Effect of clipping interval and nitrogen fertilisation on oxalate content in pot-grown napier grass (*Pennisetum purpureum*)

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Abstract

Tropical grasses sometimes contain oxalate in sufficient concentrations to induce calcium deficiency and toxicity in grazing animals. This pot study was undertaken to investigate the effects of clipping interval and N fertilisation on the oxalate concentrations in napier grass. Plants were treated with 2 rates (5 and 30 g/m^2) of N fertiliser (as urea) and harvested at 3 clipping intervals (2, 4 and 8 weeks). At all clipping intervals, both forage dry matter yield and crude protein concentration in the forage were greater ($P<0.05$) with the higher N fertiliser rate than with the lower rate. Clipping increased oxalate concentration ($P<0.05$), and oxalate levels declined as the harvest interval increased. Across all clipping regimes, concentrations of soluble (15.2 *vs* 14.9 g/kg DM), insoluble (11.1 *vs* 12.0 g/kg DM) and total (26.3 *vs* 26.8 g/kg DM) oxalates in the plant tissues were not affected significantly ($P>0.05$) by level of N fertiliser applied. These results suggest that N fertilisation using urea at the levels we used will not result in elevated oxalate levels in napier grass. Harvesting at 8-weekly intervals will lower oxalate concentrations without resulting in low CP levels, provided moderate N fertiliser rates are used.

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Introduction

Napier grass (*Pennisetum purpureum*) is a valuable forage in the tropics and subtropics because of its high biomass yield. Although napier grass can be fed safely under most conditions, it can accumulate unsafe concentrations of soluble oxalate (35.8–47.5 g/kg DM) during early summer (Rahman *et al*. 2006). Napier grass (cv. Pusa giant) poisoning has been reported in livestock (Dhillon *et al*. 1971; Sidhu *et al*. 1996), and oxalate (30.1 g/kg DM) has been identified as a primary toxin. There are numerous reports indicating mild to acute poisoning and even death of livestock following the consumption of oxalate-rich plants (James 1968; Jones *et al*. 1970; McKenzie *et al*. 1988).

The soluble oxalate content in *Setaria sphacelata* was reported to increase significantly following N fertilisation and decline with the aging of plant tissue (Jones and Ford 1972). In another study, Rahman *et al*. (2006) reported that the accumulation of soluble oxalate in napier grass decreased significantly as the seasons advanced, with the highest value (37.7 g/kg DM) in early summer and the lowest value (17.6 g/kg DM) in late autumn, while the oxalate content was higher in leaf (27.8 g/kg DM) than in stem (20.5 g/kg DM). However, no research has been carried out to evaluate the impact of N fertilisation on oxalate concentration in napier grass throughout the growing season with different clipping intervals. As such, the objective of this study was to determine the concentration of oxalate in napier grass at 2 N-fertiliser rates and 3 clipping intervals.

Materials and methods

Location and climatic conditions

Rooted tillers of the late-heading variety of napier grass (cv. Dwarf-late) were transplanted into Wagner's pots (size 1/2000 a; diameter 25 cm;

depth 30 cm) (1 plant/pot) filled with 10 kg commercial heated soil (provided by Miyazaki Shodo Co. Ltd, Miyazaki, Japan) on May 11, 2006. The pots were placed in an experimental field of the Faculty of Agriculture, University of Miyazaki, Japan, at a density of 1 pot/m^2. Climatic conditions during the plant growth period were recorded at the Miyazaki Meteorological Station about 10 km north from the experimental site (Figure 1). Mean daily temperature, monthly precipitation, solar radiation and relative humidity during the growing period were 22.9°C, 243.6 mm, 15.1 MJ/m^2/d and 75.9%, respectively, with maximum precipitation (540.5 mm) in July. The mean maximum and minimum temperatures were 27.8 and 15.6°C, recorded in August and November, respectively.

Experimental design and sampling procedure

The experimental design was a factorial arrangement with 2 N application rates (main plot, 5 and 30 g/m^2/yr) and 3 clipping intervals (subplot, 2-weekly, 4-weekly and 8-weekly) arranged in a randomised complete block layout with 4 replicates. Plant tops were harvested 15 cm above the soil surface at 10 weeks after transplanting, and dry matter (DM), N and oxalate determinations were performed on the harvested material, which was then discarded. Plants treated with the high rate of N yielded more dry matter (DM) than those at the low rate of N (121 *vs* 83 g/m^2), which contained higher crude protein (CP) (103.4 *vs* 49.7 g/kg DM), soluble oxalate (9.5 *vs* 9.4 g/kg DM) and insoluble oxalate (3.4 *vs* 2.4 g/kg DM). Based on these data, each subplot was subsequently clipped at the same height (15 cm above the soil surface). The number of harvests during the growing season, excluding the initial clipping, was 8, 4 and 2 for 2-weekly, 4-weekly and 8-weekly cutting treatments, respectively. One-third of the N fertiliser (as urea) was applied to the pots at planting, and the remainder was applied as equal split applications following each clipping for the various treatments. Water was supplied when necessary. The pots were standing in saucers which caught any water which emerged from the base of the pot after watering, and this water was reapplied to the pots. The DM concentration in the fresh biomass was determined at each defoliation date by drying the harvested material in an oven at 70ºC for 48 h.

