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Release of minerals in groundnut haulm based complete diets supplemented with organic and inorganic mineral sources by *in sacco* method in steers

K SUDHA RANI[1], J V RAMANA[2], B DEVASENA[3], A RAVI[4] and P SUDHAKARA REDDY[5]

Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh 517 502 India

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ABSTRACT

The study was carried out to evaluate the effect of organic and inorganic mineral supplementation to groundnut haulm (GNH) based complete diets on mineral release by *in sacco* method. A basal complete diet was prepared with GNH and concentrate mixture (8% maize, 10% soybean meal, 21.5% deoiled rice bran and 0.5% salt) in 60:40 ratio. The basal diet was treated as control (T_1) and 3 more diets were prepared by supplementing basal diet with commercial mineral mixture (T_2), inorganic mineral supplements (T_3) and organic mineral supplements (T_4). The commercial mineral mixture was added at the rate of 1.5% in T_2 diet by adjusting the deoiled rice bran to 20%. The inorganic mineral supplements were added at the rate of 26.4g (8.3g $ZnSO_4$, 4.0g $CuSO_4$ and 14.0 g $MnSO_4$) in the T3 diet. The organic mineral supplements were added at the rate of 72g (12g Zn-P, 10 g Cu-P and 50g Mn-P) in the T4 diet. The *in sacco* mineral release of Cu, Mn and Zn from experimental diets showed that the disappearance % was higher in commercial mineral mixture supplemented diet followed by organic mineral supplemented diet, and then inorganic mineral supplemented diet. The *in sacco* copper, manganese and zinc release from feed ingredients revealed that copper disappearance (%) was higher in all the feed ingredients followed by Mn and Zn. The NDF degradability also increased with increase in incubation time. A correlation was drawn between *in sacco* mineral release and NDF degradability of the treatment diets. Significant positive correlation was observed between the NDF degradability and mineral release in the rumen. It can be concluded that the organic mineral supplements were better bioavailable than the inorganic mineral supplements.

Key words: Copper, In organic mineral, *In sacco*, Manganese, NDF degradability, Organic mineral, Zinc

Mineral supplementation is often expensive and could lead to excessive excretion or poor utilization by the animal. Knowledge about the bioavailability of the minerals will help in providing the animals with the required minerals in the optimum quantity and right proportion.

The bioavailability of the mineral varies with the type of mineral supplement. The organic mineral chelates are more bioavailable than the inorganic mineral supplements. Lambs supplemented with Zn proteinate had higher Zn retention than lambs given a similar concentration of Zn in the form of zinc oxide, an inorganic source (Lardy *et al.* 1992). The *in sacco* studies can help in estimating the ruminal release of minerals from various sources. Presence of the anti-nutritional factors like oxalates, silicates and phytates beyond a particular level may affect the utilization of the certain minerals like Ca, P, Zn, Mn and Fe (Tripathi and Karim 2008). Minerals may be absorbed in the ionic form or as soluble complexes or chelates depending on the particular mineral (Evitayani *et al.* 2006). Field (1981) stated that the mineral absorption as well as utilization in the organism depends on solubility in the rumen to a large extent. Differences in solubility of individual mineral element can be ascribed to the ability of the cell walls to bind certain elements to their surface (cation exchange capacity) that can also be influenced by NDF concentration in feed (Emanuele and Staples 1990) or to the content of the organic acids in the plant structures underlying the ability to bind and retain ions of the metals (Van Soest 1982). So, the present study was taken up to evaluate the release of minerals in the groundnut haulms based complete diets supplemented with organic and in organic sources by *in sacco* method in steers.

MATERIALS AND METHODS

The groundnut haulms were ground in a hammer mill and a basal complete diet was prepared with a ratio of 60:40 groundnut haulms and concentrate mixture (maize 8, soybean 10, DORB 21.5 and salt 0.5%). T_1 is treated as control with complete diet without any mineral supplementation. T_2, T_3 and T_4 were prepared by supplementing the complete diet with commercially available mineral mixture (T_2), inorganic mineral supplements (T_3) and organic mineral supplements (T_4), respectively (Table 1).

