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Effect of Nitrogen Fertilization on Oxalate Content in Rhodesgrass, Guineagrass and Sudangrass

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ABSTRACT : An experiment was conducted to evaluate the effects of nitrogen (N) level on the dry matter (DM) yield, N concentration and oxalate content of some tropical grasses, namely Rhodesgrass (*Chloris gayana*), Guineagrass (*Panicum maximum*) and Sudangrass (*Sorghum vulgare*). Three levels of N as urea were applied (Standard- 260, Standard×2- 540 and Standard×4- 1,060 kg N/ha for Rhodesgrass; Standard- 380, Standard×2- 770 and Standard×4- 1,570 kg N/ha for Guineagrass and Sudangrass) in a completely randomized design and grasses were harvested twice at approximately two-month intervals. Dry matter yield tended to be higher with increased rate of N fertilizer in all species, while further additional N (Standard×2 or Standard×4) did not significantly ($p>0.05$) further increase DM yield, when compared with the Standard level of N fertilizer application. There was also a trend towards higher N concentration in plants as N fertilization increased in all species and it was increased significantly in Rhodesgrass and Sudangrass ($p<0.05$ or $p<0.01$, respectively). Further additional N (Standard×2 or Standard×4) application showed no significant ($p>0.05$) differences on oxalate content in plant tissue within species, when compared with the Standard level of N. The Rhodesgrass contained 0.11, 0.13 and 0.15% soluble oxalate and 0.23, 0.25 and 0.27% total oxalate with Standard, Standard×2 and Standard×4 level of N application, respectively. The Guineagrass contained 0.54, 0.50 and 0.42% soluble oxalate and 1.60, 1.56 and 1.45% total oxalate with Standard, Standard×2 and Standard×4 level of N application, respectively. The Sudangrass contained 0.06, 0.15 and 0.12% soluble oxalate and 0.22, 0.22 and 0.21% total oxalate with Standard, Standard×2 and Standard×4 level of N application, respectively The results from this study suggest that these grasses do not use further addition of N fertilizer (Standard×2 or Standard×4) to form high content of oxalate salts, when compared with the Standard level of N. In addition, the levels of oxalate present with these grasses are quite low as far as toxicity to animals is concerned. (**Key Words :** Nitrogen Fertilization, Oxalate Content, Dry Matter Yield, Nitrogen Concentration)

INTRODUCTION

The soluble oxalate content of forages is of great importance to ruminant animals if it is present in considerable amounts (2% or more) (McKenzie et al., 1988). Hegarty (1982) reported that forage grasses containing oxalate concentrations higher than 4% in the dry matter can be dangerous for ruminants. On the basis of the published literature, it is difficult to find any agreement regarding a safe level of oxalate content in plants for ruminants. However, if soluble oxalate is ingested rapidly by ruminants, the rumen microorganisms cannot detoxify soluble oxalate fast enough to prevent its absorption into the bloodstream, and the animal becomes intoxicated (James et al., 1967). Oxalate-producing tropical grasses, such as Setariagrass (*Setaria sphacelata*) and kikuyugrass (*Pennisetum clandestinum*), have been responsible for mortality (Jones et al., 1970; Dhillon et al., 1971; Sidhu et al., 1996), renal failure and incidences of hypocalcaemia in herbivores (Elphinstone, 1981; McKenzie, 1985; McKenzie et al., 1988). It was observed that oxalate content in Setariagrass increased from 3.3% to 5.6% with increasing levels of nitrogen (N) application from 0 to 200 kg/ha, and related to N concentration of 1.3 and 2.9%, respectively (Jones and Ford, 1972).

