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EFFECT OF HARVESTING INTERVAL ON OXALATE CONTENT OF DIFFERENT NAPIER GRASS SPECIES

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SUMMARY

Oxalates are important anti-nutritional constituents in tropical fodder grasses and may interfere with calcium metabolism in livestock when present at higher concentrations. The present study was conducted at the Veterinary College and Research Institute (VCRI), Orathanadu to evaluate the effect of harvesting days and species on oxalate content in different Napier grass varieties. Five species, namely Super napier, Red napier, CO-3, CO-4 and CO-5, were evaluated at three harvesting intervals (Day 30, Day 45 and Day 60). The experiment was carried out in a Completely Randomized Design with factorial arrangement and the data were analysed using two-way analysis of variance. Days and species affected oxalate content ($p<0.001$), whereas their interaction was not detected ($p=0.788$). Oxalate content decreased progressively with increasing harvesting interval. Higher oxalate values were observed on Day 30 (3.064 ± 0.011), followed by Day 45 (2.934 ± 0.011), while the lowest value was recorded on Day 60 (2.794 ± 0.011). Among species, Super napier recorded the highest oxalate content (3.317 ± 0.014), whereas CO-5 showed the lowest value (2.547 ± 0.014). Delayed harvesting reduced oxalate concentration in Napier grasses and CO-5 exhibited comparatively lower oxalate content, indicating better fodder suitability**.**

Key words: CO-5, cutting interval, Napier grass, oxalate, titrimetric method

Napier grass (*Pennisetum purpureum* Schumach) and its interspecific hybrids are widely used green fodder crops for dairy animals in tropical regions due to their high biomass yield, rapid regrowth and adaptability to diverse agro-climatic conditions (Libert and Franceschi, 1987, Rahman *et al.*, 2013). These grasses play a crucial role in ensuring a continuous supply of roughage in both organized dairy farms and smallholder livestock systems. Varieties such as CO-3, CO-4, CO-5 and Super Napier are extensively cultivated across South India owing to their high productivity and ease of cultivation.

However, Napier and hybrid Napier grasses are known to accumulate anti-nutritional factors, particularly oxalates, which may interfere with calcium metabolism and adversely affect animal health when present at higher concentrations (Rahman *et al.*, 2013). Considering their widespread use as a primary roughage source for dairy animals and small ruminants in Tamil Nadu, periodic evaluation of oxalate content under local conditions is essential. Although general information on oxalate levels in fodder crops is available, location-specific data for commonly cultivated varieties remain limited.

Therefore, the present study was undertaken to estimate and compare oxalate content in selected Napier and hybrid Napier fodder varieties grown at VCRI, Orathanadu, using the AOAC titrimetric method.

Oxalate in Forage Plants

Definition and Forms of Oxalate

Oxalic acid, commonly referred to as oxalate, is a naturally occurring low molecular weight organic acid widely distributed in plants (Libert and Franceschi, 1987, Rahman *et al.*, 2013). Within plant tissues, oxalate is mainly present in two forms: soluble and insoluble. Soluble oxalates readily combine with monovalent minerals such as sodium, potassium and ammonium, whereas insoluble oxalates primarily bind with divalent minerals, especially calcium and magnesium, to form calcium oxalate crystals (Goyal, 2018).This distinction is important from a nutritional standpoint, as soluble oxalates can be absorbed in the digestive tract and interfere with mineral availability, while insoluble oxalates generally pass through the digestive system without being absorbed (Messy, 2007). Calcium oxalate crystals are common biominerals in plants and have been reported in more than 200 plant families (Libert and Franceschi, 1987) These crystals occur in different shapes, including druses and raphides and are usually stored in specialized cells known as idioblasts (Tutuncu konyar *et al.,* 2014).

Biochemical Role of Oxalate in Plants

In addition to being a metabolic by-product oxalate performs several important physiological and biochemical functions in plants. It plays a role in maintaining internal calcium balance helps detoxify excess heavy metals through chelation and provides protection against herbivores by forming sharp needle-like crystals that discourage grazing. Oxalate may also contribute to structural support in plant tissues and influence light reflection. Furthermore, calcium oxalate crystals are involved in broader metabolic processes including carbon cycling and ion regulation (Nakata, 2003). The formation of oxalate in plants is linked to multiple biosynthetic pathways with ascorbic acid considered one of the major precursors involved in oxalate synthesis (Cai *et al.,* 2018).

