



## Mineral Status in Cattle Fed Rice Straw and Para Grass Combined with Different Levels of Protein Derived from Cassava Foliage

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**ABSTRACT:** Eight male cattle of the Local Yellow breed with an average live weight of 121 kg and an average age of 18 months were used to evaluate the effects of different levels of sun-dried cassava (*Manihot esculenta*) foliage supplementation on mineral metabolism in growing cattle fed rice straw and para grass as basal diet. Rice straw *ad libitum* and para grass (*Brachiaria mutica*) at 1% DM of BW comprised the basal diet. The study was arranged as a 4×4 double Latin square design, with cassava foliage contributing 0, 0.8, 1.6 or 2.4 g CP/kg BW. The cassava foliage intake was lower than the planned levels. DM consumption was significantly affected by cassava foliage supplementation, with the largest intake observed at the two highest levels of cassava foliage supplementation. Rice straw intake showed the opposite pattern, with lower intake at higher cassava foliage supplementation. No refusals occurred for para grass in any of the treatments. Ca, P, Mg, K, S and Mn intake increased significantly with increasing intake of cassava foliage, but Na intake was not affected by treatment. Faecal excretion of Ca, Mg, S and Mn increased significantly with increasing cassava foliage intake. There were no differences between P, K and Na excretion in faeces. There was a significant diet effect on Mg, S and Mn digestibility. Mg and Mn digestibility increased with increasing cassava foliage supplementation, while S digestibility decreased. Ca, P, K and Na digestibility was not affected by diet. There was a significant effect of treatment on P retention, with the highest value observed for supplementation with 1.6 g CP/kg BW cassava foliage. Ca and Mg showed similar trends, with the highest retention again for supplementation with 1.6 g CP/kg BW cassava foliage. There were weak but significant positive correlations between nitrogen retention and the macro minerals Ca, P and Mg. Furthermore, retention of all these minerals was positively correlated. Mineral losses in urine were not affected by dietary treatment with the exception of P excretion, which was affected by treatment. In conclusion, cassava foliage is a good Ca source which compensates for the low Ca content in rice straw and para grass, but P deficiency appears to be exaggerated in cattle with higher cassava intake. The results suggest that under these conditions growing cattle on a high cassava intake would benefit from P and S supplementation. (**Key Words:** Digestibility, Intake, Retention, Cassava Foliage, Rice Straw, Para Grass)

### INTRODUCTION

Tropical feed stuffs vary markedly in content of essential minerals and usually do not meet the requirement of growing cattle. Phosphorus (P) deficient soils occur in Thailand, Cambodia and many other countries in the tropical regions, resulting in P-deficient crops (Smithson and Giller, 2002). Cattle consuming diets not meeting their P requirements exhibit reduced growth and feed efficiency and decreased dry matter (DM) intake, and develop weak and fragile bones (Suttle, 2010). Cattle production in

Cambodia and other countries in tropical areas of Asia is strongly associated with rice cultivation and the by-product rice straw is a commonly used feed source (Sath et al., 2008). The level of phosphorus in rice straw is less than the level that animals need for growth (Nath et al., 1969). The content of available Ca is also generally marginal in rice straw (Joshi and Talapatra, 1968). It has recently been shown that the performance of growing cattle fed rice straw and para grass improves when the diet is supplemented with cassava foliage (Sath et al., 2012). However, it is well established that cassava contains cyanogenic glucosides, which are potentially toxic for ruminants, but dietary organic sulphur may detoxify these glucosides (Promkot and Wanapat, 2009).

Information about the mineral balance of growing cattle in South East Asia consuming diets typical for the region appears to be scarce. Thus the aim of the present study was

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Submitted Jun. 25, 2012; Accepted Sept. 18, 2012; Revised Sept. 26, 2012

to study mineral metabolism in growing cattle fed rice straw, para grass and cassava foliage.

## MATERIALS AND METHODS

### Location

The experiment was carried out at the Centre for Livestock and Agriculture Development, about 25 km south of Phnom Penh, Cambodia. The daily temperature during the experiment ranged from 26 to 32°C. The experiment was conducted from May to July, 2009.

