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Comparative evaluation of wheat roti or rice–lentil mixture as supplements for growing Asian elephants *(Elephas maximus)*

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Abstract
The feeding of supplements that supply all nutrients in optimum amounts to support normal growth is of paramount importance when feeding growing elephants in captivity. This experiment evaluated wheat roti (WR) and a rice–lentil mixture (RLM) as supplements for growing semi-captive Asian elephants. Six sub-adult (7–12 years; 1327–3288 kg) elephants were split into two groups of three each. The study period was divided into two, and each group received both supplements in a switch-over design. Elephants in treatment I and II received concentrate supplements consisting of WR and RLM respectively. They were allowed to forage in the nearby forests from 0530 to 1730. Cut grasses (narkul, *Arundo donox*) were offered ad lib (20% excess of previous day's intake) to each animal from 1730 to 0530 when they were in their enclosures. As animals were in enclosures during the night, faeces of each individual elephant could be collected separately, and elephants had no access to one another's feeds. Faeces voided during foraging were collected in full, representative samples of forages consumed were taken and intake during foraging was measured indirectly using lignin as a marker. Intake of cut grasses and forages were similar between the treatments. Apparent digestibility of CP was higher (P<0.05) in treatment II. Apparent digestibility of other nutrients was comparable in both the treatments. Intake of zinc was higher in treatment II. Intake, excretion and absorption of Ca, Co, and Cu did not differ between the treatments. Absorption of P and Fe was higher (P<0.05) in treatment II. Blood metabolites, serum enzymes activities and concentration of serum minerals were similar among the treatments. The relative population of total bacteria was numerically increased in treatment II. The results of this experiment demonstrate that RLM may be better supplement for growing elephants than WR.

Introduction

Asian elephants spend about 80% of their time feeding on grasses (Sivaganeshan, 1991; Ruggiero, 1992). Dry matter intake varies from 1–1.5% body weight (BW) in adult and up to 2% BW or more in young elephants (Ullrey et al. 1997; Loehlein et al. 2003). Mean retention time (MRT) is less and passage rate is higher in elephants than other herbivores, contributing to lower digestive efficiency (Loehlein et al. 2003). The elephant has a capacious caecum and colon where fermentation of foodstuffs takes place. Various bacteria, protozoa and fungi are present in the hindgut and help in the degradation of fibre; the type and population of microflora is influenced by diet (Cerling et al. 1999). Jenson (1986) reported that Asian elephants can derive 100% of their maintenance energy from volatile fatty acids produced in the hind gut.

Elephants require large amounts of food because of their large body size. Forages are the main component of elephant diets in most captive facilities in India (Arora 2001, Das et al., 2014), but availability and nutritional quality of forages depend on the season (Sukumar 1990). Forages consumed by elephants during the dry season are of poor quality, which is characterised by low digestibility. These forages could be deficient in one or more nutrients, and thus, deficiencies of nutrients in the diets of elephants are possible. Nutrient deficiencies or imbalances of nutrients in poor quality forages can be detected through laboratory analysis of a representative sample. These deficiencies can then be balanced with the provision of specific supplements, such as limited amounts of concentrate feeds or area-specific mineral supplements. Improper feeding of concentrates, however, may lead to obesity, chronic foot lesion and other health-related problems (Clauss et al. 2003).

The most common supplement fed to captive Asian elephants consists of various amounts of wheat roti (WR), boiled rice, finger millet or other cereals. Some pulses are fed, but not in all facilities (Arora 2001; Das et al. 2013, 2014). In most captive facilities adult and sub-adult elephants are fed on same supplement. As the nutrient requirements for adult and sub-adult elephants are different (Ullrey 1997), however, feeding the same supplement to both age groups may not be appropriate. As the requirement for crude protein (CP) is higher in growing elephants, it would be desirable to include some cereals or pulses in their diet. In the wild, elephants meet their protein requirement solely from forages when quality forages are available in abundance (Sukumar 1990). In the dry season, when forage quality is inferior, elephants raid cereal crops to meet their nutrient needs (Sukumar 1990). Given this craving of elephants for cereals during dry seasons, it seems logical to add some cereals or pulses to the diet of captive elephants. However, while free-ranging elephants have the option to select nutritious food from a wide range of plants and their parts, captive elephants have limited opportunities for diet selection, and the magnitude of any deficiencies or imbalances in nutrients may be higher compared to their wild counterparts.

