Indian Journal of Animal Sciences **85** (7): 774–780, July 2015/Article

Mineral release kinetics of common tropical green forages in the rumen of cattle

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Received: 12 January 2015; Accepted: 8 March 2015

ABSTRACT

The potential release of macro and micro minerals from six tropical green forages were determined by *in sacco* rumen degradability method. The release of minerals in the rumen were estimated in green forages (maize fodder, hybrid napier, doob grass, para grass, berseem and sunhemp) by incubating the forage samples for different periods, i.e. 10h, 24h, 48h and 72h in three adult cattle fitted with rumen cannula by *in sacco* nylon bag technique. The release of macro and micro minerals from experimental green forages were significantly ($P<0.001$) different and highest release was observed in potassium (K) and lowest in calcium (Ca) from different classes of forages. The pattern of macro minerals release from different categories of green forages was as follows: K > Mg > P > Ca. Among the green forages, berseem and sunhemp showed highest release of various minerals in the rumen at different periods of incubation. Manganese (Mn) release was lowest among the micro minerals from experimental green forages in the rumen. The pattern of micro minerals release from green forages was as follows: Cu > Zn > Mn. Results obtained from the study concluded that the potential availability of various minerals in the rumen from different classes of green forages was different and this information may be useful in strategic supplementation of minerals to animals through green forages.

Key words: Forage, Macro and micro minerals, Mineral release, Rumen

During the determination of minerals requirement, the availability of minerals from base feedstuffs is based on the certain percentage of bioavailability of minerals which is not true for all classes of feedstuffs in all conditions. Just because minerals can be found in feedstuffs does not mean that those are available to the animal, similarly, it is not always true that the minerals present in feedstuffs are not at all available to animals. Pal *et al*. (2009 and 2011) reported that there was distinct variation in release and thus potential bioavailability of minerals from a variety of feed types within the same classes in the rumen. Animal's utilization of minerals is dependent upon two major processes – solubilisation or release of minerals from feedstuffs, and absorption of released minerals from the gut. Minerals to be available for host animal and its rumen microbes have to be solubilised in digestive tract. In this aspect, the rumen has been considered to be very important site. Thus, it is important to consider the total concentration of mineral elements in feeds and fodders along with the extent to which they are solubilised and released in the rumen for their bioavailability and utilization. The site of mineral and its form in the forage structure may also influence its release (Ceresnakova *et al*. 2005). The *in vitro* and *in sacco* techniques of measuring mineral solubility and release in the rumen may be useful in predicting their absorption and potential bioavailability for utilization from base feedstuffs. The utilization of minerals from mineral supplements has been reviewed extensively but scanty information is available on the utilization of minerals from forages. Hence, the present study was carried out with the aim of determining the potential availability of minerals in the rumen from some common green forage.

MATERIALS AND METHODS

Experimental forages and sampling: Six common green forages (two nonleguminous, two grasses and two leguminous green fodders) based on their significance for ruminant feeding were studied for release of minerals in the rumen at different periods of incubation. The non-leguminous green forages were maize fodder (*Zea mays*) and hybrid napier (*Pennisetum typhoides* × *P. purpureum*); grasses were doob grass (*Cynodon dactylon*) and para grass (*Brachiaria mutica*) and leguminous forages were berseem (*Trifolium alexandrinum*) and sunhemp (*Crotalaria juncea*). The macro and micro minerals studied were calcium (Ca), phosphorous (P), magnesium (Mg), potassium (K), copper (Cu), zinc (Zn) and manganese (Mn).

The forage samples were air dried and later oven dried at 60°C for 48h and ground to pass through a 2 mm screen

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Wiley mill. The dried samples were stored in plastic containers for later analysis. For nylon bag incubation studies, the forage samples were ground to pass through a 5 mm sieve.

