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Utilization of macro-nutrients and minerals in captive greater one-horned rhinoceros fed season-specific diets

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Abstract

This experiment was conducted to study nutrient and mineral utilization in greater one-horned rhinoceros fed season-specific diets. Nine adult greater one-horned rhinoceros (774–2407 kg BW) of Sanjay Gandhi Biological Park, Patna, Bihar, India, were used in this experiment. Three digestion trials of 60-d duration were conducted per animal, out of which 55 days was adaptation period and 5 days was collection period. The amount of concentrate was kept constant during all the trials. Green fodder sources were sugarcane, berseem (*Trifolium alexndrinum*) and chari (*Sorghum bicolor*) during trials I, II and III respectively. Green fodders were offered 20% in excess of previous day's intake in the afternoon. Apparent digestibility of dry matter and crude fibre was higher ($p < .01$) in group II as compared to other groups. Apparent digestibility of crude protein was lowest ($p < .01$) in group I, followed by group III, and the highest value was observed in group II. Apparent digestibility of Ca and P was lower in greater one-horned rhinoceros fed sugarcane and sorghum as green fodder source. In spite of this, sugarcane and sorghum-based diets were able to meet the requirement of Ca, P, Fe and Mn. Berseem supplied Ca in excess of requirement. All the diets were deficient in Zn. Hence, a suitable supplement of Zn should be added to the conventional zoo diet of greater one-horned rhinoceros.

KEYWORDS

apparent absorption, forages, micronutrients, Rhinoceros unicornis, zoo

1 | INTRODUCTION

The present population of greater one-horned rhinoceros (*Rhinoceros unicornis*) is approximately 3,000 in the wild, 2,000 of which are found in Assam alone (Sarma et al., 2009). Greater one-horned rhinoceros is categorized as a vulnerable species by the International Union for Conservation of Nature (IUCN, 2011). Considering the steady decline in population of the species in its natural habitat, various organizations such as IUCN, WWF and Central Zoo Authority (CZA) of India have initiated various in situ and ex situ conservation programmes. Although these organizations have successfully implemented several programmes to conserve the species, detailed feeding recommendations for the species are missing. Adequate and balanced supply of nutrients is pre-requisite for survival and propagation of the species. However, information available on the nutrition of the species is rather scanty. The natural diet of Indian rhino consists mainly of grasses, but they can also eat branches of shrubs and trees (Brahmachary et al., 1974). Natural diets of free-ranging greater one-horned rhinoceros are characterized by lower crude protein and higher crude fibre content (reviewed by Clauss & Hatt, 2006). The absolute dry matter intake of individual rhinoceros varied from 8.8 to 28.8 kg/day (Clauss, Polster, et al., 2005). Apparent digestibility of dry matter ranged from 35% to 63% in greater one-horned rhinoceros (Clauss, Froeschle, et al., 2005). A maintenance energy requirement

TABLE 1 Details of the greater one-horned rhinoceros involved in the study

				Estimated body weight (kg) during					
				Trial I		Trial II		Trial III	
Animal no.	House name	Sex	Approximate age (years)	Start	End	Start	End	Start	End
1	Kancha	M	34	2,218	2,220	2,220	2,228	2,260	2,258
2	Rani	F	19	1,879	1,875	1,875	1,882	1,900	1,902
3	Ayodhya	M	18	2,100	2,102	2,102	2,112	2,129	2,133
4	Geri	F	10	1,655	1,660	1,660	1,667	1,685	1,685
5	Akansha	F	9	1,725	1,725	1,725	1,732	1,736	1,747
6	Ganesh	M	8	1,565	1,565	1,565	1,568	1,609	1,618
7	Suraj	M	7	1,347	1,350	1,350	1,354	1,384	1,402
8	Lali	F	7	921	925	925	939	960	972
9	Saraswati	F	6	797	805	805	822	857	870

of 0.49–0.66 MJ DE/kg $BW^{0.75}$/day has been reported for the greater one-horned rhinoceros, which correlates well with the horse maintenance requirements. However, some individuals had DE intake well above their estimated requirements even on roughage only diets (Clauss, Polster, et al., 2005). Heidegger et al. (2016) observed a correlation between the body condition score (BCS) and the amount of food offered. It was suggested that an ideal BCS can be maintained by adjusting both the amount and the nutritional quality of the feeds used for greater one-horned rhinoceros. Information on content and proportion of faecal volatile fatty acids (VFAs), mean retention time (MRT) and the ratio of particle MRT: fluid MRT of greater one-horned rhinoceros is also available (Clauss, Froeschle, et al., 2005).

Macro- and micro-minerals perform many vital functions in the body. Minerals are involved in the activation of several enzymes, physiological processes, maintaining acid–base and electrolyte balance of the body. Deficiency and imbalances of minerals have been implicated in several health issues of rhinoceros including foot lesions and infertility (Clauss, Polster, et al., 2005). There is a report that indicates that greater one-horned rhinoceros may also suffer from iron storage disease in captivity (Olias et al., 2012). Dried roughage-based diets were deficient in several minerals, such as phosphorus, copper and/or zinc (Clauss, Polster, et al., 2005), and a need for mineral supplementation is obvious when rhinoceros diets comprised of roughages that were deficient in minerals (Clauss & Hatt, 2006). Access to fresh green forages resulted in improvement of serum concentrations of Mg, I, Co, Se and Zn in greater one-horned rhinoceroses, implying browse can be expected to be richer in trace elements than grass (Bapodra et al., 2014). Clauss, Polster, et al. (2005) conducted research on mineral metabolism in greater one-horned rhinoceros in which endogenous faecal losses for Ca, P, Cu, Fe and Zn were obtained by regression analysis. It was reported that a diet formulation following mineral requirements recommended for horses should also be adequate for rhinoceros (Clauss, Polster, et al., 2005). In that study, roughage sources were either hay or silages. Considering that the availability and utilization of minerals by greater one-horned rhinoceros vary according to the forage type (Bapodra et al., 2014) and region (Dierenfeld et al., 2005), it would be desirable to study the influence of forage type on intake and apparent absorption of minerals in greater one-horned rhinoceros. This will add up to the small pool of available data on mineral metabolism that will help us in better understanding of mineral nutrition and devising feeding strategies for ex situ and in situ conservation of greater one-horned rhinoceros. Specific objective of this experiment was to evaluate the utilization of macronutrients and minerals by greater one-horned rhinoceros fed season-specific forage-based diets. Additionally, we intended to evaluate mineral adequacy of these diets so as to formulate balanced ration that will add up to health, welfare and ex situ conservation of this species.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Three digestion trials of 60-d duration were conducted (out of which 55 d was adaptation period and 5 d was collection period) on nine (4 M and 5 F) adult greater one-horned rhinoceros (6–34 years of age, 774–2407 kg BW, Table 1) at Sanjay Gandhi Biological Park, Patna, Bihar, India. The amount of concentrate (wheat grain, 40%; chickpea, 25%; maize grain, 10%; wheat bran, 10%; linseed cake, 6%; gur [jaggery, a traditional concentrated non-centrifugal product of sugarcane juice], 8%; and common salt [Nacl], 1%) was kept constant during all the trials, and wheat straw was offered for ad libitum consumption during all trials. Green fodder sources were sugarcane (*Saccharum officinarum*), berseem (*Trifolium alexandrinum*) and sorghum (*Sorghum bicolor*) during trials I, II and III respectively.

