephants (Keele and Ediger 2011). Only limited literature is available on the Asian elephant's nutrition in general and probiotics feeding in particular (Senthilkumar et al 2017; Chharang et al 2020). Due to the lack of data regarding elephants' digestive physiology and morphological likeness to that of the horse, the horse is considered a model animal to manipulate nutrient requirements and balancing diets for elephants (Oftedal et al 1996).

Constant nutritional scarcities diminish the immune system of the animal and predispose it to several pathogens (Das 2018). The mammalian gastrointestinal tract contains a complex, dynamic, and diverse community of non-pathogenic bacteria. Indeed, the supplementation of dietary probiotics can improve the balance and activities of these gut microflora via microbial production of metabolites (Chharang and Choudhary 2022). Probiotics like *Lactobacillus spp*., and *Saccharomyces spp*., generally aims the digestive compartments, principally small intestine and caecum-colon. It stabilizes the gut microbiomes and limits the exposure of enteric pathogen colonization. Fibre digestibility is increased in the colon, and it modulates the balance of hindgut microbiota through administration with live microbial cultures, consequently diminishing the risk of lactic acidosis, colic, diarrhea, and any other digestive disorders in the mammalian (Boucher et al 2024). It exerts advantageous health effects, through contributions to gut health as well as nutrients and energy utilization in the host animal (Chharang et al 2023). Wild animals in captivity are exposed to numerous sources of stress, such as constraint movement, compulsion proximity to humans, diminished feeding opportunities, lack of social groups, artificial lighting and annoying temperatures; hence, probiotics could be beneficial in captive animals (Morgan and Tromborg 2007).

Therefore, the objective of this study was to determine whether supplementation with dietary probiotics could impact the utilization of nutrients and energy in captive Asian elephants.

Material and Methods

The study was taken up in Elephant Village, Jaipur (India) (26°59'47"N 75°52'35"E) with prior approval of the Institute Animal Ethics Committee (PGIVER/IAEC/I9-05).

Animals and study design

Total eighteen healthy, adult captive female Asian elephants of 30 to 62 years of age, nearly similar body weight (3495 ± 133.34 kg), and uniform conformation were randomly selected and divided into three similar groups of six elephants each. The elephants were stall-fed a consistent feed of green pearl millet forage as basal feed throughout the research period of 60 days. Initially, an adaptation period of 10 days was observed, and then the elephants were placed for 50 days of the experimental feeding trial. Experimental probiotics, *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were administered at 1 gm 1 × 10^9 CFU /gm for every 50 kg body weight per day orally along with basal feed to all the elephants of T2 and T3 groups, respectively. The control group (T1) received no probiotic. Due to the similarities in gastrointestinal tract anatomy (Clauss et al 2003) of large hindgut fermenters like elephants, rhinos, and horses, the recommendations of the domestic horse (National Research Council 1989) have been suggested and extensively used as the suitable model for calculating probiotic requirements and designing a diet for elephants.

Digestibility of nutrients

A digestibility trial was conducted on all the experimental elephants during the last 5 days of the 50 days feeding trial to estimate the digestibility and utilization of nutrients and energy. A measured quantity of basal feed was offered to all the elephants, and residues were collected after 24 hrs, daily for chemical analysis. About 1/100th part of the offered feed was oven-dried and pooled for five days for each elephant. The dried samples were ground using a 1 mm mesh and used for further analysis.

Chemical analysis of feed and faecal samples

The ground samples of feed and faeces were analyzed for proximate principles and fibre fractions as per the standard procedures of the Association of Official Agricultural Chemists (AOAC 2016) and the method described by Van Soest et al (1991), respectively.