### Animal and experimental design

The animals used comprised eight male cattle of the Local Yellow breed with an average body weight (BW) of 121 kg (18 months of the age) at the start of the experiment. The cattle were wormed using ivermectin and vaccinated against foot-and-mouth disease prior to the experiment. They were kept in individual metabolic cages (1 m×1.8 m, with roof) allowing total collection of urine and faeces and had free access to the experimental diets. The experimental setup was a 4×4 double Latin square design with periods of 15 d on each treatment, 10 d for adaptation and 5 d for data collection.

The four diets were:

T<sub>1</sub>: Rice straw+para grass

T<sub>2</sub>: Rice straw+para grass+sun-dried cassava foliage (0.8 g CP/kg BW)

T<sub>3</sub>: Rice straw+para grass+sun-dried cassava foliage (1.6 g CP/kg BW)

T<sub>4</sub>: Rice straw+para grass+sun-dried cassava foliage (2.4 g CP/kg BW)

### Diet and feeding

The cattle were fed rice straw *ad libitum* and para grass at 1% DM of BW as the basal diet and only the level of cassava foliage differed between the four experimental diets. Rice straw was collected after harvesting of rice grown during the rainy season and stored under cover. Para grass was grown as a monoculture, collected and stored for one day prior to feeding. Cassava foliage was collected at root harvesting. The cassava leaves plus petioles were separated from the hard stems and sun-dried for 3 to 5 d by spreading them on a plastic sheet placed on the ground. After sun drying, the foliage was stored under cover in hay bags. Para grass and rice straw were cut to about 30 to 40 cm length before feeding. Water was made available during the whole period. The experimental diets were fed three times a day, at 08:00, 12:00 and 16:00, in equal portions. The composition of the feeds is shown in Table 1. At the beginning and end of each period during the trial, the cattle were weighed in the morning before feeding.

**Table 1.** Chemical composition of feeds used in the experiment

	Rice straw	Para grass	Cassava leaves	Cassava petioles
DM (g/kg)	895	194	879	886
In g/kg DM				
CP	35	146	232	88
OM	866	879	923	917
Ca	3.1	2.8	13.6	20.5
P	0.5	2.5	1.8	0.7
Mg	0.7	2.4	3.5	3.6
K	13.4	24	11.3	11.2
Na	0.8	8.3	0.05	0.1
S	0.6	3.2	1.9	0.7
Mn	0.4	0.09	0.3	0.7

DM = Dry matter, CP = Crude protein, OM = Organic matter, Ca = Calcium, P = Phosphorus, Mg = Magnesium, K = Potassium, Na = Sodium, S = Sulphur, Mn = Manganese.

### Sample collection and analyses

Feed refusals were collected and their DM weight recorded each morning for individual animals. Total collection of faeces and urine was performed on a daily basis during the last 5 d of the collection period. Sub-samples of faeces were taken from daily samples and stored in a freezer. Approximately 1% of total urine excreted was sampled and stored in a freezer for subsequent analysis. The samples from each animal were pooled for each period before analysis.

The DM of feed and faecal samples was determined as described by Undersander et al. (1993). Crude protein CP (N×6.25) and ash were determined according to AOAC (1990). Ca, P, Mg, K, Na, S and Mn concentrations in feeds and faeces were determined by inductively coupled plasma optical emission spectroscopy (Spectroflame, Spectro Analytical Instruments GmbH, Kleve, Germany) using HNO<sub>3</sub> for extraction according to Balsberg-Påhlsson (1990). Ca, P, Mg, K, Na and S concentrations in urine were analysed by the same method.

### Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of MINITAB Software, version 16.1.1 (Minitab, 2010). Treatment means that showed significant differences at probability level  $p < 0.05$  were compared using the Turkey's pair-wise comparison procedure. The following statistical model was used:

$$y_{ijkl} = \mu + T_k + P_i + A_j + S_l + e_{ijkl}$$

where  $y_{ijkl}$  = the dependent variable,  $\mu$  = overall mean value,  $T_k$  = effect of treatment,  $P_i$  = effect of period,  $A_j$  = effect of animal,  $S_l$  = effect of square and  $e_{ijkl}$  = effect of

**Table 2.** Mean values of feed and nutrient intake and daily weight gain of cattle fed different diets

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM	p
Feed intake (g DM/d)						
Para grass	1,293	1,304	1,280	1,253	20.6	0.350
Rice straw	2,173 <sup>a</sup>	1,962 <sup>ab</sup>	1,971 <sup>ab</sup>	1,824 <sup>b</sup>	69.9	0.017
Cassava foliage	0 <sup>d</sup>	460 <sup>c</sup>	875 <sup>b</sup>	1,149 <sup>a</sup>	28.7	<0.001
Total DMI	3,466 <sup>b</sup>	3,727 <sup>b</sup>	4,125 <sup>a</sup>	4,225 <sup>a</sup>	72.4	<0.001
CPI (g/d)	266 <sup>d</sup>	345 <sup>c</sup>	422 <sup>b</sup>	467 <sup>a</sup>	6.9	<0.001
OMI (g/d)	3,019 <sup>c</sup>	3,269 <sup>b</sup>	3,636 <sup>a</sup>	3,738 <sup>a</sup>	62.9	<0.001
Total DM (% BW)	2.7 <sup>b</sup>	2.8 <sup>b</sup>	3.1 <sup>a</sup>	3.2 <sup>a</sup>	0.04	<0.001
Daily gain (g/d)	155 <sup>b</sup>	251 <sup>b</sup>	469 <sup>a</sup>	492 <sup>a</sup>	52.2	<0.001

<sup>a,b,c</sup> Means within rows with different subscript letters are significantly different ( $p < 0.05$ ).

DMI = Dry matter intake, CPI = Crude protein intake, OMI = Organic matter intake.

random error.

On an individual basis, partial correlations were estimated among the variables Ca, P, Mg and N retention using the basic statistics option correlation in MINITAB Software, version 16.1.1 (Minitab, 2010).

## RESULTS

### Feeds and mineral intake

The actual intake of the different dietary components is shown in Table 2. The cassava foliage intake was lower

than the planned levels of 0.8, 1.6 and 2.4 g CP/kg BW. Dry matter consumption was significantly affected by cassava foliage supplementation, with the largest intake observed at the two highest levels of cassava foliage supplementation (treatments T<sub>3</sub> and T<sub>4</sub>). Rice straw intake showed the opposite pattern, with lower intake at higher cassava foliage supplementation. No refusals were seen for para grass, which was fed at 1% DM of BW in all treatments. Ca, P, Mg, K, S and Mn intake increased significantly with increasing intake of cassava foliage, but Na intake was not affected by treatment (Table 3). The total intake of P and S

