Ruminal Solubility of Trace Elements from Selected Philippine Forages

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ABSTRACT : The ruminal solubility of copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn) in eight Philippine forages was studied. These forages were: 1) grasses: paragrass (*Brachiaria mutica* (Forsk.) Stapf), stargrass (*Cynodon plectostachyum* Pilger), and napiergrass (*Pennisetum purpureum* Schumach.); 2) creeping legumes: calopogonium or calopo (*Calopogonium muconoides* Desv.) and centrocema (*Centrocema pubescens* Benth.); 3) tree lequmes: gliricidia or kakawate (*Gliricidia sepium* (Jacq.) Walp.), leucaena or ipil-ipil (*Leucaena leucocephala* (Lam.) de Wit.), and sesbania or katuray (*Sesbania grandiflora* (L.) Poir). Nylon bags with samples were incubated for 0, 3, 6, 12, 24, 48 and 72h in three rumen cannulated sheep fed with timothy hay (*Phleum pratense* L.) at 2% body weight/d. The 0-h bags were washed with deionized water. There were species differences ($p < 0.05$) on the different solubilities of trace elements. At O-h incubation, the trend of solubility was Mo (54%) > Zn (43%) > Cu (38.7%) > Mn (29.5%). At 3 -h incubation, all the elements except Mn had an average solubility above 50%. Combining particulate passage rate (1.9%/h) and various trace elements disappearance rates, the effective ruminal solubilization (ERS) of trace elements were computed. The ERS across species ranged from 44.6 to 89.9% for Cu, 29.9 to 84% for Mn, 66 to 95.1% for Mo, and 30.1 to 82.3% for Zn resulting to a trend of Mo > Zn > Cu > Mn.

(**Key Words** : Tropical Forage, Philippine Forage, Trace Element, Solubilization, In Situ, Grass, Legume)

INTRODUCTION

Both the amount of mineral in the forage and their bioavailability are important in assessing the mineral requirements of the animal. The former can be determined chemically while the latter is much more difficult to assess. One technique that can be used is in situ (in sacco) or nylon bag technique. The technique has been used with varying success in the determination of dry matter (DM), protein and fiber degradabilities but very few studies deal with ruminal solubility of minerals (Field, 1981). Earlier reports dealing on this topic can be found in the literature: Playne et al. 1978; Rooke et al. 1983; van Eys and Reid, 1987; Emanuele and Staples, 1990; Ledoux and Martz, 1991; Serra et al. 1996a. On these previous studies, only Rooke et al. (1983) and Emanuele and Staples (1990) included trace elements. The former included copper (Cu) and zinc (Zn) while the latter included only Cu.

In the present study the effective ruminal solubilization (ERS) of Cu, manganese (Mn), molydenum (Mo) and Zn of some Philippine forages were determined by incubating them in nylon bags in the rumen of sheep. The bags and their contents were those used by Serra et al. (1996a) to measure the ruminal solubilization of macrominerals in selected Philippine forages. Using a multi-spectrometer, the Inductively Coupled Plasma Emission Spectrometer, several elements can be determined simultaneously.

MATERIALS AND METHODS

Forage samples

The forage samples were the same to our two previous reports (Serra et al., 1996a. 1996b). These forages were: 1) grasses: paragrass (*Brachiaria mutica* (Forsk.) Stapf), stargrass (*Cynodon plectostachyum* Pilger), and napiergrass (*Pennisetum purpureum* Schumach.); 2) creeping legumes: calopogonium or calopo (*Calopogonium muconoides* Desv.) and centrocema (*Centrocema pubescens* Benth.); 3) tree legumes: gliricidia or kakawate (*Gliricidia sepium*(Jacq.) Walp.), leucaena or ipil-ipil (*Leucaena leucocephala* (Lam.) de Wit.), and sesbania katuray (*Sesbania grandiflora* (L) Poir.). All the samples were air dried and later oven dried at 60℃ for 2 d and ground to pass in a 2-

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mm screen Wiley mill. All the samples were kept properly for later analysis.

Experimental procedures

Three ruminally cannulated Japaness Corriedale wethers, average body weight of 45 kg, were fed timothy hay (*Phleum pratense* L.) at 2% body weight (DM basis). Water and mineralized salt blocks were available ad libitum throughout the duration of experiment.