Choosing the right kind of supplement that can supply all nutrients in the proper amounts to support normal growth in young elephants is of paramount importance. The specific objective of this experiment was therefore to evaluate wheat roti (WR) or a rice–lentil mixture (RLM) as a supplement for sub-adult elephants in captivity.

Methods

This experiment was conducted at Dudhwa National Park, Lakhimpur Kheeri, Uttar Pradesh, India (152–183 m asl; 28°18′–29°42′N, 80°28′–80°57′E). The average temperature was 32–40º C during the period of the study. All the animals were dewormed with albandazole (Cadila Pharmaceuticals Private limited, India) at 7.5 mg/kg BW, one month prior to the beginning of the trial. Proper management and sanitary guidelines as suggested by the Central Zoo Authority were followed during the course of the study (CZA, 2000).

Six sub-adult elephants (7–12 years of age; 1327–3288 kg BW) were randomly allocated into two groups of three each. The experiment used a switch-over design with two time periods, two treatments and three animals per treatment during each period. Two feeding trials of 30 days adaptation and 5 days of collection period were conducted.

Elephants received either WR (treatment I) or RLM (treatment II) as a supplement. Wheat roti was prepared by mixing 4 kg of wheat flour and 150 g of common salt in 2 litres of water to make dough. The dough was spread into a thick (~2–3 cm), circular (~20–25 cm diameter) pancake, which was baked over a flame. About 500 g of jaggery was provided to each of the elephants along with WR. Rice–lentil was mixed in a proportion of 2:1 and was soaked in water for 12 h. The diets were switched over after 35 days.

All the elephants were provided with night shelters (10×8×6 m) from 1730 to 0530. This facilitated the collection of individual elephants' faeces separately and elephants had no access to the feeds of others. Cut grass (narkul, *Arundo donox*) was offered *ad libitum* to all the elephants during the night.

The feeding cycle started at 0530 when elephants were hand-fed 4 kg of the appropriate supplement. After that they were allowed to forage in the nearby grasslands/forests, returning by 1300. The elephants were allowed to bathe from 1300 to 1500, and then were again allowed to forage till1730. After this final foraging session, all elephants were returned to their individual night shelters. At 1930 elephants were handfed the remaining portion (2 kg) of their respective supplement.

After a preliminary period of 30 days, a digestion trial over a 5-day collection period was conducted. Faeces voided from 1730 to 0530 (when all elephants were in their individual night shelters) was collected directly and measured. When animals were out (either to forage or bathe), they were accompanied by one researcher and a mahout. Whenever an elephant defaecated, the mahout dismounted to collect all the faeces voided. The researcher then measured, noted and sampled the faeces. Faecal samples for analysis were collected with care after removing the outer layer of dung to avoid any soil contamination. Thus, the total volume of faeces voided for 24 h was collected.

We accurately sampled the forages consumed by each of the elephants during the daily periods of foraging using the "trunk grab" method. Sampling was done at 10 min intervals and each day, 25–30 "plucks" were collected directly from the trunk before the elephant put the forage into its mouth. An aliquot (1/10) of the "pluck" was sampled to calculate intake and the remainder was returned to the elephant.

Feed consumption during foraging was measured indirectly by using lignin as an internal marker. The amounts of cut grass and concentrate offered to the elephants and their refusals were measured directly.

Body measurements were taken on days 0, 30 and 35 of the trial (during both trials) to calculate body weight as per Hile et al. (1997) using the formula: Body weight (kg) = 18.0 × Heart girth (cm) – 3336. From this, average daily gain (ADG) was calculated.