Estimation of gross energy

The gross energy (GE) content of feed and faecal samples was estimated using a fully automatic digital bomb calorimeter (Span Automation, Model: SABC-01). Various components of the energy balance profile were calculated using the following formulas;

- Metabolizable energy (ME) =Digestible Energy × 0.87 (Pagan and Hintz 1986)
- Energy Density =ME Intake/ DM Intake
- Energy requirement/ Maintenance Metabolic Rate = ($BW^{0.75}$ × 140 Kcal × 4.182)/ 1000 {To calculate the energy requirement for each elephant, the Kleiber formula was used (Dierenfeld 1994)}.
- Relative difference (%) =Offered energy (ME)/ MMR ×100

Table 1. Chemical composition of the experimental feed (per cent DM basis)			
Ingredients	T_1	T_2	T_3
Dry matter %	17.19	17.70	17.02
Organic matter %	87.70	90.63	91.47
Crude protein %	5.84	5.55	5.84
Ether extract %	1.60	1.29	1.07
Crude fibre %	36.67	37.24	36.96
Nitrogen free extract %	43.59	46.55	47.60
Total carbohydrates %	80.26	83.79	84.56
Total ash %	12.30	9.37	8.53
Acid-insoluble ash %	5.33	5.61	4.53
#Neutral detergent fibre %	69.40	69.15	71.00
##Acid detergent fibre %	46.45	44.96	47.42
Acid detergent lignin %	7.20	7.95	7.08
*Neutral detergent soluble %	30.60	30.85	29.00
**Hemicelluloses %	22.95	24.19	23.58
Cellulose %	36.36	34.02	34.70
NDF_{ash} %	1.80	2.00	1.78
ADF_{ash} %	2.89	2.99	2.64
Gross energy MJ/kg DM	18.33	18.79	19.84

#Neutral detergent fiber (NDF) is the most common measure of plant fiber (i.e. lignin, hemicellulose and cellulose) in animal feed analysis. ##Acid-detergent fiber (ADF) is the portion of fiber that is composed of cellulose and lignin. *Neutral detergent soluble was determined as; NDS (%) = 100- NDF (%). **Hemicellulose was determined as; HC = NDF – ADF

Statistical analysis

The experimental data were subjected to statistical analysis (SPSS version 24) using a one-way analysis of variance described by Snedecor and Cochran (2004). Significance was defined at $p < 0.05$. All the values represent mean ± standard errors of the mean. Difference between means was compared using Duncan's multiple range test.

Results

The chemical composition, which is usually considered as basic criteria for the assessment of the quality of the feed, was determined for the basal feed i.e., green pearl millet forage and probiotics, used for feeding elephants during this experiment. The chemical composition of the experimental basal feed was found to be nearly similar in all three groups (Table 1).

Utilization of nutrients

The average daily intake of proximate principles, fibre fractions and other gross nutrients expressed as per cent body weight was recorded during the digestibility trial (Table 2). Statistically, the observed mean values of all the proximate principles other than ether extract (EE) and total ash were found to be nearly similar in all three groups and could not reveal any significant effect of treatments. Higher intake for the proximate principles other than EE and total ash was observed in probiotics-fed groups as compared to the control group and supports the fact that the supplementation of probiotics in the basal diet increased the voluntary intake of these nutrients. The results of ether extract and total ash revealed a significant effect of treatments and indicated significantly lower mean values in elephants of the T2 and T3 groups as compared to the control (T1) group. Intake of fibre fractions such as NDF, ADF, ADL, NDS, hemicelluloses, cellulose, NDFash and ADFash, were found to be nearly similar in all three groups. Though the differences were non-significant, intake of NDF was recorded to be higher in the T3 group, followed by the T2 group and then in the T1 group whereas, the intake of ADF and cellulose was recorded to be higher in the T3 group, followed by the T1 group and then T2 group. In contrast, the intake of ADL and hemicelluloses was recorded to be higher in the T2 group, followed by the T3 group, and then in the T1 group. Higher intake was observed in probiotics-fed groups as compared to the control group. It is noteworthy to mention that the intake of dry matter in different groups was in accordance with the recommendation of the National Research Council (1989).

The statistical analysis of data obtained for the digestibility coefficients of proximate constituents except for total ash and acid-insoluble ash as well as various fibre fractions, as shown in Table 3, could not reveal any significant effect of treatments. The average values of the digestibility of total ash and acid-insoluble ash exhibited a highly significant effect of treatments.