2.2 | Feeding and housing management

The amount of concentrate allocated to each rhinoceros was decided according to the metabolic body weights (27.4 ± 0.37 g/kg

$BW^{0.75}$/day); however, the amount of concentrate fed to a particular animal was kept constant during all the trials. Concentrate was offered at 9 a.m. every day. Green forages were offered at 3 p.m. and made available to the animals till 8 a.m. in the next morning. Green fodder was offered 20% in excess of previous day's intake in the afternoon. Wheat straw was offered at 10 a.m. and was made available for rest of the day. All the animals had free access to clean and fresh drinking water. Other managemental practices such as deworming, general hygiene and sanitation were maintained as per the CZA norms. Animals had regular (from 10:00 till 15:00 hr) access to the outside enclosure (50 × 40 m), which was cleaned of any potential food items. They were kept confined to their indoor enclosures (5 × 3 × 2.5 m) from 15:00 hr to 10:00 hr. During the collection period, animals had no access to their outer enclosure.

2.3 | Measurements and sampling

Body measurement of all animals was taken on day 1 and 60 of each experimental period. Shoulder height was measured by placing a measuring tape at the base of one of the forelimbs and pulling it up to the spine of the scapulae (caudal). All measurements were taken by the same person (AK). Body weight of animals was estimated by assuming body mass: shoulder height ratio (kg/cm) of 11.6 and 10.5 for male and female individuals respectively (Heidegger et al., 2016).

During the digestion trials, the animals were housed separately so that food intake and faeces voided by each animal could be recorded. The total amount of faeces voided during a 24-hr feeding cycle was collected, weighed and sampled for five consecutive days. After thorough mixing, an aliquot was taken for the estimation of dry matter (DM) in duplicate. Samples of feed, refusals and faeces were dried in a hot-air oven (®YSI) at 100°C for 24 hr to determine the DM content. Sub-samples of feed, faeces and refusals were dried in a hot-air oven at 50°C for 4 days. Another aliquot was composited in a separate container under acidic condition (mixed with 25% sulphuric acid) to prevent losses of N in the form of ammonia from the samples. Each individual's refusals and faeces were pooled across the 5-day trial period. Before drying, samples of forages were cut into approximately half-inch pieces using a laboratory forage chopper. Dried samples of feed, refusals and faeces were initially ground using a 6-mm screen made of stainless steel with wear-resistant coating and then ground again using the 1-mm screen (stainless steel with wear-resistant coating) in a grinding mill (®RETSH GmbH) and stored at room temperature in air-tight plastic containers (®Tarsons Products) for further laboratory analysis.

2.4 | Proximate and lignin analysis

Ground samples of feed, refusal and excreta were analysed for ash (AOAC, 2000, procedure 942.05), nitrogen (N) (AOAC, 2000, 990.03), ether extract (EE) (AOAC, 2000, 920.29) and crude fibre (CF) (AOAC, 2000, 962.09). Organic matter (OM) content was calculated by subtracting ash from DM content. Nitrogen-free extract (NFE) was determined by subtracting ash, EE, CP and CF from dry matter. Ash-corrected acid detergent lignin [Lignin (sa)] was determined as per the method described by Van Soest et al. (1991) using 72% H_2SO_4 as oxidizing agent.

2.5 | Mineral estimation

Calcium content in feed, refusals and faeces was determined according to Talapatra et al. (1940). Calcium was precipitated as insoluble calcium oxalate by adding saturated solution of ammonium oxalate under acidic condition, dissolved in dilute sulphuric acid, heated, and the oxalic acid thus released was titrated against standard solution of potassium permanganate. Estimation of phosphorus was done by the spectrophotometric method of AOAC (2000; 965.17). Concentrations of Mg, Fe, Cu, Zn and Mn were determined by atomic absorption spectrophotometry (®AAS 4141; ECIL) following wet digestion according to Kolmer et al. (1951) using nitric, sulphuric and perchloric acid in a volume ratio of 3:1:1.

2.6 | Calculation and statistical analysis

Apparent digestibility (%) of nutrients (the DM, OM, CP, EE, CF and NFE) was calculated as [(nutrient intake-nutrient in faeces)/nutrient intake] × 100. In order to calculate total digestible nutrients (TDNs) of the feed, apparently digestible EE was multiplied by 2.25, which was then added to the sum of apparently digestible CP, CF and NFE. Minimum dietary requirement (MDR) of nutrients was calculated as per the model given value:

$$\text{MDR for Ca and P (\% DM)} = \left[\{(\text{EFL}/\text{TABS}) * \text{BW}\}/1000\right]/\text{DMI (g/d)} * 100,$$

$$\text{MDR for Fe, Cu, Mn and Zn (mg/kg DM)} = \left[(\text{EFL}/\text{TABS}) * \text{BW}\right]/\text{DMI(kg/d)},$$

[EFL, endogenous faecal losses (mg or mg/kg BW); TABS, coefficient of absorption; BW, body weight].