Pedometers were used to monitor the distance travelled during each foraging day. Pedometers were calibrated regularly against a known distance.

For both periods, blood was collected from all animals on day 35 (at the end of the digestion trial) at 0500 by puncturing an ear vein. Serum was separated and stored at -20°C in clean sterile vials and analysed for serum Ca and P, blood metabolites and enzymatic profile using commercial diagnostic kits (Span Diagnostics Ltd, Surat, India). All samples of feed, forages, faeces and refusals were dried at 100±2º C to determine the dry matter (DM) content. At the end of each trial, the individual animals' feeds, refusals and faeces were pooled across the 5-day collection period, ground and stored for further analysis. The ground samples of feeds, refusals and faeces were analysed for organic matter (OM), crude protein (CP) and gross energy (GE) as per the method described in AOAC (2005). Samples were also analysed for neutral detergent fibre (NDF) and acid detergent fibre (ADF) as per the method described by Van Soest et al. (1991). Acid detergent lignin (ADL) was determined according to the method of Van Soest et al. (1991) using 72% (w/w) H_2SO_4 as an oxidising agent. Residual ash was subtracted from ADL. Hemicellulose was calculated as the difference between NDF and ADF. Similarly, cellulose was calculated as difference between ADF and lignin. Estimation of Ca and P in feed samples was done as per the methods of Talapatra et al. (1940) and AOAC (2005), respectively. Concentration of Co, Cu, Fe and Zn in feed, faeces and serum samples were determined by using an atomic absorption spectrophotometer (AAS 4141, ECIL, Hyderabad, India) following the wet digestion method (Kolmer et al. 1951).

Gut microbes were quantified by real time PCR (q-PCR) using faecal genomic DNA as a template (Denman and McSweeney, 2005; Chen et al. 2008; Agarwal et al. 2008) using KAPA SYBR® Fast qPCR Master Mix (®KAPA Biosystems) for SYBR Assay and specific primers for total bacteria, *Fibrobacter succinogenes, Ruminococcus flavefaciens,* methanogens and total fungi. PCR reaction was carried out in real time PCR (Biorad, Applied Biosystems, Hercules, CA 94547, USA) programmed as denaturation of DNA at 95º C for 10 min, followed by 40 cycles of 30 sec each for denaturation at 95º C, annealing at 60º C and extension at 72º C. The amplified product specificity was determined by dissociation curve obtained by the cycle of 2 min at 95º C, 15 sec at 60º C and 15 sec at 95º C. The results were presented as a change in microbial populations using the $2^{-\Delta\Delta Ct}$ (threshold cycle) method relative to control, taken as one (Livak and Schmittgen 2001).

Table 1. Nutritional characteristics of feeds and forages fed to semi-captive Asian elephants during both treatment periods.

	Period 1				Period 2			
	Narkul	RLM	WR	Forages	Narkul	RLM	WR	Forages
DM (%)	28.30	60.2	68.80	36.70	28.80	58.60	64.00	37.35
Nutrient content (%DM)								
OM	90.42	95.82	96.47	89.28	89.71	95.22	97.15	90.04
CP	7.45	20.14	11.42	9.67	7.32	19.84	10.82	9.58
EE	1.55	3.32	2.30	1.64	1.80	3.14	2.10	1.40
NDF	66.12	26.0	30.13	62.14	65.44	24.85	29.20	63.03
ADF	40.83	19.82	17.28	44.56	38.33	18.34	16.72	44.76
Hemicellulose	25.3	6.18	12.85	17.68	27.11	6.51	12.48	18.27
Cellulose	26.33	11.30	14.52	32.56	26.33	13.52	13.30	32.56
Ca (%)	0.52	0.14	0.25	0.62	0.60	0.12	0.24	0.68
P (%)	0.24	0.97	0.68	0.27	0.23	0.95	0.64	0.23
Mineral content (ppm)								
Co	3.78	12.04	3.34	4.88	3.84	3.12	11.82	5.63
Cu	8.72	14.02	16.40	9.88	8.54	15.41	16.78	9.23
Fe	83.14	34.76	53	98.56	88.27	31.50	50.20	105.48
Zn	13.88	78	46	32.20	12.02	74.10	41.34	35.33
GE (kcal/kg DM)	3530.04	4053.47	4238.76	3642	3418	4215	4088	3684

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; GE, gross energy; ppm, parts per million; WR, wheat roti; RLM, rice–lentil mixture.

