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Effects of Calcium Fertilization on Oxalate of Napiergrass and on Mineral Concentrations in Blood of Sheep

M. M. Rahman[1,2], T. Nakagawa[3], M. Niimi[1], K. Fukuyama[4] and O. Kawamura[1,]*
[1] Faculty of Agriculture, University of Miyazaki, Japan

ABSTRACT : An experiment was conducted to investigate the effects of calcium (Ca) fertilization on oxalate content in napiergrass (*Pennisetum purpureum*) and on some blood parameters of sheep. Re-growth napiergrass was grown with or without Ca fertilizer and offered to sheep. Eight sheep, divided into two groups of 4 animals each were used. Calcium-fertilized napiergrass was offered daily to the animals as experimental treatment, whereas without Ca-fertilized napiergrass was given to the animals as control. Results showed that there was a trend to lower soluble and total oxalate concentrations in Ca-fertilized grass than control. The Ca-fertilized grass contained ($p<0.05$) lower concentrations of K and Mg than control, though Ca fertilization had no affect ($p>0.05$) on Ca and Na concentrations in plants. Feeding of Ca-fertilized grass had no affect on the feed consumption, blood Mg level and daily gain of sheep. However, sheep fed control grass had lower ($p<0.05$) blood Ca level than sheep fed Ca-fertilized grass. Our findings suggest that Ca fertilization might minimize the negative effects of oxalate. (**Key Words :** Blood, Calcium Fertilizer, Mineral Status, Napiergrass, Oxalate, Sheep)

INTRODUCTION

Oxalic acid is synthesized by a wide range of plants and its concentration in animal feed is of great concern because of health-related hazard. Oxalic acid occurs in plants as soluble and/or insoluble salts. Previous studies suggest that insoluble oxalate, which presents mostly as a calcium (Ca) complex, is not thought to have a harmful effect on the body's metabolism as it seems to pass through the digestive tract (Ward et al., 1979), whereas soluble oxalate is likely to be absorbed in the intestine and cause chronic Ca deficiency (Blaney et al., 1982). McKenzie et al. (1988) reported that levels of 2.0% or more soluble oxalate can lead to acute toxicosis in ruminants. In a previous study, we observed that sheep fed high oxalate-containing grass had lower blood Ca levels than sheep fed low oxalate-containing grass (Rahman et al., 2011). Although soluble oxalate is degraded by rumen microflora (Allison et al., 1977) to some extent, there has been no attempt to assess the potential of using Ca fertilizer in grass to reduce the toxicity of soluble oxalate in ruminants.

Among the tropical grasses, setaria contained high levels of oxalate (Jones and Ford, 1972) and napiergrass contained medium levels of oxalate (Rahman et al., 2006). Cattle mortality occurred on setaria pasture due to acute hypocalcaemia (Jones et al., 1970). Although napiergrass is well cultivated in the tropical and subtropical areas of the world due to its high biomass yield, mortality of cattle and buffalo calves has also been reported following feeding on napiergrass (cv. Pusa giant) that contained 30.1 g/kg oxalic acid (Dhillon et al., 1971; Sidhu et al., 1996).

The previous results of our study have shown that the concentration of soluble oxalate in pot-grown napiergrass can be partially replaced by formation of insoluble oxalate using Ca fertilizer (Rahman et al., 2009a). However, evaluation should be conducted in large plots so that their effect can be studied on animals is of agricultural importance. The objective of this experiment was to evaluate the potential of Ca fertilizer to decrease soluble oxalate content in napiergrass and its subsequent effects on the chemical composition of tissue, voluntary intake and blood parameters of sheep.