Rumen incubation of forages in nylon bags

Animals and their diet: The animal experimentation was approved by the Institutional Ethics Committee for feeding and management of animals during trial period. The release of mineral elements from green forages was determined using *in sacco* nylon bag technique following the procedure of Ørskov *et al*. (1980). The trial was carried out on 3 adult male cattle, fitted with large rumen canula and housed in individual pens. The average body weight of animals was 450 kg and was maintained on maintenance diet (1kg concentrate mixture with *ad lib.* nonleguminous fodder) and vitamin-mineral mixture premix for 2 weeks before and during the trial. Animals have free access to water throughout the feeding period.

In sacco *nylon bag technique:* The feedstuffs (5g) were placed into the nylon bags (pore size, 50 mm; internal dimensions, 5 cm × 10 cm) and after 14 days adaptation period to the diet, nylon bags containing test feed samples (5g) were placed into the rumen of each animal. Three bags were used as replicate for each forage, animal and incubation time. Loaded bags were introduced in the rumen just before the morning feeding and were removed after each incubation time, determined per feedstuff according to a preliminary dry matter degradation study. The forages were incubated in the rumen for 10, 24, 48, 72h. Immediately after removal from the rumen, the bags were kept in the ice and then washed with continuous flow of water for 3 min in low speed of washing machine. To remove the forage released minerals, bags were dipped in acidic water (0.5% HCl) for half an hour followed by final dipping in distilled water for hour. The bags were then dried in hot air oven at 70^{o}C for 48h and the residues were ground to pass through a 1 mm sieve in a laboratory mill. The quantity of a mineral remaining in each bag at each incubation time was expressed as a percentage of mineral originally present in the bag prior to rumen incubation.

Mineral analysis: The test feeds were analysed for macro and micro mineral estimation by Inductively Coupled Plasma (ICP-OES) spectrophotometer. The samples were processed by wet oxidation method using nitric and perchloric acids (AOAC 1990). The prepared extractions were analysed for calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), manganese (Mn) and zinc (Zn) using ICP-OES. The phosphorous (P) content in feeds and fodders was analysed in UV-visible spectrophotometer by metavandate method of Fiske and Subbarow (1925). Mineral standards (National Institute of Standards and Technology, NIST certified) were run in each analysis to ensure the accuracy of estimation.

Statistical analysis: The data on the observed losses of mineral elements from forages were evaluated by calculation of basic variation statistical characteristics. The influence of forage and time of observations was evaluated by two-way analysis of variance. The dependences of the analysed traits on time were evaluated by estimation of parameters of linear functions and polynomial of the second and third (cubic) degree. Mathematical and statistical processing was done on the basis of statistical methods of Grofik and Fl'ak (1990) and by help of statistical program package (SPSS.ver.15).

RESULTS AND DISCUSSION

The macro and micro mineral content in experimental forages are given in Table 1. Legume forages (berseem and sunhemp) contained highest content of Ca and P than nonlegume fodder and grasses.

The minimum, maximum and mean values of release percentage of minerals from all incubation periods are presented in Table 2a-b. The maximum release of Ca was observed in legume forages and minimum in grasses. The Ca release in the rumen varied from 13.30 to 39.60% in nonlegume forages and 21.60 to 48.30% in legume fodders on different period of incubation whereas, P release ranged from 48.10 to 75.80% in nonlegume forages and 66.10 to 78.30% in legume forages. The Mg and K release in the rumen from all green forages were found high and it ranged from 67.80 to 94.80% for Mg and from 95.70 to 100% for K. The cultivated fodder like maize and hybrid napier showed significantly ($P<0.001$) higher release of Mg than grasses. Almost all the K (>99%) was released from all the forages at minimum period of incubation (10h) in the rumen (Table 2a). The release of Ca, P, Mg and K in the rumen was significantly ($P<0.01$) different among the green forages and among the incubation periods (Table 3). Amongst the micro minerals, Cu showed highest release followed by Zn and Mn (Table 2b). The release of Cu and Mn was significantly ($P<0.01$) higher in legume forages than nonlegume forages. Berseem showed the highest