Data obtained were analysed for a switch-over design (Snedecor and Cochran 1989) and treatment means were separated by applying Tukey's test using the SPSS software package, version 13 (SPSS, Chicago, IL, USA). Various microbial populations in the samples were analysed considering total bacteria as a 'house keeping gene'. Gene expression level = $2^{-\Delta\Delta Ct}$, ΔCt of the samples were calculated as per Livak and Schmittgen (2001).

Results

The chemical compositions of narkul, forages and concentrates offered were similar during the two experimental periods (Table 1). The RLM was characterised by higher CP, GE and mineral contents. Composite samples of forages consumed contained 9.67% CP and 3642 kcal/kg DM of GE. Narkul was characterised by lower CP, GE and higher NDF content. Intake (kg/d) of narkul and forages was similar in both the treatments (Table 2). Consequently, total dry matter intake (DMI) was comparable among the treatments. Apparent digestibility of CP was higher ($P<0.05$) in elephants fed RLM. Apparent digestibility of other nutrients was comparable in both the treatments.

In this study, all elephants were allowed to forage for 8 h/d during which elephants in treatments I and II covered 14±2 and 13.8±3 km respectively, as measured by pedometer. Intake, excretion and absorption of Ca, Co and Cu did not differ significantly between the treatments. Absorption of P and Fe was greater ($P<0.05$) in RLM-fed elephants compared to WR-fed elephants (Table 3). Blood metabolites, serum enzymes activities and concentration of serum minerals were not altered by feeding the different supplements (Table 4). The relative population of total bacteria was numerically increased 10.25-fold in RLM-supplemented elephants. The relative populations of *Ruminococcus flavefaciens* and total fungi were also increased numerically by 1.18 and 1.02-fold, respectively, in elephants supplemented with RLM. The relative populations of *Fibrobacter succinogenes* and methanogens were, however, numerically reduced in elephants supplemented with RLM.

Discussion

The rice–lentil mixture fed to the Asian elephants in this study contained more proteins and micro-minerals (Combe et al. 2004) than the wheat roti. Composite samples of forage materials contained moderate amounts of CP and energy. Nutrient and mineral concentrations of forages are known to differ (Minson 1990) according to season and location (Karadavut and Palta 2010). However, we did not observe any change in chemical composition of composite forages during the experimental periods. Nutrient composition remains similar during a particular season of the year (Watts and Chatterton 2004), and as both trials were conducted in the same season, similar nutrient composition was expected. The NDF content of the cut grass narkul (*Arundo donox*) observed during the present experiment was higher than the value reported previously for the same grass (Coulson et al. 2004). This could be attributed to stage of maturity (Arthington and Brown 2005); the narkul used in this experiment was cut at maturity in contrast to growing stage in the earlier study, resulting in more NDF content.

Average daily dry matter intake (DMI) ranged from 1.15 to 2.81% BW, very similar to the range of 1.13 to 2.57% BW reported in sub-adult Asian elephants fed hay-based diets (Clauss et al. 2003). From the results of the present experiment and from a review of the literature it seems that sub-adult elephants consume more DM than adults (Ullrey et al. 1997; Loehlein et al. 2003). Feeding of RLM resulted in more CP intake in elephants as lentils contain higher CP (Costa et al. 2006) than WR. This increased CP intake was also reflected in increased apparent digestibility of CP in elephants fed RLM. The digestibility of nutrients is influenced by various factors such as age, level of concentrates, fibre content in diets, environmental temperature (Jorgensen et al. 1996), rate of

Table 2. Effect of different types of supplement on nutrient intake and diet digestibility in semi-captive Asian elephants.