Chemical analysis

The dried samples were ground in a Wiley mill through a 1 mm screen and preserved in plastic bottles for chemical analysis. These samples were

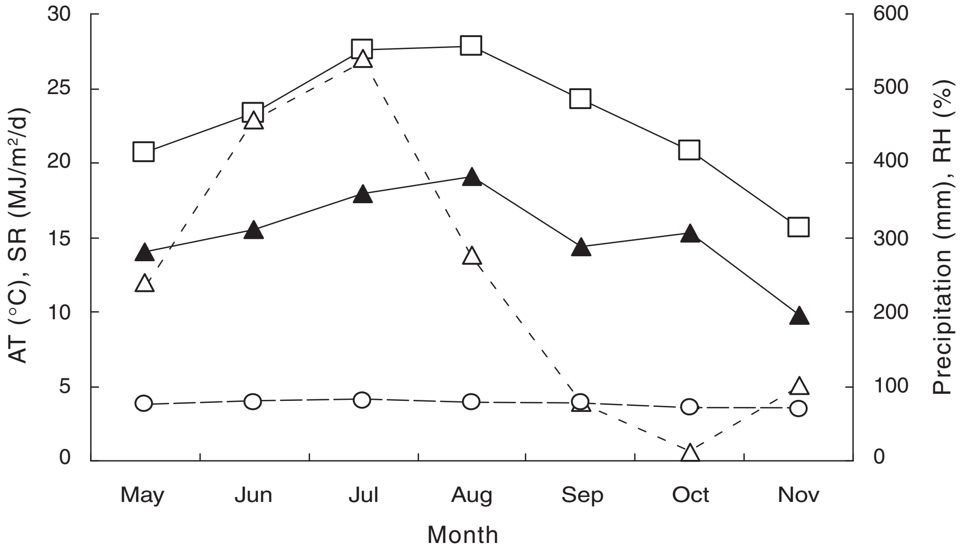

Figure 1. Monthly variation in air temperature (AT, □), precipitation (△), solar radiation (SR, ▲) and relative humidity (RH, ○) during the experimental period in 2006.

analysed for N concentration by the Kjeldahl method (AOAC 1990), and oxalate concentration by the method of Rahman *et al*. (2007). Crude protein concentration was calculated as N × 6.25.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) to determine the effect of clipping interval on DM yield and CP and oxalate concentrations in plant tissue by using the general linear model procedure of SPSS (version 12.0, Statistical Package for the Social Sciences software, SPSS Inc, Chicago, USA) and the differences between the means were determined by the least significant difference method. Statistically significant differences between the oxalate concentrations for the 2 fertiliser levels were determined by the Student's *t* test (Steel and Torrie 1984).

Results

Dry matter yield and crude protein concentration in plant tissue

At all clipping intervals, both DM yield and CP concentration in the plant tissue were higher (P<0.05) with the high rate of N fertiliser than with the low rate (Table 1). At the low fertiliser rate, clipping interval had no significant effect (P>0.05) on DM yield of napier grass, while clipping 4-weekly produced higher yields than clipping 2-weekly at the higher fertiliser level (Table 1). Crude protein concentration declined (P<0.05) as the clipping interval increased. Highest CP concentration (236.0 g/kg DM) was observed with high N and 2-weekly clipping and the lowest (71.6 g/kg DM) with low N and 8-weekly clipping.

Oxalate concentration in plant tissue

Overall, soluble oxalate concentrations in harvested material were about 15 g/kg DM, while insoluble and total oxalate concentrations were about 11.5 and 26.6 g/kg DM, respectively. Clipping interval had a significant (P<0.05) effect on both soluble and total oxalate concentrations in plant tissues at both N fertiliser rates, with oxalate levels declining as clipping interval increased (Figure 2). While insoluble oxalate levels also tended to decline as clipping interval increased at both N fertiliser rates, differences failed to reach significance (P>0.05).

There was no consistent effect of N fertilisation on oxalate concentrations in napier grass across all clipping frequencies (Figure 2).

