Effects of Cultivar and Plant Fraction on Oxalate Content and Nutritional Composition of Napier Grass and Selected Non-Napier Forages

Siti Nur Haslina Mamat[1], Mohammad Mijanur Rahman[2*], Rovina Kobun[2], Candyrine Su Chui Len[2], and Rohaida Abdul Rasid[2]

[1]Faculty of Agro based Industry, Universiti Malaysia Kelantan, Jeli 17600, Kelantan, Malaysia
[2]Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan 90509, Sabah, Malaysia
[3]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10, Makassar 90245, South Sulawesi, Indonesia.

Abstract. Napier grass (*Pennisetum purpureum*) is an important tropical forage valued for its high biomass yield and carbohydrate content, which supports efficient rumen fermentation. However, its utilisation is limited by antinutritional factors, particularly oxalic acid, which is generally higher in Napier grass than in other tropical forages. Excessive oxalate intake impairs calcium and magnesium metabolism, which can lead to metabolic disorders and renal dysfunction in animals. In this study, the effects of cultivar and botanical fraction on the oxalate content and proximate composition of Napier grass and selected non-Napier forages were investigated. Seven Napier cultivars (Dwarf, Taiwan, Zanzibar, King, Purple, Pakchong, and Indian), as well as *Indigofera tinctoria* and *Trichanthera gigantea*, were collected from farms in Kedah and Kelantan, Malaysia. The leaves and stems were analysed separately for soluble oxalate, total oxalate, and composition of constituents. The results showed that Dwarf Napier had the highest crude protein content, but also an increased content of soluble oxalate, especially in the leaves. For all forage varieties, the oxalate content was consistently higher in the leaves than in the stems, highlighting the importance of variety selection and the ratio of leaves to stems in feed formulation to optimise nutritional value while minimising the risks associated with oxalate in ruminant diets.
Keywords: Botanical fraction, cultivar, Napier grass, oxalate.

1 Introduction

Global population growth has increased the demand for animal products, placing pressure on livestock systems to deliver sustainable and cost-effective feeding strategies. Feeding practices play a critical role in livestock productivity and the nutritional quality of animal-derived foods, with direct implications for economic efficiency and animal health [1]. Although modern production systems increasingly use formulated feeds, forage remains the foundation of ruminant nutrition due to its availability, affordability and contribution to

* Corresponding author: mijanur.r@ums.edu.my

rumen function [2]. In forage-based systems, particularly those relying on high-yield tropical grasses such as Napier grass, improving forage utilisation and managing associated nutritional constraints, rather than simply increasing concentrate inclusion, is key to reducing production costs and ensuring safe, sustainable livestock production [3].

Among tropical forages, Napier grass (*Pennisetum purpureum*) is widely cultivated in Asia and Africa due to its rapid growth, adaptability across agro-ecological conditions and high biomass production. It is also well suited for silage making because of its favourable carbohydrate profile that supports effective fermentation [3]. A wide range of cultivars, including tall and dwarf types, have been developed, which differ mainly in growth habit, leaf-to-stem ratio, dry matter (DM) yield and crude protein (CP) concentration. In general, dwarf cultivars are characterised by higher CP content and a greater proportion of leaves, whereas tall cultivars tend to produce higher DM yields. These varietal differences influence both nutritional value and suitability for specific feeding and production systems [4].

Forage legumes complement grasses by providing higher protein and mineral concentrations. *Indigofera tinctoria* has attracted attention as a protein-rich legume that thrives in low-water environments, making it a reliable alternative in the dry season [5]. Its high digestibility and mineral content support optimal animal growth, although it is best utilised in combination with grasses. Similarly, *Trichanthera gigantea* has been investigated in smallholder systems for its potential as a supplementary protein source. Taken together, these forages offer the opportunity to reduce reliance on expensive concentrates and improve the sustainability of ruminant production.

Despite their benefits, many tropical forages contain anti-nutritional factors such as oxalic acid, which pose a risk to animal health. Oxalic acid occurs in both soluble and insoluble forms. While insoluble oxalates are relatively harmless and pass through the digestive tract unabsorbed, soluble oxalates can be absorbed in the intestine and combine with calcium and magnesium, leading to mineral deficiency, hypocalcaemia, and in severe cases, kidney damage. Concentrations below 2% of DM are considered safe, but higher concentrations can cause oxalate toxicosis in ruminants [6]. Reported oxalate levels in commonly used tropical forages, including Napier grass and forage legumes, often approach or occasionally exceed this threshold depending on cultivar, maturity and plant fraction, highlighting the need for species- and fraction-specific evaluation when formulating rations. Among tropical grasses, *Setaria sphacelata* is known for high oxalate content, while Napier grass accumulates oxalates in medium content, with levels varying according to cultivar, plant maturity and botanical fractions [7].