Table 1. Macro and micro minerals content in experimental green forages

Forage	Ca	P	Mg	K	Cu	Zn	Mn
	(%)				(µg/g)		
Maize fodder	0.21	0.22	0.42	1.35	8.9	40.3	26.8
Hybrid napier	0.34	0.26	0.31	3.11	13.1	55.9	16.5
Doob grass	0.44	0.25	0.39	2.81	6.3	63.4	21.4
Para grass	0.30	0.28	0.32	2.21	11.6	41.0	14.7
Berseem	1.37	0.33	0.47	2.36	11.4	71.1	29.5
Sunhemp	1.25	0.31	0.41	1.72	11.3	51.4	24.0

Table 2a. Basic statistical characteristics of macro minerals release (%) from green forages over all times in the ruminal incubation

Forage	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
	Ca				P			
Maize fodder	31.50	9.432	18.30	39.60	63.3	12.32	48.10	75.60
Hybrid Napier	23.08	4.758	16.20	26.80	65.13	8.067	53.30	70.70
Doob grass	22.10	6.511	13.30	27.60	65.30	8.619	52.80	72.40
Para grass	23.50	6.201	15.30	29.50	66.25	12.47	48.20	75.80
Berseem	40.43	10.79	24.70	48.30	73.40	5.375	66.10	78.30
Sunhemp	38.30	11.50	21.60	46.20	70.00	2.342	67.40	72.40
	Mg				K			
Maize fodder	88.60	4.027	82.70	91.70	99.63	0.330	99.30	100.0
Hybrid Napier	82.43	9.927	68.70	90.30	99.20	0.627	98.40	99.90
Doob grass	79.98	8.774	67.80	87.10	99.02	0.512	98.30	99.50
Para grass	79.65	7.569	68.70	85.70	97.45	1.179	95.70	98.20
Berseem	91.02	6.125	81.90	94.80	99.93	0.095	99.80	100.0
Sunhemp	86.50	11.42	70.20	94.80	99.35	0.300	99.10	99.70

Table 2b. Basic statistical characteristics of micro minerals release (%) from green forages over all times in the ruminal incubation

Forage	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
	Cu				Zn			
Maize fodder	56.28	12.50	39.50	67.80	79.40	4.048	73.70	83.10
Hybrid Napier	52.38	8.102	42.30	61.30	52.98	4.931	47.60	58.50
Doob grass	73.45	14.78	51.30	81.80	52.80	5.503	46.00	57.80
Para grass	52.23	7.163	42.70	59.80	53.33	5.413	46.20	59.10
Berseem	87.50	7.287	76.80	92.70	73.35	5.708	65.10	77.80
Sunhemp	79.83	9.960	65.60	88.00	68.88	7.459	58.60	75.70
	Mn							
Maize fodder	49.03	7.388	38.20	53.80				
Hybrid Napier	45.28	9.858	31.10	52.60				
Doob grass	42.50	10.13	28.20	51.00				
Para grass	46.53	11.24	30.90	55.70				
Berseem	61.85	13.89	42.70	72.10				
Sunhemp	56.10	15.28	34.80	69.10				

Table 3. Two-way ANOVA of macro and micro minerals release with the test of non-additivity of group (forage) × time of incubation interactions

Minerals		Group ($f_g = 5$)	Time ($f_t = 3$)	Residual ($f_r = 15$)	P-value Group	P-value Time
Ca	MS	265.8***	399.2***	8.394	P < 0.0001	P < 0.0001
P	MS	56.24*	390.5***	18.09	P < 0.0402	P < 0.0001
Mg	MS	89.17***	376.6***	8.055	P < 0.0001	P < 0.0001
K	MS	3.005***	0.9726*	0.2563	P < 0.0001	P < 0.0331
Cu	MS	938.8***	567.7***	15.26	P < 0.0001	P < 0.0001
Zn	MS	566.0***	176.6***	2.383	P < 0.0001	P < 0.0001
Mn	MS	215.2***	761.6***	9.107	P < 0.0001	P < 0.0001

$F_{0.05}$ (5, 15) = 2.90; $F_{0.01}$ (5, 15) = 4.56; $F_{0.05}$ (3, 15) =3.29; $F_{0.01}$ (3, 15) = 5.42.

release of Cu and Mn whereas Zn release was highest from Maize fodder. The Zn and Mn release from berseem and sunhemp ranged from 69-78% at 72h of incubation in the rumen. Cu, Zn and Mn release in the rumen was significantly (P<0.0001) different among the green forages and among the periods of incubation.