* Corresponding Author : Osamu Kawamura. Tel: +81(0)985-58-7148, Fax: +81(0)985-58-7259, E-mail: kawamura@cc.miyazaki-u.ac.jp
[2] Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia.
[3] Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Japan.
[4] Sumiyoshi Livestock Science Station, University of Miyazaki, Japan.
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MATERIALS AND METHODS

Site and field design

Two 0.01 ha adjacent paddocks had been established on 8-year-old perennial napiergrass (*Pennisetum purpureum* cv. dwarf-late) in Sumiyoshi Field at the University of Miyazaki. Prior to the start of the field experiment, the experimental area was grazed intensively by cows. After grazing, each plot was fertilized by N (75 kg/ha), P_2O_5 (150 kg/ha) and K_2O (300 kg/ha) on 6 September 2010. Two experimental treatments were set-up in paddock 1 (0.01 ha) and paddock 2 (0.01 ha) comprising applications of: i) no Ca (0 kg/ha) and ii) Ca (500 kg/ha), respectively. The fertilizers used were sodium nitrate for N, super-phosphate for P_2O_5, potassium chloride for K_2O and calcium hydroxide for Ca.

Soil properties

Soil samples were collected from each plot with 3 replications at 5-10-cm depths of the soil surface on 7 December 2010. The soil pH was determined on a 1:5 soil water (w/v) suspension. Soil pH was affected ($p<0.05$) by application of Ca fertilizer and it increased from 5.06 to 5.23 as Ca fertilizer level increased from 0 to 500 kg/ha, respectively (Table 1). Similarly, the potassium (K) and Ca concentrations in soil were affected by application of Ca fertilizer. The Ca-fertilized soil contained lower ($p<0.05$) K and higher ($p<0.05$) Ca than soil fertilized without Ca. However, sodium (Na) and magnesium (Mg) concentrations in soil were not affected by application of Ca fertilizer.

Animals

The animal experiment was conducted in the Sumiyoshi Livestock Science Station at the University of Miyazaki according to the procedures approved by the University of Miyazaki Animal Care and Use Committee. The experiment consisted of 8 sheep (Suffolk, 2 years of age, 51.01±9.35 kg body weight) obtained from a local supplier. Upon arrival, all sheep were dewormed with 500 μg per kg of body weight (BW) ivermectin solution (Fujita Pharmaceutical Co., Ltd, Tokyo, Japan). These sheep were randomly divided into two groups of 4 animals each. Sheep in group 1 were fed the control grass, whereas those in group 2 were given the experimental grass. The two groups were kept separately for the whole experimental period in semi-open stalls with yards to facilitate movement of the animals. Group feeding was applied. Prior to beginning the experimental feeding, all sheep were fed alfalfa cubes for 14 d. The experimental period, during which the measurements were taken, lasted for 54 d, i.e. from the 21st of Oct. to the 13th of Dec.

Table 1. The pH and mineral concentration (g/kg) of soil fertilized with or without calcium (Ca) (date of collection: Dec. 7, 2010)

Parameter	Control (Mean±SE)	Ca-fertilized (Mean±SE)
pH	5.06^a±0.04	5.23^b±0.05
Potassium (K)	2.08^b±0.04	1.90^a ±0.05
Sodium (Na)	0.69±0.04	0.69±0.06
Calcium (Ca)	0.80^a±0.02	1.14^b±0.05
Magnesium (Mg)	3.38±0.11	3.27±0.03

SE = Standard error.
[a, b] Means in the same row with different superscripts differ significantly ($p<0.05$).

Diets

The control grass for the sheep was without Ca-fertilized napiergrass, whereas that for the experimental sheep was Ca-fertilized napiergrass. The grasses were cut daily by cut-and-carry method, and offered to sheep in the morning. Animals had access to grass and water *ad libitum*. Amount of grasses offered were adjusted to every 3-4 d to maintain the preferred daily refusal rate of 10%. No concentrate was offered to the animals.

Average feed intake of the sheep was similar between the two groups (Table 2). During the feeding period from late Oct. to mid Dec., the treatments imposed had no effect on BW for all measuring dates, and the final BW were similar ($p>0.05$) on both treatments.