Previous studies have reported that oxalate concentrations in Napier grass are generally higher in the leaves than in the stems, with dwarf Napier grass having higher oxalate levels than other varieties despite its better nutrient profile. Seasonal variations also influence oxalate accumulation, with higher soluble oxalate levels observed in early summer [8]. While nitrogen fertilisation does not appear to have a significant effect on oxalate concentration, the harvest date influences both oxalate content and forage quality. A shorter cutting interval reduces DM yield, while a longer harvest date increases fibre content and reduces CP [3]. These trade-offs emphasise the importance of finding optimal harvesting strategies to balance yield, nutritional value and oxalate safety.

Despite the widespread use of Napier grass and the increasing importance of *Indigofera* and *Trichanthera* in tropical livestock production, there is little information on their comparable oxalate content under uniform management conditions in Malaysia. In addition, the relationship between the botanical fractions (leaf and stem) and proximate components of the different forage species has not been sufficiently explored.

Therefore, this study was conducted to investigate the effects of forage type and botanical fractions on the soluble, insoluble and total oxalate contents as well as proximate composition of *Pennisetum purpureum*, *Indigofera tinctoria* and *Trichanthera gigantea*. The results of this work will provide regionally specific data to develop strategies for feed utilisation, optimise nutrient intake, and minimise the risks of oxalate toxicity in ruminant feeding systems.

2 Materials and methods

2.1 Collection of forage samples

Representative forage samples of six Napier grass varieties (dwarf, Indian, Taiwan, Zanzibar, Purple and Pakchong) were collected from three independent local farms practising cut-and-carry feeding systems in Alor Setar, Kedah, Malaysia. For each Napier grass variety, three biological replicates ($n = 3$) were obtained, with one replicate collected from each farm when the forage was ready for animal feeding. In addition, *Indigofera tinctoria* and *Trichanthera gigantea* were sampled using the same replication scheme ($n = 3$ per species) to allow cross-species comparison. Samples were collected randomly within each farm.

All forages were harvested at the pre-flowering stage, which corresponds to the growth stage commonly offered to animals under local farm conditions. The Napier grass varieties were grown under typical farm management practices; however, soil conditions differed among farms, and no attempt was made to standardise fertilisation, irrigation or cutting frequency. This approach was adopted to reflect on-farm production conditions and to capture variability representative of practical feeding systems. After harvest, each forage sample was manually separated into two botanical fractions (leaves and stems), except for *Indigofera tinctoria* and *Trichanthera gigantea*, for which only leaves were collected.

2.2 Preparation of samples

The freshly harvested samples were weighed and chopped by hand into approximately 2–3 cm pieces. The chopped material was oven-dried at 70 °C for 48 h to determine dry weight. The dried samples were ground using a laboratory blender and passed through a 1 mm sieve. The ground samples were stored in plastic bottles with tight-fitting mouths at room temperature for two weeks until further chemical analysis, to minimize moisture reabsorption.

2.3 Determination of oxalate content

For oxalate determination, 0.5 g of ground sample was extracted with either 15 mL of distilled water for soluble oxalate or 15 mL of 1 mol L^{-1} HCl for total oxalate. The mixture was heated on a stirring hot plate for 18 min, then cooled, filtered, rinsed, and diluted to a final volume of 50 mL. For total oxalate determination, the filtrate was further adjusted to pH 3.0 using 5 mol L^{-1} NaOH. All extracts were filtered through a 0.45 µm hydrophilic membrane filter and transferred into 1.5 mL HPLC vials for analysis [8]. Insoluble oxalate was calculated as the difference between total and soluble oxalates contents.

2.4 Standard calibration

A stock standard solution of oxalic acid was prepared by dissolving 1.41 g of commercial oxalic acid in 100 mL of distilled water. Serial dilutions were then prepared to obtain five calibration standards (0.5, 1.0, 2.0, 3.0, and 4.0 mL aliquots diluted to 100 mL with distilled water). These standards were used to construct calibration curves for oxalate quantification. Oxalate extraction and quantification were performed according to the method described by

Rahman et al. [8]. This method was selected due to its suitability for determining soluble and total oxalate contents in forage samples.