Present address: [2]Professor and Head (jvenkataramana @rediffmail.com), Department of Animal Nutrition, College of Veterinary Science.

Table 1. Ingredient composition (%) of complete rations

Parameter	T_1	T_2	T_3	T_4
Groundnut haulms	60	60	60	60
Maize	8	8	8	8
Soybean meal	10	10	10	10
Deoiled ricebran	21.5	20.0	21.5	21.5
Commercial mineral mixture	-	1.5	-	-
$ZnSO_4$ (8.3 g) + $CuSO_4$ (4.0 g) + $MnSO_4$ (14.0 g)	-	-	0.0264	-
Zn-P (12 g) + Cu-P (10 g) + Mn-P (50 g)	-	-	-	0.072
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100

The *in sacco* study was conducted using 3 rumen cannulated adult steers (400±10 kg) housed in individual pens to evaluate the DM, CP, NDF degradability and *in sacco* mineral release from T_1, T_2, T_3 and T_4 feeds as well as from GNH and other concentrate feed ingredients.

The animals were fed with 4 kg groundnut haulms, 2 kg concentrate (maize, DORB, soybean meal, salt) and 15 kg green fodder (APBN-1) per day. The drinking water was made available all the times. The feed samples were ground in a Wiley mill with 2mm mesh screen. About 5 g of dried sample was placed into nylon bag (15×7 cm with a pore size of 25–28µ which provides 21 cm^2 area/g sample). The nylon bags were tied with a nylon string (60 cm), after placing a marble inside and then incubated in the ventral sac of the rumen. Incubation times were 0, 3, 6, 9, 12, 24, 48, 72 h post feeding. At 0 h the bags were not incubated in the rumen but were inserted into the rumen for 1 min and removed. After removal from the rumen, the bags were washed thoroughly under a gentle stream of running tap water. The process was repeated for additional 4 times (Vanzant *et al.* 1998) and a final rinse with distilled water. The bags were dried at 60°C for 48 h. The feed ingredients were analyzed for proximate principles as per AOAC (2007) methods (Table 2). Cell wall constituents of groundnut haulms were analyzed as per Goering and Van Soest (1970). The samples were digested by wet digestion method in CEM microwave accelerated digestion system, using 0.5 g of sample and 15 ml of nitric acid. The samples were aspirated in air acetylene flame in atomic absorption spectrophotometer and mineral content was estimated. The data were subjected to statistical analysis as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Ingredient composition of complete ration: Four complete rations were formulated with same basal diet consisting of roughage (GNH) to concentrate in the ratio of 60:40 supplemented with commercial mineral mixture (T_2), inorganic mineral supplements (T_3), and organic mineral supplements (T_4) and without mineral supplements (T_1). The DORB content varied slightly to adjust the mineral supplementation (Table 1). All the diets were isonitrogenous and isocaloric. The commercial mineral mixture was supplemented as per the company's specification. The organic and inorganic supplements were included in the rations as per the NRC (2007) taking into consideration the available mineral content present content in the supplements.

Chemical composition of complete rations: The chemical composition of complete rations presented in Table 2 revealed that all the rations contain same chemical composition, as all the 4 rations contain same basal diet. The addition of mineral supplements to the basal diet did not alter the chemical composition of the rations as they are added in very small amounts. The basal or control ration (T_1) contained 31, 49 and 149 mg/Kg of Cu, Zn and Mn respectively. The mineral content of the basal diet was taken into consideration while calculating the quantity of organic and inorganic mineral supplementation.

Cell wall constituents of complete rations: Since all the 4 treatments contain the same basal diet the cell wall constituent composition was similar in all the 4 treatments. The NDF, ADF, cellulose, HC, ADL and silica content of the T_1 were 40.3, 33.3, 23.3, 7.0, 12.1, and 3% respectively (Table 3).