After a 7-d adaptation period to the diet, nylon bags containing forage sample (5 g) were placed into the rumen of each animal following the procedure of Ørskov et al. (1980). The time of incubation were 3, 6, 12, 24, 48 and 72h. The bags were placed into the rumen at time 08:30. Only six bags (representing the various time) per incubation period were placed in the rumen of each animal. This was replicated twice such that there were six bags (three animals × two sets of incubations) for each incubation time in every forage species. Each species was incubated separately from each other.

After retrieval, the bags were washed individually with deionized water until the rinsing water cleared. The 0 bags were not placed in the rumen. Disappearance at 0 h was determined from DM and mineral loss after washing. All bags and contents were dried at 60℃ for 2 d. The residues were stored in a plastic container for later analysis.

Laboratory analysis

The DM content of all forages and residues collected in the in situ experiment were determined. Then, they were digested with nitric and perchloric acids for the determination of trace elements. The prepared solutions were analyzed for Cu, Mn, Mo and Zn using an Inductively Coupled Plasma Emission Spectrometer (ICPS-2000, Shimadzu Co., Kyoto, Japan).

Calculations and statistical analysis

The quantity of a trace element remaining in each bag after each incubation time was expressed as a proportion of that trace element originally present in the bag prior to rumen incubation. The fractional disappearance rate of each trace element from nylon bags (K_d) was calculated from the slope of the linear regression (Rooke et al., 1983) of the natural logarithm of the proportion of each trace element remaining in the bag (y) on the length of incubation in hours (X). From the intercept (c) of the linear regression equation the proportion of each trace element rapidly released from the forage (a) was determined. These values then were used in the following formula used by Rooke et al. (1983) to calculate effective ruminal solubilization (ERS):

$$ERS = a + (1 - a) \times K_d/(K_p + K_d), \qquad \text{[Equation 1]}$$

The average particulate passage rate of 1.9%/h which was previously reported (Serra et al., 1996a) was used as particulate passage rate constant (Kp).

The data were analyzed as a randomized complete block design in which the variance components between forage species (main factor) and between animals (blocking factor) were estimated, and the residual variance was obtained by subtracting both from the total. Means were compared with Duncan's multiple range test (Snedecor and Cochran, 1980) at $p < 0.05$ unless otherwise specified.. All analyses were done using statistical software, Statistica for the Macintosh™ Release 4.1, Statsoft, Inc., Tulsa, OK.

RESULTS AND DISCUSSION

Trace element concentrations of forages

Table 1 shows the trace elements concentrations of selected Philippine forages. There were differences ($p < 0.01$) existed in terms of various trace elements concentrations across species. The Cu concentration of centrocema and sesbania was highest and lowest respectively. However, sesbania appeared to have the highest Mn conncentration followed by centrocema and the least was seen in leucaena. Gliricidia contained the highest Mo concentration followed by leucaena. Zinc concentration of leucaena was markedly higher compared to other forage species; it was followed by paragrass, stargrass and the lowest was sesbania.

Trace element disappearance at 0-h

The proportion of each trace element disappearing from nylon bags due to water rinsing gives an idea of the proportion of each trace element which is readily solubilized. This is presented in table 2 expressed as a percentage of original concentration in the forage. There were differences ($p < 0.05$) existed in terms of DM and trace elements disappearances across species. The DM disappearance varied from 5.8 to 33.3%. The lowest value was observed in calopo while the highest was observed in sesbania.

The ranking of the trace elements disappeared due to water rinsing averaged across forages was Mo > Zn > Cu > Mn. The reverse was found by Emanuele and Staples (1990), on the trend of Cu (50.6 to 88.9%) and Zn (7.3 to 43.1%) in some temperate and subtropical forages. The solubility in water of the various trace elements under study could be explained partly on their location in the plant cell. Our previous findings (Serra et al., 1996b) showed a percentage of total trace element located in the cell wall and the trend was Cu (16.4%) > Mo (9.3) > Mn (5.6) > Zn (2.6%). Thus Cu is associated to the fiber component of the forages especially in legumes where its correlation coefficient (r) to NDF and ADF was 0.90 and 0.93 resepctively (Serra et al., 1996b). It was also presented by Robson et al. (1981) that Cu deficiency decreased the lignin and hemicellulose of cell walls in severely deficient wheat plants. Whitehead et al. (1985) observed that trace elements, i. e. Fe. Mn. Zn and Cu were present in the cell wall fractions of some temperate grasses and legumes.