Table 2. Average daily intake of proximate principles, fibre fractions and other gross nutrients in Asian elephants

Ingredients in (% BW)	T_1	T_2	T_3	Overall	*p* Value
Dry matter	1.73 ± 0.12	1.75 ± 0.19	1.74 ± 0.21	1.74 ± 0.10	0.999
Organic matter	1.52 ± 0.10	1.58 ± 0.17	1.59 ± 0.19	1.56 ± 0.09	0.944
Crude protein	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.974
Ether extract	0.03^b± 0.002	0.02^a ± 0.002	0.017^a ± 0.003	0.02 ± 0.002	0.044*
Crude fibre	0.64 ± 0.04	0.65 ± 0.07	0.64 ± 0.08	0.64 ± 0.04	0.986
Nitrogen free extract	0.76 ± 0.05	0.81 ± 0.09	0.83 ± 0.10	0.80 ± 0.04	0.812
Total carbohydrates	1.39 ± 0.09	1.46 ± 0.16	1.47 ± 0.18	1.44 ± 0.08	0.917
Total ash	0.21^b ± 0.01	0.17^a ± 0.02	0.15^a ± 0.02	0.18 ± 0.01	0.030*
Acid-insoluble ash	0.09 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.348
Neutral detergent fibre	1.20 ± 0.08	1.21 ± 0.13	1.23 ± 0.15	1.21 ± 0.07	0.982
Acid detergent fibre	0.81 ± 0.05	0.78 ± 0.09	0.82 ± 0.10	0.80 ± 0.04	0.937
Acid detergent lignin	0.12 ± 0.01	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.633
Neutral detergent soluble	0.53 ± 0.03	0.54 ± 0.06	0.50 ± 0.06	0.52 ± 0.03	0.891
Hemicelluloses	0.40 ± 0.03	0.42 ± 0.05	0.41 ± 0.05	0.41 ± 0.02	0.934
Cellulose	0.63 ± 0.04	0.59 ± 0.06	0.65 ± 0.08	0.63 ± 0.03	0.796
NDF_{ash}	0.031 ± 0.002	0.035 ± 0.004	0.031 ± 0.004	0.032 ± 0.002	0.624
ADF_{ash}	0.050 ± 0.003	0.052 ± 0.006	0.046 ± 0.006	0.049 ± 0.003	0.662

*Means superscripted with a different letter within a column differ significantly from each other

The average values of digestible nutrients such as % digestible crude protein (DCP), % total digestible nutrients (TDN), and nutritive ratio (NR) as well as the intake of digestible nutrients in terms of digestible dry matter, digestible organic matter, DCP and TDN intakes have been presented in Table 3. The statistical analysis of the data showed a non-significant effect on the practical nutritional worth and plane of nutrition. Though the differences were non-significant in statistical terms but apparently on observing the data, the mean values for TDN%, NR, digestible DM intake, digestible OM intake and TDN intake were obtained to be higher for probiotics-supplemented groups than the control group.

Utilization of energy

The intake of gross energy, digestible energy, metabolizable energy and energy losses (per cent BW) in faeces were recorded during the digestibility trial of the study in all the groups. The samples were analyzed for gross energy estimation, and data obtained were compiled for the calculation of the digestibility coefficients of gross energy for assessment of gross energy availability from the experimental feed. The results of the effect of supplementation of probiotics on intake and the digestibility coefficient of energy have been presented in Table 4 and the statistical analysis revealed non-significant differences among the groups due to treatment. Though the differences were non-significant but apparently on observing the data, the mean values were obtained to be higher for probiotics-supplemented groups than the control group.

The present study revealed an increased digestibility and intake of energy values with an increase in NDF digestibility and DM intake. However, it is noteworthy to mention that the results of the present study regarding energy intake in different groups were in accordance with the recommendation of the National Research Council (1989) as well as the findings of Koirala et al (2019).

The assessment of the energy balance profile to determine the effect of supplementation of probiotics has been presented in Table 4. The results of the statistical analysis of the data could not reveal any significant effect of the treatment. Though the differences were non-significant but apparently on observing the data, the mean values were obtained to be higher for probiotics-supplemented groups than the control group.