Table 1. Effects of clipping interval on cumulative dry matter yield (g/m^2) and mean crude protein concentration (g/kg DM) in napier grass at 2 rates of N fertiliser.

Parameter	Rate of N fertiliser (g/m^2)	Clipping interval		
		2 weeks	4 weeks	8 weeks
Total dry matter yield (g/m^2/yr)	5	59A[1] (144)[2]	64A (152)	53A (128)
	30	92aB (181)	152bB (259)	125abB (263)
Crude protein (g/kg DM)	5	150.9cA	96.0bA	71.6aA
	30	236.0cB	197.9bB	143.4aB

[1] Means within rows followed by different lower case letters and within columns and parameters followed by different upper case letters differ (P<0.05).
[2] Figures in parentheses represent annual total DM yield including the initial clipping.

Discussion

This study has shown that the oxalate levels in napier grass can be manipulated by varying the harvesting interval, and that fertilising with N might not result in unacceptably high oxalate concentrations in forage. The decline in oxalate levels of plant material as harvest interval increased agreed with the observations of Jones and Ford (1972), who reported that the oxalate concentrations in *Setaria sphacelata* declined with the age of the plant material. While we did not sort material into leaf and stem, the decline with age could be explained by a decrease in leaf: stem ratio in the harvested material as harvest interval increased, as stems contain less oxalate than leaves (Rahman *et al*. 2006).

Although plants can tolerate significant amounts of oxalate in tissues during the different stages of growth, high oxalate levels in plants have long been a significant concern for animal nutritionists, because of the negative health effects associated with high intake of oxalate. Soluble oxalate can bind with calcium in the intestines and the blood to form insoluble calcium oxalate crystals, lowering serum calcium levels. Horses appear to be unable to utilise