**Table 3.** Mean mineral intake, excretion to faeces and digestibility in cattle fed the four diets

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM	p
Intake (g/d)						
Ca	10.3 <sup>d</sup>	17 <sup>c</sup>	23.4 <sup>b</sup>	27 <sup>a</sup>	0.46	<0.001
P	4.4 <sup>c</sup>	5 <sup>b</sup>	5.6 <sup>a</sup>	5.8 <sup>a</sup>	0.09	<0.001
Mg	4.7 <sup>d</sup>	6.3 <sup>c</sup>	7.8 <sup>b</sup>	8.7 <sup>a</sup>	0.13	<0.001
K	60 <sup>c</sup>	62.8 <sup>bc</sup>	66.9 <sup>ab</sup>	67.4 <sup>a</sup>	1.11	<0.001
Na	12.3	12.3	12.2	11.9	0.20	0.304
S	5.4 <sup>c</sup>	6 <sup>b</sup>	6.6 <sup>a</sup>	6.9 <sup>a</sup>	0.11	<0.001
Mn	1.1 <sup>c</sup>	1.2 <sup>b</sup>	1.3 <sup>a</sup>	1.4 <sup>a</sup>	0.03	<0.001
Faeces (g/d)						
Ca	7.4 <sup>d</sup>	11.7 <sup>c</sup>	15.3 <sup>b</sup>	20.3 <sup>a</sup>	0.70	<0.001
P	2.8	2.8	2.8	3.5	0.21	0.079
Mg	3.5 <sup>c</sup>	4.2 <sup>bc</sup>	4.6 <sup>b</sup>	5.8 <sup>a</sup>	0.21	<0.001
K	7.1	8.1	7.8	9.4	0.74	0.207
Na	2.4	1.8	1.8	1.8	0.26	0.271
S	2 <sup>c</sup>	2.5 <sup>bc</sup>	2.6 <sup>b</sup>	3.4 <sup>a</sup>	0.13	<0.001
Mn	0.9 <sup>b</sup>	0.9 <sup>ab</sup>	0.9 <sup>ab</sup>	1.1 <sup>a</sup>	0.04	0.013
Digestibility (g/kg intake)						
Ca	281	315	346	263	25.6	0.135
P	362	427	502	414	37.9	0.104
Mg	241 <sup>b</sup>	320 <sup>ab</sup>	408 <sup>a</sup>	344 <sup>ab</sup>	32.4	0.013
K	883	871	885	863	9.8	0.367
Na	807	852	853	851	21.4	0.365
S	623 <sup>a</sup>	587 <sup>a</sup>	606 <sup>a</sup>	519 <sup>b</sup>	16.4	0.001
Mn	181 <sup>b</sup>	222 <sup>ab</sup>	313 <sup>a</sup>	236 <sup>ab</sup>	30.7	0.044

<sup>a,b,c</sup> Means within rows with different subscript letters are significantly different ( $p < 0.05$ ).

showed the same trend as the total intake of Mn, i.e. significant differences between T<sub>1</sub> and T<sub>2</sub> and the two highest cassava levels (T<sub>3</sub>+T<sub>4</sub>) but with no significant differences between T<sub>3</sub> and T<sub>4</sub>. Ca and Mg intake showed the same significant trend among treatments (Table 3).

### Mineral digestibility and retention

Faecal excretion of Ca, Mg, S and Mn increased significantly with increasing intake of cassava foliage intake. There were no differences between P, K and Na excretion in faeces. Faecal excretion of Mg and S showed the same trend, i.e. significant differences between the group fed no cassava foliage (T<sub>1</sub>) and the two groups with the highest cassava foliage intake (T<sub>3</sub>+T<sub>4</sub>) but no difference between T<sub>2</sub> and T<sub>3</sub> diets. Mn faecal excretion only differed between the T<sub>1</sub> and T<sub>4</sub> diets (Table 3). However, there was a significant effect of diet on Mg, S and Mn digestibility. Mg and Mn digestibility increased with increasing cassava foliage supplementation, while S digestibility decreased (Table 3). The highest digestibility of both Mg and Mn was observed for diet T<sub>3</sub> and the lowest for diet T<sub>1</sub>. The lowest S digestibility was observed with diet T<sub>4</sub>, which was significantly lower than for the other three diets (T<sub>1</sub>-T<sub>3</sub>). Ca, P, K and Na digestibility was not affected by diet. There was a significant effect of treatment on P retention, with the highest value observed for treatment T<sub>3</sub>. Ca and Mg showed similar trends, with the highest retention also for treatment T<sub>3</sub> (Table 4). There were weak but significant positive correlations between nitrogen retention and the macro minerals Ca, P and Mg. Furthermore, the retention values of all these minerals were positively correlated (Table 5). Mineral losses in urine were generally not affected by diet with the exception of P excretion, which was higher for treatment T<sub>4</sub> than for T<sub>1</sub> (Table 4).

**Table 5.** Correlations (r, p-value) between N, Ca, P and Mg retention

	N retention (g)	Ca retention (g)	P retention (g)
Ca retention (g)	0.516***	-	-
P retention (g)	0.357*	0.870***	-
Mg retention (g)	0.498***	0.859***	0.864***

\* p<0.05, \*\*\* p<0.001.