Discussion

The average values of NDF, ADL, NDS and ADF ash contents of basal feed were found nearly similar to that reported by Harinarayana et al (2005) whereas, the ADF and cellulose contents were reported to be higher than Harinarayana et al (2005). However, Stevenson and Walter (2006) quoted that the elephant's natural forage ranged between 13 to 62 per cent crude fibre and suggested that hays with an Acid Detergent Fibre (ADF) of over 30 per cent should be fed to the elephants to prevent colic. The dry matter and CP contents were found to be lower as compared to Harinarayana et al (2005). Chaves et al (2002) opined that lignin was the prime factor, which was associated with the digestibility of the plant cell wall material. The differences in DM, CP, ADF and cellulose might be due to agronomic conditions (Ball et al 2001). The gross energy value of pearl millet forage was found nearly similar to Civiero et al (2021).

The intake is probably the first and most important parameter in ascertaining the utilization of feed. Among

Table 3. Digestibility coefficient of gross nutrients, practical nutritional worth and plane of nutrition in Asian elephants

Ingredients	T_1	T_2	T_3	Overall	*p* Value
Digestibility coefficient					
Dry matter	56.10 ±2.99	58.27 ±2.73	57.85 ±3.19	57.40 ±1.63	0.862
Organic matter	54.36 ±3.40	58.25 ±2.90	58.22 ±3.45	56.94 ±1.82	0.634
Crude protein	75.73 ±2.59	72.10 ±3.76	76.39 ±0.92	74.74 ±1.53	0.494
Ether extract	64.41±3.46	55.43±4.28	47.64±9.77	55.83±3.88	0.219
Crude fibre	54.65±3.24	55.92±3.42	55.75±3.44	55.44±1.83	0.959
NFE	50.89±4.20	58.54±2.70	58.14±3.93	55.86±2.17	0.282
Total carbohydrates	52.61±3.70	57.38±2.96	57.09±3.69	55.69±1.95	0.563
Total ash	68.47^b ±2.54	58.47^a ±2.37	53.93^a ±2.21	60.29±1.96	0.002^{**}
Acid-insoluble ash	57.51^b ±2.94	61.02^b ±3.23	46.22^a ±2.38	54.92±2.18	0.006^{**}
NDF	49.70±3.72	53.37±2.90	53.83±3.95	52.30±1.98	0.673
ADF	48.07±3.66	45.62±3.55	48.75±4.46	47.48±2.15	0.840
Acid detergent lignin	23.08±6.87	30.91±4.89	21.40±7.20	25.13±3.61	0.546
Neutral detergent soluble	70.62±2.61	69.25±2.67	67.70±2.02	69.19±1.36	0.708
Hemicelluloses	52.99±6.45	67.78±2.31	64.04±3.19	61.60±2.81	0.074
Cellulose	54.58±3.72	49.97±3.81	55.46±4.35	53.34±2.23	0.586
NDF_{ash}	39.58±4.87	41.93±13.73	32.36±5.76	37.96±5.00	0.742
ADF_{ash}	28.34±5.92	35.30±5.36	26.15±1.96	29.93±2.74	0.387
Practical nutritional worth					
DCP%	4.42±0.15	4.00±0.21	4.46±0.05	4.30±0.10	0.091
TDN%	48.96±2.96	53.69±2.67	53.89±3.19	52.18±1.69	0.430
NR	10.11±0.68	12.52±0.65	11.07±0.67	11.23±0.43	0.064
Plane of nutrition					
Digestible DM intake	1.73 ± 0.12	1.75 ± 0.19	1.74 ± 0.21	1.74 ± 0.10	0.999
Digestible OM intake	1.52 ± 0.10	1.58 ± 0.17	1.59 ± 0.19	1.56 ± 0.09	0.944
DCP intake	0.08±0.01	0.07±0.01	0.08±0.01	0.07±0.00	0.863
TDN intake	0.86± 0.10	0.96± 0.15	0.96± 0.16	0.93± 0.08	0.848

*Means superscripted with a different letter within a column differ significantly from each other

the intake of estimated fibre fractions such as NDF, ADF, ADL, NDS, hemicelluloses, cellulose, NDFash and ADFash, importance was always paid to the lignin content in the feeds. In the present study, the differences in intake of EE and total ash may be attributed to the stage of harvesting and soil contamination of green forage.