The use of excessive amounts of nitrogen fertilizer to stimulate dry matter yield from grassland is often justified,

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Table 1. Rate of fertilizer application (kg/ha)

Species	Treatment	Amount of fertilizer		
		Nitrogen (N)	Phosphorus (P_2O_5)	Potassium (K_2O)
Rhodesgrass	Standard	260	300	290
	Standard×2	540	300	290
	Standard×4	1,060	300	290
Guineagrass	Standard	380	260	430
	Standard×2	770	260	430
	Standard×4	1,570	260	430
Sudangrass	Standard	380	150	380
	Standard×2	770	150	380
	Standard×4	1,570	150	380

Table 2. Dry matter yield (t/ha) as affected by forage species, N fertilization and harvesting frequency

Species	Harvesting frequency	N fertilization rates ^{X}			SEM	Harvest mean^Y
		Standard	Standard×2	Standard×4		
Rhodesgrass	1st	6.79	9.12	8.82	0.71	8.24
	2nd	6.04	6.86	9.48	0.65	7.46
	Average	6.42	7.99	9.15	0.48	7.85
Guineagrass	1st	12.62^B	12.95^B	14.15^B	0.44	13.24^B
	2nd	4.73^{aA}	5.82^{aA}	9.20^{bA}	0.70	6.58^A
	Average	8.67	9.39	11.67	0.80	9.91
Sudangrass	1st	8.47^B	8.28	9.40	0.66	8.72^B
	2nd	4.70^{aA}	5.76^a	9.16^b	0.70	6.54^A
	Average	6.59	7.03	9.28	0.52	7.63
Overall effect of N fertilization^Z		7.23^a	8.13^a	10.03^b	0.37	

^{X} Standard, 380 kg N/ha for each grass except Rhodesgrass (260 kg N/ha); Standard×2, two times the Standard; Standard×4, four times the Standard.
^{Y} Contrasts testing the effect of harvesting frequency across all species and fertilization rates ($p<0.01$).
^{Z} Contrasts testing the effect of fertilization across all species and harvesting frequency ($p<0.01$).
$^{a, b}$ Within a row, means without a common superscript differ ($p<0.05$).
$^{A, B}$ Within a column for each species, means without a common superscript differ ($p<0.05$). SEM = Standard error of the mean.

but it is important to ensure that in the quest for increased yields, forage toxin concentrations do not become dangerously high. However, there is very limited information on the rate of oxalate accumulation in tropical forages in response to heavy nitrogen fertilizer application. Therefore, the present study was conducted to investigate the effect of nitrogen fertilization on oxalate content in some tropical grasses.

MATERIALS AND METHODS

Experimental design

This experiment was carried out at the experimental field station of the Faculty of Agriculture, University of Miyazaki, Japan from 10 May to 1 September 2004. The seeds of Rhodesgrass (*Chloris gayana* cv. Katambora), Guineagrass (*Panicum maximum* cv. Natsukaze) and Sudangrass (*Sorghum sudanense* cv. Haysudan) were sown in a burned soil of 1/2,000 in Wagner pots at a seeding rate of 25, 70 and 55 kg/ha, respectively. Three levels of nitrogen (N) fertilizer (standard level, standard level×2 times, standard level×4 times) were applied by dividing 4 times. The rate of fertilization is shown in Table 1. Standard level of fertilizer in all species was designed as recommended by Inosaka (1989). The fertilizer used was urea for N, super phosphate for P_2O_5 and potassium chloride for K_2O. Each treatment of N fertilizer was replicated four times using a completely randomized design. The plants were cut twice at approximately two-month intervals. The cutting height was 5 cm above the ground.

Analysis

The harvested samples were chopped and then dried at 70°C for 48 h in a forced-air oven for determination of dry matter (DM) yield. The dried samples were ground in a Wiley mill through a 1-mm screen. Nitrogen content was measured by the Kjeldahl method (AOAC, 1990). Soluble oxalate and total oxalate in plant tissues were determined with high-performance liquid chromatography (Shimadzu Co. Ltd., Japan) by the method of Rahman et al. (2006) and Rahman et al. (2007), respectively. The amount of insoluble oxalate was evaluated as the difference between the total and the soluble.