Among the forage species, gliricidia and calopo had the highest and lowest ($p < 0.05$) Cu solubility respectively. These two species are both legumes where the former is a tree legume and latter is a creeping legume. It is expected in calopo to have a low release of Cu including its Mn, Mo and Zn concentrations due to its low DM disappearance rate. Manganese in grasses especially in napiergrass and paragrass was better (p <

Table 1. Dry matter and trace element concentration of selected Philippine forages

Forage	DM (%)	Cu	Mn	Mo	Zn
		.... mg/kg DM			
Grasses					
Para	90.1	9.0^{ab}	54^{d}	14.7^{c}	82.8^{b}
Star	92.4	8.8^{ab}	69^{c}	14.9^{c}	77.2^{b}
Napier	89.0	7.1^{bc}	33^{f}	14.4^{c}	50.4^{cd}
Creeping legumes					
Calopo	88.3	10.0^{ab}	54^{d}	16.2^{c}	60.8^{c}
Centrocema	88.3	11.2^{a}	77^{b}	15.1^{c}	40.4^{d}
Tree legumes					
Gliricidia	92.4	6.6^{bc}	43^{e}	33.0^{a}	55.1^{c}
Leucaena	90.7	7.1^{bc}	36^{f}	20.6^{b}	101.3^{a}
Sesbania	89.0	5.0^{c}	99^{a}	15.3^{c}	30.0^{e}
Mean		8.1	58	18.0	62.2
SEM		0.7	1.2	0.5	2.1

^{a,b,c,d}Means within column having different superscripts differ ($p < 0.01$).

Table 2. Dry matter and trace element disappearance from nylon bags washed with deionized water (Oh bags)

Forage	DM (%)	Cu	Mn	Mo	Zn
		.... %			
Grasses					
Para	17.8^{c}	36.7^{bc}	42.9^{a}	53.1^{c}	44.1^{cd}
Star	24.0^{b}	32.0^{bc}	32.3^{bc}	66.2^{b}	46.8^{bcd}
Napier	17.6^{c}	39.8^{b}	43.9^{a}	67.8^{b}	60.6^{a}
Creeping legumes					
Calopo	5.8^{d}	18.7^{c}	1.6^{e}	23.3^{e}	21.7^{e}
Centrocema	18.5^{c}	39.9^{b}	36.5^{ab}	40.3^{d}	54.9^{abc}
Tree legumes					
Gliricidia	22.7^{b}	85.5^{a}	25.0^{cd}	88.1^{a}	58.4^{ab}
Leucaena	23.9^{b}	20.1^{c}	35.9^{b}	51.7^{cd}	18.0^{e}
Sesbania	33.3^{a}	36.8^{bc}	18.2^{d}	41.5^{cd}	39.1^{d}
Mean	20.4	38.7	29.5	54.0	43.0
SEM	0.2	4.4	2.9	2.9	3.3

^{a,b,c,d,e}Means within column having different superscripts differ ($p < 0.05$).

0.05) solubilized than those Mn in legumes. Molybdenum in gliricidia was markedly soluble ($p < 0.05$) but not Zn in leucaena ($p < 0.05$).

Trace element disappearance at 3-h

The DM and trace elements disapperances after 3 h incubation in the rumen are presented in table 3 expressed as a percentage of the original concentration in the forage. Differences ($p < 0.05$) across species were noted on their DM and trace elements disappearances. The average DM disappearance was 29.1% and ranged from 16.4 to 44.7%. Furthermore, it could be observed that DM disappearance rate was lowest ($p < 0.05$) in centrocema and highest ($p < 0.05$) in sesbania. An improvement of 9 percentage unit in DM disappearance across species at 3-h incubation when compared to 0-h incubation.

Table 3. Dry matter and trace element disappearance from nylon bags placed in the rumen for 3h

Forage	DM (%)	Cu	Mn	Mo	Zn
		....	.... %	....	....
Grasses					
Para	22.1^{cd}	51.8^{c}	63.1^{a}	74.0^{b}	70.1^{a}
Star	38.4^{a}	42.4^{cd}	54.8^{ab}	83.9^{a}	55.1^{bc}
Napier	23.4^{bcd}	49.3^{c}	43.8^{b}	87.4^{a}	62.6^{ab}
Creeping legumes					
Calopo	19.7^{cd}	30.0^{d}	-0.8^{d}	58.4^{c}	23.4^{d}
Centrocema	16.4^{d}	75.5^{b}	52.8^{ab}	54.6^{c}	57.9^{bc}
Tree legumes					
Gliricidia	36.3^{ab}	88.2^{a}	1.7^{d}	71.0^{b}	58.7^{bc}
Leucaena	32.1^{abc}	11.1^{e}	-2.7^{d}	59.8^{c}	30.7^{d}
Sesbania	44.7^{a}	66.2^{b}	22.9^{c}	10.8^{d}	48.4^{c}
Mean	29.1	51.8	29.5	62.5	50.9
SEM	1.6	5.0	5.5	4.7	3.3

^{a,b,c,d,e}Means within column having different superscripts differ ($p < 0.05$).