	Treatments			
	I	II	SEM	p-value
Feed consumption (kg/d)				
Grass	17.25±0.74	17.47±0.95	0.590	0.851
Concentrates	3.39±0.05	3.26±0.04	0.036	0.070
Browses	21.50±0.17	22.10±1.05	0.526	0.583
Total DMI	42.13±0.82	42.83±1.73	0.937	0.720
DMI (%BW)	2.08±0.15	2.14±0.14	0.102	0.763
Nutrient intake (g/kgBW$^{0.75}$)				
DM	138.78±7.78	141.96±6.41	4.942	0.755
OM	126.28±7.10	129.10±5.85	4.509	0.762
CP*	13.82±0.74	15.39±0.79	0.472	0.047
NDF	82.68±4.63	83.80±3.65	2.883	0.851
ADF	56.27±3.13	58.05±2.69	2.029	0.672
Hemicellulose	26.64±1.54	25.98±1.04	0.910	0.724
Cellulose	40.25±2.26	41.23±1.84	1.429	0.739
EE	2.42±0.14	2.60±0.12	0.092	0.325
GE (kcal/kg BW0.75)	503.32±28.06	514.30±23.50	17.933	0.767
DE (kcal/kg BW0.75)	227.68±13.67	236.88±10.50	8.483	0.599
Digestibility of nutrients (%)				
DM	44.12±0.69	45.17±1.15	0.667	0.441
OM	45.81±0.69	47.17±1.09	0.648	0.304
CP*	47.31±0.43	50.72±0.86	0.616	0.012
NDF	34.91±0.75	35.75±1.38	0.774	0.600
ADF	34.57±1.04	35.58±1.74	0.997	0.625
Hemicellulose	36.24±0.83	36.80±0.86	0.589	0.647
Cellulose	32.37±1.10	33.63±1.65	0.980	0.532
EE	61.00±0.44	62.22±0.73	0.436	0.167
GE	45.18±0.78	46.16±0.75	0.537	0.371
Nutrient content of diet (%)				
CP*	9.95±0.06	10.82±0.12	0.111	0.001
NDF	59.57±0.18	59.08±0.22	0.148	0.094
Ca	0.58±0.01	0.57±0.01	0.007	0.669
P**	0.28±0.004	0.30±0.006	0.004	0.003
Co (ppm)**	4.55±0.052	5.18±0.055	0.076	0.001
Cu (ppm)	9.73±0.054	9.59±0.063	0.043	0.106
Fe (ppm)	91.31±0.86	90.07±0.93	0.634	0.341
Zn (ppm)**	26.11±0.27	28.58±0.32	0.329	0.001
Body weight (kg)				
Initial	2150.31±183.9	2117.20±195.9	131.450	0.903
Final	2166.00±183.9	2133.00±195.8	131.446	0.905
ADG (g/d)	448.00±0.001	451.04±0.009	0.005	0.762

Means (±standard error); *P<0.05; **P<0.01; SEM, standard error of mean; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; GE, gross energy; DE, digestible energy; ppm, parts per million; ADG, average daily gain; elephants in treatment I received the wheat-roti supplement and in treatment II the rice–lentil mixture.

passage and mean retention time (Clauss et al. 2007) and exercise (Katole et al. 2014). The amount of concentrate offered in both the treatments was similar; the proportion of supplement was 7.6 and 8.1% of total DMI in elephants fed WR and RLM, respectively. It was therefore unlikely that passage rate, mean retention time and digestibility of nutrients would differ between WR and RLM fed elephants.

When protein content of the diet is very low, DM digestibility has been reported to be low. In this experiment, CP content of WR was lower than RLM. However, WR was able to supply the minimum CP recommended for optimal fibre degradation (Leng 1990). Thus, similar digestibility of nutrients observed in both the treatments was expected. Digestibility of CP was higher in RLM-fed elephants, which might be partly attributable to the higher CP content of lentils and higher intake of CP (15.4g/kgBW$^{0.75}$; Table 2) in elephants fed on RLM. Pulses (lentils) contain 20% CP, which is almost double that of wheat. Utilisation of protein of pulses is also higher than cereals (Naik and Gleason 1988). It is evident that RLM is a better supplement for growing elephants than WR, because RLM not only provided more protein but also improved its utilisation.