Blood sampling and sample analysis

Body weight was recorded at 2-week intervals. Daily records were kept on feed consumption. Representative samples of offered grass and orts were taken at approximate 7-d intervals for chemical analysis and dry matter (DM) determination. Approximately 10 ml of blood was collected via the jugular vein using heparinized collection tubes in the

Table 2. Mean feed intake and daily gain of sheep fed napiergrass

Parameter	Control	Ca-fertilized	SEM	Significance
Feed intake (kg/d)[1]	1.21	1.22	-	-
Initial body weight (kg)	51.0	51.1	3.57	NS
Final body weight (kg)	54.5	53.2	4.49	NS
Daily gain (g/d)	67.0	41.0	20.32	NS

NS = Not significant ($p>0.05$); SEM = Standard error of mean.
[1] Since group feeding was performed, no statistical analysis was done for feed intake.

Table 3. Oxalate concentration (g/kg) of napiergrass fertilized with or without calcium (Ca)

Sampling date	Soluble oxalate		Total oxalate	
	Control (Mean±SE)	Ca-fertilized (Mean±SE)	Control (Mean±SE)	Ca-fertilized (Mean±SE)
	%			
Oct. 21, 2010	23.4±1.11	22.3±2.10	27.6±1.37	27.1±2.05
Nov. 9, 2010	17.3^{b}±0.13	14.4^{a}±0.07	20.1^{b}±0.58	18.2^{a}±0.02
Nov. 16, 2010	14.4^{b}±0.34	9.04^{a}±0.04	19.9^{b}±0.19	15.2^{a}±1.04
Nov. 19, 2010	11.9^{a}±0.10	14.3^{b}±0.08	16.5^{a}±0.06	20.2^{b}±0.03
Nov. 26, 2010	15.9^{b}±0.16	14.8^{a}±0.04	20.3±0.17	19.6±0.30
Dec. 4, 2010	16.1^{b}±0.02	13.5^{a}±0.12	21.8^{b}±0.14	17.7^{a}±0.01
Mean	16.5±0.87	14.7±0.99	21.0±0.84	19.7±0.95

SE = Standard error. [a, b] Means within the same oxalate type and sampling date followed by the different superscripts differ significantly (p<0.05).

morning before feeding on d 0, 15, 29, 43 and 53. Blood was centrifuged at 1,006×*g* for 15 min at room temperature after which the plasma was transferred into eppendorf tubes and stored at -20°C for analysis of plasma constituents.

Concentrations of K, Na, Ca and Mg in forage and soil were determined by flame atomic absorption spectrophotometer after wet digestion with nitric acid and hydrogen peroxide (Laboratory of Agricultural Chemistry, the University of Tokyo, 1978). Oxalate concentration in grass was measured following the method of Rahman et al. (2007).

Blood samples were diluted 1:1 with an aqueous solution of 5% lanthanum chloride, then diluted 50-fold with water. Subsequently, concentrations of Ca and Mg in blood were determined by flame atomic absorption spectrophotometer, following the method of Thomas and Skujins (1999).

Statistical analysis

The standard *t*-test was used to estimate the significance of difference of means for various characteristics between the two dietary treatments. The statistical analysis of experimental data was done by using the SPSS for Windows Release (version 12.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Oxalate and mineral concentrations in napiergrass

There was a trend of lower soluble and total oxalate concentrations in Ca-fertilized grass than in the control for all sampling dates except for 19th Nov. harvest (Table 3). Irrespective of sampling dates, the difference was not found to be significant (p<0.05). For all sampling dates, Ca-fertilized grass had significantly (p<0.05) lower concentration of K than control grass (Table 4). Similarly, there was a trend to lower Na concentration in Ca-fertilized grass than in the control for all sampling dates (except for sampling date of 26th Nov.), though the differences were not found to be significant (p>0.05). Irrespective of sampling dates, Ca-fertilized grass contained lower Ca and Mg concentrations than control grass and the observed

Table 4. Potassium (K) and sodium (Na) concentrations (g/kg) of napiergrass fertilized with or without calcium (Ca)

