ORIGINAL RESEARCH

Accumulation of Oxalate and Nitrate in Hybrid Napier var. CO -3 (*Pennisetum perpureum X P. americarnum*) and Wild Guinea Grass (*Panicum maximum*)

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Abstract

Excess amounts of soluble oxalate and nitrate cause poisonous effects on ruminants, fed mainly on forages. Oxalate and nitrate accumulation in forages depend on many factors such as soil, climate, agronomy and plant factors.

A field experiment was conducted as a Complete Randomized Design (CRD) in 4 factor factorial treatment arrangement with three replicates to evaluate the effect of location (Animal experimental farm *Gannoruwa*, Artificial Insemination Centre, Kundasale), species (hybrid Napier variety CO-3, wild Guinea grass), plant parts (stem, leaf) and growth stages (2, 4 and 6 weeks) on nitrate and soluble oxalate accumulation.

There was a four way interaction among the factors in nitrate accumulation *($p<0.05$)*. Higher nitrate contents were observed in stems compared to the leaves and declined with maturity in hybrid Napier variety CO-3. A similar trend was observed in wild Guinea grass with a location effect. Three way interactions of location × species × plant part and species × plant part × growth stage were significant *($p<0.05$)* on oxalate accumulation. In wild Guinea grass, soluble oxalate concentration of leaves were higher than stems and declined in both parts with maturity while similar trend was followed by hybrid Napier variety CO-3.

Thus, it can be concluded that nitrate and soluble oxalate accumulation in forages depend on plant factors like species, plant parts and maturity.

Key words: CO-3, Guinea, Nitrate accumulation, Oxalate accumulation, Plant factors

Introduction

Pasture and fodder play a vital role in livestock nutrition, especially in ruminants because it is the cheapest source of animal feed compared to the concentrates. In Sri Lanka, approximately 12,000 km^2 of the total land area of 65,000 km^2 are under grasslands [1,] which consist of natural and improved grasses. However, many small and medium scale farmers fulfill their pasture requirement from natural grasslands. Guinea grass, Brachiaria spp, fodder sorghum, maize and Napier and its hybrids are the main pasture and fodder species used by Sri Lankan farmers.

Many farmers have already accepted to cultivate hybrid Napier variety CO-3 under the government promotion programs due to its characteristic features such as profuse tillering, high yield potential, high dry matter and crude protein content, quick regeneration capacity, high leaf to stem ratio, high palatability, resistance to pest and diseases and low adverse factors [2.] However, Guinea grass (Panicum maximum) has become naturalized in most ecological zones, ecosystems and habitats except in hilly and semi-arid parts of the country [3.]. Some forages contain anti-nutrition factors such as oxalate, nitrates and cyanides etc. which can greatly affect on animals' health and performances. It has been reported that when ruminants consume plants containing more oxalate for a long period of time, soluble oxalate may combine with calcium (Ca) and form Ca-oxalate which is insoluble, is excreted in feces and also reduces absorption of Ca. Feeding forages that containing 2.0% or more soluble oxalate in dry basis can lead to acute toxicities in ruminants [4]. One of the main reasons for nitrate toxicity is consumption of fodder containing high amounts of nitrate. Plants containing more than 1.76% nitrate is dangerous [5]. In ruminants, some amount of nitrate is reduced to ammonia by rumen microbes but excessive nitrate get accumulated in rumen and absorbed into blood stream which combines with ferrous ion. This creates oxygen deficiency by converting hemoglobin to met-hemoglobin which result mortality. Moreover, nitrate toxicity causes weight loss and abortions which result in economical losses.

Oxalate and nitrate accumulation in forage plants are influenced by many factors such as soil, climate, agronomy and plant factors. When the plant factors are considered, plant maturity (young and mature), parts of the plant (leaf and stem/culm), plant species and plant variety have a larger impact on oxalate and nitrate accumulation [6]. However, data for oxalate and nitrate accumulation in wild Guinea grass and hybrid Napier var. CO-3 under local conditions are not available. Therefore, this field research was conducted to evaluate the nitrate and oxalate accumulation in hybrid Napier var. CO-3 and in wild Guinea grass.