Pearson's regression analysis was done by plotting the dietary content of CP, CF and ADL on the *x*-axis and the apparent digestibility of OM on the *y*-axis, and also by plotting the dietary content ADL on *x*-axis and CF digestibility on *y*-axis. Considering that the linear relationship of dietary CP content was not a good fit, they were fitted into a model of second-degree quadratic function: $f(x) = ax^2 + bx + c$, where a, b and c are real numbers, and $a \neq 0$. Regression analysis was done by plotting the intake of nutrients (mg or μg/kg BW/day) on the *x*-axis and the apparent retention of the nutrient (mg or μg/kg BW/day) on the *y*-axis to estimate the basal endogenous losses and coefficient of retention. Data obtained were subjected to analysis of variance (ANOVA) followed by Tukey's post hoc test using statistical software package SPSS 17.0 (SPSS). The significance level was set to 0.05.

3 | RESULTS

3.1 | Chemical composition of feeds and forages

The chemical composition of feeds and forages fed to greater one-horned rhinoceros is presented in Table 2. Contents of CP, EE, Ca, P, Fe and Zn were higher, whereas that of CF and ADL were lower in berseem than other two roughages. Nutritional composition of wheat straw and concentrate was similar across the trial periods and was comparable to published value (Ranjhan, 2001).

3.2 | Feed consumption and diet digestibility

Data pertaining to feed consumption and nutrient content of the individual rhinoceros are presented in Table 3. Consumption of concentrates and forages was similar among the groups. Consumption of wheat straw and average daily dry matter intake (DMI) was higher ($p < .002$) in group II as compared to other groups. Apparent digestibility of CP, DCP intake and DCP contents of the diet was lowest ($p < .01$) in group I, followed by group III, and the highest value was observed in group II. Apparent digestibility of CF and DM, TDN intake and its dietary concentration was higher ($p < .01$) in group II as compared to other groups (Table 4). Apparent digestibility of OM increased with increased level of CP in the diet ($r = .73$; $p < .001$); however, a decline in digestibility of OM was observed with increased level of CF ($r = -.53$; $p = .0012$) and ADL ($r = -.71$; $p < .001$) (Figure 1). Apparent digestibility of CF decreased with ADL ($r = .79$; $p < .001$) content of the diet. Digestibility of CF increased when CP content of the diet was increased from 7% to 9%, beyond which there was no further improvement (Figure 2).

3.3 | Minerals intake, faecal excretion and apparent absorption

Data pertaining to intake, faecal excretion and apparent absorption of minerals are presented in Table 5. Apparent absorption of Ca and P was higher ($p < .01$) in group II than in the other two groups. Apparent absorption of Fe and Cu was not significantly different among the groups. Apparent absorption of Zn was lower ($p < .01$) in group I than in the other two groups. The linear relationships between the mineral intake and amount apparently absorbed are as expressed on mg or μg/kg BW basis (Table 6; Figure 3).

3.4 | Nutritional adequacy of diets

All the three diets fed to the respective groups were adequate to fulfil the dietary concentration of Ca, P, Fe and Mn. All diets were deficient in Zn (Table 7).

4 | DISCUSSION

4.1 | Feed consumption and nutrient utilization

Consumption of wheat straw was higher when berseem was used as roughage source. This corroborates well with results reported in

TABLE 2 Nutritional characteristics of feeds and forages fed to greater one-horned rhinoceros

	Concentrate[a]	Wheat straw	Sugarcane	Berseem	Sorghum
DM (g/kg as fed)	897	904	345	175	225
Macronutrients and lignin (g/kg DM)					
Organic matter	890	903.7	938	898	898
Ether extract	45.7	11.2	17.4	32.4	22.8
Crude fibre	84.5	394.5	302.5	271.4	268.7
Crude protein	136.5	21.8	38.8	164.0	78.0
Nitrogen-free extract	623.7	476.2	579.1	431.2	528.9
Acid detergent lignin	12.5	114.5	84.7	64.8	78.9
Minerals					
Calcium (g/kg DM)	0.21	0.28	0.38	1.46	0.46
Phosphorus (g/kg DM)	0.43	0.08	0.18	0.31	0.19
Iron (mg/kg DM)	104	168	75	393	884
Copper (mg/kg DM)	10	05	09	11	12
Manganese (mg/kg DM)	38	59	65	61	76
Zinc (mg/kg DM)	40	17	22	37	31

Abbreviation: DM, dry matter.

[a]Concentrate mixture comprised of wheat grain (40%), chickpea (25%), maize grain (10%), wheat bran (10%), linseed cake (6%), gur (8%) and salt (1%).

TABLE 3 Feed consumption, nutrient intake and apparent digestibility in greater one-horned rhinoceros fed season-specific diets

Animal number	Forage source	Feed consumption on DM basis (kg/day)				Nutrient content of consumed diet (%DM)			
		Wheat straw	Concentrates	Forages	DMI	CF	CP	EE	NFE
1	Sugarcane	8.36	8.52	8.28	25.16	26.6	6.62	2.51	55.27
1	Berseem	9.26	8.74	10.15	28.16	25.2	10.87	2.96	50.35
1	Sorghum	7.68	8.30	10.34	26.32	25.5	8.00	2.66	53.28
2	Sugarcane	5.87	8.07	6.90	20.85	24.7	7.18	2.68	56.42
2	Berseem	6.78	7.85	9.10	23.72	24.3	11.43	3.08	50.69
2	Sorghum	6.33	7.85	9.22	23.39	24.7	8.24	2.73	53.82
3	Sugarcane	6.78	8.52	7.94	23.23	25.5	6.97	2.61	55.94
3	Berseem	7.46	8.52	9.63	25.60	24.2	11.34	3.07	50.91
3	Sorghum	6.55	8.30	9.67	24.51	24.6	8.28	2.74	53.90
4	Sugarcane	5.42	7.62	7.59	20.64	25.4	7.04	2.64	56.90
4	Berseem	6.10	8.07	7.53	21.70	22.9	11.38	3.14	52.11
4	Sorghum	5.20	7.85	7.64	20.69	23.1	8.61	2.86	55.01
5	Sugarcane	5.42	7.62	6.90	19.95	24.9	7.15	2.67	56.30
5	Berseem	5.87	7.62	7.88	21.37	23.3	11.51	3.13	51.52
5	Sorghum	5.20	7.40	8.54	21.14	24.1	8.47	2.80	54.20
6	Sugarcane	4.07	6.73	6.56	17.35	24.7	7.27	2.71	56.44
6	Berseem	4.97	7.62	6.83	19.42	22.0	11.68	3.22	52.63
6	Sorghum	4.29	7.17	7.64	19.11	23.1	8.73	2.88	54.89
7	Sugarcane	3.84	5.83	6.21	15.88	25.6	7.06	2.65	55.95
7	Berseem	4.52	5.38	7.35	17.25	24.8	11.82	3.10	49.83
7	Sorghum	3.84	5.38	9.67	18.89	26.2	8.32	2.70	52.38
8	Sugarcane	2.26	4.48	5.52	12.26	25.7	7.14	2.68	55.93
8	Berseem	2.94	4.48	5.25	12.67	23.2	12.13	3.22	51.05
8	Sorghum	2.71	4.48	8.54	15.74	26.0	8.50	2.73	52.36
9	Sugarcane	1.81	4.04	4.49	10.33	24.6	7.40	2.76	56.52
9	Berseem	2.26	4.04	4.55	10.84	22.4	12.41	3.29	51.50
9	Sorghum	2.03	4.04	6.07	12.14	24.3	8.80	2.85	53.66