The precise energy requirements of captive Asian elephants are not known. Hence, we used the energy requirement of horses to test the adequacy of the diet. The digestible energy (DE) requirement for maintenance of horses is reported to be 144 kcal/kgBW$^{0.75}$/d (Meyer and Coenen 2002). Energy is also required for locomotion during foraging (Lachica and Aguilera 2005). Net energy (NE) expenditure in elephants during walking is 0.186 cal/kgBW/m (Langman et al. 1995). Using a conversion factor of 0.80 from ME to NE (McDonald et al. 2002) and 0.90 from DE to ME (Robbins 1993; Reid and White 1978; Pagan and Hintz 1986), the DE requirement for grazing elephants would be 0.258 cal/kgBW/m. Applying this factor to the BW of the animals in the present experiment, elephants fed WR and RLM would require 24.86 and 24.45 kcal DE/kgBW$^{0.75}$, respectively, for locomotion in addition to their maintenance requirement.

In the present experiment, ADG was 448 and 451 g/d in treatments I and II, respectively (Table 2). The DE requirement for an ADG of 450 g is reported to be 10,256 kcal for horses (NRC 1989). It is assumed that an elephant growing at the same rate would require the same amount of DE. If we divide 10,256 kcal by respective metabolic body size (315.28 and 311.07 kgBW$^{0.75}$), the DE requirement for growth would be 32.53 and 33 kcal/kgBW$^{0.75}$ in treatments I and II, respectively. Thus, the calculated DE (kcal/kgBW$^{0.75}$) requirements would be 201.39 (144+24.86+32.53) and 201.46 (144+24.45+33) for treatments I and II, respectively. Elephants fed on WR and RLM supplemented diets consumed 227.68 and 236.88 kcal DE/kgBW$^{0.75}$, respectively. Both the diets therefore supplied adequate amounts of DE.

Similar to horses, bioavailability of Ca from forages seems to be higher in elephants (Schryver 1975; Kienzle and Zorn 2006) and the addition of concentrates to the diet does not influence its absorption. Concentration of serum Ca was also similar in both treatments. The present results are in accordance with earlier findings that indicated that plasma Ca was not affected by concentrate feeding in horses (Cymbaluk and Christison 1989). Serum concentration of Ca was, however, within the normal physiological range reported for Asian elephants (Fowler 1986; Tuntasuvan et al. 2002). Both the diets supplied adequate Ca to meet the minimum recommended requirement of 0.5–0.7% for growing elephants (Ullrey et al. 1997). Trees and forages are the main components of semi-captive elephants' diets in most of the National Parks (Arora 2001) and are rich sources of Ca. Thus, in practical feeding situations, Ca deficiency is unlikely to occur in semi-captive elephants. Intake and apparent absorption of P was higher in RLM-fed elephants compared to WR-fed elephants.

Table 3. Effect of different types of supplement on minerals intake, excretion and apparent absorption in semi-captive Asian elephants.