There were significant (P<0.0001) differences in the release of minerals among the periods of incubation in the rumen as well as among the green forages (Table 3). The dependence of minerals released on incubation time was evaluated by estimation of parameters of linear and polynomial functions and the most suitable polynomial was of the second order and third (cubic) degree (Table 4a-b and Fig. 1-2). The mineral release pattern of studied green forages at different period of incubation in the rumen are shown in Fig 1 and 2 by cubic polynomials. Minerals released from the forages increased on advanced period of incubation in the rumen and highest release was found at 72h. Amongst the green forages, berseem and sunhemp showed highest release of Ca (46-48%), P (72-78%), Mg (94.8%), K (99.7-100%), Cu (66-77%), Zn (76-78%) and Mn (69-72%) at 72h of incubation in the rumen. The pattern of macro minerals release from different categories of green forages was as follows: K > Mg > P > Ca and of micro minerals release it was as follows: Cu > Zn > Mn.

Considerable variations in mineral content were observed in selected green forages and their release during incubation in the rumen also found different. Almost all the K (> 99%) was released from all the forages at minimum period of incubation (10h), thereafter no significant changes in the release of K was detected. Similar releasing pattern of K was reported by Pal *et al.* (2011) from dry fodders. Emanuele *et al.* (1991) reported a complete release of K in the rumen from forages. The amount and rate of release of minerals were not connected with their original concentration. This difference may be due to large differences between forages in the content of cell walls and of individual fractions (like cellulose, hemicelluloses and lignin) and release of mineral elements (Ledoux and Martz 1991, Flachowsky *et al.* 1994).

The linear functions of the release of individual elements from forages in dependence on incubation time were non-significant except for P and Zn, which indicated that the pattern of mineral release in the rumen were not linear with the incubation time. Hence, the data were further analysed for estimation of polynomial functions. Polynomial functions of the release of individual minerals from forages in dependence on incubation time had high values of R^2 which indicated that the release of minerals from forages follow some specific non-linear pattern as related to dry matter degradability in the rumen (Fig.1 and 2).

The release of Ca from grasses was lower than non-leguminous and leguminous forages but the release of Ca was statistically similar within the same forage classes. The lower values of Ca release from grasses can be explained by its function in the plant in which it is important for the stability of cell walls and membranes (Flachowsky *et al.* 1994). Mc Manus *et al.* (1979) reported that Ca accounted for 34% and 64% of all minerals formed in the cell walls of grasses and legumes, respectively. Hence, the amount of released Ca does not increase before the onset of cell wall degradation. Forages with highest neutral detergent fibre (NDF) concentration sequestrate Ca in the intestine (Emnuele *et al.* 1991, Ward *et al.* 1979, Van Soest 1982). There are large differences between soluble nutrient content