## DISCUSSION

### Ca and P intake and metabolism

A number of minerals are essential in the animal diet. In growing cattle, the maintenance and growth requirements of essential minerals must be covered by the diet provided. The concentration of minerals in different forages varies greatly. In the present study rice straw, para grass and varying amounts of sun-dried cassava foliage were offered to growing cattle in Cambodia. The different dietary combinations gave rise to significant variations in intake of all minerals studied except Na. As expected, the concentration of Ca in both rice straw and para grass was low. Thus cattle only consuming rice straw and para grass had a deficient intake of Ca based on current recommendations for growing cattle (Nielsen and Volden, 2011). On the other hand, the content of Ca in cassava leaves and petioles was high. This confirms results presented by Gomez and Valdivieso (1985) showing high levels of Ca in dried cassava foliage. Cassava proved to be a good Ca source and can be used in order to supplement feeds with a poor Ca content. However, the P content was rather low in cassava foliage. The Ca/P ratio increased in treatments with higher cassava inclusion. In the diet with the highest cassava inclusion, the Ca/P ratio was 4.7, far above the generally accepted safe range of 1:1 to 2:1 (ARC,

**Table 4.** Mineral excretion to urine, retention and concentration in blood plasma of cattle fed the four diets

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM	p
Urine (g/d)						
Ca	0.02	0.02	0.02	0.02	0.002	0.146
P	0.01 <sup>b</sup>	0.01 <sup>ab</sup>	0.01 <sup>ab</sup>	0.02 <sup>a</sup>	0.001	0.028
Mg	0.5	0.5	0.7	0.6	0.072	0.286
K	34.5	36.9	36.9	39.8	2.19	0.414
Na	5.3	5.8	5.1	5.8	0.43	0.582
S	1.7	2.0	1.8	1.9	0.12	0.280
Retention (g/d)						
Ca	2.9 <sup>c</sup>	5.3 <sup>b</sup>	8.1 <sup>a</sup>	6.7 <sup>ab</sup>	0.59	<0.001
P	1.6 <sup>b</sup>	2.1 <sup>ab</sup>	2.8 <sup>a</sup>	2.4 <sup>a</sup>	0.18	0.002
Mg	0.7 <sup>c</sup>	1.5 <sup>b</sup>	2.5 <sup>a</sup>	2.3 <sup>ab</sup>	0.20	<0.001
Ca/P	2.2	2.5	2.8	2.9	0.3	0.339
Blood plasma (mmol/L)						
Ca	1.8	1.8	1.8	1.9	0.05	0.737
Mg	0.7	0.7	0.6	0.7	0.02	0.289

<sup>a,b,c</sup> Means within rows with different subscript letters are significantly different (p<0.05).

1980). All diets were deficient in P in relation to the actual requirements of the animals (NRC, 2000; Nielsen and Volden, 2011). Growing calves of dairy breeds require 0.30 to 0.34% P in the diet DM, while the dietary P requirement of growing Zebu steers is even higher, (Silva de Almeida et al., 2009). In the present study the P content was about 0.13% of diet DM, i.e. markedly lower than recommended. While low urinary P excretion can be expected for ruminants, according to Puls (1994) a urine output below 3.6 mg/L indicates deficiency. In the present study the average urinary output was lower than this level in all dietary treatments, indicating that the cattle were P-deficient. Chizzotti et al. (2009) reported a significant linear relationship between retained Ca and retained P such that:  $\text{Ca (g/d)} = 2.57 \times \text{retained P (g/d)} - 0.44$  ( $r^2 = 0.93$ ). In the present study a corresponding, but weaker, relationship was observed:  $\text{retained Ca (g/d)} = 3.01 \times \text{retained P (g/d)} - 0.89$  ( $r^2 = 0.76$ ). This relationship indicates that less P was retained in relation to Ca than observed in the study by Chizzotti et al. (2009). Prolonged P deficiency results in clinical symptoms (Suttle, 2010). However, although the results clearly indicate that the cattle in the present study received too little dietary P, there were no obvious effects on their performance.