	Treatments			
	I	II	SEM	p-value
Calcium (Ca)				
Intake (mg/kg BW)	121.03±9.50	122.13±7.19	5.828	0.927
Excretion (mg/kg BW)	75.93±6.17	77.08±5.67	4.096	0.893
Apparent absorption (%)	37.36±0.68	37.45±1.60	0.853	0.960
Phosphorus (P)				
Intake (mg/kg BW)*	57.50±3.97	64.61±5.22	3.291	0.029
Excretion (mg/kg BW)	41.16±2.87	45.93±3.79	2.376	0.327
Apparent absorption (%)*	28.42±0.43	29.02±0.51	0.330	0.040
Cobalt (Co)				
Intake (µg/kg BW)	94.72±7.20	111.07±7.31	5.299	0.125
Excretion (µg/kg BW)	78.63±6.01	92.73±6.19	4.466	0.116
Apparent absorption (%)	16.90±0.66	16.56±0.43	0.385	0.671
Copper (Cu)				
Intake (µg/kg BW)	202.33±14.31	206.10±14.60	10.005	0.855
Excretion (µg/kg BW)	177.90±12.87	182.99±13.44	9.116	0.787
Apparent absorption (%)	12.19±0.35	11.37±0.43	0.285	0.153
Iron (Fe)				
Intake (µg/kg BW)	1902.23±143.37	1923.36±118.06	90.847	0.910
Excretion (µg/kg BW)	1737.72±130.03	1736.00±105.05	81.744	0.992
Apparent absorption (%)*	8.59±0.14	9.67±0.23	0.174	0.038
Zinc (Zn)				
Intake (µg/kg BW)*	543.48±30.14	614.36±33.78	21.971	0.045
Excretion (µg/kg BW)	481.17±35.82	537.82±39.07	26.586	0.297
Apparent absorption (%)	11.52±0.29	12.57±0.29	0.228	0.076

Means (±standard error); *P<0.05); *(P<0.01); SEM, standard error of mean; elephants in treatment I received the wheat-roti supplement and in treatment II the rice–lentil mixture.

Lentils are a fairly good source of phosphorus (Ravindran et al. 1994) and usually contain more available P than cereals (Yadav et al. 2007). Porres et al. (2004) also reported that lentil feeding increased intake and net absorption of P in growing rats. This improved intake and absorption of P was, however, not reflected in serum P concentration. Serum concentration of Ca and P is a hormonally regulated process (Potts and Gardella 2007; Peacock 2010). Serum concentration of P in both the treatments was within the normal physiological range reported for Asian elephants (Fowler 1986; Tuntasuvan et al. 2002). The phosphorus content of the diet of elephants fed WR was unable to supply an adequate amount of P to fulfill the recommended requirement of 0.3% (Ullrey et al. 1997), but the RLM supplied sufficient P to reach this requirement.

Intake, excretion, apparent absorption and serum concentration of Co and Cu were similar in both the treatments. Serum concentrations of Co and Cu were within the normal range reported for Asian elephants (Fowler 1986). Ullrey et al. (1997) recommended 0.1 ppm Co and 10 ppm Cu as a minimum maintenance requirement for young elephants. Both the diets supplied adequate amounts of Co and Cu to meet the recommended requirement. Lentils are a good source of Cu, Fe and Zn (Iqbala et al. 2006; Erskine et al. 2009).

Absorption of Fe was greater in RLM-fed elephants compared to WR-fed elephants. Intake of Zn was also more in elephants fed on RLM, which might be attributed to higher Zn content of RLM, but excretion and apparent absorption of Zn was similar in both the treatments. Serum concentrations of Fe and Zn were comparable in both the treatments and were within the normal ranges reported for Asian elephants (Fowler 1986). The iron content of both the diets was sufficient to supply the minimum maintenance requirement of 50 ppm (Ullrey et al. 1997).

Zinc content was 28 and 26 ppm in the diets of elephants fed RLM and WR, respectively. Neither of the diets therefore fulfilled the requirement of 40 ppm recommended for young elephants (Ullrey et al. 1997), but research conducted on Zn requirements of hindgut fermenters does not agree with such high levels of dietary Zn (Kienzle and Zorn 2006). Irrespective of these controversies, the higher Zn supply in RLM-supplemented elephants could be beneficial for normal growth, immune function and hoof health. However, it would be desirable to include a supplementary source of Zn in the diet.