Abbreviations: CF, crude fibre; CP, crude protein; DM, dry matter; DMI, average daily dry matter intake; EE, ether extract; NFE, nitrogen-free extract.

ruminants (Das & Singh, 1999). This increase, however, did not cause any change in average daily dry matter intake. The low CP digestibility as observed in the sugarcane-based diets could be attributed to lower CP content of sugarcane. As the obligatory endogenous losses in the form of MFN remain constant, increasing the CP content of the diet results in increased apparent digestibility of CP. This corroborates well with findings reported earlier in greater one-horned rhinoceros (Clauss, Froeschle, et al., 2005) and Asian elephants (Das et al., 2015). For optimal microbial activity, fibrinolytic organisms need a regular supply of degradable N, peptides, essential minerals and vitamins (Leng, 1990). Additionally, actively growing bacteria in the hindgut compete among themselves for peptides and free amino acids (Cummings & Macfarlane, 1997). Thus, an inadequate supply of protein and other essential nutrients may have adverse impact on survival and propagation of fibrinolytic micro-organisms (Hussein et al., 2004). In the present experiment, CP content of the diets distinctly differed from each other; sugarcane-based diet was not able to supply 8% of CP, recommended for the maintenance of adult captive Asian elephants (Ullrey, 1997). Lower CF digestibility in the sugarcane-based diet could be partially attributed to inadequate supply of nitrogen to hindgut micro-biota. However, the influence of dietary CP on digestibility of CF was quadratic. Although the CP content of the sorghum-based diet was lower than that of the berseem-based diet, the sorghum-based diet supplied the adequate amount of protein. As a result, the digestibility of CF was similar in rhinoceros fed either sorghum- or berseem-based diets. The digestibility of fibre in herbivores depends upon several factors such as mean retention time, fibre content and extent of its lignifications (Steuer et al., 2013). The results of the present experiment show that digestibility of CF decreased linearly with the increased level of ADL in the diet. The content of ADL was highest in sugarcane, whereas berseem contained the least. Although the dietary content of both CP and ADL influenced the digestibility of CF, a stronger correlation was observed between ADL content and CF digestibility. Changes in

TABLE 4 Feed consumption, nutrient intake and apparent digestibility in greater one-horned rhinoceros fed season-specific diets

Parameters	Groups* I	II	III	*SEM*	*p*-value
Feed consumption (g DM/kg BW)					
Wheat straw	$2.96^a \pm 0.16$	$3.41^b \pm 0.13$	$2.93^a \pm 0.11$	0.078	.032
Concentrate	4.41 ± 0.12	4.43 ± 0.14	4.23 ± 0.13	0.075	.509
Roughage	4.45 ± 0.28	4.88 ± 0.17	5.01 ± 0.31	0.150	.303
DMI	$11.82^a \pm 0.27$	$12.72^b \pm 0.15$	$11.47^a \pm 0.35$	0.157	.012
Nutrient intake					
DCP (mg/kg BW)	$0.28^a \pm 0.02$	$0.80^c \pm 0.02$	$0.47^b \pm 0.03$	0.019	.001
DCP (mg/kg $BW^{0.75}$)	$1.73^a \pm 0.06$	$5.00^c \pm 0.06$	$2.90^b \pm 0.11$	0.540	.001
TDN (g/kg BW)	$5.38^a \pm 0.15$	$6.42^b \pm 0.22$	$5.90^a \pm 0.29$	0.128	.011
TDN (g/kg $BW^{0.75}$)	$32.90^a \pm 0.78$	$39.91^c \pm 0.34$	$36.82^b \pm 1.03$	0.445	.001
Digestibility of nutrients (%)					
Dry matter	$46.51^a \pm 0.65$	$51.97^b \pm 1.44$	$47.19^a \pm 0.92$	0.614	.002
Crude fibre	$38.76^a \pm 0.41$	$43.15^b \pm 0.27$	$42.51^b \pm 0.17$	0.173	.001
Crude protein	$33.09^a \pm 1.12$	$54.24^c \pm 0.49$	$43.54^b \pm 0.88$	0.502	.001
Ether extract	53.94 ± 1.91	60.27 ± 2.35	56.44 ± 1.94	1.189	.112
Nitrogen-free extract	54.16 ± 0.79	58.20 ± 2.36	53.21 ± 1.45	1.146	.1023
Nutritive value (% dry matter) of the consumed diet					
Total digestible nutrients	$45.46^a \pm 0.49$	$50.41^b \pm 1.23$	$45.97^a \pm 0.85$	0.53	.001
Digestible crude protein	$2.35^a \pm 0.08$	$6.30^c \pm 0.11$	$3.68^b \pm 0.1$	0.06	.001

Abbreviations: DM, dry matter; DMI, average daily dry matter intake; *SEM*, standard error of mean.
[abc]Mean (±standard error) with different superscripts in a row differs significantly.
*Fodder source in groups I, II and III was *Saccharum officinarum*, *Trifolium alexandrium* and *Sorghum bicolor* respectively.