The trend on the average trace element ruminal disappearance across species was: Mo > Cu > Zn > Mn. There was an increased on the average ruminal disappearance of Cu, Mo and Zn but not in Mn compared to their 0-h incubation. Among the forage groups, creeping and tree legumes except in centrocema and sesbania showed a decrease values on their Mn solubilization when compared to their 0-h incubation (washed with water only). A negative release value of Mn was even observed in leucaena and calopo which means the attachment of free Mn from the rumen environment to their particulate material. Therefore, Mn in any type of legumes is not readily released in the rumen. Aside from this element, Cu in leucaena and Mo in gliricidia and sesbania were also lowered their solubilities at 3-h incubation period. Some trace elements in legumes have low solubilities probably because of their affinities to cell wall fractions as explained earlier or because of the presence of tannin compounds. It is well known that this group of plants especially from those tree legumes contain tannins. As reviewed by Norton (1994) that gliricidia and leucaena contained a tannin of 20 to 30 and 37 to 55g/kg DM respectively. However, tannin was not detected in the leaves of sesbania. Tannins found in some tree legume leaves form complexes with plant proteins which decrease their rate of digestion (degradability) in the rumen (Norton, 1994), somehow some mineral fractions are possibly being covered or trapped or they have an affinity to this compound thereby their release are affected.

Trace element disappearance at 48-h

The DM and trace elements disappearances after 48 h incubation in the rumen are presented in table 4 expressed as a percentage of the original concentration in the forage. Differences ($p < 0.05$) on the DM and trace elements disappearances existed across species. The average DM disappearance was 71.0% and ranged from 49.2 to 93.6%. It was lowest ($p < 0.05$) in calopo and highest ($p < 0.05$) in sesbania. There were similarities between the DM and trace elements disappearances of 48 and 72 h incubation periods.

Table 4. Dry matter and trace element disappearance from nylon bags placed in the rumen for 48h

Forage	DM (%)	Cu	Mn	Mo	Zn
		....	%	....	
Grasses					
Para	66.0[e]	62.0[c]	78.4[b]	81.9[c]	78.0[bc]
Star	78.9[b]	78.8[b]	88.1[a]	92.5[b]	89.9[a]
Napier	69.9[d]	75.8[b]	65.1[c]	94.5[b]	84.5[ab]
Creeping legumes					
Calopo	56.2[f]	50.4[d]	30.2[e]	77.1[d]	31.1[d]
Centrocema	49.2[g]	91.2[a]	62.6[c]	66.0[e]	73.2[c]
Tree legumes					
Gliricidia	78.6[b]	90.3[a]	66.7[c]	99.0[a]	83.7[ab]
Leucaena	75.4[c]	44.2[d]	43.7[d]	83.3[c]	78.5[bc]
Sesbania	93.6[a]	91.1[a]	69.8[bc]	91.2[b]	84.3[ab]
Mean	71.0	73.0	63.1	85.7	75.4
SEM	2.7	3.7	3.7	2.1	3.7

[a,b,c,d,e,f,g] Means within column having different superscripts differ ($p < 0.05$).

The trend on mean trace elements disappearances was highest in Mo followed by Zn, Cu and the lowest was Mn. At this point of incubation (48 h) and even at 72 h, all the trace elements in grasses had a disappearances above 60% while legumes either creeping or trees had variable trace elements disappearances. A very high disappearance was seen in Cu from centrocema, gliricidia and sesbania but very low disappearances in Mn and Cu of calopo and leucaena and Zn of calopo.

Generally, Mo was the most available in all forages than Zn, Cu and Mn because of their high ruminal release. The lower ruminal release of Zn, Cu and especially in Mn were probably due to their affinities to cell wall fractions. Also, the presence of tannins could not be ruled out on their influence to ruminal release of trace elements in some forage species.

Comparing the different forage groups, grasses tended to release its Cu, Mn, Mo and Zn consistently than legumes. The two creeping legumes had a ruminal release of trace elements different ($p < 0.05$) from each other. The tree legumes, gliricidia and sesbania were higher ($p < 0.05$) in their ruminal release of the aforementioned trace elements than that of leucaena. Thus ruminal release of trace elements can not be generalized in legumes groups.

