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Short Communication

Oxalate and silica contents of seven varieties of Napier grass *(Pennisetum purpureum)*

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Abstract

Oxalate and silica are considered antinutrients. Large quantities of oxalate and silica in plants can interfere with the uptake of essential minerals in ruminants. Therefore, the aim of this study was to compare the total silica and oxalate contents of seven varieties of Napier grass to find out which is best for cultivation. Taiwan, Zanzibar, Pakchong, Purple, Kobe, Indian, and Dwarf Napier grass were grown in a completely randomized design with three replications to determine their soluble oxalate, total oxalate, and silica contents. Plants were harvested at two months of plant maturity. Whole plant of the Dwarf Napier grass contained significantly higher soluble oxalate content than tall varieties. Total oxalate content in whole plant differed significantly among varieties. Dwarf showed the highest total oxalate content (3.23% dry matter (DM)) followed by Kobe (2.61%), Zanzibar (2.60%), Purple (2.44%), Taiwan (2.43%), Indian (2.15%), and Pakchong (1.95%). Regardless of variety, leaf tissue contained significantly higher soluble oxalate and total oxalate than stem tissue. There were no differences in silica content among them. In conclusion, the tall varieties could produce lower levels of soluble oxalate than the Dwarf variety, whereas silica content might not vary among them.

Keywords: botanical fractions, mineral bioavailability, ruminant
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In many countries, the most popular grass that is used by smallholder farmers is Napier grass *(Pennisetum purpureum)* because of its high DM yield, moderate nutritional values, and ease of propagation (Wangchuk *et al.*, 2015). However, this grass contains antinutrients such as oxalates, phytates, and tannins (Okaraonye & Ikewuchi, 2009). Antinutrients exist commonly in plants, interfering with the absorption of nutrients in animals. For example, soluble oxalate can bind with blood calcium and other minerals, leading to the inhibition of absorption by the animal body. Rahman *et al.* (2013) suggested that less than 2.0% soluble oxalate of DM intake would be a safe level to avoid oxalate toxicity in ruminants, whereas 0.5% soluble oxalate is acceptable for non-ruminants. Rahman *et al.* (2006) reported that when Napier grass is grown during the early summer, levels of oxalate may reach a critical toxicity level for grazing ruminants.

Silica is another type of antinutrient that can be found in the plant cell wall that may cause lower feed intake and physical damage to the tongues of animals because of its hairy structure. It may also reduce forage digestibility by reducing accessibility to rumen microflora (Widyastuti & Abe, 1989). Pathan *et al.* (2014) observed that pearl millet and Napier grass hybrid contained about 2.43% silica. Thus, Napier grass varieties that have a lower tendency to accumulate antinutrients might be selected for cultivation. In general, Napier grass has a range of phenotypic variations from dwarf to tall genotypes. Antinutrients vary with climatic conditions, soil type, plant maturity and fertilizer application (Rahman & Kawamura, 2011), which indicates that management could help to better understand how to handle these varieties to limit the negative implications. So far, studies on antinutrients in the varieties of Napier grass are scarce. Therefore, this study was designed to find out the contents of oxalate and silica in seven varieties of Napier grass.

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This study was conducted at Agro Techno park, University of Malaysia Kelantan, Jeli Campus (N 05°44.46′ E 101°52.31′), Kelantan, Malaysia, from July to September 2018. Daily temperatures during the experimental period ranged from 24 °C to 32 °C, whereas monthly rainfall for July, August and September was 115, 142 and 174 mm, respectively.

Before planting, land was cleared and ploughed with a tractor to soften the soil. The soil type was clay and its pH was 5.31. Before fertilization, the soil contained 22.6 g nitrogen (N)/kg and 46.4 g organic matter/kg. Seven varieties of Napier grass (Taiwan, Zanzibar, Pakchong, Purple, Kobe, Indian and Dwarf) were purchased from a local supplier (Adi Farm, Segamat, Malaysia). Stem cuttings were planted in rows with spacing 0.5 m x 0.5 m, laying them horizontally on the ground and covering with soil. Three plots for each variety were established with an area of 2.5 m × 2.5 m per plot, separated by a 1.0 m alley between the plots.