Anti-nutritional Significance of Oxalate

From a nutritional perspective, oxalate is regarded as an anti-nutritional factor because of its ability to bind essential minerals such as calcium and magnesium forming insoluble compounds that reduce their absorption (Massey, 2007, Rahman *et al.,* 2013). In forage crops particularly those with a high proportion of leaves elevated levels of soluble oxalate can disrupt mineral utilization in animals and lead to imbalances in mineral metabolism. Prolonged consumption of fodder containing high oxalate levels may result in health problems such as hypocalcaemia, reduced growth performance and decreased milk yield (Rahman *et al.,* 2013). In severe cases, chronic oxalate intake has been associated with conditions such as urolithiasis and renal damage (Rahman and Kawamura, 2011). Numerous forage-related studies have consistently shown that soluble oxalate plays a significant role in reducing mineral availability in grazing livestock (Massey, 2007, Rahman and Kawamura, 2011).

Oxalate Toxicity in Livestock

Oxalate toxicity in livestock occurs when animals consume forages containing high levels of soluble oxalate. After ingestion, oxalate binds with divalent minerals such as calcium and magnesium in the digestive tract, forming insoluble complexes that reduce mineral absorption (Goyal, 2018, Massey, 2007, Rahman *et al.*, 2013). Severe mineral depletion, particularly calcium deficiency, can result in hypocalcaemia and associated metabolic disorders. In addition, absorbed oxalate may precipitate in the kidneys as calcium oxalate crystals, leading to nephrosis and, in chronic cases, renal failure (Herbert and Dittmer, 2017, Rahman *et al.,* 2013, Rahman and Kawamura, 2011). Such toxic effects have been reported in both ruminant and non-ruminant animals fed oxalate-rich tropical forages, including Napier grass (Panir Choudhury *et al.,* 2023).

Ruminants possess partial protection against oxalate toxicity due to oxalate-degrading microorganisms in the rumen, which convert oxalate into less harmful compounds. Gradual adaptation to oxalate-containing feeds enhances this microbial activity and improves tolerance, whereas sudden consumption of large quantities of high-oxalate forage can overwhelm this detoxification mechanism, resulting in systemic absorption and metabolic disturbances (Rahman *et al.,* 2013, Rahman and Kawamura, 2011). The principal toxic effect of oxalate is disruption of calcium metabolism. Reduced intestinal calcium absorption leads to low blood calcium levels, impairing neuromuscular and cardiac function. Clinical signs include muscle tremors, weakness, incoordination, tetany, recumbency and, in severe cases, sudden death. Chronic exposure may cause bone demineralization, poor growth, reduced productivity and progressive renal damage (Goyal, 2018, Rahman *et al.,* 2013).

Susceptibility to oxalate toxicity varies among animal species. Ruminants typically show signs such as hypocalcaemic tetany, weight loss, reduced feed intake and lethargy, whereas non-ruminants, particularly horses, are more sensitive due to limited oxalate degradation in the digestive system (Herbert and Dittmer, 2017, Rahman *et al.,* 2013). As a general guideline, ruminants can tolerate soluble oxalate levels below about 2.0% of dietary dry matter; while, concentrations above approximately 0.5% may pose a risk to non-ruminants. These limits are influenced by dietary composition, mineral balance and degree of adaptation (Rahman *et al.,* 2013).

Factors Affecting Oxalate Accumulation in Fodder Crops

Oxalate accumulation in fodder crops is influenced by a combination of genetic makeup, plant physiology, environmental conditions and management practices. Wide variation in oxalate concentration has been observed not only between different forage species but also among varieties within the same species, as well as between plant parts and growth stages. A clear understanding of these factors is essential to manage fodder crops effectively and reduce the risk of oxalate toxicity in livestock.