CF digestibility in greater one-horned rhinoceros fed different kind of forages can thus be better explained on the basis of their lignin content. Increased apparent digestibility of CP and CF in captive greater one-horned rhinoceros fed berseem as forage source was also reflected in higher apparent digestibility of dry matter. An increase in intake and apparent digestibility of CP resulted in increased DCP intake in berseem fed animals. The increased apparent digestibility of CP, CF and DM also resulted in increased intake and dietary supply of TDN when berseem was used as forage source.

4.2 | Intake and apparent absorption of minerals

4.2.1 | Calcium

Berseem contained 3.8 and 3.2 times more Ca than sugarcane and sorghum respectively. Further, the Ca:P ratio was wider in berseem than in the other two fodders, which resulted in increased Ca:P ratio in berseem fed group. Several earlier works (Cymbaluk, 1990; Pagan, 1998 and Cymbaluk & Christison, 1989) have indicated that increasing the ratio of Ca:P in the diet improved the apparent absorption of Ca in the horse. In the present study, the apparent absorption of Ca ranged from 7.5% to 44.4%. Clauss, Polster, et al. (2005) measured coefficients of 40%–60% in greater one-horned rhinoceros. Apparent absorption of Ca was 44% for horse (Pagan, 1998) fed on a mixed diet containing 0.89% calcium. Crozier et al., (1997) reported that apparent absorption of Ca was 46% in horse raised on the alfalfa hay-based diet containing 0.94% Ca, which was quite similar to the value observed in greater one-horned rhinoceros fed the berseem-based diet. The lower apparent digestibility of Ca in sugarcane and sorghum fed group could be attributed to the lower intake of absolute amount of Ca and also to the decreased Ca:P (Cymbaluk, 1990; Hagen et al., 2015).

True absorption of Ca was 68.4% in the present study. Clauss, Polster, et al. (2005) reported that true absorption of Ca was 83% in greater one-horned rhinoceros. True absorption of Ca was 41% in domestic horses (Clauss et al., 2007). Pagan (1998) reported that true absorption of Ca was 74% in the horse. From the result of the

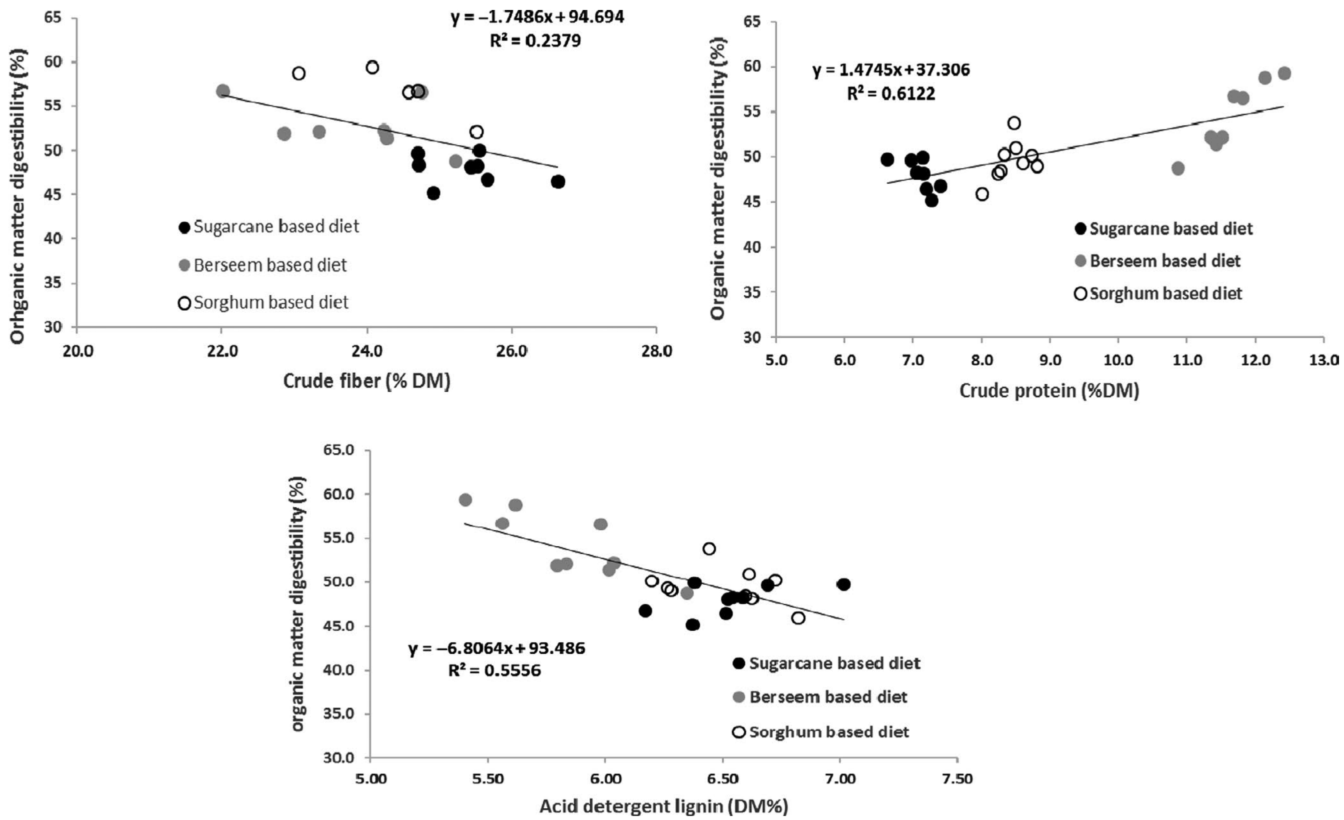

FIGURE 1 Regression of organic matter digestibility (%) on crude fibre, crude protein and acid detergent lignin content of the diet

present experiment and review of literature, it seems that rhinoceros-like horses absorb Ca in excess. The rhinoceros-like equids and the elephant do not regulate Ca uptake in gut as other animals do, but absorb it in great quantities and excrete the excess amount in urine (Clauss, Polster, et al., 2005). Endogenous faecal loss of Ca was estimated to be 22.1 mg/kg BW in the present study. According to the estimate of Clauss, Polster, et al. (2005), faecal endogenous loss of Ca was 5.1 mg/kg BW in greater one-horned rhinoceros. Pagan (1998) reported that endogenous faecal loss of Ca was 31 mg/kg BW in horse. From the result of present experiment and review of literature, it seems that rhinoceros can absorb Ca as efficiently as horses. However, endogenous losses of Ca could be lower in rhinoceros than horses. Such differences could be due to more muscular activity in horses than rhinoceros. It is well known that muscular activity requires Ca (Fleckenstein, 1977). Applying the true absorption and endogenous faecal losses, Ca requirement for greater one-horned rhinoceros weighing 1,650 kg would be 53.3 g or 32.3 mg/kg BW. If they are fed on diet, which is consumed at the rate of 1.15%–1.27% BW, a diet containing 0.25%–0.28% Ca would be sufficient to fulfil the requirement. Nutrition Advisory Group (Lintzenich