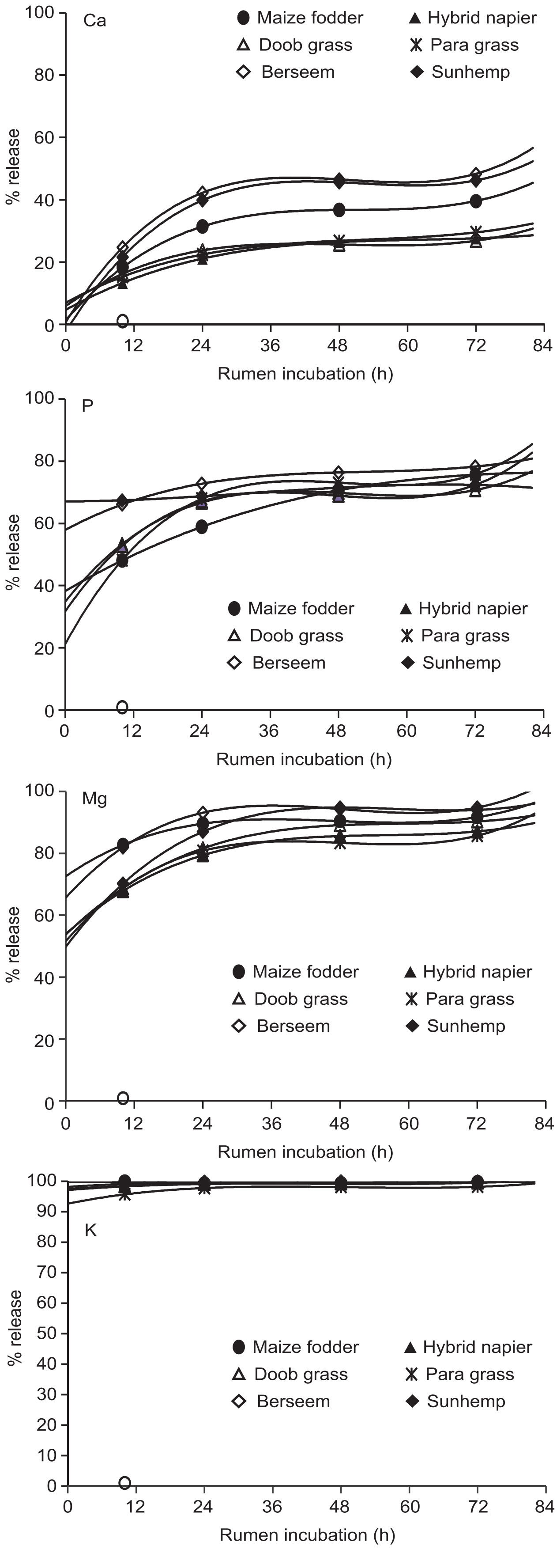

Fig. 1. Cubic polynomials of macro minerals release from green forages in relation to rumen incubation time.

Table 4a. Parameters estimates of the linear function $y = b_0 + b_1t$ of macro minerals release (%) from green fodders in the time of ruminal incubation

Feedstuffs	b_0	b_1	r^2	P-value	b_0	b_1	r^2	P-value
	Ca				P			
Maize fodder	19.47 ± 4.71	0.3125 ± 0.1043	0.8177	0.0957	46.45 ± 3.49	0.4378 ± 0.0772	0.9414	0.0298*
Hybrid Napier	17.29 ± 2.83	0.1502 ± 0.0626	0.7423	0.1384	55.73 ± 5.33	0.2440 ± 0.1179	0.6815	0.1744
Doob grass	13.65 ± 2.99	0.2194 ± 0.0662	0.8461	0.0802	54.93 ± 5.26	0.2694 ± 0.1164	0.7281	0.1467
Para grass	15.20 ± 2.28	0.2157 ± 0.0504	0.9012	0.0507	51.17 ± 7.52	0.3917 ± 0.1664	0.7349	0.1427
Berseem	27.61 ± 6.82	0.3329 ± 0.1508	0.7088	0.1581	66.35 ± 2.31	0.1832 ± 0.0511	0.8654	0.0697
Sunhemp	24.72 ± 7.36	0.3527 ± 0.1631	0.7005	0.1631	66.77 ± 0.57	0.08394 ± 0.0126	0.9567	0.0219*
	Mg				K			
Maize fodder	83.87 ± 2.61	0.1229 ± 0.0578	0.6933	0.1673	99.66 ± 0.38	-0.001007 ± 0.0085	0.006916	0.9168
Hybrid Napier	69.84 ± 5.10	0.3268 ± 0.1129	0.8073	0.1015	98.35 ± 0.19	0.02219 ± 0.004211	0.9328	0.0342*
Doob grass	68.82 ± 4.45	0.2897 ± 0.0986	0.8121	0.0989	98.39 ± 0.28	0.01662 ± 0.006167	0.7841	0.1145
Para grass	70.46 ± 4.50	0.2388 ± 0.0997	0.7414	0.1389	96.14 ± 0.85	0.03405 ± 0.01879	0.6214	0.2117
Berseem	84.35 ± 4.55	0.1734 ± 0.1007	0.5971	0.2273	99.80 ± 0.05	0.003199 ± 0.0010	0.8318	0.0880
Sunhemp	72.94 ± 7.20	0.3523 ± 0.1595	0.7093	0.1578	99.10 ± 0.28	0.006398 ± 0.00632	0.3389	0.4179