2.5 High-Performance Liquid Chromatography (HPLC)

Oxalate analysis was conducted using a Synergi 4 µm Hydro-RP 80 Å column (250 × 4.6 mm, Phenomenex, USA) maintained at 30 °C. The mobile phase comprised solvent A (20 mM methanol) and solvent B (2% formic acid buffer), delivered at a flow rate of 0.7 mL min^{-1}. A 1.5 mL sample was injected, and oxalates were detected at 220 nm using a UV–Vis detector. Quantification was performed with reference to calibration curves obtained from commercial oxalic acid standards.

2.6 Proximate analysis

Forage samples were analysed for DM, nitrogen (N), ash, crude fibre (CF), and ether extract (EE) following standard procedures of the AOAC [9]. Crude protein (CP) was calculated as N × 6.25. Crude fibre was determined according to AOAC method 978.10, and EE was determined using AOAC method 920.39.

2.7 Statistical analysis

Data on oxalate fractions and proximate composition were analysed using IBM SPSS Statistics (version 25). One-way analysis of variance (ANOVA) was used to assess the effect of forage variety on oxalate concentrations and proximate components separately for leaves and stems. Assumptions of normality and homogeneity of variance were assumed for ANOVA, and no data transformation was applied. Significant differences among means were determined using Tukey's test at $p < 0.05$.

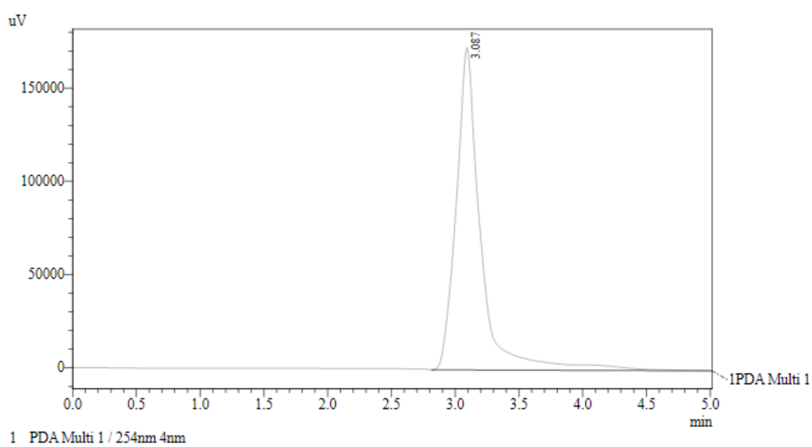

Fig. 1. Chromatogram of an authentic oxalic acid (1%) by high performance liquid chromatography.

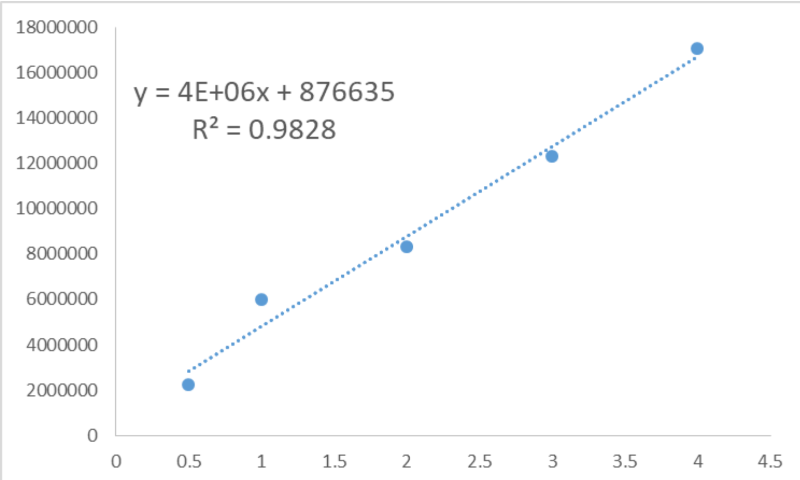

Fig. 2. Calibration curve using different concentrations of authentic oxalic acid.

3 Results and discussion

3.1 Soluble oxalate content

In the current study, soluble oxalate in leaves of dwarf Napier grass ranged from 0.08% to 0.58% of DM (Table 1). Dwarf Napier contained higher soluble oxalate in leaves (0.37%) than King (0.08%), Taiwan (0.33%), and Pakchong (0.23%), but lower than Indian (0.44%) and Purple Napier (0.44%). In stems, Indian, Taiwan, and Zanzibar Napier had higher soluble oxalate than in leaves, consistent with previous reports that thicker stems tend to accumulate more oxalate. Although the observed values were lower than some reported thresholds, they remain relevant because tropical grasses can contain soluble oxalate levels sufficient to induce calcium deficiency in grazing animals [8].