Mineral content of feed grade mineral supplements: The copper content of $CuSO_4$ and copper propionate were 20 and 26 mg/kg respectively. The zinc content of $ZnSO_4$ and zinc propionate were 30 and 27 mg/Kg, respectively. The manganese content of manganese sulphate and manganese propionate were 26 and 11 mg/kg respectively. The Cu, Zn and Mn contents of commercial mineral mixture were 1,000, 2,200, 1,200 mg/Kg respectively (Table 4). The mineral

Table 2. Chemical composition (%) of complete rations

Particulars	T_1	T_2	T_3	T_4
Dry matter	93.4	93.4	93.4	93.5
Organic matter	92.2	92.4	92.2	92.2
Crude protein	15.8	15.6	15.8	15.7
Crude fiber	20.0	21.0	20.0	20.0
Ether extract	1.4	1.4	1.4	1.3
Total ash	17.8	17.6	17.8	17.8
AIA	9.7	9.7	9.7	9.7
NFE	45.0	44.4	45.0	45.2
Trace mineral (mg/kg)				
Copper	31	46	41	56
Zinc	49	214	79	82
Manganese	149	170	186	204

Table 3. Cell wall constituents (%) of complete rations (dry matter basis)

Parameter	T_1	T_2	T_3	T_4
NDF	40.3	40.3	40.3	40.2
ADF	33.3	33.3	33.3	33.2
Cellulose	23.3	23.1	23.3	23.3
Hemi cellulose	7.0	7.0	7.0	7.0
ADL	12.1	12.2	12.1	12.1
Silica	30	3.1	3.0	3.1

Table 4. Mineral composition of the feed grade mineral supplements

Mineral supplement	Copper (mg/Kg)	Zinc (mg/Kg)	Manganese (mg/Kg)
Copper sulphate ($CuSO_4$)	20	-	-
Zinc sulphate ($ZnSO_4$)	-	30	-
Manganese sulphate ($MnSO_4$)	-	-	26
Copper propionate	26	-	-
Zinc propionate	-	27	-
Manganese propionate	-	-	11
Commercial mineral mixture	1000	2200	1200

The *In sacco* manganese disappearance (%) for T_1, T_2, T_3 and T_4 rations between 0 to 72 h of ruminal incubation ranged from 12 to 64%, 22 to 71%, 26 to 72% and 24 to 71% respectively (Table 6). The *in sacco* Mn disappearance (%) at 0, 3, 6, 9, 12, 24, 48 and 72 h of incubation for T_1, T_2, T_3 and T_4 rations is presented in Fig 2. the mineral supplemented groups showed better disappearance than the control group. The commercial mineral mixture supplemented group showed better Mn disappearance than organic, while inorganic mineral supplemented groups showed similar Mn disappearance (%). The mineral release was rapid between 3 to 24 h of incubation.

Table 5. *In sacco* mineral release of feed ingredients

Feed ingredient	Cu disappearance (%)		Mn disappearance (%)		Zn disappearance (%)	
	0h	72h	0h	72h	0h	72h
Maize	21	56	24	56	19	38
Soybean meal	24	58	28	52	14	41
DORB	28	62	21	49	20	42
GNH	21	60	20	42	11	45

content of the organic, inorganic and commercial mineral mixtures were in accordance with their specified mineral content given by the manufacturer.

In sacco mineral release of feed ingredients: The *in sacco* mineral release of feed ingredients is presented in Table 5. The Cu, Mn and Zn disappearance (%) of maize, soybean meal, deoiled rice bran and GNH was around 50% even up to 72 h of ruminal incubation. The *in sacco* mineral disappearance (%) of Cu from maize, soyabean meal, DORB and GNH after 72 h of ruminal incubation was higher than Mn and Zn in that order. This is supported by the studies carried out by Emanule and Staples (1990) in steers on ruminal release of minerals from 6 forage species which indicated that the ranking of the minerals for immediate release in the rumen averaged among forages was K (100%) >Mg (82%)> Cu (71%) >P (66%)> Ca (29%)> Zn (26%). Ceresnakova *et al.* (2005) studied the *in sacco* mineral release of 6 forages in the rumen and ranked the mineral release as Mg >Fe> Cu >Ca >Zn.