Table 4b. Parameters estimates of the linear function $y = b_0 + b_1t$ of micro minerals release (%) from green fodders in the time of ruminal incubation

Feedstuffs	b_0	b_1	r^2	P-value	b_0	b_1	r^2	P-value
	Cu				Zn			
Maize fodder	39.75 ± 5.100	0.4291 ± 0.1129	0.8784	0.0628	74.26 ± 2.069	0.1334 ± 0.04579	0.8093	0.1004
Hybrid Napier	41.19 ± 1.952	0.2905 ± 0.04321	0.9576	0.0214*	46.07 ± 0.6699	0.1794 ± 0.01483	0.9865	0.0068**
Doob grass	58.74 ± 12.27	0.3820 ± 0.2715	0.4973	0.2948	45.41 ± 1.981	0.1919 ± 0.04384	0.9054	0.0485*
Para grass	42.68 ± 2.756	0.2479 ± 0.06099	0.8920	0.0556	46.06 ± 1.940	0.1888 ± 0.04295	0.9062	0.0481*
Berseem	79.55 ± 5.401	0.2066 ± 0.1195	0.5990	0.2261	66.42 ± 3.400	0.1800 ± 0.07526	0.7410	0.1392
Sunhemp	67.45 ± 5.525	0.3213 ± 0.1223	0.7754	0.1194	59.17 ± 3.377	0.2520 ± 0.07476	0.8503	0.0779
	Mn							
Maize fodder	40.53 ± 5.011	0.2206 ± 0.1109	0.6641	0.1851				
Hybrid Napier	33.31 ± 5.878	0.3108 ± 0.1301	0.7405	0.1395				
Doob grass	29.71 ± 5.295	0.3323 ± 0.1172	0.8007	0.1052				
Para grass	32.25 ± 5.706	0.3709 ± 0.1263	0.8117	0.0990				
Berseem	44.52 ± 7.578	0.4502 ± 0.1677	0.7827	0.1153				
Sunhemp	36.58 ± 7.573	0.5071 ± 0.1676	0.8206	0.0941				

of cell walls, and in the representation of individual fractions that influence the degradability of cell walls (Ceresnakova et al. 2000) and release of mineral elements (Ledoux and Martz 1991, Flachowsky *et al*. 1994).

Rumen P availability could play an important role in dietary P quality and quantity (Bravo *et al.* 2000). There was significant ($P<0.001$) differences in the release of P at different periods of incubation though no significant difference in the release of P in the rumen from different forages observed at 72h of incubation time. Similarly Pal *et al.* (2011) reported a non significant release of P in the rumen from various dry fodders at advanced period of incubation. In contrary to this finding, Emnuele *et al*. (1991) reported that grasses released more P in the intestine than legumes. However, P release was significantly ($P<0.05$) higher than Ca from experimental forages. Leguminous forages showed significantly ($P<0.001$) higher release of Mg than nonlegume forages and grasses. Ceresnakova *et al*. (2005) reported that the release of Mg from legumes was higher than grasses and dry forages. The rumen is a major site of Ca and Mg release from the forages because there are highly related to NDF degradation (Ceresnakova *et al*. 2005). More than 60% of P, Mg and K were solubilised as early as the feeds were washed (in nylon bags) by *in sacco* method (Ledoux and Martz 1991). The release of Zn and Mn was comparatively slower than Cu from different forages. Surprisingly, maize fodder showed the highest release of Zn (83%) amongst all forages which is difficult to explain from the present study. Kabaija and Smith (1988) explained low water solubility and release of Zn from plants by its bond to various complexes from which it was released in the rumen gradually. Ceresnakova *et al.* (2005) suggested