Methodology

Sample collection

Already established fields of hybrid Napier var. CO-3 and natural fields of wild Guinea grass were selected from two farms; Animal Experimental Farm(AEF), Gannoruwa and Artificial Insemination Center (AIC), Kundasale. Recommended plot areas for each forage was demarcated. All plots were harvested and recommended fertilizers (Urea: 40 kg/Ac, TSP: 35 kg/Ac and MOP: 25 kg/ac) were applied. Harvests were then obtained from each plot at 14 day intervals at 2, 4 and 6 weeks from 1st cutting and random samples were taken for the analysis.

The experiment was conducted as a Complete Randomized Design (CRD) in 4 factor factorial treatment arrangement with three replicates. Factors considered were locations (Gannoruwa and Kundasale), species (hybrid Napier var. CO-3 and wild Guinea grass), plant parts (stem and leaf) and growth stages (2, 4 and 6 weeks).

Sample preparation and laboratory analysis

The harvested forage samples were separated as leaf and stem and cut into small pieces before drying. Samples were then dried at 100^{0}C until these reached to a constant weight. The dried samples were ground into powder form and stored in glass bottles for laboratory analysis. For each forage sample, sub samples were analyzed for nitrate and soluble oxalate contents by Devada's alloy [7] and Potassium permanganate reduction [8] methods respectively. Nitrate and oxalate concentrations were calculated as percentage in dry weight.

Data analysis

The General Linear Model (GLM) procedure of SAS (Ver. 9.0, SAS) was used to analyze the data statistically. Analysis of variance (ANOVA) was carried out to determine the effect of treatment on soluble oxalate and nitrate and the means were separated by Tukey's studentized test. Statistical significance was declared at $p<0.05$.

Results and Discussion

Nitrate accumulation

There was a significant association ($p<0.05$) among the four factors namely location, species, plant part and growth stage on nitrate accumulation. Nitrate accumulation in plant parts of hybrid Napier var.

CO-3 and wild Guinea grass in all 3 growth stages were higher ($p<0.05$) in AEF at Gannoruwa than AIC farm at Kundasale. The reason for higher nitrate contents of plants obtained from Gannoruwa than Kundasale could be due to presence of climatic parameters that helped for maximum growth of grasses in Gannoruwa which increase the uptake of nitrate from soil. Further, nitrate contents in stem parts of hybrid Napier (during all growth stages) were significantly different between the two locations ($p<0.05$, Figure 1). In each growth stage, higher levels of nitrate ($p<0.05$) were observed in stems than leaves in both forages despite the locations. Rasby et al[9] and Sidhu *et-al.*[10] also reported that nitrate content is highest in stem tissues than in leaf tissues. These findings confirmed that nitrate content differs with the plant parts.

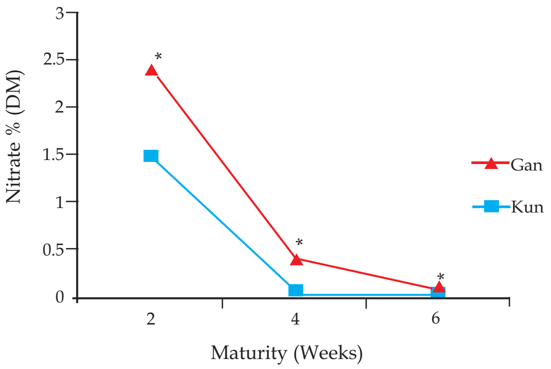


Figure 1. Effect of location on nitrate contents in stem parts of hybrid Napier var. CO -3