Before planting, goat manure was applied to the land at a rate of 10 ton/ha as a basal fertilizer and incorporated into the soil. Similarly, slaked lime ($Ca(OH)_2$) was applied and incorporated into the soil at a rate of 50 kg $Ca(OH)_2$/ha. The N, phosphorus (P) and potassium (K) fertilizers were applied as NPK fertilizer (15:15:15) at the rate of 50 kg/ha during grass establishment. The nutritional content per kg of the manure was 22.0 g N, 25.1 g calcium (Ca), 3.6 g magnesium (Mg), 13.4 g P, 2.6 g K, and 1.1 g sodium (Na). Irrigation water was applied immediately after planting the stem cuttings. Subsequent irrigations were applied, depending on weather conditions. Weeds were controlled manually when necessary. At two months of plant maturity, the Napier grass was cut at 10 cm above ground level. The stem and leaf parts were separated from the plant and chopped into small pieces (2 - 3 cm) manually, and then dried in an oven for 48 hours at 70 °C. Dried samples were ground with a coffee grinder and passed through a 1-mm sieve and stored. Oxalate content was determined as described by Rahman *et al.* (2007) on a column Synergi 4 µm Hydro-RP 80 Å, LC Column 250 x 4.6 m, Ea (Phenomenex, USA). Insoluble oxalate was estimated by subtracting the soluble oxalate from total oxalate. Silica content was determined as described by Widyastuti and Abe (1989).

The data were analysed with a one-way analysis of variance using the general linear model procedure of SPSS software (version 12, SPSS Inc., Chicago, IL, USA), whereas Tukey's test was used to separate the treatment means at P <0.05.

The soluble oxalate content in plant parts is shown in Table 1. In the leaf tissue, the Dwarf variety contained significantly (P <0.05) higher soluble oxalate content than the tall varieties (except Taiwan). In the stem tissue, no difference was observed in soluble oxalate content among varieties. For the whole plant, the Dwarf variety showed significantly (P <0.05) higher soluble oxalate content than the tall varieties.

Table 1 Effect of botanical fraction on soluble oxalate content (%DM) in seven varieties of Napier grass

Botanical fractions	Napier grass varieties							SE
	Taiwan	Zanzibar	Kobe	Pakchong	Purple	Indian	Dwarf	
Leaf	2.73^{ab}	2.09^{a}	2.22^{a}	2.64^{a}	2.44^{a}	2.48^{a}	3.45^{b}	0.12
Stem	1.85	2.11	1.86	1.21	1.60	1.84	1.66	0.12
Whole*	2.29^{a}	2.04^{a}	2.00^{a}	1.80^{a}	1.97^{a}	2.13^{a}	3.00^{b}	0.11

DM: dry matter
^{a,b} Within a row, means with a similar superscript are not different with probability ≤0.05
*Soluble oxalate content of whole plant = soluble oxalate content of leaf × weight ratio of leaf + soluble oxalate content in stem × weight ratio of stem

The soluble oxalate content in the leaves of Napier grass in Table 1 ranged from 2.09% to 3.45% DM, which is in line with the findings of Rahman and Kawamura (2011), in which the content ranged from 1.8% to 3.8% DM. One of the factors that affect oxalate accumulation is plant maturity. Early harvesting leads to higher oxalate content compared with late harvesting (the optimum maturity). In this study, the plant was harvested at 60 days, which is the optimum plant maturity for harvesting in DM yield and nutritive value (Halim *et al.*, 2013). However, most of the varieties (except Pakchong and Purple) in this study contained 2.0% or more soluble oxalate. Consequently, feeding ruminants solely with these varieties might produce hypocalcaemia (Rahman *et al.*, 2013).

Table 2 Effect of botanical fraction on insoluble oxalate content (% DM) in seven varieties of Napier grass

Botanical fractions	Napier grass varieties							SE
	Taiwan	Zanzibar	Kobe	Pakchong	Purple	Indian	Dwarf	
Leaf	0.34[ab]	0.57[b]	0.29[ab]	0[a]	0.08[ab]	0[a]	0[a]	0.09
Stem	0	0.25	0.82	0.37	0.78	0.17	0.92	0.14
Whole*	0.13[a]	0.56	0.61	0.15	0.47	0.02	0.23	0.08

DM: dry matter
[a,b] Within a row, means with a similar superscript are not different with probability ≤0.05
* Insoluble oxalate content of whole plant = insoluble oxalate content in leaf × weight ratio of leaf + insoluble oxalate content in stem × weight ratio of stem

The Ca or Mg oxalate usually exists in all plant tissues as an insoluble salt (Savage *et al.*, 2000). Therefore, it cannot be absorbed and utilized in the animal's body, and passes through the digestive tract. Although insoluble oxalate has no impact on ruminant health, it reduces the bioavailability of some minerals for animals (Rahman *et al.*, 2013). There were no significant (P >0.05) differences in insoluble oxalate content among varieties (Table 2).

Total oxalate content was (P <0.05) affected by botanical fractions among varieties. In the leaf, Dwarf showed the highest value of total oxalate content (3.45%), whereas Indian showed the lowest (2.41%). For the stem, Kobe had the highest content of total oxalate (2.68%), and Pakchong had the lowest (1.58%). For the whole plant, Dwarf showed the highest content of total oxalate (3.23%), and Pakchong the lowest (1.95%). Total oxalate content in these varieties of Napier grass is shown in Table 3.