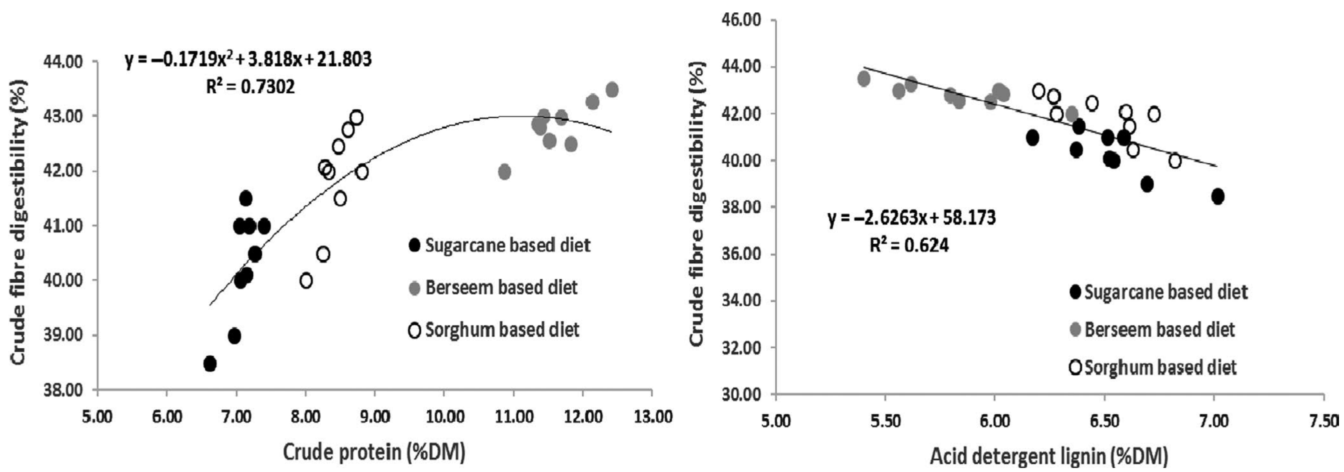

FIGURE 2 Regression of crude fibre digestibility (%) on crude protein and acid detergent lignin content of the diet

TABLE 5 Minerals intake, faecal excretion and apparent absorption in greater one-horned rhinoceros fed season-specific diets

Parameters	Groups* I	II	III	*SEM*	*p*-value
Calcium (Ca)					
Intake (mg/kg BW)	33.87[a] ± 0.96	90.93[c] ± 2.59	42.77[b] ± 2.28	1.192	.001
Faecal excretion (mg/kg BW)	32.14[a] ± 0.71	50.43[c] ± 2.25	36.95[b] ± 0.64	0.814	.001
Apparent absorption (%)	7.48[a] ± 2.31	44.39[b] ± 1.89	13.71[a] ± 2.92	3.435	.001
Phosphorus (P)					
Intake (mg/kg BW)	27.60[a] ± 0.52	34.55[b] ± 0.61	29.45[a] ± 0.83	1.070	.044
Faecal excretion (mg/kg BW)	27.92[a] ± 0.54	32.09[b] ± 0.68	30.72[b] ± 1.27	0.720	.048
Apparent absorption (%)	4.08[a] ± 0.68	9.36[b] ± 0.52	4.41[a] ± 1.04	0.640	.002
Iron (Fe)					
Intake (mg/kg BW)	1.29[a] ± 0.02	2.71[b] ± 0.05	5.47[c] ± 0.41	0.136	.001
Faecal excretion (mg/kg BW)	1.13[a] ± 17	2.47[b] ± 0.05	4.75[c] ± 0.32	0.109	.001
Apparent absorption (%)	12.41[b] ± 4.14	8.87[a] ± 2.93	12.81[b] ± 4.27	0.497	.006
Copper (Cu)					
Intake (µg/kg BW)	99.17[a] ± 2.98	114.89[b] ± 1.96	125.44[b] ± 6.35	2.432	.001
Faecal excretion (mg/kg BW)	89.67[a] ± 1.35	102.88[b] ± 1.09	110.56[b] ± 3.45	1.290	.001
Apparent absorption (%)	8.97 ± 1.91	10.7 ± 1.57	10.5 ± 1.74	1.006	.742
Manganese (Mn)					
Intake (µg/kg BW)	504.66[a] ± 14.3	552.63[a] ± 9.74	669.44[b] ± 41.6	15.012	.001
Faecal excretion (µg/kg BW)	453.44[a] ± 6.67	489.21[b] ± 8.13	572.11[b] ± 26.2	9.424	.001
Apparent absorption (%)	9.83 ± 1.82	11.5 ± 1.24	13.9 ± 1.44	0.875	.185
Zinc (Zn)					
Intake (µg/kg BW)	326.86[a] ± 15.1	433.71[b] ± 11.6	450.04[b] ± 35.3	13.412	.002
Faecal excretion (µg/kg BW)	347.29[a] ± 9.52	429.57[b] ± 8.07	443.03[b] ± 26.0	9.623	.001
Apparent absorption (%)	−6.96[a] ± 1.98	0.72[b] ± 0.92	0.59[b] ± 1.77	0.936	.003

Abbreviation: *SEM*, standard error mean.

[abc]Mean (±standard error) with different superscripts in a row differs significantly.

*Fodder source in groups I, II and III was *sugarcane*, *berseem* and *sorghum* respectively.