1. Species and Genotypic Differences

Different fodder species differ greatly in their capacity to accumulate oxalate. Several tropical grasses, including *Pennisetum*, *Setaria*, *Cenchrus* and *Panicum*, are known oxalate accumulators (Goyal, 2018, Rahman and Kawamura, 2011). Within Napier grass and its hybrids, considerable genotypic variation has been reported (Pathmasiri *et al.,* 2014, Rahaman *et al.,* 2006). Studies comparing dwarf and non-dwarf Napier types indicate that dwarf and leafy varieties often contain higher oxalate levels, mainly because of their greater leaf proportion (Rahaman *et al.,* 2006). Similar varietal differences have been documented in pearl millet × Napier hybrids such as CO-3 (Pathan *et al.,* 2014, Pathmasiri *et al.,* 2014). These findings suggest that oxalate accumulation is strongly influenced by genetic traits as well as plant morphology (Rahman and Kawamura, 2011).

2. Plant Parts (Morphological Fractions)

Oxalate distribution within the plant is uneven. Leaves generally contain much higher oxalate concentrations than stems and leaf sheaths (Pathmasiri *et al.,* 2014, Rahman *et al.,* 2006). This is due to the greater metabolic activity of leaves and their role in calcium regulation and organic acid metabolism (Cai *et al.,* 2018, Libert and Dittmer, 2017). Consequently, fodder crops with a high leaf-to-stem ratio, such as hybrid Napier grasses, may contribute to higher oxalate intake by animals (Pathmasiri *et al.,* 2014, Rahaman *et al.,* 2006).

3. Stage of Growth and Plant Maturity

Plant age is one of the most consistent factors affecting oxalate levels. Young, fast-growing plants usually have higher oxalate concentrations, which gradually decline as the plant matures (Jones and Ford, 1972, Pathmasiri *et al.,* 2014, Rahaman *et al.,* 2006). This reduction is typically faster in stems than in leaves (Rahaman *et al.,* 2006). Elevated oxalate content in young plants is associated with active metabolism and rapid synthesis of organic acids during early growth stages (Cai *et al.,* 2018, Rahaman and Kawamura, 2011). In addition, shorter cutting intervals that maintain plants in a younger physiological stage have been shown to increase oxalate accumulation, whereas longer intervals allow maturation and reduce oxalate levels (Pathan *et al.,* 2014).

4. Seasonal and Climatic Factors

Seasonal and climatic conditions play a major role in oxalate accumulation. Oxalate levels are generally highest during periods of rapid vegetative growth, particularly in warm seasons with high light intensity. As the season progresses and growth slows, oxalate concentration tends to decrease (Rahaman and Kawamura, 2011, Rahman *et al.,* 2006). Factors such as temperature, solar radiation, rainfall and day length influence plant growth rate and metabolic activity, thereby indirectly affecting oxalate synthesis (Goyal, 2018, Rahaman and Kawamura, 2011).

5. Soil and Mineral Nutrition

Soil fertility and mineral nutrition significantly influence oxalate content in fodder crops. High applications of nitrogen and potassium fertilizers are commonly associated with increased oxalate accumulation, especially in Napier and hybrid Napier grasses (Govindasawamy and Manickam, 2018, Pathan *et al.,* 2014, Rahaman and Kawamura, 2011). In contrast, adequate calcium availability can reduce the proportion of soluble oxalate by promoting the formation of insoluble calcium oxalate within plant tissues (Libert and Franceschi, 1987, Nakata, 2003).

6. Agronomic Management Practices

Crop management practices such as cutting interval, harvest frequency and fertilizer application have a strong impact on oxalate levels. Frequent harvesting and short cutting intervals maintain plants in a young, actively growing state, which generally results in higher oxalate concentrations (Pathan *et al.,* 2014, Rahaman and Kawamura, 2011). In contrast, longer cutting intervals allow plants to mature, leading to lower oxalate content (Jones and Ford, 1972, Pathan *et al.,* 2014). Similarly, intensive fertilization aimed at maximizing biomass production can increase oxalate accumulation by stimulating rapid vegetative growth (Govindasawamy and Manickam, 2018, Pathan *et al.*, 2014, Rahaman and Kawamura, 2011).