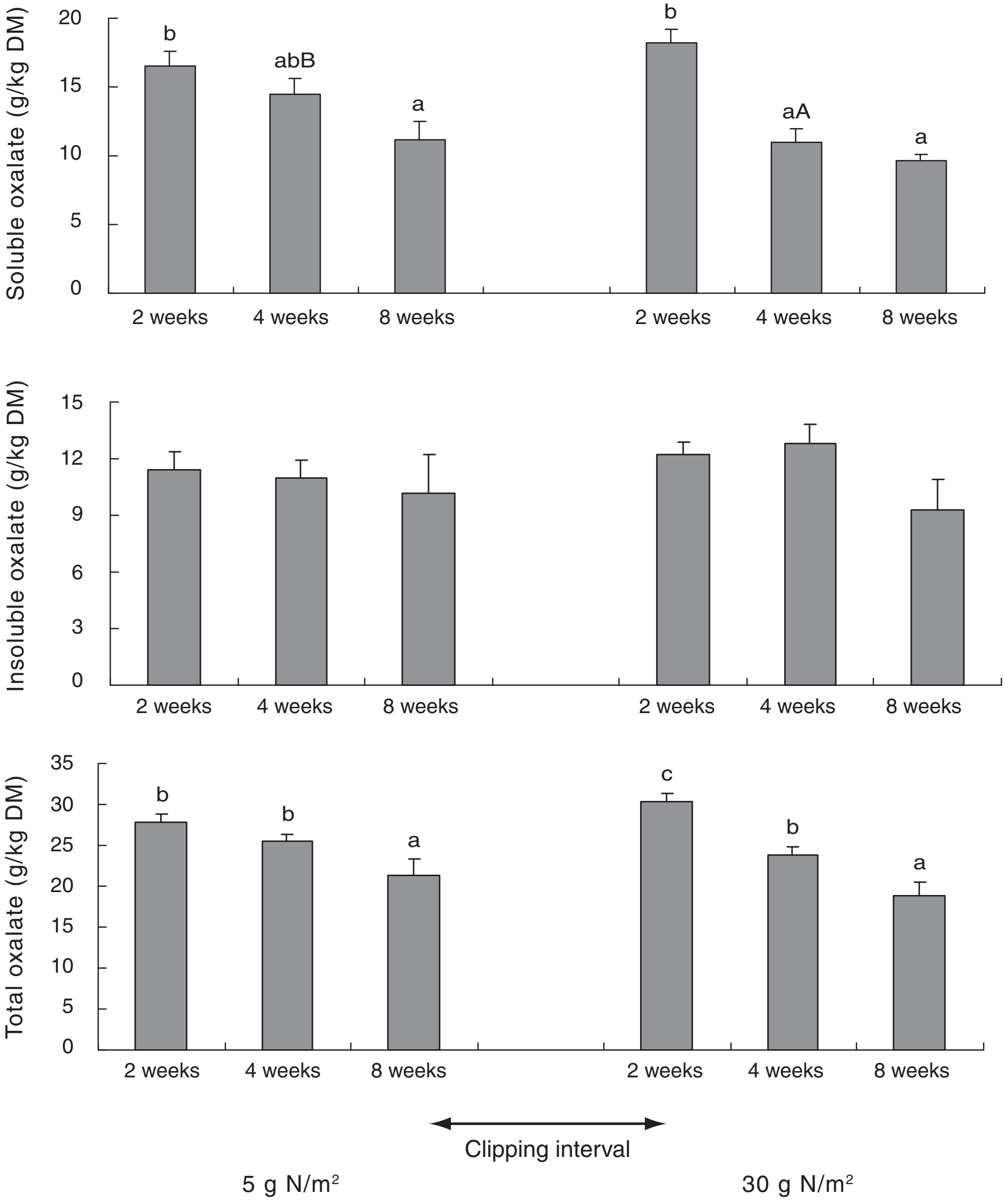

Figure 2. Effects of rates of nitrogen fertiliser and clipping interval on soluble, insoluble and total oxalate concentrations in napier grass. Within parameters and N levels, columns carrying the same lower case letters do not differ (P>0.05). Different upper case letters on columns indicate significant differences within clipping interval between fertiliser levels by Student's *t*-test (P<0.05). Vertical bars indicate standard errors.

calcium oxalate in forages (Blaney *et al*. 1981). Levels of 0.5% or more soluble oxalate in forages may induce nutritional hyperparathyroidism in horses (Cheeke 1995), and calcium deficiency can result from consumption of low calcium/ high oxalate grasses resulting in bone deformity and weakness. McKenzie *et al*. (1988) reported that 2% or more soluble oxalate can lead to acute toxicosis in ruminants. Although rumen bacteria can adapt to high levels of soluble oxalate in the diet (Allison *et al*. 1977), acute toxicity occurred even in adapted ruminants fed with kikuyu grass containing relatively low oxalate (3.9–24.4 g/kg DM) concentrations (Marais 2001). While soluble oxalate is destroyed in the rumens of adapted animals, insoluble oxalate seems to pass through the digestive tract (Ward *et al*. 1979), though Libert and Franceschi (1987) reported that a substantial portion of calcium oxalate in the feed could be dissolved during digestion in ruminants. The role of insoluble oxalate in animals is not well understood.

Since napier grass is such an important forage species for animals throughout the tropics and sub-tropics, its soluble oxalate concentrations are of concern, being reported as relatively high (<3%) (Garcia-Rivera and Morris 1955; Lal *et al*. 1966; Dhillon *et al*. 1971), and sometimes exceeding 4% of dry matter (Rahman *et al*. 2006). The levels of soluble oxalate in the plants grown in pots in this study (9.2–18.2 g/kg DM) were somewhat lower than those recorded above in plants grown in the field and we have no explanation for this situation. However, levels in our study were in the range where toxicity has been recorded in stock as mentioned earlier. Thus, it is desirable to find harvesting practices that would reduce the oxalate concentration in forages to acceptable levels. The decline in oxalate concentration when clipping interval was increased from 2 to 8 weeks shows that a harvesting strategy can be devised to produce high DM yields of forage, while maintaining good CP levels (14%) and acceptable total oxalate levels (about 2%). While harvesting at 4-weekly intervals would elevate CP levels without any appreciable drop in DM yield, total oxalate levels would be increased to about 2.5%.

The failure of N fertiliser to affect the oxalate concentration in the plant tissues of napier grass supports the previous findings of Williams *et al.* (1991), who reported that fertilisation with urea produced no significant differences in soluble oxalate concentration between treated and untreated kikuyu grass. In contrast, Jones and Ford (1972) observed that the oxalate concentration in *Setaria sphacelata* increased from 3.3 to 5.6% as N fertiliser (as urea) level increased from 0 to 20 g/m^2. In another study, Smith (1978) reported that different species use different organic anions (*e.g.* malate, citrate, oxalate etc.) for maintenance of their ionic balance, and inorganic nutrition can have an impact on the relative contribution of different organic anions within a species. It seems that accumulation of oxalate in plants in the presence of N fertiliser might vary between plant species.

We conclude that fertilising napier grass with urea might not result in excessive oxalate accumulation in the forage. However, the more frequently the material is harvested, the higher the oxalate levels in the forage will be. It is inadvisable to feed large quantities of young napier grass to ruminants (unless they are gradually introduced to the forage to allow the build-up of oxalate-degrading bacteria). Based on oxalate concentrations in the forage in this study, horses should not be fed a diet of predominantly napier grass as nutritional disorders can result.

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