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Interactive Effects of Nitrogen and Potassium Fertilization on Oxalate Content in Napiergrass (*Pennisetum purpureum*)

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ABSTRACT : Ingestion of forage containing a large quantity of soluble oxalate can result in calcium deficiency and even death of livestock. Fertilization is one of the most practical and effective ways to improve yield and nutritional quality of forage. An experiment was conducted to determine the effects of nitrogen (N) fertilization (150, 300 and 600 kg/ha) across varying levels (150, 300 and 600 kg/ha) of potassium (K) on oxalate accumulation in napiergrass (*Pennisetum purpureum*). Application of N at 300 kg/ha produced higher dry matter yield than at 150 or 600 kg/ha, while K fertilization had no effect on yield. In general, N fertilization did not affect the soluble and total oxalate contents, but slightly affected the insoluble oxalate content. Soluble oxalate content showed an increasing trend and insoluble oxalate content showed a decreasing trend with increasing K level, but total oxalate content remained relatively constant. There were significant interactions between N and K fertilization for the content of soluble and insoluble oxalate fractions. The greatest increase in soluble oxalate content with N level at 300 kg/ha was found at the high level (600 kg/ha) of K application. The greatest increase in insoluble oxalate content with N level at 600 kg/ha was found at the low level (150 kg/ha) of K application. These results indicated the possibility of controlling the content of soluble and insoluble oxalate fractions in forage by fertilization. (**Key Words :** Napiergrass, Oxalate Accumulation, Fertilization, Nitrogen, Potassium)

INTRODUCTION

In Asian countries, intensive animal production systems are developing fast due to shortage of grazing land (Sheen and Hong, 1999). Concentrated animal operations generate substantial amounts of manure that are rich in plant nutrients including nitrogen (N) and potassium (K) (Harada, 1992). Excessive manure is often applied on limited areas of forage crop fields resulting in high to excessively high applications of N and K. Forage crops take up N and K easily, and accumulate them abundantly in the aboveground parts (Harada, 1992). Experiments carried out with tropical grasses showed that soluble oxalate content increased with increasing levels of N fertilizer (Jones and Ford, 1972; Mani and Kothandaraman, 1980). It was also shown that soluble oxalate content correlates highly with K concentration in napiergrass (*Pennisetum purpureum*) (Rahman et al., 2008b). These findings suggest that high application rates of animal manure may enhance the soluble oxalate content that can cause oxalate toxicity in animals (Dhillon et al., 1971; Sidhu et al., 1996).

Non-ruminants are usually more susceptible to oxalate toxicity than ruminants. When a ruminant consumes an oxalate-containing plant, the oxalate is metabolized in four possible ways. First, soluble oxalate may be degraded by rumen microflora (Allison et al., 1977). Second, soluble oxalate may combine chemically with calcium (Ca) to become insoluble oxalate, which is excreted in feces. As a result, absorption of Ca is reduced. Third, soluble oxalate may be absorbed from the rumen into the blood stream where it can combine with serum Ca to form insoluble oxalate crystals, leading to hypocalcemia (Blaney et al., 1982). This crystal may then precipitate in the kidneys and can cause kidney failure (Lincoln and Black, 1980). Fourth, ingested insoluble oxalate from plants may pass through the digestive tract without a harmful effect on body metabolism (Ward et al., 1979).

While applications of N and K play a vital role in enhancing forage yield, little information exists about oxalate accumulation by forage in relation to N and K fertilization. The objective of this study was to investigate whether application of N with varying levels of K can influence oxalate accumulation in napiergrass.

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MATERIALS AND METHODS

Plant cultivation, experimental design and sampling procedure

Rooted tillers of the late-heading variety of napiergrass (cv. dwarf-late) were transplanted (1 plant/pot) into Wagner's pots (Wagner's pot size 1/2,000a; 25 cm in diameter and 30 cm in height) filled with sandy soil (a commercial burned soil for horticultural use (Miyazaki Shodo, Miyazaki, Japan)) on May 15, 2008. The pots were placed in an experimental house (to protect from runoff of nutrients by rainfall) of the Faculty of Agriculture, University of Miyazaki, Japan, at a density of 1 pot/m^2. Mean daily temperature during the growing period (May to November) was 26.1°C. The mean maximum and minimum temperatures were 40.9 and 14.6°C, recorded in July and November, respectively.

A factorial experiment (3 N levels×3 K levels) in a Completely Randomized Design with four replications was used. Nitrogen levels were 150, 300 and 600 kg N/ha and potassium levels were 150, 300 and 600 kg K/ha. One-third of the N and K fertilizers were applied to the pots at planting, and the remainder was applied as equal split applications following each cutting. The phosphorus (P) fertilizer (300 kg P/ha) was applied in all treatment groups at planting. The medium level of N fertilizer was designed as recommended by Wadi et al. (2003) who reported that the appropriate N fertilization for dry matter production of napiergrass was 300 kg N/ha/yr. The fertilizers used were urea for N, super-phosphate for P and potassium carbonate for K. Plant tops were harvested (15 cm above the soil surface) 3 times at approximately 8-week intervals: July 10, September 5 and November 4. Watering was done on alternate days. Dry matter (DM) yield was measured after oven-drying (70°C) of samples for 48 h.