Sampling date	K		Na	
	Control (Mean±SE)	Ca-fertilized (Mean±SE)	Control (Mean±SE)	Ca-fertilized (Mean±SE)
	%			
Oct. 21, 2010	41.0^{b}±0.30	33.1^{a}±1.73	1.15±0.06	1.06±0.01
Nov. 9, 2010	38.6^{b}±1.76	28.1^{a}±0.81	1.15±0.01	1.16±0.01
Nov. 16, 2010	35.2^{b}±0.26	25.0^{a}±0.19	1.35±0.11	1.13±0.02
Nov. 19, 2010	31.6^{b}±0.27	29.2^{a}±0.69	1.19±0.03	1.13±0.03
Nov. 26, 2010	35.0^{b}±0.30	30.3^{a}±0.36	1.09±0.03	1.19±0.03
Dec. 4, 2010	33.7^{b}±0.14	28.9^{a}±0.90	1.15±0.01	1.12±0.01
Mean	35.8^{b}±0.79	29.1^{a}±0.67	1.18±0.03	1.13±0.01

SE = Standard error.
[a, b] Means within the same mineral (K or Na) type and sampling date followed by the different superscripts differ significantly (p<0.05).

Table 5. Concentrations (g/kg) of calcium (Ca) and magnesium (Mg) in napiergrass fertilized with or without Ca

Sampling date	Ca		Mg	
	Control (Mean±SE)	Ca-fertilized (Mean±SE)	Control (Mean±SE)	Ca-fertilized (Mean±SE)
	%			
Oct. 21, 2010	2.18±0.06	2.51±0.20	1.53±0.06	1.60±0.06
Nov. 9, 2010	2.45±0.09	2.62±0.08	1.63±0.05	1.72±0.05
Nov. 16, 2010	2.87±0.11	2.62±0.14	1.94^{b}±0.07	1.72^{a}±0.02
Nov. 19, 2010	2.59±0.06	2.41±0.03	2.12^{b}±0.09	1.17^{a}±0.01
Nov. 26, 2010	2.83^{b}±0.05	2.54^{a}±0.08	1.56^{b}±0.02	1.49^{a}±0.01
Dec. 4, 2010	2.77^{b}±0.08	2.35^{a}±0.07	1.56^{b}±0.01	1.33^{a}±0.04
Mean	2.62±0.07	2.51±0.05	1.72^{b}±0.06	1.50^{a}±0.05

SE = Standard error.

$^{a, b}$ Means within the same mineral (Ca or Mg) type and sampling date followed by the different superscripts differ significantly (p<0.05).

difference for Mg was found to be significant (p<0.05) (Table 5).

Calcium and Mg status in the blood

There was a trend of lower Ca concentration in blood of sheep fed control grass than in sheep fed Ca-fertilized grass, but the difference was mostly not significant except for Nov. 5 harvested napiergrass fed sheep (Table 6). However, the treatments imposed had no effect on blood Mg concentration, and the Mg concentrations remained fairly uniform on both treatments for all sampling dates.

DISCUSSION

Calcium fertilizer has been shown to have an influence on the concentrations of soluble and total oxalate in grass for almost all sampling dates. In a previous study, the concentration of soluble oxalate decreased from 11.52 to 7.98 g/kg DM as the application rate of Ca increased from 0 to 485 kg/ha, and this reduction might have occurred by increasing the insoluble oxalate in grass (Rahman et al., 2009a). Although Ca concentration was higher in Ca-fertilized soil than control soil, Ca concentration in plants was not affected by Ca fertilization in this study. This implies that absorption of Ca by grass was similar in both treatments. Calcium fertilizer may have reduced the soluble oxalate concentration due to a low K concentration in the grass, because K concentration in plants is positively correlated with soluble oxalate concentration (Rahman et al., 2008). The Ca-fertilized grass contained lower K concentration than control grass, suggesting that Ca fertilizer inhibits the absorption of K from the soil. This result is consistent with the previous study of Rahman et al. (2009a) who observed that K concentration in plants gradually decreased with an increased rate of Ca application.