Statistical analysis

All data were analyzed statistically by analysis of variance and statistically significant differences between the oxalate content of harvest 1 and harvest 2 were determined

Table 3. Nitrogen concentration (% DM) as affected by forage species, N fertilization and harvesting frequency

Species	Harvesting frequency	N fertilization rates[X]			SEM	Harvest mean[Y]
		Standard	Standard×2	Standard×4		
Rhodesgrass	1st	0.95[aA]	1.18[aA]	1.67[bA]	0.11	1.27[A]
	2nd	1.78[B]	1.84[B]	2.25[B]	0.10	1.96[B]
	Average	1.36[a]	1.51[ab]	1.96[b]	0.10	1.61
Guineagrass	1st	1.30[aA]	1.57[aA]	2.05[bA]	0.11	1.64[A]
	2nd	2.41[aB]	2.78[abB]	3.10[bB]	0.12	2.76[B]
	Average	1.86	2.17	2.57	0.14	2.20
Sudangrass	1st	1.47[a]	1.83[b]	2.24[c]	0.11	1.85
	2nd	1.78	1.96	2.26	0.09	1.99
	Average	1.62[a]	1.90[b]	2.25[c]	0.07	1.92
Overall effect of N fertilization[Z]		1.61[a]	1.86[a]	2.26[b]	0.07	

[X] Standard, 380 kg N/ha for each grass except Rhodesgrass (260 kg N/ha); Standard×2, two times the Standard; Standard×4, four times the Standard.
[Y] Contrasts testing the effect of harvesting frequency across all species and fertilization rates ($p<0.01$).
[Z] Contrasts testing the effect of fertilization across all species and harvesting frequency ($p<0.01$).
[a, b, c] Within a row, means without a common superscript differ ($p<0.05$).
[A, B] Within a column for each species, means without a common superscript differ ($p<0.05$). SEM = Standard error of the mean.

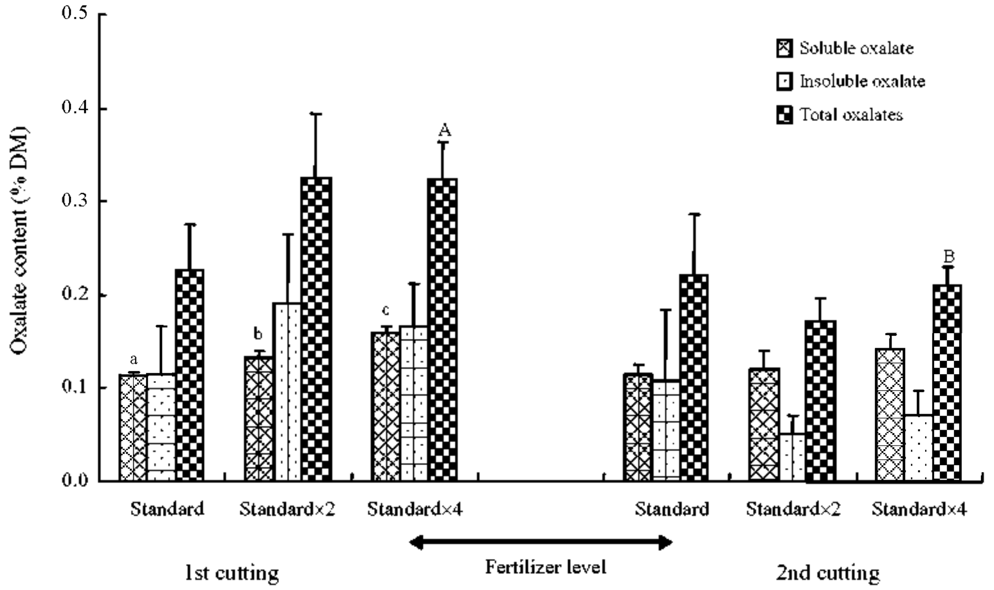

Figure 1. Effects of level of nitrogen fertilizer and cutting frequency on oxalate content in Rhodesgrass. Standard, 260 kg N/ha; Standard ×2, two times the Standard; Standard×4, four times the Standard. Means in a bar of soluble oxalate content with different superscript ([a, b, c]) under 1st cutting differ significantly ($p<0.05$) from each other. [A, B] Comparison of total oxalate content between 1st cutting and 2nd cutting within the same level of N fertilizer application by Student's t-test ($p<0.05$). Error bars indicate standard errors.

by the Student's *t*-test. The difference between mean values and among variants was calculated using the LSD method with a 5% level of significance (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