Table 3 Effect of botanical fractions on total oxalate content (% DM) in seven varieties of Napier grass

Botanical fractions	Napier grass varieties							SE
	Taiwan	Zanzibar	Kobe	Pakchong	Purple	Indian	Dwarf	
Leaf	3.07[bc]	2.66[ab]	2.51[a]	2.46[a]	2.52[a]	2.41[a]	3.45[c]	0.09
Stem	1.80[ab]	2.36[bc]	2.68[c]	1.58[a]	2.38[bc]	2.01[abc]	2.59[c]	0.11
Whole*	2.43[bc]	2.60[bc]	2.61[c]	1.95[a]	2.44[bc]	2.15[ab]	3.23[d]	0.10

DM: dry matter
[a,b,c,d] Within a row, means with a similar superscript are not different with probability ≤0.05
*Total oxalate content of whole plant = total oxalate content in leaf × weight ratio of leaf + total oxalate content in stem × weight ratio of stem

Soluble oxalate is the main concern in forage grasses. It has a negative influence on animal performance as it can bind with the blood Ca or Mg and form insoluble oxalate crystals. Calcium oxalate can be responsible for the formation of kidney stones and for hypocalcaemia as the Ca level in the body decreases. Oxalate can cause acute poisoning and death when the level ranges from 7% to 16.6%, as reported by El-khodery *et al.* (2008). These authors also reported that ewes that fed on beet tops (*Beta vulgaris*) exhibited a high oxalate content with increased deposition of Ca oxalate crystal formation, as observed microscopically. To prevent the excess Ca oxalate formation, the safety level for the ruminant to consume forages containing soluble oxalate is less than 2.0% of DM intake (Pathmasiri *et al.*, 2014). The soluble oxalate is degraded by the rumen bacteria in the ruminant, but non-ruminants cannot degrade more than 0.5% oxalate of DM intake owing to absence of rumen bacteria (Rahman *et al.*, 2013).

The effect of botanical factions on soluble, insoluble and total oxalate contents in Napier grass is illustrated in Figure 1. The botanical fractions contribute to animal nutrition as a function of the oxalate content and the botanical ratio depending on animal preferences. In general, they tend to consume leaf more than stem. The total oxalate and soluble oxalate contents were significantly higher in the leaf compared with the stem, which may have a negative effect on animal performance. Pathmasiri *et al.* (2014) also found that the soluble oxalate content was higher in the leaf compared with the stem in Guinea grass. The leaf proportion in the Dwarf variety was significantly higher than the stem proportion. In contrast, the stem proportions in Kobe and Pakchong were significantly higher than the leaf proportion (Figure 2).

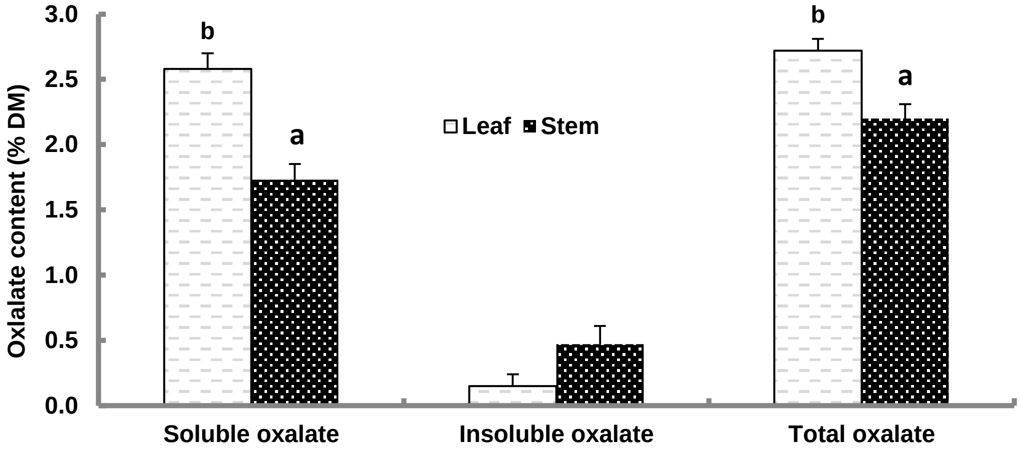

Figure 1 Average effect of botanical fractions on soluble, insoluble and total oxalate contents in Napier grass.

Means in a bar with different superscripts differ significantly (P <0.05).
Error bars indicate standard errors.