In agreement with this study, Morgan et al (2007) observed non-significant effects of yeast supplementation on DM, NDF and ADF intake in horses. In other studies, no differences were also evidenced in total per cent feed intake in S. cerevisiae-fed horses (Agazzi et al 2011) and *L. acidophilus*-fed rabbits (Bhatt et al 2017).

Contrary to the above findings, Jouany (2008) found that the addition of yeast in diets had positive effects on DM, OM, NDF and hemicelluloses in horses whereas, its negative effect on NDF intake negatively affected fibrolytic bacteria in the rabbits (Campos-Morales et al 2015). Iwu et al (2015) also observed significantly increased daily feed intake in probiotics-phytase mixture-fed Californian rabbits.

Besides the physiological form, quantitative and qualitative attributes of the feed, the digestibility is also considered to be important in assessing the nutritional worth of the feed. The differences in the apparent digestibility of total ash and acid-insoluble ash may be accounted for soil contamination of green forage, type of probiotics microorganisms, intestinal microbiota condition, etc. Though the differences were non-significant but the apparent digestibility of DM, OM, CF, NFE, total carbohydrates, NDF and hemicelluloses, were obtained to be higher whereas, the apparent digestibility of EE and NDS were obtained to be lower for probiotics-supplemented groups than the control group. The trend in NDF digestibility in this study suggests that live yeast has been observed to enhance fiber digestion.

In agreement with the present study, Jounay et al (2008) found that yeast supplementation had no significant effect on the digestibility of dietary components such as DM, OM, CP, NDF, cellulose and hemicelluloses in horses however; it increased the digestibility in the whole digestive tract of the ADF fraction significantly. Similarly, Swyers et al (2008) showed that supplementation of *L. acidophilus* and

Table 4. Average intake, digestibility coefficient and balanced profile of energy in Asian elephants

Components	T_1	T_2	T_3	Overall	*p* Value
Energy intake per cent BW					
GE intake	31.75 ± 2.13	32.75± 3.53	34.49 ± 4.13	33.00 ± 1.85	0.846
DE intake	19.37 ± 2.00	20.87 ± 3.28	22.29 ± 3.49	20.84 ± 1.65	0.790
ME intake	16.85 ± 1.74	18.16 ± 2.85	19.40 ± 3.03	18.13 ± 1.44	0.790
Digestibility coefficient of energy					
GE	60.32 ± 2.74	62.47 ± 3.08	63.28 ± 2.85	62.02 ± 1.60	0.760
Energy balance profile					
Energy offered ME MJ/Day	580.30 ± 79.38	619.84 ± 84.74	675.56 ± 108.11	625.23 ± 50.57	0.765
DM intake kg/day	59.26 ± 5.64	59.71 ± 5.28	60.60 ± 6.79	59.84 ± 3.22	0.986
Energy density ME MJ/kg DM	9.62 ± 0.44	10.21 ± 0.50	10.92 ± 0.49	10.25 ± 0.29	0.191
Required MMR ME MJ/Day	261.30 ± 12.46	265.19 ± 11.10	270.42 ± 17.55	265.64 ± 7.64	0.899
Relative difference %	219.89 ± 23.66	236.64 ± 35.73	253.13 ± 37.41	236.55 ± 18.11	0.777

a mixture of *L. acidophilus, L. casei, Bifidobacterium bifidum,* and *Enterococcus faecium* had no effect on the digestibility of DM, OM, CP, NDF and ADF but the significant effect was seen on EE digestibility in horses. In growing rabbits, Oso et al (2013) reported that the apparent nutrient digestibility values of DM, OM, EE, CP, NDF and ADF were not affected by dietary inclusion of probiotics. Stercova et al (2016) observed that the digestibility of DM, total ash, crude fibre, CP, and fat were not influenced, whereas the apparent digestibility of NDF was observed to be highly significant in live yeast-supplemented dogs. Wang et al (2023) noticed no interactive effects of probiotics in the nutrient digestibility in weaning pigs.