Oxalate analysis

Dried samples were milled to pass through a 1 mm screen using a Wiley mill. Samples were analyzed for total oxalate and soluble oxalate according to Rahman et al. (2007). Insoluble oxalate was estimated by subtracting soluble oxalate from total oxalate.

Statistical analysis

The data on DM yield and oxalate content were analyzed by analysis of variance using the general linear model of SPSS (version 12.0, SPSS Inc., Chicago, IL, USA) for a 3×3 factorial experiment in a Completely Randomized Design with 4 replications. The differences between the means were determined by the least significant difference method, and significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Dry matter yield

Dry matter yields of napiergrass as affected by N and K fertilization are presented in Table 1. Total annual DM yield was significantly ($p<0.001$) affected by N fertilization. As N fertilizer application was increased from 150 to 300 kg/ha, total annual DM yield was increased significantly ($p<0.001$), whereas increasing N fertilizer from 300 to 600 kg/ha did not increase ($p>0.05$) it further. These results are in agreement with Rahman et al. (2008a) who observed that DM yield of some tropical grasses had peaked at N application of 300 kg/ha. Total annual DM yield was not significantly ($p>0.05$) affected by K fertilization. Although soil K was not determined in the present study, the range in

Table 1. Dry matter yield (t/ha) of napiergrass as affected by nitrogen (N) and potassium (K) fertilization

N level (kg/ha)	K level (kg/ha)	Cutting times			Total yield
		First cutting	Second cutting	Third cutting	
150	150	1.34	2.36	1.18	4.88
	300	1.29	2.48	1.20	4.96
	600	1.30	2.63	1.33	5.26
300	150	1.49	3.11	1.42	6.02
	300	1.33	3.32	1.52	6.16
	600	1.50	2.95	1.40	5.85
600	150	1.53	2.68	1.18	5.38
	300	1.40	3.19	1.30	5.89
	600	1.37	2.51	1.11	4.99
F-values and level of significance					
N		2.5NS	8.5**	4.3*	10.2***
K		1.5NS	0.9NS	0.3NS	0.3NS
N×K		1.3NS	3.1*	1.2NS	3.5*

NS: Not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

the levels of K fertilizer applications was likely to be similar to the study of Dampney (1992) who reported that there was no herbage yield response to K fertilizer when soil K was above 120 mg/L. There was a significant ($p<0.05$) interaction between N and K fertilization for total annual DM yield, and the greatest increase in total annual DM yield with N level at 300 kg/ha was found at the medium level (300 kg/ha) of K application.

Soluble oxalate content

The effects of levels of N and K fertilizer on soluble oxalate content are presented in Table 2. Soluble oxalate content in the first-cut napiergrass was affected ($p<0.01$) by the N treatments, but this effect was not consistent in the subsequent cuttings and the average soluble oxalate content of 3 cuttings was not altered ($p>0.05$) by the N treatments. This is in agreement with the results of Rahman et al. (2008a) who observed that N fertilization with urea either had no effect on the accumulation or even reduced the level of soluble oxalate. However, in a nutrient solution study, Rahman (2009) has shown that nitrate treated napiergrass contained significantly higher soluble oxalate content than ammonium treated napiergrass. It seems that oxalate accumulation in napiergrass may be affected by form of N application.

The soluble oxalate content in all cut napiergrass was significantly affected by K fertilization. The soluble oxalate content appeared to increase with increasing K level, and the difference was statistically significant between K levels of 150 and 300 kg/ha. This is in good agreement with our previous report that there was high correlation between soluble oxalate and K concentrations in napiergrass (Rahman et al., 2008b).

There was a significant interaction between N and K fertilization for soluble oxalate content in all cuttings, and the greatest increase in soluble oxalate content with N level at 300 kg/ha was found at the high level (600 kg/ha) of K application. The medium level (300 kg/ha) of N and low level (150 kg/ha) of K produced comparatively lower content of soluble oxalate without resulting in lower DM yield.

Many researchers have observed various toxic levels of soluble oxalate for ruminants. For example, McKenzie et al. (1988) reported that forage containing soluble oxalate (20.0 g DM/kg) can lead to acute toxicosis in ruminants. In another study, WeinChang et al. (2004) reported that the concentration of calcium ions in the blood of cattle and goats fed napiergrass containing high soluble oxalate (17.4-17.7 g DM/kg) was lower than in animals fed napiergrass containing low soluble oxalate (9.5-12.6 g DM/kg). In the present study, the levels of soluble oxalate in all samples were more than 20.0 g DM/kg. Furthermore, when napiergrass was grown in early summer (first cutting) and/or fertilized with a high level of K (600 kg/ha), the levels of soluble oxalate were 30.0 g DM/ kg or more.