The present data are broadly consistent with earlier studies showing that soluble oxalate declines with plant maturity and varies among species and plant parts [7]. For example, Rahman et al. [7] reported higher soluble oxalate in young dwarf Napier leaves, decreasing with age, which aligns with the pattern observed here. The higher stem oxalate in Indian, Taiwan, and Zanzibar varieties also supports previous findings that oxalate accumulation can differ between leaves and stems [8].

While seasonal effects and fertilisation can influence oxalate levels [10], the focus of the current study confirms that variety and plant part are major determinants of soluble oxalate content in Napier grass, extending knowledge by providing comparative values across six local varieties.

3.2 Insoluble oxalate content

The insoluble oxalate content (Table 1) was significantly affected ($p < 0.05$) by both variety and botanical fraction. Higher insoluble oxalate concentrations were generally observed in stems compared to leaves, although this pattern was more pronounced in certain varieties, such as Indian (2.58% in stems vs. 0.55% in leaves) and King (2.03% in stems vs. 0.25% in leaves). Zanzibar Napier exhibited the highest overall insoluble oxalate (2.02%) content, surpassing all other varieties. This trend is consistent with Rahman et al. [8], who reported higher stem oxalate during early summer and increased leaf oxalate during late summer. Although insoluble oxalates are less bioavailable, they generally pass through the digestive tract without adverse effects on animal metabolism [10]. The higher stem oxalate may also relate to structural support, but this appears to vary among varieties rather than being a uniform feature.

Table 1. Soluble, insoluble and total oxalates contents (%, dry matter) in leaves and stems of Napier grass varieties and *Trichantera gigantea*.

Plant species and variety	Soluble oxalate		Insoluble oxalate		Total oxalate	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
Dwarf	$0.37^{c}\pm0.06$	$0.36^{ab}\pm0.01$	$1.98^{b}\pm0.36$	$0.98^{ab}\pm0.16$	$2.36^{b}\pm0.33$	$1.34^{b}\pm0.17$
Indian	$0.44^{d}\pm0.02$	$0.86^{d}\pm0.03$	$0.55^{a}\pm0.41$	$2.58^{d}\pm0.57$	$0.99^{a}\pm0.40$	$3.44^{cd}\pm0.57$
King	$0.08^{a}\pm0.06$	$0.76^{cd}\pm0.35$	$0.25^{a}\pm0.05$	$2.03^{cd}\pm0.23$	$0.34^{a}\pm0.06$	$2.79^{c}\pm0.35$
Pakchong	$0.23^{b}\pm0.06$	$0.22^{a}\pm0.08$	$0.29^{a}\pm0.07$	$0.30^{a}\pm0.06$	$0.52^{a}\pm0.07$	$0.52^{a}\pm0.07$
Taiwan	$0.33^{c}\pm0.06$	$0.37^{ab}\pm0.11$	$0.78^{a}\pm0.07$	$0.62^{a}\pm0.35$	$1.12^{a}\pm0.07$	$1.0^{ab}\pm0.27$
Purple	$0.44^{d}\pm0.04$	$0.41^{ab}\pm0.03$	$1.81^{b}\pm0.56$	$0.40^{a}\pm0.14$	$2.25^{b}\pm0.52$	$0.80^{ab}\pm0.13$
Zanziber	$0.37^{c}\pm0.04$	$0.57^{bc}\pm0.44$	$2.02^{b}\pm0.10$	$1.54^{bc}\pm0.81$	$2.38^{b}\pm0.07$	$2.11^{c}\pm0.83$
Significance level	0.000	0.001	0.000	0.000	0.000	0.000

^{abcde}Means with different superscripts in a column differ significantly ($p<0.05$).

3.3 Total oxalate content

Total oxalate content (Table 1) also varied significantly ($p < 0.05$) with variety and fraction, in line with earlier findings [8]. Zanzibar Napier leaves contained the highest total oxalate (2.38%), followed closely by dwarf Napier (2.36%) and Purple Napier (2.25%). In contrast, King Napier leaves recorded the lowest total oxalate (0.34%). Consistent with previous reports [8], leaves contained higher oxalate levels than stems, reflecting their greater metabolic activity and higher proportion of photosynthetic tissue. The relatively high total oxalate in leaves of dwarf, Purple, and Zanzibar Napier suggests that these varieties should be carefully managed in grazing systems. This may include mixing with low-oxalate forages or limiting grazing time on these varieties to reduce the risk of calcium deficiency in animals.