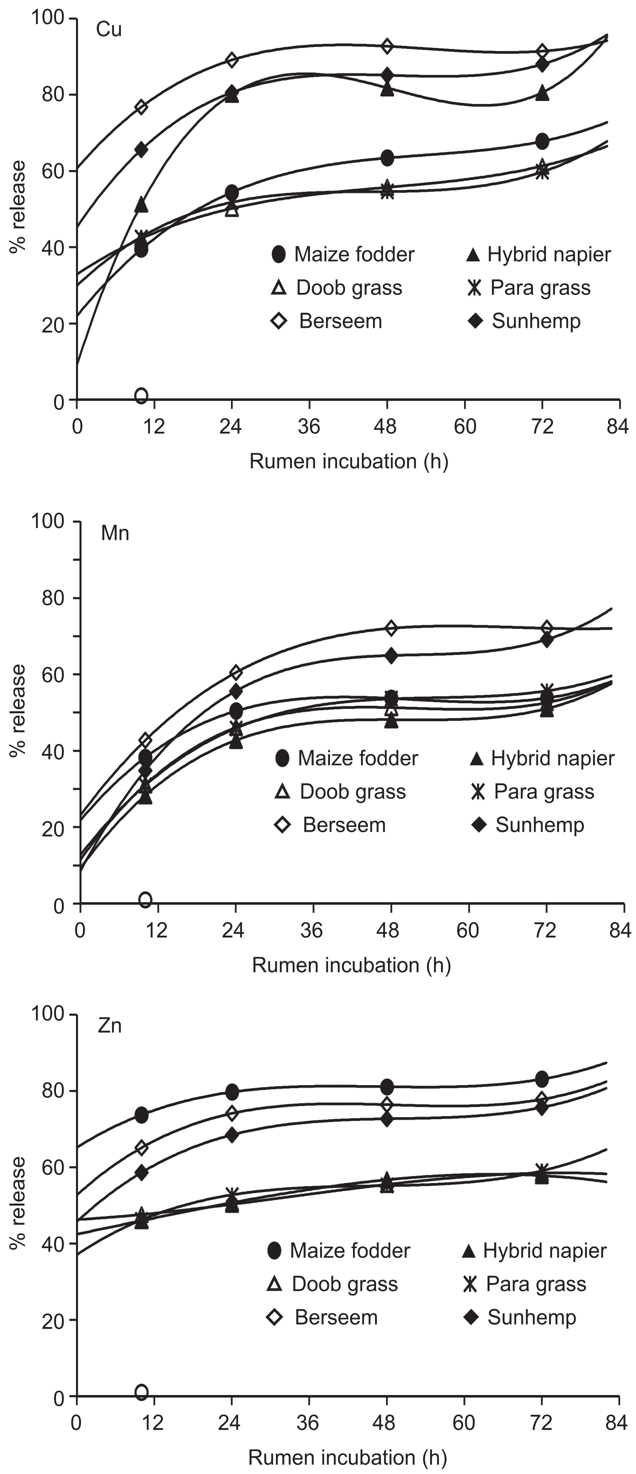

Fig. 2. Cubic polynomials of micro minerals release from green forages in relation to rumen incubation time.

that the low release of Zn in the rumen from forages and Cu from grasses must be taken into account with Zn and Cu requirements of animals. Other authors (Flachowsky *et al*. 1994, Gralak *et al*. 1997 and Trinacty *et al*. 2000) found a similar tendency and they also described differences between the feeds. The overall mineral release pattern in the rumen from green forages tested was K>Mg>P>Ca>Cu>Zn>Mn at 72h of incubation.

Based on findings of the study it may, therefore, be concluded that both the type of forage and the incubation time influences the proportion of minerals released in the rumen. Among the green forages tested, leguminous fodders showed the highest release of all the minerals as compared to nonleguminous fodders and grasses in the rumen. This information may be useful in strategic supplementation of minerals to animals through green forages.

ACKNOWLEDGEMENT

The authors are highly thankful to the Director, National Institute of Animal Nutrition and Physiology for funding the project and providing all necessary facility for carrying out the research work. The financial support from Indian council of Agricultural research (ICAR), New Delhi is duly acknowledged. The authors are highly thankful and wish to express appreciation to NDDB, Gujarat for assistance in ICP spectrometry.

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