7. Physiological and Metabolic Factors

At the physiological level, oxalate accumulation is closely linked to metabolic pathways such as ascorbate breakdown, glyoxylate metabolism and organic acid synthesis (Cai *et al.*, 2018, Rahman *et al.,* 2013). Plants with high growth rates and elevated photosynthetic activity tend to accumulate more oxalate (Rahaman and Kawamura, 2011). Oxalate also serves important functions in plants, including calcium regulation, detoxification of excess metals and structural defence (Cai *et al.,* 2018, Libert and Franceschi, 1972, Nakata, 2003). which explains its variation under different growth conditions and physiological states.

MATERIALS AND METHODS

The study was conducted at the Veterinary College and Research Institute (VCRI), Orathanadu, Tamil Nadu. The experiment was carried out in a completely randomized design (CRD) with factorial arrangement comprising two factors, namely variety and cutting interval. The fodder varieties included CO-3, CO-4, CO-5, Super Napier and Red Napier, while the cutting intervals were 30, 45 and 60 days. Each treatment combination was replicated five times.

Fresh green fodder samples were collected from well-established Napier and hybrid Napier plots maintained at VCRI, Orathanadu. Sampling was carried out at the specified cutting intervals of 30, 45 and 60 days to study the effect of plant maturity on oxalate content. Samples were collected randomly from different locations within the plots to ensure representativeness, avoiding periods immediately following irrigation to minimize variability due to surface moisture. The collected samples were composited and proportioned to contain approximately 75% leaves and 25% stems, reflecting the typical fodder consumed by livestock, and were immediately transported to the laboratory for further processing.

The fodder samples were chopped into small pieces and dried in a hot air oven at 110°C for 8 hours to arrest enzymatic activity and remove moisture. The dried samples were cooled in a desiccator and ground using a laboratory grinder. The ground material was passed through a 1 mm sieve to obtain uniform particle size and stored in airtight containers until chemical analysis.

Estimation of Oxalic Acid in Fodder Samples

Procedure

A 2 g dried and ground fodder sample was extracted with 190 ml distilled water and 10 ml of 6 N HCl by boiling for 1 hour. The extract was filtered and made up to 250 ml. An aliquot of 50 ml was taken, acidified, concentrated and neutralized using ammonia with methyl red indicator. Calcium oxalate was precipitated by adding 5% calcium chloride and allowed to stand overnight. The precipitate was filtered, washed, dissolved in dilute sulfuric acid, and titrated against N/20 $KMnO_4$, at 70°C until a persistent pink endpoint was obtained. (Abaza *et al.,* 1968).

Statistical analysis

The experiment was conducted in a Completely Randomized Design (CRD) with factorial arrangement. The experimental data were subjected to two-way analysis of variance (ANOVA) using the General Linear Model procedure of SPSS to evaluate the effects of days, species and their interaction on oxalate content. When the F-test indicated significant differences, the treatment means were compared using Tukey's Honestly Significant Difference (HSD) test and Least Significant Difference **(**LSD**)** test as appropriate. A probability level of $p < 0.05$ was considered statistically significant, while $p < 0.01$ was considered highly significant.

RESULTS

The effects of days and species on oxalate content were detected (p<0.001), whereas the interaction between days and species was not detected (p=0.788) (Table 1). The model explained a major proportion of variability (R^2=0.974).

Oxalate content decreased with increasing duration (Table 2). Values were greater on Day 30 (3.064 ± 0.011) than on Day 45 (2.934 ± 0.011) and Day 60 (2.794 ± 0.011) (p<0.05).

Differences among species were also observed (Table 3). Super napier recorded greater oxalate content (3.317 ± 0.014), whereas CO-5 showed the lowest value (2.547 ± 0.014) (p<0.05).

TABLE 1
Two-way ANOVA for oxalate content

Source	df	Mean Square	F value	p-value
Days	2	0.456	156.98	<0.001
Species	4	1.424	490.46	<0.001
Days × Species	8	0.002	0.583	0.788
Error	60	0.003	-	-

df = degree of freedom; mean square = sum of squares divided by respective df.

