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Nutritional, mineral, and antinutritional components of five cultivars of napier grass (*Pennisetum purpureum* Schumach)

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Abstract: This study aims to evaluate the nutrient content, digestibility, mineral composition, and anti-nutritional compounds in five cultivars of Napier grass (*Pennisetum purpureum* Schumach) in Indonesia. The study utilized five Napier grass cultivars: 'Gama Umami', 'Pakchong', 'Mott', 'Purple', and 'Local'. A total of 20 replicates per cultivar were planted using a randomized group design. Harvesting was conducted 70 days after planting, and the measured parameters included morphological characteristics, production, nutrient content, fiber components, in vitro digestibility, mineral content, and anti-nutrient content. Morphological measurements exhibited significant differences ($P < 0.05$) for nearly all variables among the cultivars. The 'Gama Umami' cultivar showed significantly higher fresh production, leaf production, and leaf-to-stem ratio ($P < 0.05$). At the same time, nutrient and chlorophyll contents varied significantly ($P < 0.05$) across cultivars. In terms of fiber composition, significant differences ($P < 0.05$) were found for neutral detergent fiber (NDF) and acid detergent fiber (ADF). However, lignin content did not differ significantly ($P > 0.05$) among the cultivars. Likewise, both *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) varied significantly among the cultivars ($P < 0.05$). The mineral composition and anti-nutrient levels of each cultivar also showed significant differences ($P < 0.05$), with each cultivar exhibiting distinct characteristics and advantages. Overall, the study's results emphasize the importance of selecting appropriate Napier grass cultivars to optimize forage nutritional quality, enhance livestock productivity, and promote sustainability in tropical farming systems.

Keywords: Cultivars; Forage; Napier grass; *Pennisetum purpureum*; Productivity.
Abbreviations: GU_Gama Umami, PC_Pakchong, MO_Mott, PU_Purple, LC_Local.

Introduction

Napier grass (*Pennisetum purpureum* Schumach) is one of the main forage grasses for livestock in tropical regions, including Indonesia. It is known for its high biomass yield, wide adaptability to various environmental conditions, and excellent nutritional quality (Sathees and Santhiralingam, 2022). As a C4 perennial grass, Napier grass does not need replanting each growing season, which lowers maintenance costs compared to other forage crops (Gondo et al., 2017). These qualities allow Napier grass to play a key role in supporting ruminant farming systems, especially in tropical environments.

Various cultivars of napier grass have been developed to improve productivity, nutritional quality, and resistance to environmental challenges. Genetic selection and mutation breeding are the main methods used to create cultivars with better traits (Sanjaya et al., 2022). In Indonesia, cultivars such as Afrika, Hawaii, Trinidad, Merkeri, King, Taiwan, and Mott have been introduced (Budiman et al., 2011). Although these cultivars are closely related genetically, there are notable differences in productivity, nutrient content, and environmental adaptability (Budiman et al., 2012; Kamruzali et al., 2021). Therefore, careful selection and evaluation of each cultivar's potential are crucial for maximizing its use as high-quality livestock feed.

Numerous studies have assessed the characteristics of Napier grass (*Pennisetum purpureum* Schumach) worldwide. For instance, research in Ethiopia (Aleme et al., 2024) and Tanzania (Maleko et al., 2019) compared various cultivars in terms of productivity and nutrient content. Conversely, studies in Kenya (Nyambati et al., 2010), Thailand (Rengsirikul et al., 2013), and Mexico (Rueda et al., 2016) have shown notable differences in morphology and nutrient quality among cultivars.

In Malaysia, evaluations of nine and four cultivars by Halim et al. (2013) and Zailan and Jusoh (2018), respectively, confirmed significant variations in nutrient levels. Despite extensive global research on Napier grass productivity and quality, systematic evaluation of cultivars grown in Indonesia's tropical environment remains very limited.

The nutrient composition of Napier grass is affected by various factors, including its growth stage, the part harvested, location, and weather conditions (Nasution et al., 2025). Besides its nutrient profile, Napier grass is a good source of essential minerals for livestock, which are vital for growth, metabolism, and overall health. However, the mineral content can differ among cultivars depending on soil type, environmental factors, and genetic traits (Lee, 2018). Additionally, anti-nutritional compounds—such as tannins, saponins, and oxalates—impact its overall nutritional value as livestock feed (Onyeonagu et al., 2013). These compounds can hinder nutrient absorption and reduce feed efficiency (Tadele, 2015); their levels are influenced by environmental conditions and the genetic features of each cultivar (Love et al., 1994; Onyeonagu and Ukwueze, 2012; Pandey et al., 2011). Therefore, thorough assessments of the nutritional makeup, mineral levels, and anti-nutritional compounds of different Napier grass cultivars are crucial to ensure they are suitable as high-quality feed for livestock.

Although many studies have assessed Napier grass biomass productivity, few have explored the relationships among its morphological features, overall yield, nutrient quality, mineral content, and anti-nutritional factors. Additionally, comparative data on Napier grass cultivars grown under uniform environmental conditions in Indonesia are lacking. This study examines and compares the nutritional content, mineral composition, and anti-nutritional factors of five Napier grass cultivars in Indonesia. The findings are expected to offer new insights for selecting cultivars with high productivity and optimal nutrient levels, thereby aiding the development of sustainable livestock farming systems in Indonesia.