Sheep fed high oxalate-containing grass had lower blood Ca level than sheep fed low oxalate-containing grass (Rahman et al., 2011), because oxalic acid binds with Ca to form Ca oxalate, a non-soluble, non-digestible compound (Blaney et al., 1982). The present study showed that sheep fed Ca-fertilized grass had higher blood Ca level than sheep fed control grass, even though total Ca intake was similar between the treatments. This result may be due to the presence of a lower concentration of soluble oxalate in Ca-fertilized grass than in the control grass, because soluble oxalate combines chemically with free Ca to become

Table 6. Concentrations (mg/dl) of calcium (Ca) and magnesium (Mg) in blood of sheep fed napiergrass

Sampling date	Ca		Mg	
	Control (Mean±SE)	Ca-fertilized (Mean±SE)	Control (Mean±SE)	Ca-fertilized (Mean±SE)
	mg/dl			
Oct. 21, 2010	13.0±0.28	13.3±0.49	1.49±0.11	1.85±0.13
Nov. 5, 2010	11.4^{a}±0.13	13.3^{b}±0.49	1.56±0.17	1.61±0.24
Nov. 19, 2010	12.4±0.26	13.6±0.56	1.52±0.10	1.70±0.16
Dec. 3, 2010	12.7±0.57	13.4±0.39	1.57±0.10	1.65±0.15
Dec. 13, 2010	12.9±0.27	13.3±0.21	1.54±0.05	1.63±0.11
Mean	12.5^{a}±0.19	13.4^{b}±0.18	1.54±0.04	1.69±0.07

SE = Standard error.

$^{a, b}$ Means within the same mineral (Ca or Mg) type and sampling date followed by the different superscripts differ significantly (p<0.05).

insoluble oxalate in the rumen, reducing absorption of Ca (Blaney et al., 1982). On the other hand, soluble oxalate may also be absorbed from the rumen into the blood stream where it can combine with serum Ca to form insoluble Ca oxalate crystals and then precipitate in the kidneys (Lincoln and Black, 1980). No effect on blood Mg concentration was observed between the treatments, which is in agreement with the observations of James and Butcher (1972) and Rahman et al. (2011).

No toxicological symptoms were observed in the sheep, probably because blood Ca concentrations of all sheep in both treatments (11.4-13.6 mg/dl) were in the normal range of Ca (9.0-12.0 mg/dl) as described by Kincaid (1993). This normal range of blood Ca may be due to a lower soluble oxalate concentration in the grass. McKenzie et al. (1988) reported that levels of 2.0% or more soluble oxalate can lead to acute toxicosis in ruminants. In the present study, re-growth napiergrass grown in autumn contained more than 2.0% soluble oxalate only for the sampling date of 21 Oct. and then declined. This decline might be due to the age of plant materials and seasonal effect, because oxalate concentration declines as the harvest interval increases (Rahman et al., 2009b), and oxalate concentration in plants decreases with the advancing of the season with the highest value (3.77%) being associated with early summer samples and the lowest value (1.76%) with late autumn samples (Rahman et al., 2006).

In conclusion, Ca fertilizer decreased the concentrations of K and Mg in plants, while the Ca concentration in Ca-fertilized grass was similar to that of control grass. There was a trend to decrease the concentrations of soluble and total oxalate using Ca fertilizer for almost all sampling dates. Feeding of Ca-fertilized grass had no effect on the feed consumption, blood Mg level and daily gain of sheep. There was a trend of lower Ca concentration in the blood of sheep fed control grass than in sheep fed Ca-fertilized grass, but the difference was mostly not significant ($p>0.05$) except for Nov. 5 harvested napiergrass fed sheep. Our findings thus support the hypothesis that feeding of Ca-fertilized grass can minimize the negative effects of oxalate.

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