Dry matter yield

Dry matter yield (t/ha) as affected by forage species, N fertilization and harvesting frequency is shown in Table 2. It was found that the overall effect of N fertilization on DM yield varied significantly ($p<0.01$) among the treatment groups when averaged across all species, and an increasing trend was observed with an increase of N fertilizer. The highest DM yield was found in standard×4 level of fertilizer (10.03 ton/ha/cutting) and the lowest in standard level of fertilizer (7.23 ton/ha/cutting). However, N fertilization had no significant ($p>0.05$) effect on average DM yield (harvest 1 and harvest 2) within species, while an increasing trend was observed with an increase of N fertilizer in all species. The results indicated that DM yield had peaked at standard levels of fertilizer in all species. The present findings follow the pattern reported by Lee et al. (1996) and Johnson et al. (2001).

Nitrogen concentration in plant tissues

Nitrogen concentration (% DM) as affected by forage

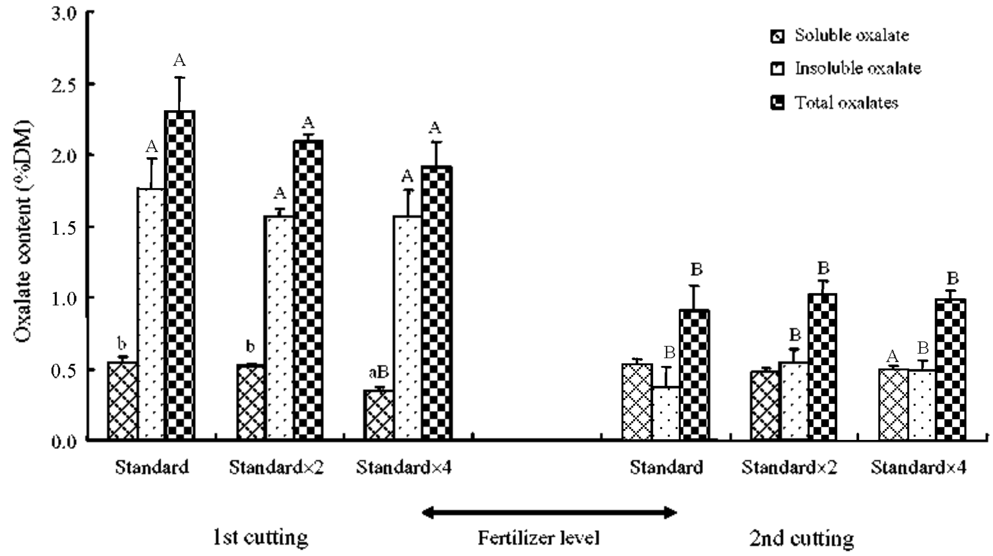

Figure 2. Effects of levels of nitrogen fertilizer and cutting frequency on oxalate content in Guineagrass. Standard, 380 kg N/ha; Standard×2, two times the Standard; Standard×4, four times the Standard. Means in a bar of soluble oxalate content with different superscript ($^{a, b}$) under 1st cutting differ significantly ($p<0.05$) from each other. $^{A, B}$ Comparison of respective oxalate content between 1st cutting and 2nd cutting within the same level of N fertilizer application by Student's t-test ($p<0.05$). Error bars indicate standard errors.