In sacco mineral release of experimental diets: The *in sacco* copper disappearance (%) from 0 to 72 h of incubation ranged from 16 to 63%, 31 to 75%, 22 to 71% and 18 to 69% respectively for T_1, T_2, T_3 and T_4 rations (Table 6). The *in sacco* copper disappearance at 0, 3, 6, 9, 12, 24, 48 and 72 h of incubation for T_1, T_2, T_3 and T_4 rations is depicted in Fig 1. The mineral supplemented groups indicated better Cu disappearance than the control group. Among the supplemented groups commercial mineral mixture followed by organic and then inorganic mineral supplemented groups showed better Cu disappearance. These findings were in accordance with the findings of Ivan and Grieve (1976) who reported that supplementation of the ration with copper resulted in an increase (from 33% to 60%) (P<0.01) in the total net absorption.

Table 6. *In sacco* mineral release and NDF degradability of experimental

Particulars	Incubation period (h)							
	0	3	6	9	12	24	48	72
Cu disappearance (%)								
T_1	16	27	31	37	40	44	52	63
T_2	31	38	41	52	57	62	72	75
T_3	22	36	43	50	57	62	67	71
T_4	18	26	35	46	51	58	64	69
Mn disappearance (%)								
T_1	12	21	31	40	49	54	60	64
T_2	22	36	43	50	57	62	66	71
T_3	26	30	40	51	59	65	70	72
T_4	24	34	41	52	56	63	69	71
Zn disappearance (%)								
T_1	11	20	29	38	46	52	60	67
T_2	26	34	41	49	56	61	65	70
T_3	16	29	35	49	57	65	71	72
T_4	23	30	39	47	57	64	73	75
NDF degradability (%)								
T_1	-	9	14	30	41	46	51	56
T_2	-	10	16	31	42	45	51	56
T_3	-	9	14	34	41	45	50.6	55
T_4	-	10	15	32	43	46	51	57

Table 6a. Correlation between mineral release and NDF degradability

	Cu	Mn	Zn	NDF
Cu	0.000	0.9599^{**}	0.9904^{**}	0.9016^{**}
Mn	-	0.000	0.9632**	0.8856^{**}
Zn	-		0.0000	0.8815^{**}
NDF	-			0.0000

Mean values bearing different superscript indicators in a row differ significantly (P<0.01).

The *in sacco* Mn disappearance (%) for T_1, T_2, T_3 and T_4 rations between 0 to 72 h of ruminal incubation ranged from 12 to 64%, 22 to 71%, 26 to 72% and 24 to 71% respectively (Table 6). The *in sacco* Mn disappearance (%) at 0, 3, 6, 9, 12, 24, 48 and 72 h of incubation for T_1, T_2, T_3 and T_4 rations is presented in Fig 2. the mineral supplemented groups showed better disappearance than the control group. The commercial mineral mixture supplemented group showed better Mn disappearance than organic, while inorganic mineral supplemented groups showed similar Mn disappearance (%). The mineral release was rapid between 3 to 24 h of incubation.

Ivan and Grieve (1976) observed that supplemental Zn increased the net absorption of Mn in the gastrointestinal tract. In the present study, the Zn supplementation might have increased Mn release in the rumen.

The *in sacco* Zn disappearance (%) of T_1, T_2, T_3 and T_4 rations between 0 to 72 h of incubation ranged from 11 to 67%, 26 to 70%, 16 to 72% and 23 to 75%, respectively (Table 6). The Zn disappearance values (%) of T_1, T_2, T_3 and T_4 rations at 0, 3, 6, 9, 12, 24, 48, and 72 h of incubation is presented in Fig. 3. Similar trend was observed as it was observed with Cu and Mn. The commercial mineral mixture supplemented groups showed better Zn disappearance followed by organic and inorganic in that order. Initially, the commercial mineral mixture showed better Zn disappearance but at 72 h of incubation the organic and commercial mixture supplemented groups showed similar disappearance values. The release of minerals increased with the increase in concentration of the mineral in the feed.

The present findings were supported by findings of Arelovich *et al*. (2000) who reported that the concentration of zinc in ruminal fluid increased linearly (P<0.01) with zinc supplementation. The zinc concentration in ruminal fluid was 2.26, 7.60 and 11.6 ppm zinc with 30, 250 and 470 ppm supplemental dietary Zn, respectively. All the 3 minerals (Cu, Mn and Zn) disappeared more rapidly within 24 h of incubation for all the treatments. Playne *et al.* (1978) also opined that the minerals are released between 0 to 48 h of *in sacco* incubation and the elements not released from feed stuffs in nylon bags after 48 h of incubation in the rumen are probably unavailable for absorption of animal.