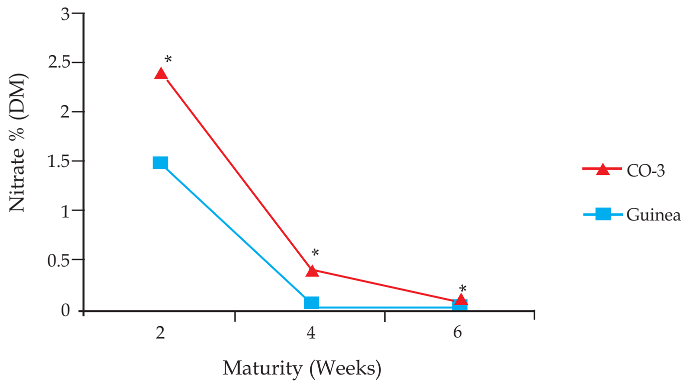


Figure 2. Effect of species on nitrate contents in stem parts at Gannoruwa farm

Moreover, nitrate contents in stems were significantly higher in hybrid Napier var. CO-3 than in wild Guinea grass at all growth stages in Gannoruwa farm ($p<0.05$, Figure 2). Sidhu *et-al.*[10] also reported that there are differences in nitrate accumulation among the species of forages. Plant species having a lower tendency to accumulate nitrate might be selected for cultivation, that will reduce the risk of nitrate poisoning.

Hybrid Napier and wild Guinea grass samples taken from three cuttings revealed significantly higher nitrate contents in both leaves and stems at 1st cutting compared to 2nd and 3rd cuttings (Table 1). The reasons for higher nitrate levels in 1st cutting could be due to plentiful growth of forages during the period and application of fertilizer (urea) into the fields when fields were prepared for the experiment. Lower nitrate levels in mature plants may be due to decreased uptake or increased enzyme activity to convert the nitrate into intermediate compounds ready for evaporation or used by the plants. These results confirmed the previous findings that had been reported,[9,10] where lower nitrate levels were observed with plant maturity. Moreover, data suggested that nitrate contents decreased with plant maturity and use of nitrate containing fertilizer influenced the nitrate accumulation in plants. Hence delaying of harvesting time may reduce the toxic effect of nitrate. However, nitrate levels in both parts of selected species at both locations and at all growth stages did not exceed the poisonous level of 1.76% (DM) nitrate, which is safe for feeding.

Effect on soluble oxalate accumulation

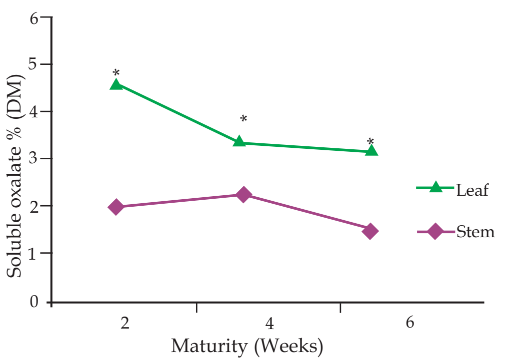
There was no significant association *($p>0.05$)* between four factors namely location, species, plant part and growth stage on soluble oxalate accumulation. However, three way interactions of location × species × plant part and species × plant part × growth stage were significant *($p<0.05$)*.

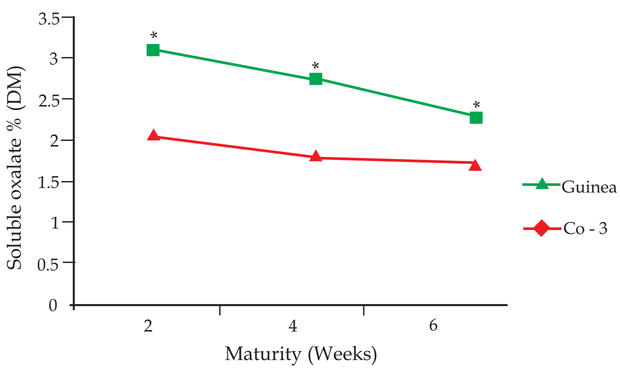
Higher soluble oxalate contents (p<0.05) were observed in leaf tissues compared to stem tissues in Guinea grass at *Gannoruwa* farm at all growth stages (Figure 3). The difference between oxalate contents of stems and leaves in hybrid Napier var. CO-3 were significant (p<0.05) at Gannoruwa except at 3rd growth stage. Differences in soluble oxalate accumulation among the plant parts of forages have also been reported.[11],[12] However under local conditions, values were higher than the recorded values which have a potential to be toxic to the animals fed on studied forages, especially leaves of wild Guinea grass. Further, higher soluble oxalate contents (*p*<0.05) were recorded in wild Guinea grass than hybrid Napier var. CO-3 in both plant parts at all growth stages at Kundasale farm (Figure 4). A similar trend was observed at Gannoruwa farm. Research studies on oxalate contents in various plants have shown significant genotypic variation on oxalate accumulation [13]. Plants having a lower tendency to accumulate soluble oxalate might be selected for cultivation since feeding forages with higher oxalate create adverse effects on the animal. Therefore, consumption of plants containing oxalate by ruminants should be carefully monitored.