#### **Mg and S intake and metabolism**

The Mg content was relatively high in both cassava foliage and para grass, but low in rice straw, and Mg intake increased with increased cassava intake. However, even in cattle consuming no cassava, Mg intake was above the level recommended for cattle by NRC (2000). It is generally accepted that a surplus of absorbed Mg is rapidly excreted with the urine (Martens and Schweigel, 2000). The minimal inevitable loss of Mg with urine is 3 to 4 mg/kg BW (Guéguen et al., 1989). The average BW of the cattle in the present study was 131 kg and the minimal Mg losses with urine would thus be about 0.5 g/d. The average Mg excretion did not fall below that level in any of the dietary treatments and there was no difference in daily urinary Mg excretion.

The required dietary content of S has been set to 1.5 g of the whole diet in growing cattle, which is normally assumed to satisfy the high requirements of rumen microbiota (NRC, 2000). In the present study the average intake was close to this level in all four treatments. However the S requirement may increase in ruminants fed cassava and other feeds containing cyanogenic glucosides (Onwuka et al., 1992; Promkot and Wanapat, 2009). An increased level of S supplementation from 1.5 to 4 g/kg DM can be beneficial to cows consuming cassava foliage in terms of DM intake and it has been suggested that the increased S intake facilitates cyanide detoxification (Promkot and Wanapat, 2009). In the present study the

apparent S digestibility decreased significantly in cattle receiving the highest level of cassava supplementation. S is mainly absorbed as S-containing amino acids in the small intestine (Doyle and Moir, 1980). Our results may reflect use of S for detoxification and thus reduced intestinal uptake of these amino acids. However, it is also possible that the lower S digestibility reflected the overall reduced nutrient digestibility related to increased cassava consumption (Sath et al., 2012).

#### **K and Na intake and metabolism**

The K intake was mainly related to DM intake and was well above the requirement of growing cattle (Puls, 1994). More than 80% of the K consumed was excreted with urine, indicating that the capacity for absorption of K was high. There was a marked difference in Na content between the different feed sources. As expected, the Na content in both cassava foliage and rice straw was too low to satisfy the requirement of growing cattle (Fasuyiet, 2005). However, the Na content of para grass was higher and compensated for the low Na content in cassava and rice straw. The content of Na was efficiently absorbed and the surplus excreted with urine.

#### **Ca, P, Mg and N correlations**

Daily retention rates of Ca, P and Mg were all strongly correlated with each other ( $r = 0.86-0.87$ ). The correlations were expected, since the main fraction of these minerals is found in bone and their net retention presumably reflects bone mineralisation. However, it has been shown that the rate of bone mineralisation does not follow the rate of protein retention (Chizzotti et al., 2009). This lack of correlation is probably explained by the fact that soft tissues, which are the main pool of protein, contain only small amounts of Ca and P. Chizzotti et al. (2009) also suggested that the requirement for mineralisation diminishes relative to the requirement for protein as cattle grow heavier. In the present study, protein retention showed a moderate correlation to Ca retention and Mg retention (0.52 and 0.50, respectively). On the other hand, the correlation between protein retention and P retention was poorer (0.36), although still significant. It is possible that the poorer correlation of P to protein retention reflected the observed inadequate dietary P supply regardless of treatment. However, the significant relationship between bone mineralisation and protein accretion in the present study suggests that the skeletal tissue was still growing.

## **CONCLUSIONS**

From the findings of this study, it can be concluded that cassava foliage is a good Ca source which compensates for the low Ca content in rice straw and para grass. However all

feeds used here had a low P content and thus none of the diets provided enough P for the animals. The P deficiency appeared to be exaggerated in cattle with higher cassava intake. The results suggest that growing cattle, especially those on a high cassava intake, would benefit from supplementation with P and S under these conditions.

## ACKNOWLEDGEMENTS

The authors are grateful to the Swedish International Development Agency for Research Cooperation with Developing Countries (Sida/SAREC), through the regional programme Mekong basin Agriculture Research Network (MEKARN), for financial support for this research.

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