3.4 Proximate components

Among Napier grass varieties (Table 2), dwarf Napier leaves contained the highest CP (11.8%), followed by Purple Napier (11.5%) and Zanzibar Napier (11.0%). King Napier exhibited the lowest protein level (8.2%). These values are comparable to those reported by Haryani et al. [2], who found no significant differences among tall Napier grass varieties in nutritive quality. The higher protein content in dwarf Napier is likely due to its leafier morphology and denser tillering, which enhance nutritive value relative to tall varieties typically favoured for yield. In Pakchong Napier, the CP level (8.7%) was lower than previously reported (10.2% at nine weeks; [4]), confirming that protein concentration declines with plant maturity.

Non-Napier forages exhibited superior protein content (Table 3). *Indigofera* contained 20.3% CP, consistent with values reported by Nurhayu et al. [11], where levels approached 28%. Fertilisation strongly influenced *Indigofera* protein yield, with N application significantly increasing plant height, leaf number, and biomass [12]. *Trichanthera gigantea* contained 14.9% CP, consistent with earlier studies [13]. Although it has good nutritive potential, fresh leaves are less palatable to goats. Its crude fibre (16.2%) and ash content (22%) also matched previously reported values [13].

Table 2. Proximate components (%, dry matter) in leaves and stems of Napier grass varieties.

Plant species and variety	Leaf					Stem			
	CP	EE	CF	Ash		CP	EE	CF	Ash
Dwarf	11.8	1.44	22.0	6.8		5.3	1.76	28.06	8.4
Indian	10.9	1.68	37.1	7.0		7.9	1.68	37.10	7.0
King	8.2	1.21	30.6	4.3		2.1	1.09	39.00	3.9
Pakchong	8.7	1.99	39.7	7.4		2.2	1.25	41.54	5.2
Taiwan	10.2	1.91	28.8	4.9		2.9	1.69	40.07	2.7
Purple	11.5	1.73	28.0	4.7		8.8	3.81	38.97	9.8
Zanziber	11.0	1.69	31.33	9.0		3.5	0.87	41.92	7.5

CP, crude protein ; EE, ether extract ; CF, crude fibre.

Table 3. Proximate composition (% dry matter) of non-Napier grass leaves.

Plant species	Proximate components					Type of oxalate		
	CP	EE	CF	Ash		Soluble	Insoluble	Total
Trichantera	14.9	2.86	16.2	22.0		0.58±0.02	1.62±1.07	2.20±1.06
Indigofera	20.3	2.37	12.74	10.5		N.D.	N.D.	N.D.

CP, crude protein ; EE, ether extract ; CF, crude fibre ; N.D., not detected.

3.5 Integrated interpretation

Overall, dwarf Napier combined the highest CP content with relatively elevated soluble oxalate levels, as demonstrated by the CP and oxalate concentrations measured in the present study, highlighting a trade-off between nutritional value and anti-nutritional factors within the observed oxalate range of this experiment. As soluble oxalates tend to concentrate in leaves [7] and CP is also leaf-associated, our results confirm that leafier cultivars, such as dwarf Napier, exhibited both higher protein and higher soluble oxalate concentrations. Tall Napier varieties, which showed comparatively lower soluble oxalate concentrations in this study, although lower in protein, may therefore be preferable when a reduction in oxalate intake is desired; however, this recommendation is based on relative differences observed here rather than species-specific safety thresholds.

Non-Napier species such as *Indigofera* and *Trichanthera* provided valuable protein supplementation, with *Indigofera* showing the highest CP values among the non-Napier forages evaluated in this experiment. While the implications of these oxalate and CP levels for specific animal species require interpretation alongside published oxalate tolerance thresholds, the present findings underscore the importance of balancing forage quality with oxalate content when formulating ruminant diets. Cultivar selection, harvest interval, fertilisation practices, and seasonal growth patterns are known from previous studies to influence the nutritive and anti-nutritive value of Napier grass and its alternatives, and should be considered when extrapolating the present results to broader feeding recommendations.