Correlation was drawn between the NDF degradability and mineral release from the rations (Table 6a). The NDF degradability showed highly significant (P<0.01) positive correlation to Cu, Mn and Zn disappearance from T_1, T_2, T_3 and T_4 rations. The release of dietary NDF also increased the mineral release. This may be due to release of bound minerals with cell wall constituents. Ceresnakova *et al.* (2005) also reported that there was large difference (P<0.01) in element release in the rumen between experimental forage and the particular element. Calcium and zinc were found to have longer time for their maximum release. Evitayani *et al.* (2006) reported that the micromineral solubility increased as increasing incubation period from 0 to 72 h. they also reported that at 72 h of incubation, the average solubility of Zn, Fe, Mn and Cu in grass was 53.5, 61.4, 54.4 and 51.7% respectively while in legumes, the values were 57.6, 64.1, 47.2 and 57.7% respectively. It was concluded by giving the trend of the percentage average micromineral solubility in the rumen at 72 h incubation period for grass and legumes as Fe> Zn> Cu >Mn.

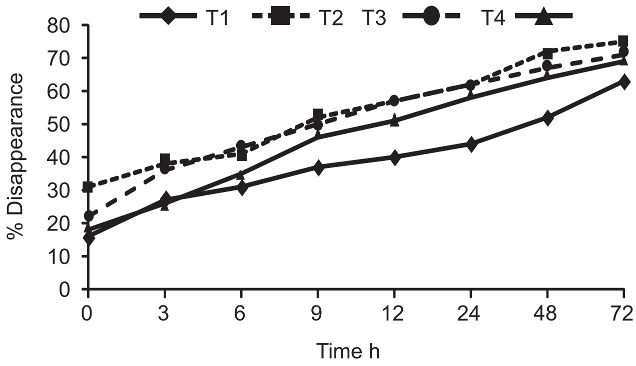

Fig. 1. *In sacco* copper release from experimental diets at different hours of incubation.

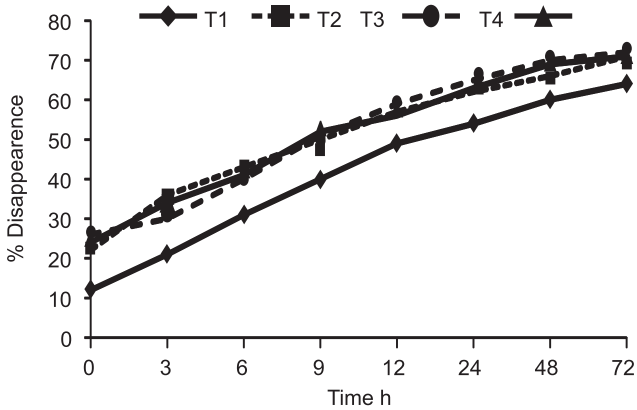

Fig. 2. *In sacco* manganese release from experimental diets at different hours of incubation.

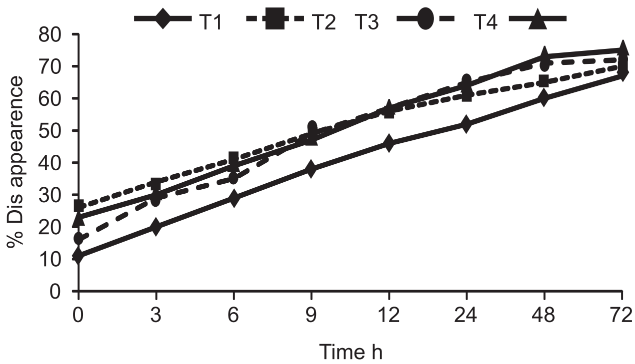

Fig. 3. *In sacco* zinc release from experimental diets at different hours of incubation.

The *in sacco* mineral (Cu, Zn, Mn) release was higher in the organic mineral supplemented diet than inorganic mineral supplemented diets. The results of the present investigation revealed that organic mineral supplements have better bioavailability than inorganic mineral supplements.

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