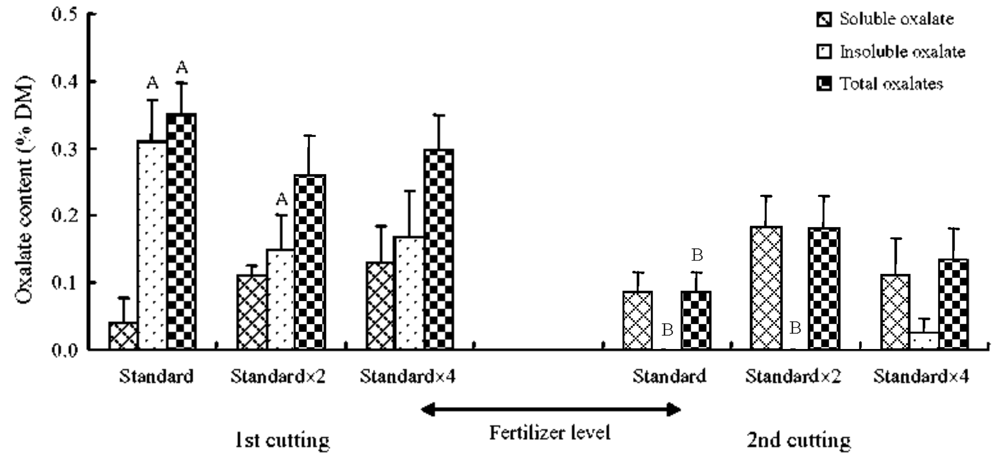

Figure 3. Effects of levels of nitrogen fertilizer and cutting frequency on oxalate content in Sudangrass. Standard, 380 kg N/ha; Standard ×2, two times the Standard; Standard×4, four times the Standard. $^{A, B}$ Comparison of respective oxalate content between 1st cutting and 2nd cutting within the same level of N fertilizer application by Student's t-test ($p<0.05$). Error bars indicate standard errors.

species. N fertilization and harvesting frequency is presented in Table 3. Total N concentration increased ($p<0.01$) as N fertilization increased when overall effects of N fertilization across both harvest dates and all species were observed. Similar results were found within species except Guineagrass for the average of both harvest dates. However, an increasing trend of N concentration was found with increased rate of N fertilizer in all species. The present results follow the same pattern reported by earlier researchers (Penney et al., 1990; Khan et al., 1999; Sarwar and Mahr-un-Nisa, 1999; Johnson et al., 2001).

Oxalate content in plant tissues

The effects of nitrogen fertilization on oxalate content in Rhodesgrass, Guineagrass and Sudangrass are illustrated in Figure 1, 2 and 3, respectively. Oxalate content of Rhodesgrass was not increased significantly ($p>0.05$) with increased rate of N fertilizer at both harvests, except for soluble oxalate content at harvest 1 which increased significantly ($p<0.05$) with the increased rate of N fertilizer (Figure 1). Total oxalate content tended to be higher as N fertilization increased at harvest 1, whereas no such trend was found at harvest 2 and the causes of this dissimilar trend were unknown. In addition, oxalate levels in plants between harvest 1 and harvest 2 were not significantly ($p>0.05$) different for any treatment, except for the standard×4 treatment for total oxalate. Irrespective of N fertilizer levels, the mean total oxalate content (harvest 1 and harvest 2) of Rhodesgrass was 0.25%, and consisted of 48 and 65% soluble oxalate for harvest 1 and harvest 2, respectively.

In Guineagrass, compared to Standard level of N, further addition (Standard×2 or Standard×4) of N fertilization did not significantly increase ($p>0.05$) the oxalate content, apart from soluble oxalate content at harvest 1 which tended to decrease with increased rate of N fertilizer but differences between Standard and Standard×2 level of N were not significant ($p>0.05$) (Figure 2). Oxalate content of Guineagrass tended to be lower as N fertilization increased at harvest 1, whereas no trend was found at harvest 2 and the causes of this dissimilar trend were unknown. In all treatments, plants at harvest 1 had significantly ($p<0.05$) higher insoluble and total oxalate content in plant tissue than at harvest 2, whereas there was no significant ($p>0.05$) difference in soluble oxalate content except for Standard×4 treatment which produced a significantly ($p<0.05$) lower soluble oxalate content at harvest 1 (0.35%) than harvest 2 (0.50%). Irrespective of N fertilizer level, the mean total oxalate content (harvest 1 and harvest 2) of Guineagrass was 1.53%, and most of the oxalate content seemed to be in an insoluble form (78% of total oxalate) at harvest 1, while the content of insoluble oxalate was 48% of total oxalate at harvest 2.