On the contrary, Morgan et al (2007) observed that yeast supplemented horses trended to have greater DM, CP, NDF, hemicelluloses and cellulose digestibility. There was no difference in the apparent digestibility of ADF. Lizardo et al (2012) found a favourable effect of live yeast supplementation on NDF degradation in piglets. In another study, Senthilkumar et al (2017) noticed statistically highly significant variations in DM digestibility before and after the supplementation of a probiotics mixture of Lactobacillus and Bifiodobacterium in adult elephants whereas; they also observed significant variations between young and adult elephants.

Similarly, Phuoc and Jamikorn (2017) in rabbits and Daraaz et al (2018) in sheep observed non-significant effects on per cent DCP and TDN. However, later noticed significantly lower values of NR and higher values of intake of digestible nutrients such as digestible DM intake, digestible OM intake, DCP intake and TDN intake in terms of gm/day, whereas values when expressed in percentage of body weight and/kg $W^{0.75}$, could not reveal any significant difference between the experimental groups.

On the contrary to these results, Senthilkumar et al (2017) observed statistically a highly significant effect and significant variations in digestible DM intakes on supplementation of a probiotics mixture in adult elephants and between the selected age groups of elephants, respectively.

The results of the present study coincide with Morgan et al (2007); Wang et al (2023) who found non-significant differences in energy intake and the digestibility of gross energy as a result of probiotics-supplementation in horses and weaning pigs, respectively. In contrast to the present results, Phuoc and Jamikorn (2017) recorded improvements in the digestibility of energy in *L. acidophilus*-fed rabbits, whereas Campos-Morales et al (2015) observed that supplementation of S. cerevisiae negatively affected the digestibility of energy in rabbits.

No reliable equations for estimating maintenance energy requirements for elephants are available. The allometric functions, derived from Kleiber formulas for placental mammals, are often used as a reference to estimate the maintenance energy recommendations for elephants. Energy density is the amount of energy per kg of feed whereas, the relative difference is the difference between the offered (ME) and required energy (MMR). If the per cent relative difference is 100%, it means the amount of energy offered is the same as the amount of energy required (MMR) and it is considered the optimal situation (Baarlen and Gerritsen 2012). The closer the relative difference is to 100%, the more the offered amount matches the requirement.

The present study showed that per cent relative differences were associated with increased DM intake and NDF digestibility. Overall, all the elephants were trending with higher per cent of the relative difference. Simplistically stated, more than 100 per cent of the relative difference will result in body weight gain in elephants; this might be attributed to a greater amount of feed offered and lesser energy expenditure due to the captive nature of elephants. It is noteworthy to mention that the result of this study regarding energy density in different groups was in accordance with the husbandry guidelines of elephant nutrition (Dierenfeld 1994). Kristensen et al (2014) demonstrated no effect, whereas Leicester (2015) observed a significant positive effect on energy balance in yeast-based DFM-supplemented high-producing cows.

Conclusion

It is concluded that the supplementation of dietary probiotics could not exhibit any significant effect on the utilization of nutrients and energy; practical nutritional worth; plane of nutrition and energy balance profile in the Asian elephant. However, the intakes of EE and total ash revealed a significant whereas the digestibility coefficients of total ash and acid-insoluble ash revealed a highly significant effect of treatments. Increasing trend of intake and the digestibility of energy; and the relative difference was associated with increased DM intake and NDF digestibility. TDN%, NR, digestible DM and OM intakes and TDN intake were obtained to be higher for probiotic-supplemented groups. It is suggested that further research needs to be conducted to solidify the results and examine the effect of larger doses, strains and viability of the probiotics in the land's largest living mammalian.

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Conflict of Interest

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Ethical Approval

The authors confirm that the ethical policies of the journal, have been adhered to and the appropriate institute animal ethics committee approval has been received (PGIVER/IAEC/I9-05).