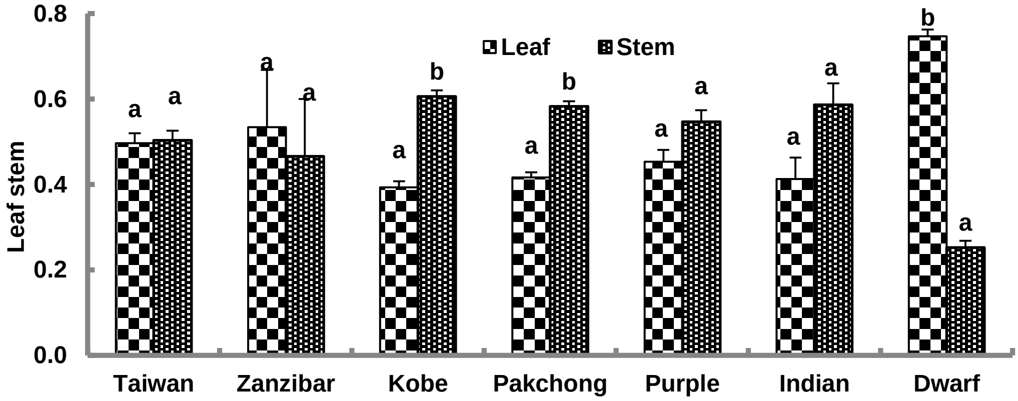

Figure 2 Proportions of leaf and stem in seven Napier grass varieties.

Within a variety, means with a similar superscript are not different with probability ≤0.05.
Error bars indicate standard errors.

No significant (P >0.05) differences were observed in silica content among the varieties (Figure 3). The silica content ranged from 3.14% to 4.19%. Massey and Hartley (2006) stated that the grasses are regarded as silica-accumulating plants, which accumulate silica phytoliths in their tissue as it absorbs the silicon in silicic acid form in the soil. Quigley & Anderson (2014) reported that bunch grasses such as *Themeda triandra* and lawn-grass *Digitaria macroblephara* had silica contents of 3.7% and 2.7%, respectively. Pathan *et al.* (2014) reported that pearl millet and Napier hybrid contained 2.43% silica at 45 days cutting interval. The silica content in this study is in line with the findings of Jusdado (2011), who reported that among the plant species the silica content varied from 0.1% to 10.0% of dry weight. Silica urolithiasis in sheep and cattle is related to silica excretion in which the silica absorbed by the kidney passes through the urine (Jugdaohsingh, 2007). The abundance of the hairy structure of silica on the leaf surface causes refusal of forage by animals when fed in huge amounts.

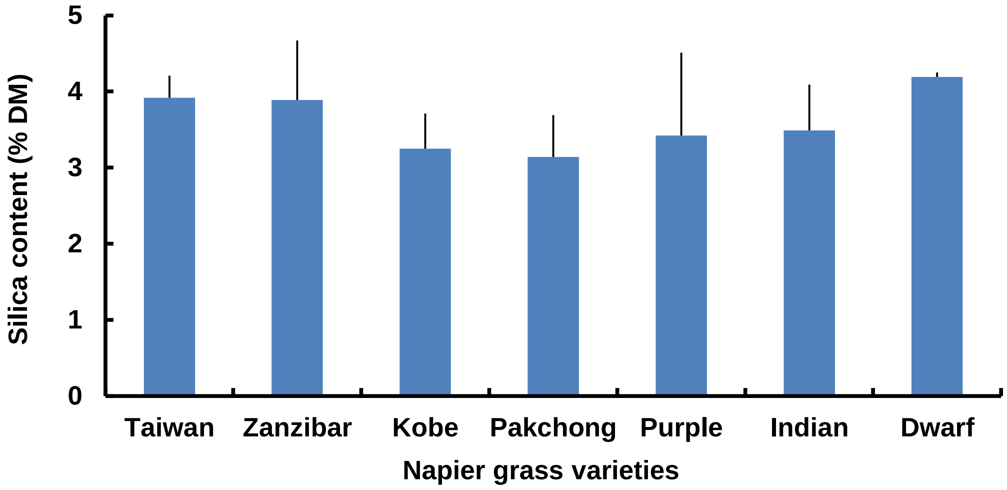

Figure 3 Silica content of seven Napier grass varieties.

Error bars indicate standard errors

Conclusion

Oxalate content varied among these varieties of Napier grass. However, their silica content did not differ. Overall, tall varieties produced lower levels of soluble oxalate than the Dwarf variety. Leaf tissue contained more soluble oxalate and total oxalate than stem tissue. Thus, because of these antinutrient qualities, these varieties cannot be used in isolation without mineral supplements or a more diverse forage base. However, further study is required to substantiate these preliminary results. Subsequent production phase studies should be carried out at multiple locations and over multiple years to allow farmers to choose the most suitable varieties of Napier grass, based on localized needs for a higher quality forage.

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Authors' Contributions

MMR, RA and MNM designed the experiment. MSN carried out the analysis. MMR and MNM assisted with data analysis. TG and MSN arranged the scientific content, and MMR and TG drafted the manuscript. All authors contributed editorial suggestions and supported the final manuscript draft.

Conflict of Interest Declaration

None of the authors has declared any conflict of interest.

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