TABLE 2
Effect of days on oxalate content

Days	Oxalate (Mean ± SE)
Day 30	3.064 ± 0.011C^{a}
Day 45	2.934 ± 0.011G^{b}
Day 60	2.794 ± 0.011œ^{c}

Values are expressed as mean ± standard error. Means with different superscripts within a column differ ($p < 0.05$) according to Tukey/LSD test.

TABLE 3
Effect of species on oxalate content

Species	Oxalate (Mean ± SE)
Super napier	3.317 ± 0.014C”
Red napier	3.137 ± 0.014G”
CO-3	2.923 ± 0.014œ”
CO-4	2.730 ± 0.014H”
CO-5	2.547 ± 0.014I”

Values are expressed as mean ± standard error. Means with different superscripts within a column differ significantly ($p < 0.05$) according to Tukey/LSD test.

TABLE 4
Interaction effect (Days × Species)

Days	Super napier	Red napier	CO-3	CO-4	CO-5
Day 30	3.450	3.272	3.032	2.858	2.710
Day 45	3.308	3.142	2.942	2.738	2.538
Day 60	3.194	2.996	2.796	2.594	2.392

The interaction between days and species was not detected (Table 4), indicating a similar pattern of oxalate reduction across species over time.

DISCUSSION

Varietal Differences in Oxalate Content

The differences in oxalate content observed among the fodder varieties in the present study can primarily be attributed to genetic factors. Among the Cumbu Napier hybrids, CO-5 recorded the lowest oxalate concentration, indicating its suitability as a safer fodder with respect to oxalate intake. In contrast, Super Napier exhibited the highest oxalate level, which may be associated with its vigorous growth and higher leaf proportion higher leaf proportion as reported in earlier studies.

These findings are consistent with earlier reports that indicate significant varietal variation in oxalate accumulation in Napier grass and related species (Pathmasiri *et al.*, 2014, Rahman *et al.*, 2006). Studies have also shown that dwarf and leafy genotypes tend to accumulate higher oxalate due to increased leaf biomass (Rahman *et al.*, 2006). Since oxalate is predominantly concentrated in leaf tissues, varieties with a higher leaf-to-stem ratio are expected to exhibit greater oxalate content.

Effect of Growth Stage and Plant Parts

The decrease in oxalate content with increasing cutting interval (maturity) is consistent with earlier findings that younger plants contain higher concentrations of soluble oxalates, which decline as the plant matures. This reduction may be due to dilution effects caused by increased accumulation of structural carbohydrates such as cellulose and hemicellulose, along with reduced metabolic activity in older tissues.

The non-significant interaction between variety and cutting interval indicates that all varieties followed a similar decreasing trend with maturity, suggesting that the effect of cutting interval on oxalate content is consistent irrespective of genotype.

This observation is in agreement with earlier studies, which reported that oxalate content decreases with plant maturity and is generally higher in younger tissues (Jones and Ford, 1972, Pathmasiri *et al.*, 2014, Rahman *et al.*, 2006). Additionally, the higher oxalate concentration in leaves compared to stems observed in the present study aligns with previous findings that attribute this difference to higher metabolic activity in leaf tissues.

Nutritional Significance and Feeding Implications

Oxalate levels exceeding 3.0% dry matter, as observed in Super Napier (3.20–3.45%) and Red Napier (3.00–3.25%) are of nutritional concern when these fodders are used as the primary roughage source. Oxalates can bind dietary calcium and reduce its bioavailability, potentially leading to hypocalcaemia, reduced milk production and poor growth in livestock if fed continuously without adequate mineral supplementation.

In contrast, relatively lower oxalate levels observed in CO-4 (2.60–2.85%) and CO-5 (2.40–2.65%) indicate their suitability as safer fodder options. Furthermore, harvesting at a later stage (60 days) significantly reduces oxalate content, thereby minimizing potential anti-nutritional effects.

These findings are supported by previous studies, which have highlighted the adverse effects of high oxalate intake on mineral metabolism and animal performance (Goyal, 2018, Rahman *et al.*, 2013). It has been reported that oxalate-rich diets can impair calcium absorption and lead to metabolic disorders in both ruminants and non-ruminants (Massey, 2007, Rahman and Kawamura, 2011). Conversely, the lower oxalate content observed in CO-5 suggests that this variety is more suitable for routine feeding, particularly under smallholder conditions where mineral supplementation may be limited.