Regarding Sudangrass, compared to Standard level of N, further addition of N fertilizer (Standard×2 or Standard×4) application did not affect ($p>0.05$) the content of soluble, insoluble or total oxalate in the plants either at harvest 1 or harvest 2 (Figure 3). However, soluble oxalate content at harvest 1 tended to increase with increased N fertilization, while no such trend was found at harvest 2 and the causes of this dissimilar trend were unknown. In all treatments, plants at harvest 1 had significantly ($p<0.05$) higher insoluble oxalate content than at harvest 2, apart from Standard×4 treatment. There was no significant ($p>0.05$) difference in total oxalate content between harvest 1 and harvest 2 within the same level of N fertilizer application, except for the Standard treatment which produced a significantly ($p<0.05$) higher total oxalate content at harvest 1 (0.35%) than harvest 2 (0.09%). The mean total oxalate content (harvest 1 and harvest 2) of Sudangrass was 0.22% across all N fertilizer levels and most of the oxalate content seemed to be in an insoluble form (70% of total oxalate) at harvest 1, while insoluble oxalate content was very negligible at harvest 2.

The data from the present study indicates that further addition of N fertilizer (Standard×2 or Standard×4) does not affect either the content of soluble, insoluble or total oxalate in plant tissue, when compared with the Standard level of N fertilizer application. This is partially supported by the work of Williams et al. (1991) who found that a high level of N did not significantly increase soluble oxalate content in kikuyugrass. Our results are also in agreement with earlier reports on the effects of total N on oxalate content in *Tetragonia tetragonioides* (Ahmed and Johnson, 2000). The results of the present study may be explained by ammonium activity in plants, which may have served as a negative signal to inhibit oxalate accumulation (Ji, 2004; Palaniswamy et al., 2004; Xu et al., 2006). Xu et al. (2006) observed that glyoxalate accumulated in ammonium-fed rice leaves and was negatively correlated with oxalate, which suggested that downstream glyoxalate metabolism, including oxidation to oxalate, could be interrupted under ammonium treatment. Plants can absorb N both as NO_3^- and NH_4^+ forms. In the present study, plants absorbed N as urea and it is commonly accepted that urea converts quickly to ammonium nitrogen. Ammonium nitrogen can be directly used by plants in the synthesis of amides and amino acids, whereas NO_3^--N has to be reduced in the shoots (nitrate reduction by nitrate reductase) before absorption of N by plant. Ammonium nitrogen as a sole source of N acidifies the rhizosphere due to the excretion of H^+ from plant roots, and can be deleterious to plant growth (Weir et al., 1972). Schubert and Yan (1997) also suggested that the low oxalate found in plants grown with ammonium was due to ammonium assimilation favoring a slight acidification of the cytoplasm. In the present study, the absence of significant differences in the oxalate content of experimental plant species with different levels of N fertilizer as urea may be due to slight acidification of the rhizosphere or the cytoplasm by ammonium assimilation. In another study, Xu et al. (2006) reported that nitrate fertilizer stimulated oxalate accumulation in rice leaves, while ammonium fertilizer reduced its level. However, this observation contradicts the results of Jones and Ford (1972) who reported that the oxalate content in Setariagrass increased ($p<0.01$) significantly from 3.3% to 5.6% as N fertilizer (as urea) level increased from 0 to 200 kg/ha, respectively. This suggests that accumulation of oxalate in plant species by N forms is likely to be genetically controlled.

High levels of soluble oxalate (0.5% or more) cause severe calcium deficiencies by binding calcium in non-ruminants (McKenzie, 1985). However, ruminants can adapt to diets containing soluble oxalate (Allison et al., 1977). While soluble oxalate is utilized by rumen microbes of adapted animals, insoluble oxalate seems to pass through the digestive tract and is eliminated in the faeces (Ward et al., 1979). According to McKenzie et al. (1988), levels of 2% or more soluble oxalate can lead to acute toxicosis in ruminants. Cymbaluk et al. (1986) pointed out that certain prairie forages with moderate amounts of oxalate (13-18 g/kg DM) could cause sub-clinical bone diseases. In the present study, the soluble oxalate content of the experimental grasses in all treatments was far lower than the critical levels cited in the literature.

CONCLUSIONS

Forage DM yield tended to increase with increased rate of N fertilizer application in all forage species, but the differences were not statistically significant within species. Increasing N application rate produced higher N content in all forage species and the differences were statistically significant in Rhodesgrass and Sudangrass. However, high N application rates did not significantly increase the oxalate content in plant tissue. The levels of oxalate present in the experimental grasses were quite low in terms of toxicity to animals.

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