4 Conclusion

This study showed that Napier grass varieties contained variable oxalate concentrations, generally high in comparison with most non-Napier forages, although *Trichanthera gigantea* leaves also exhibited total oxalate values comparable to the higher Napier cultivars. Soluble oxalate in Napier grass leaves ranged from 0.08 to 0.58% of DM, insoluble oxalate from 0.25 to 2.58% of DM, and total oxalate from 0.34 to 2.38% of DM, while crude protein ranged from 8.2 to 11.8% DM. Excessive intake of high-oxalate forages poses health risks, particularly for non-ruminants such as horses, which cannot utilise calcium oxalate and may develop nutritional hyperparathyroidism and calcium deficiency when consuming forages with soluble oxalate levels above 0.5%. While ruminants such as goats, cattle, and sheep show greater tolerance, prolonged grazing of high-oxalate tropical grasses can still result in severe hypocalcaemia. Therefore, selecting cultivars with lower oxalate accumulation, such as King and Pakchong Napier, and careful feeding management are essential for the safe and sustainable use of Napier grass and similar forages. Further research on feeding trials, plant maturity, and soil moisture effects on oxalate accumulation will provide valuable insights to improve forage quality and livestock nutrition.

References

1. Sapkota, A. R., Lefferts, L. Y., McKenzie, S., & Walker, P. (2007) What do we feed to food-production animals? A review of animal feed ingredients and their potential impacts on human health. Environmental Health Perspectives, 115(5), 663-670.
2. Haryani, H., Norlindawati, A.P., Norfadzrin, F., Aswanimiyuni, A., & Azman, A. (2018). Yield and nutritive values of six napier (*Pennisetum purpureum*) cultivars at different cutting age. Malaysian Journal of Veterinary Research, 9(2), 6–12.
3. M.Z. Zailan, M.Z., Yaakub, H., & Jusoh, S. (2018). Yield and nutritive quality of napier (*Pennisetum purpureum*) cultivars as fresh and ensiled fodder. The Journal of Animal and Plant Sciences, 28(1), 63-72.
4. Kathiraser, T., Zakaria, S. H., Mohamed, W. A. G., Hamidan, M. F. R., Azira, N. R., Nasir, N. R. A. M. A., & Shaari, M. R. (2019). Rumput Napier Pakchong sebagai sumber protein ternakan ruminan (Napier Pakchong grass as protein source for ruminants). Buletin Teknologi MARDI Bil. 16, Khas Ternakan Lestari: 53 – 61. http://ebuletin.mardi.gov.my/buletin/16/Thayalini.pdf
5. Joynim Farm. (2025). *Indigofera tinctoria plant for goat, cattle, don't for poultry*. Retrieved August 21, 2025, from https://kambingjoynim.com/en/indigofera-plants/
6. Rahman, M. M., Nakagawa, T., Niimi, M., Fukuyama, K., & Kawamura, O. (2011a). Effects of calcium fertilization on oxalate of napiergrass and on mineral concentrations in blood of sheep. Asian-Australasian Journal of Animal Sciences, 24(12), 1706–1710.
7. Rahman, M. M., & Kawamura, O. (2011b). Oxalate Accumulation in Forage Plants: Some Agronomic, Climatic and Genetic Aspects. Asian-Australasian Journal of Animal Sciences, 24(3), 439–448.
8. Rahman, M. M., Niimi, M., Ishii, Y., & Kawamura, O. (2006). Effects of season, variety and botanical fractions on oxalate content of napiergrass (*Pennisetum purpureum* Schumach). Grassland Science, 52(4), 161–166.
9. AOAC. (2005). *Official methods of analysis* (18th ed.). Association of Official Analytical Chemists, Washington, DC, USA.
10. Goyal, M. (2018). Oxalate accumulation in fodder crops and impact on grazing animals-a review. Forage Research, 44(3), 152-158.
11. Kaur, G., Mandhania, S., & Chaudhary, D. P. (2012). Calcium oxalate in Napier Bajra Hybrid: trends. Indian Journal of Animal Nutrition, 29(1), 58-62.
12. Nurhayu, A., & Ishak, A. B. L. (2015). 6th Int. Sem. Trop. Anim. Prod.: Integr. Approach Dev. Sustain. Trop. Anim. Prod., October 20-22, 2015, Yogyakarta, Indonesia. Pp. 228-232.

13. Rafaat, J. G., Hussein, R. A., & Ahmad, M. M. (2016). Effect of fertilizer application on vegetative growth characters of broad bean (*Vicia faba* L.). International Journal of Plant, Animal and Environmental Science, **6**(1), 103-108 (2016).