Insoluble oxalate content

The effects of levels of N and K fertilizer on insoluble oxalate content are presented in Table 3. At almost all the cuttings, the insoluble oxalate content was affected by N and K fertilization and their interaction. Application of N at 600 kg/ha produced significantly higher content of insoluble oxalate than at 150 or 300 kg/ha, which is in agreement with our previous study (Rahman et al., 2008b). The insoluble oxalate content in plants appeared to decrease with increasing K level, and the difference was statistically significant between K levels of 150 and 600 kg/ha. There was a significant interaction between N and K fertilization

Table 2. Soluble oxalate content (g/kg, dry matter basis) in napiergrass as affected by nitrogen (N) and potassium (K) fertilization

N level (kg/ha)	K level (kg/ha)	Cutting times			Mean
		First cutting	Second cutting	Third cutting	
150	150	32.7	21.1	23.1	25.6
	300	35.5	28.0	27.9	30.5
	600	34.3	35.2	29.6	33.0
300	150	30.8	24.9	21.7	26.2
	300	36.4	33.6	27.2	32.4
	600	35.4	35.0	31.2	33.9
600	150	28.2	29.5	26.9	28.2
	300	30.2	32.1	28.0	30.1
	600	32.3	33.8	31.5	32.4
F-values and level of significance					
N		6.5**	1.0NS	0.5NS	0.5NS
K		4.6*	10.9***	10.8***	18.2***
N×K		4.3**	4.3**	3.3*	5.0***

NS: Not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 3. Insoluble oxalate content (g/kg, dry matter basis) in napiergrass as affected by nitrogen (N) and potassium (K) fertilization

N level (kg/ha)	K level (kg/ha)	Cutting times			Mean
		First cutting	Second cutting	Third cutting	
150	150	7.3	4.1	9.2	6.9
	300	7.8	4.8	7.5	6.7
	600	7.7	0.0	7.7	5.1
300	150	10.9	1.5	12.9	8.0
	300	6.6	0.0	8.4	5.0
	600	7.7	0.0	5.1	4.3
600	150	23.0	5.6	5.1	11.2
	300	15.5	4.3	5.8	9.2
	600	10.3	2.5	5.5	7.0
F-values and level of significance					
N		13.6***	6.9**	2.9*	4.5*
K		2.7*	4.2*	1.8NS	3.5*
N×K		10.2***	4.1**	2.9*	2.1*

NS: Not significant (p>0.05); * p<0.05; ** p<0.01; *** p<0.001.

for insoluble oxalate content, and the greatest increase in insoluble oxalate content with N level at 600 kg/ha was found at the low level (150 kg/ha) of K application. Ward et al. (1979) reported that ingested insoluble oxalate from plants may pass through the digestive tract without a harmful effect on body metabolism.

Total oxalate content

The effects of levels of N and K fertilizer on total oxalate content are presented in Table 4. Total oxalate content in the first-cut napiergrass was affected (p<0.05) by the N treatments, but this effect was not consistent in the subsequent cuttings and the average total oxalate content of 3 cuttings was not altered (p>0.05) by the N treatments. Similarly, the average total oxalate content of 3 cuttings was not significantly (p>0.05) altered as a result of the level of K fertilizer application and there was also no N×K fertilizer interaction effect (p>0.05). Although the soluble and insoluble oxalate fractions were affected by fertilizer treatments as described above, total oxalate content remained relatively constant. This is in agreement with the study of Rahman et al. (2008a) who reported that the total oxalate content of plants was not affected by N fertilization. These findings indicate that oxalic acid biosynthesis in plants is not affected by N (as urea) and K fertilization and their interaction, but synthesized oxalic acid forms as soluble and/or insoluble salts based on the availability of nutrient ions. Oxalic acid biosynthesis, however, may be elevated by nitrate application as reported by Rahman (2009).

Table 4. Total oxalate content (g/kg, dry matter basis) in napiergrass as affected by nitrogen (N) and potassium (K) fertilization

N level (kg/ha)	K level (kg/ha)	Cutting times			Mean
		First cutting	Second cutting	Third cutting	
150	150	40.0	25.2	32.2	32.5
	300	43.3	32.8	35.4	37.1
	600	42.0	35.2	37.3	38.2
300	150	41.7	26.4	34.6	34.2
	300	42.9	33.6	35.5	37.3
	600	43.1	35.0	36.3	38.1
600	150	51.1	35.1	32.0	39.4
	300	45.6	36.4	33.7	39.3
	600	42.6	36.3	37.1	39.3
F-values and level of significance					
N		4.2*	2.4NS	0.5NS	2.5NS
K		0.5NS	6.0**	4.4*	2.1NS
N×K		2.7*	3.9**	1.2NS	1.6NS

NS: Not significant (p>0.05); * p<0.05; ** p<0.01.

CONCLUSIONS

In general, N fertilization using urea did not affect the soluble and total oxalate contents in napiergrass, but slightly affected the insoluble oxalate content. With increasing K level, soluble oxalate content showed an increasing trend and insoluble oxalate content showed a decreasing trend, but total oxalate content remained relatively constant. There were significant interactions between N and K fertilization for the content of soluble and insoluble oxalate fractions. The medium level (300 kg/ha) of N and low level (150 kg/ha) of K produced a comparatively lower content of soluble oxalate without resulting in a lower DM yield. The results of this study suggest that the content of soluble and insoluble oxalate fractions in forage can be controlled by fertilization.

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