Effective ruminal solubilization of the trace elements

The ERS of some trace elements in selected Philippine forages are shown in table 5. The ERS was calculated based on Equation 1 where particulate passage rate and disappearance rate of each trace element were included in the equation, The average particulate passage of 1.9%/h (Serra et al., 1996a) was used in the equation. The average disappearance rate of various trace elements was 1.28%/h for Cu, 1.01%/h for Mn, 1.76%/h for Mo, and 1.28%/h for Zn. Based on the table, differences ($p < 0.05$) existed on various ERS of trace elements across species.

The order of ERS across species was Mo > Zn > Cu > Mn. It ranged from 44.6 to 89.9, 29.9 to 84, 66 to 95.1, and 30.1 to 82.3% in Cu, Mn, Mo and Zn respectively. The ERS of trace elements in this study were much lower than those found by Rooke et al. (1983) in silages with an ERS value of 90 and 88% in Cu and Zn respectively, using a particulate passage rate of 2%/h. Our study implies that solubilization is not a factor limiting the ruminant's ability to absorb trace elements except those in calopo (Cu, Mn and Zn) and leucaena (Cu and Mn). It is the ruminal environment, however, that affects most the bioavailability of trace elements, e.g the action of ruminal microbes can render trace elements unavailable in the rumen (Durand and Kawashima, 1980). Even dietary protein lowers the ruminal solubility of Cu but not in Fe, Mn and Zn (Ivan and Veira, 1981). The formation of complexes between trace elements and organic ligands tend to make trace elements insoluble like formation of Cu thiomolybdate complexes (Grace, 1991). However, not all complexes are insoluble because some trace elements combined to amino acids for readily absorption in the gut which may result for the trace elements competing for amino acids or other potential carriers (Butler and Jones, 1973). With all these conditions, *in vivo* apparent digestibilities are expected to be low as observed by Perdomo et at. (1977). They obtained a range of 60.2 to 73.7% and 27.6 to 55.1% *in vivo* apparent digestibility of Cu and Zn, respectively, of some tropical grasses.

Those fractions of trace elements which are insoluble in the rumen especially in calopo and leucaena will probably solubilize in the lower gut due to lower pH. Solubilities of trace elements are increased by decreasing pH as shown *in vitro* (Keith and Bell, 1981). The protein of leuacena, a high quality forage in the tropics tend to bypass the rumen (Aii and Stobbs, 1990) and possibly including some of its trace elements.

Table 5. Effective ruminal solubilization of some trace elements in selected Philippine forages[1]

Forage	Cu	Mn	Mo	Zn
	....	.... %	....	....
Grasses				
Para	60.8^{e}	76.9^{b}	80.7^{d}	76.8^{b}
Star	74.2^{c}	84.0^{a}	91.7^{bc}	80.5^{ab}
Napier	70.5^{d}	64.0^{cd}	92.9^{ab}	77.0^{b}
Creeping legumes				
Calopo	54.5^{f}	29.9^{f}	75.9^{e}	30.1^{d}
Centrocema	89.9^{a}	60.3^{d}	66.0^{f}	70.5^{c}
Tree legumes				
Gliricidia	88.0^{ab}	65.9^{c}	95.1^{a}	81.7^{a}
Leucaena	44.6^{g}	40.6^{e}	79.7^{d}	71.6^{c}
Sesbania	86.1^{b}	64.1^{c}	89.3^{c}	82.3^{a}
Mean	71.1	60.7	83.9	71.3
SEM	1.5	2.8	1.7	1.4

[1] Effective ruminal solubilization (%) was calculated according to Rooke et al. (1993): ERS = a+(1 − a) × kd/(kp+kd), where a is the proportion of minerals rapidly solubilized, kd is the fractional rate of mineral disappearance from nylon bags and kp is the rate of passage of particulate material. Average particulate passage rate, 1.9%/h (Serra et al., 1996a); average of disappearance rate: 1.28%/h for Cu, 1.01%/h for Mn, 1.76%/h for Mo, and 1.28%/h for Zn.

[a,b,c,d,e,f,g] Means within column having different superscripts differ ($p < 0.05$).

CONCLUSION

This study concludes that species differences exist on the solubilities of trace elements (Cu, Mn, Mo, and Zn) in Philippine forages. Combining particulate passage rate and trace elements disappearance rates, this study indicates that the trend of ERS is Mo > Zn > Cu > Mn. Furthermore, ERS data inconjunction to their initial concentration, forages can be classified base on their contribution to the trace element requirements of the animals.

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