CONCLUSION

It can be concluded that oxalate content in Napier grass is significantly influenced by both variety and cutting interval. Among the varieties studied, Super Napier recorded the highest oxalate content, whereas, CO-5 recorded the lowest.

Oxalate content decreased significantly with advancing maturity, indicating that harvesting at later stages (60 days) can help reduce oxalate levels in fodder.

Since high oxalate levels may adversely affect mineral availability in animals, the use of low-oxalate varieties such as CO-4 and CO-5, along with optimum cutting intervals, is recommended for safe and efficient feeding.

REFERENCES

Abaza, R. H., J. T. Blake and E. J. Fisher, 1968 : Determination of oxalates in feeds and fodders. J. Assoc. Off. Anal. Chem., 51: 963.

Cai, X., C. Ge, C. Xu, X. Wang, S. Wang and Q. Wang, 2018 : Expression analysis of oxalate metabolic pathway genes reveals oxalate regulation patterns in spinach. *Molecules*, **23**(6): 1286.

Govindaswamy, M. and T. S. Manickam, 1988 : Effect of nitrogen on the content of oxalic acid in Bajra-Napier hybrid grass BN 2. *Madras Agric. J.*, **75**(5-6): 219-220.

Goyal, M., 2018 : Oxalate accumulation in fodder crops and impact on grazing animals - A review. *Forage Research*, **44**(3): 152-158.

Herbert, E. W. and K. E. Dittmer, 2017: Acute and chronic oxalate toxicity in miniature horses associated with soursob (Oxalis pes-caprae) ingestion. Equine Vet. Educ., **29**(10): 549-557.

Jones, R. J. and C. W. Ford, 1972 : Some factors affecting the oxalate content of the tropical grass Setaria sphacelata. Aust. J. Exp. Agric. Anim. Husb., **12**(57): 400-406.

Libert, B. and V. R. Franceschi, 1987 : Oxalate in crop plants. *J. Agric. Food Chem.*, **35**(6): 926-938.

Massey, L. K., 2007: Food oxalate: factors affecting measurement, biological variation and bioavailability. *J. Am. Diet. Assoc.*, **107**(7): 1191-1194.

Nakata, P. A., 2003: Advances in our understanding of calcium oxalate crystal formation and function in plants. *Plant Sci.*, **164**(6): 901-909.

Panir Choudhury, M., S. M. Hossain, S. Akter and M. Tasmirul Islam, 2023 : A narrative review on effects of Napier grass oxalate accumulation on ruminant animals. *Curr. Nutraceuticals*, **4**(1).

Pathan, S. H., A. D. Tumbare and A. B. Kamble, 2014 : Effect of agronomic management on oxalate and silica content in pearl millet × Napier hybrid. *Indian J. Agron.*, **59**(3): 415-420.

Pathmasiri, P. G. R. P., G. C. C. Premalal and W. A. D. Nayananjalie, 2014: Accumulation of oxalate and nitrate in hybrid Napier var. CO-3 (*Pennisetum purpureum* × *Pennisetum americanum*) and Guinea grass (*Panicum maximum*). Rajarata Univ. Sri Lanka.

Rahman, M. M. and O. Kawamura, 2011: Oxalate accumulation in forage plants: some agronomic, climatic and genetic aspects. Asian-Australasian J. Anim. Sci., **24**(3): 439-448.

Rahman, M. M., M. Niimi, Y. Ishii and O. Kawamura, 2006 : Effects of season, variety and botanical fractions on oxalate content of Napiergrass (*Pennisetum purpureum* Schumach). *Grassl. Sci.*, **52**(4): 161-166.

Rahman, M. M., R. B. Abdullah and W. E. Wan Khadijah, 2013 : A review of oxalate poisoning in domestic animals: tolerance and performance aspects. *J. Anim. Physiol and Anim. Nutr.*, **97**(4): 605-614.

Tütüncü Konyar, S., N. Öztürk and F. Dane, 2014 : Occurrence, types and distribution of calcium oxalate crystals in leaves and stems of some species of poisonous plants. *Bot. Stud.*, **55**(1): 32.