The concentration of haemoglobin was similar in both the treatments and was within the normal ranges reported for Asian elephants (Fowler 1986; Weerakhun et al. 2010). Research conducted previously has indicated that feeding of lentils does not

Table 4. Effect of different types of supplement on blood biochemical, enzymatic and serum mineral profile in semi-captive Asian elephants

	Treatments		SEM	p-value
	I	II		
Serum metabolites				
Haemoglobin (g%)	11.71±0.17	12.10±0.14	0.122	0.102
Glucose (mg/dl)	70.33±3.05	69.00±2.42	1.868	0.739
Total proteins (g/dl)	8.30±0.13	8.59±0.14	0.102	0.156
Albumin (g/dl)	2.17±0.07	2.37±0.18	0.094	0.325
Globulin (g/dl)	6.28±0.25	6.18±0.20	0.154	0.759
A:G ratio	0.35±0.02	0.39±0.04	0.024	0.456
Urea (mg/dl)	24.04±0.89	23.88±0.48	0.509	0.884
Cholesterol (mg/dl)	72.35±2.20	71.69±1.18	1.194	0.798
Creatinine (mg/dl)	1.33±0.12	1.32±0.07	0.065	0.915
Serum enzymes				
ALT (IU/l)	10.24±0.52	10.09±0.65	0.396	0.862
AST (IU/l)	47.42±1.47	43.55±1.88	1.279	0.136
ALP (IU/l)	89.66±5.42	90.03±5.94	3.834	0.964
Serum minerals				
Ca (mg/dl)	11.11±0.28	10.57±0.36	0.235	0.271
P (mg/dl)	5.38±0.14	5.87±0.12	0.115	0.065
Co (ppm)	1.17±0.07	1.43±0.09	0.069	0.072
Cu (ppm)	1.64±0.04	1.78±0.06	0.041	0.107
Fe (ppm)	2.54±0.18	2.20±0.06	0.102	0.102
Zn (ppm)	1.21±0.10	1.20±0.11	0.070	0.938

Mean ± standard error; SEM, standard error of mean; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; elephants in treatment I received the wheat-roti supplement and in treatment II the rice–lentil mixture.

influence haemoglobin concentration in rats (Tarwid et al. 1985). Serum concentrations of glucose, total protein, albumin, globulin, A:G ratio, urea, cholesterol and creatinine, and activity of ALT, AST and ALP were not affected by type of supplement and were within normal physiological ranges reported for elephants (Fowler 1986; Tuntasuvan et al. 2002). Similarly, no significant differences were observed in serum concentration of glucose, protein, creatinine and ALT activity in rats fed on lentils (Tarwid et al. 1985). Feeding of lentils did not show significant effects on total cholesterol in rats (Al-Tibi et al. 2010). Similarly, activity of ALP was unaffected in horses supplemented with concentrates (Cymbaluk and Christison 1989). Normal serum metabolite profiles and enzyme activities would indicate that both the supplements tested can be fed to growing Asian elephants without any immediate threat to health. However, further studies involving a greater number of individuals and more sensitive markers are warranted to examine the suitability of these supplements.

Ruminococcus flavefaciens and *Fibrobacter* spp. are among the major cellulolytic bacteria present in horses (Julliand et al. 1999; Lin and Stahl 1995). Molecular analysis of faecal samples in the present study indicates that the gut microbial profile of elephants is similar to that of horses. A higher degree of genetic diversity has been reported for the bacterial community in the equine hindgut (Daly et al. 2001; Milinovich et al. 2006), and a similar diversity of micro-biota in the hind gut of elephants was expected as the digestive physiology of elephants is similar to that of horses (Clements and Maloiy 1982; Schmidt 1986; Dierenfeld 1994; Ullrey et al. 1997). However, further study is required for a better understanding of the gut microbial profile of elephants.

Conclusion

Dry matter intake and digestibility were similar in elephants fed either WR or RLM. However, intake and utilisation of CP and apparent absorption of P and Fe were better in elephants fed RLM than those fed WR. Intake of zinc was also higher in elephants fed RLM. The results of this experiment demonstrate that rice–lentil mixture could be a better supplement than wheat roti for growing Asian elephants in captivity. However, further research involving more replicates is warranted to examine whether or not long-term feeding of RLM can support the normal growth and health of captive Asian elephants.

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