Table 1. Nitrate content (% DM) in forage samples at different growth stages

Sample	Stage 2 weeks	Stage 4 weeks	Stage 6 weeks
Gannoruwa / CO-3/stem	2.384 ^{a}	0.402 ^{b}	0.072 ^{b}
Gannoruwa / CO-3/leaf	0.723 ^{a}	0.032 ^{b}	0.009 ^{b}
Gannoruwa / Guinea/stem	1.417 ^{a}	0.071 ^{b}	0.012 ^{b}
Gannoruwa / Guinea /leaf	0.065 ^{a}	0.009 ^{b}	0.008 ^{b}
Kundasale / CO-3/stem	1.486 ^{a}	0.055 ^{b}	0.019 ^{b}
Kundasale / CO-3/leaf	0.562 ^{a}	0.028 ^{b}	0.008 ^{b}
Kundasale / Guinea/stem	0.098 ^{a}	0.018 ^{b}	0.013 ^{b}
Kundasale / Guinea/leaf	0.006 ^{a}	0.007 ^{a}	0.006 ^{a}

$^{a, b}$ Means in the same row with different superscripts differ significantly (*p<0.05*).





*Soluble oxalate content is significantly different at α =0.05 level.

Figure 3. Effect of plant parts on oluble oxalate contents in Guinea grass at Gannoruwa farm

Figure 4. Effect of species on soluble oxalate contents in leaves at Kundasale farm

The results revealed that, young plants contain more soluble oxalate than older plants (Table 2). Even though soluble oxalate levels decreased with the maturity, poisonous effect was not changed in leaf part of wild Guinea grass. Oxalate content of forages can be manipulated by changing the harvesting intervals, and further oxalate content declined as the harvesting interval increased[12, 14]. Therefore, it is desirable to find harvesting practices in forages that would reduce the soluble oxalate content to the acceptable level (< 2% DM) by choice of appropriate growth stages for harvest.

Table 2**.** Soluble oxalate content (% DM) in forage samples at different growth stages

Sample	Stage 2 weeks	4 weeks	6 weeks
Gannoruwa / CO-3/stem	1.342 [a]	2.582 [b]	1.203 [a]
Gannoruwa / CO-3/leaf	2.024 [a]	1.980 [b]	1.679 [a]
Gannoruwa / Guinea/stem	2.437 [a]	2.137 [ab]	1.589 [b]
Gannoruwa / Guinea /leaf	4.867 [a]	3.760 [b]	3.345 [c]
Kundasale / CO-3/stem	1.500 [a]	1.319 [a]	1.312 [a]
Kundasale / CO-3/leaf	2.077 [a]	1.814 [a]	1.709 [a]
Kundasale / Guinea/stem	2.197 [a]	1.987 [a]	1.627 [a]
Kundasale / Guinea/leaf	3.097 [a]	2.745 [a]	2.288 [b]

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

Conclusion

The results of the experiment concluded that nitrate and soluble oxalate content in fodder plants may vary over wide range, mainly depending upon the forage species, parts of the plant and growth stages. There is no single factor which could be used to control nitrate and soluble oxalate accumulation in forage plants. Since species of fodder has a higher effect on oxalate and nitrate accumulation, forage species having a lower tendency to accumulate nitrate and soluble oxalate should be selected for cultivation. Hybrid Napier var. CO-3 is safer than wild Guinea grass for feeding of ruminants.

With respect to the plant parts, in fields reported with higher nitrate levels, lower part of the grasses near the ground should not be used as feeds for ruminants because stem stores more nitrate than leaves. However, careful attention should be paid to plant materials consumed by grazing animals because soluble oxalate content is higher in leaves than stem parts. Although nitrate and oxalate content in forages adversely affect the nutritional quality in animal feeds, studies on nitrate and oxalate accumulation in forage plants have received little attention. Given it as a multi-factorial problem, there is an urgent need to carry out further research on the nitrate and oxalate toxicity.

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