TABLE 6 Linear regression analysis of daily mineral intake (x) and amount apparently absorbed (y) on a mg/kg BW basis according to y = ax − b

					Reference value			
					Greater one-horned rhinoceros[a]		Horse[b]	
Minerals	EFL (95% CI)	TABS (95% CI)	R^2	*p*-value	EFL	TABS	EFL	TABS
Ca	22.14 (18.8–25.5)	0.68 (0.63–0.74)	0.97	<.01	5.10	0.83	31.6	0.75
P	9.12 (7.3–11.0)	0.36 (0.30–0.41)	0.88	<.01	10.00	0.64	8.55	0.25
Fe	0.09 (0.02–0.16)	0.15 (0.13–0.17)	0.91	<.01	0.62	0.18	-	-
Cu	0.03 (0.02–0.04)	0.38 (0.27–0.45)	0.68	<.01	0.02	0.40	0.069	0.40
Mn	0.13 (0.09–0.17)	0.36 (0.28–0.42)	0.92	<.01	-	-	0.20	0.29
Zn	0.11 (0.10–0.12)	0.26 (0.23–0.29)	0.99	<.01	0.06	0.30	0.098	0.21

Abbreviations: CI, confidence interval; EFL, endogenous faecal losses (mg/kg BW); TABS, true absorption coefficient.

[a]Clauss, Polster, et al. (2005).

[b]Pagan (1998).

& Ward, 1997) recommends that rhinoceros diet should contain 0.20%–0.65% Ca. The dietary concentration of 0.24% has been recommended for horses (NRC, 1989). The results of the present experiment indicate that Ca requirement for greater one-horned rhinoceros is in the range of the recommendation of Nutrition Advisory Group (Lintzenich & Ward, 1997) for rhinoceros and NRC (1989) for horses. Therefore, recommendations of Nutrition Advisory Group (Lintzenich & Ward, 1997) and NRC (1989) in this regard could be used safely in greater one-horned rhinoceros as well. Diets of all the groups were found adequate to fulfil these requirements.

4.2.2 | Phosphorus, iron, copper, manganese and zinc

Apparent absorption of P, Fe, cu, Mn and Zn ranged from −1.88 to 18.75, 6.23 to 22.77, 1.56 to 21.99, −29.8 to 61.00 and 5.31% to 21.30% respectively. These values collate well with the labels reported in the literature for greater one-horned rhinoceros (Clauss, Polster, et al., 2005) and horses (Pagan, 1998). From the regression analysis, the endogenous faecal losses of the greater one-horned rhinoceros were calculated to be 9.11 mg P/kg BW/day, 92 μg Fe/

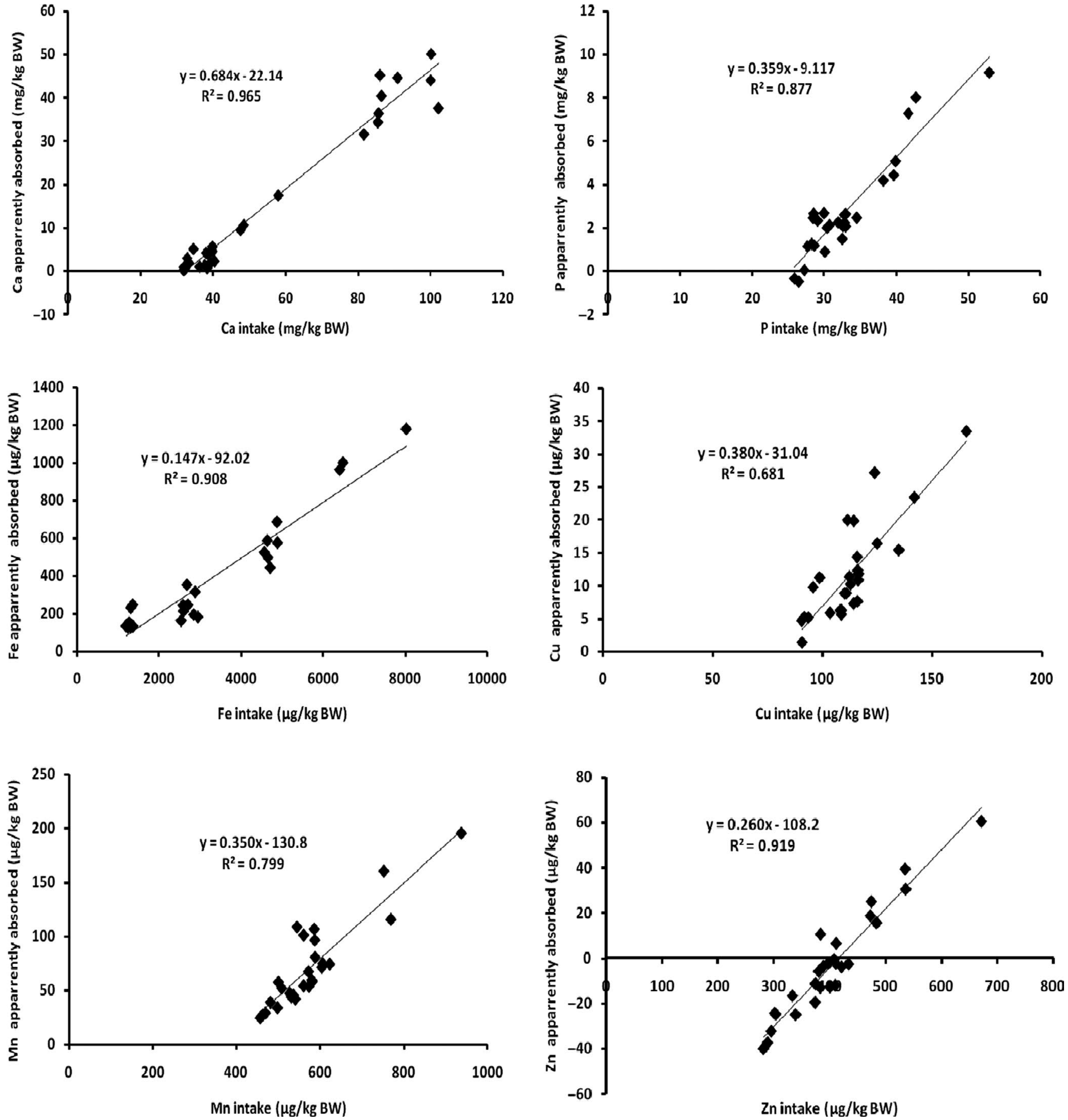

FIGURE 3 Linear regression analysis of daily mineral intake (mg or μg/kg BW) and amount apparently absorbed (mg or μg/kg BW)

TABLE 7 Nutritional adequacies of three season-specific diets currently being fed to greater one-horned rhinoceros at Sanjay Gandhi Biological Park, Patna

Parameters	Nutrient supplied in the diet of groups					(%) Deficit or surplus		
	I	II	III		Recommended requirement			
Nutrient content of the diets				*SEM*		I	II	III
Ca (%)	$0.28^{a} \pm 0.01$	$0.69^{c} \pm 0.02$	$0.33^{b} \pm 0.00$	0.037	0.24*	+16	+187	+37
					0.27**	3.6	+156	+22
P (%)	$0.25^{a} \pm 0.01$	$0.29^{b} \pm 0.01$	$0.25^{a} \pm 0.01$	0.004	0.17*	+47	+70	+47
					0.19**	+32	+53	+32
Fe (ppm)	$110.93^{a} \pm 1.09$	$229.28^{b} \pm 3.05$	$442.84^{c} \pm 11.52$	27.207	40*	+177	+472	+1,107
					41.6**	+167	+451	+1,065
Cu (ppm)	$9.01^{a} \pm 0.08$	$9.00^{a} \pm 0.09$	$9.65^{b} \pm 0.10$	0.077	10.0*	−9	−10	−3.5
					10.2**	−12	−12	−5
Mn (ppm)	$53.09^{a} \pm 0.27$	$52.33^{a} \pm 0.23$	$58.72^{b} \pm 0.43$	0.590	40.0*	+33	+31	+47
					31**	+71	+69	+89
Zn (ppm)	$27.53^{a} \pm 0.27$	$32.57^{c} \pm 0.32$	$30.85^{b} \pm 0.27$	0.441	40.0*	−31	−19	−23
					34.4**	−20	−5	−10

Note: Fodder source in groups I, II and III was *sugarcane*, *berseem* and *sorghum* respectively.

Abbreviation: *SEM*, standard error mean.

[abc]Mean (±standard error) with different superscripts in a row differs significantly.

*NRC (1989)recommendation for adult horse maintenance.

**Requirement estimated using endogenous losses and true absorption of nutrients in the present study.

kg BW/day, 31 μg Cu/kg BW/day and 108 μg Zn/kg/BW/day. Hypothetical endogenous faecal losses of P were comparable to the values reported in the literature for greater one-horned rhinoceros (Clauss, Polster, et al., 2005) and horses (Pagan, 1998). However, endogenous faecal losses of Fe were lower and that of Cu and Zn were higher as compared to the values reported earlier in greater one-horned rhinoceros (Clauss, Polster, et al., 2005). The coefficients of true absorption of P, Fe, Cu, Mn and Zn were 35.9%, 14.7%, 31.0%, 35.0% and 26.0% respectively. The coefficient of true absorption of P was lower, and that of Cu and Zn was comparable, whereas that of Fe was higher as compared to the values reported earlier in the greater one-horned rhinoceros.

Applying the true absorption and endogenous faecal losses, daily requirement of greater one-horned rhinoceros weighing 1,650 kg would be 37.6 g P, 827 mg Fe, 202 mg Cu, 617 mg Mn and 685 mg Zn. If they are fed on diets which is consumed at the rate of 1.15%–1.27% BW, dietary concentrations of 0.18%–0.20% P, 39.5–43.6 ppm Fe, 9.65–10.65 ppm Cu, 29.4–32.5 ppm Mn and 32.7–36.1 ppm Zn would be sufficient to fulfil these requirement. The result of the present experiment suggests that the requirements of greater one-horned rhinoceros for P, Fe, Cu, Mn and Zn are in the range of the recommended value for horses (NRC, 1989) and white rhinoceros (Lintzenich & Ward, 1997). Thus, the recommendation of NRC (1989) and Nutrition Advisory Group (Lintzenich & Ward, 1997) in this regard could be used safely in greater one-horned rhinoceros as well. Diets of all the groups were found adequate to fulfil the minimum dietary concentrations of P, Fe, Cu and Mn recommended by NRC (1989) and Nutrition Advisory Group (Lintzenich & Ward, 1997). In the present study, Zn was deficient by 28%, 17% and 21% in groups I, II and III respectively. Irrespective of fodder sources, all the diets were deficient in Zn in the present study. Zn deficiency had been reported earlier in greater one-horned rhinoceros kept in European zoos (Clauss, Polster, et al., 2005). Zn deficiency is widespread among livestock (Sharma & Joshi, 2004; Tiwary et al., 2007) and other mega-herbivores in India (Katole, 2011). Thus, a suitable supplementation with adequate amount of zinc would be necessary.

5 | CONCLUSIONS

Apparent digestibility of CP, DCP intake and DCP contents of the diet was lowest when sugarcane was used as a forage source, followed by sorghum and the highest values with respect to these parameters were observed when berseem was used as forage source. Apparent digestibility of CF and DM, TDN intake and its dietary concentration was also higher in berseem-based diets. Apparent digestibility of Ca and P was lower in greater one-horned rhinoceros fed *sugarcane* and *sorghum* as green fodder source. In spite of this, *sugarcane*- and *sorghum*-based diets were able to meet the requirement Ca and P. All the diets supplied adequate amount of Fe and Mn. *Berseem* supplied Ca in excess of requirement. All the diets were deficient in Zn. Hence, a suitable supplement of Zn should be added to the conventional zoo diet of greater one-horned rhinoceros. Basic data regarding basal endogenous faecal losses of Ca, P, Fe, Cu, Mn and Zn in greater one-horned rhinoceros have been generated which would add to the small pool of available data in this species and could be used as reference value for future research.

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CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest.

ANIMAL WELFARE STATEMENT

It is certified that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and ethical approval of Sanjay Gandhi Biological Park, Patna, India, was obtained. It is confirmed that the animals were cared for according to the guidelines for the accommodation and care of animals used for experimental and other scientific purposes set by the Central Zoo Authority of India.

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