Result and Discussion

Morphological characterization

Table 2 displays the measured morphological traits of five Napier grass cultivars. Statistical analysis showed significant differences ($P < 0.05$) in plant height, leaf length, leaf width, number of leaves, stem diameter, and leaf area among these cultivars. The 'Gama Umami' cultivar had the tallest plants (251.90 ± 9.63 cm), followed by 'Pakchong' (218.40 ± 33.11 cm), while 'Mott' had the shortest height (95.90 ± 4.40 cm). Similarly, 'Gama Umami' had the longest leaves (111.88 ± 2.02 cm), with 'Mott' having the shortest (63.69 ± 1.54 cm). For leaf width, 'Gama Umami' produced the widest leaves (4.61 ± 0.30 cm), closely followed by 'Pakchong' (4.59 ± 0.36 cm), while the 'Purple' cultivar had the narrowest leaves (3.79 ± 0.16 cm). Regarding leaf count, 'Gama Umami' had the highest (100 ± 4.03 leaves), and Local had the fewest (50 ± 4.70 leaves). 'Gama Umami' also showed the largest stem diameter (30.65 ± 3.43 mm), while 'Local' had the smallest (23.22 ± 1.84 mm). Lastly, 'Gama Umami' exhibited the greatest leaf area (350.28 ± 20.85 cm^2), followed by 'Pakchong' (332.40 ± 34.14 cm^2), with 'Mott' having the smallest (180.10 ± 10.35 cm^2). These results suggest that the 'Gama Umami' cultivar excels in nearly all measured morphological traits.

Fig. 2 shows notable differences in leaf color among the five Napier grass cultivars, based on the RHS Color Chart. 'Gama Umami', 'Pakchong', 'Mott', and 'Local' cultivars exhibited shades of moderate olive green (RHS 137-A or 137-B). Meanwhile, the 'Purple' cultivar displayed a distinct dark greyish-purple hue (RHS N186-C), clearly setting it apart from the other cultivars.

Research findings show notable differences in the morphological traits among Napier grass cultivars. Plant morphology is affected by both internal factors, such as genetic determinants that control cell division, reproduction, and growth, and external factors, including soil conditions, climate, and nutrient availability. Napier grass grows best in moist environments with fertile, nutrient-rich soils that support the absorption of essential nutrients by the roots (Prasojo et al., 2023; Wen et al., 2024). Moreover, differences among cultivars lead to variations in the formation and development of new cells at the intercalary meristem, located at the base of the internodes (Budiman et al., 2012).

The 'Gama Umami' cultivar showed the highest values for plant height, leaf length, leaf width, number of leaves, stem diameter, and leaf area among the examined cultivars. Its superior morphological performance indicates better genetic adaptability under the experimental environmental conditions. The optimal plant height observed in this cultivar is likely due to genetic factors that enable more vertical growth compared to other cultivars. Additionally, Evitayani et al. (2022) reported that sufficient soil nutrient availability supports increased plant height by promoting overall plant growth.

The correlation between high biomass yield and longer leaves observed in our study aligns with Nyambati et al. (2010), who reported that cultivars with high biomass yields often have longer leaves. Longer and wider leaves can improve photosynthetic capacity by increasing the area available for sunlight absorption, supporting the buildup of carbohydrates and proteins vital for plant growth (Halim et al., 2013). Additionally, the notable leaf width seen in the 'Gama Umami' and 'Pakchong' cultivars indicates an enhanced photosynthetic ability. These findings agree with those of Pinchi-Carbajal et al. (2024), who state that wider leaves absorb solar energy more efficiently, speeding up the rate of photosynthesis and leading to greater biomass accumulation.

A larger leaf area enhances the photosynthetic process, ultimately boosting plant productivity. Cultivars that produce more leaves tend to show higher overall quality compared to those with fewer leaves. Since leaves are the primary organs for photosynthesis, generating the sugars that support plant growth and development, the total number of leaves is crucial in determining a plant's photosynthetic efficiency (Ernawati et al., 2023).

Additionally, the larger stem diameter observed in the 'Gama Umami' cultivar indicates a greater capacity to store nutrient reserves, support higher biomass production, and enhance resistance to mechanical stresses such as wind (Nguyen et al., 2021). A larger stem diameter is also generally linked to more efficient water and nutrient transport throughout the plant.

Table 1. Soil analysis at the experimental site.

Test parameters	Value
pH (H_2O)	7.3
C-organic (%)	2.41
Organic matter (%)	5.61
N total (%)	0.35
Cation Exchange Capacity (cmol (+) Kg-1)	19.46

Table 2. Morphological characteristics of five Napier grass cultivars.

Variables	Cultivars				
	GU	PC	MO	PU	LC
Plant height (cm)	251.90 ± 9.63^{a}	218.40 ± 33.11^{b}	95.90 ± 4.40^{c}	204.40 ± 15.91^{b}	180.30 ± 9.67^{d}
Leaf length (cm)	111.88 ± 2.02^{a}	106.19 ± 4.48^{b}	63.69 ± 1.54^{d}	105.57 ± 4.52^{bc}	101.79 ± 6.76^{c}
Leaf width (cm)	4.61 ± 0.30^{a}	4.59 ± 0.36^{a}	4.16 ± 0.24^{b}	3.79 ± 0.16^{c}	3.86 ± 0.26^{c}
Number of leaves	100 ± 4.03^{a}	71 ± 3.30^{c}	87 ± 3.66^{b}	73 ± 8.75^{c}	50 ± 4.70^{d}
Stem diameter (mm)	30.65 ± 3.43^{a}	25.82 ± 2.59^{b}	25.90 ± 3.09^{b}	23.89 ± 2.23^{ba}	23.22 ± 1.84^{a}
Leaf area (cm^2)	350.28 ± 20.85^{a}	332.40 ± 34.14^{a}	180.1 ± 10.35^{c}	272.72 ± 19.67^{b}	268.17 ± 31.83^{b}
Leaf colour	Moderate Olive Green (RHS 137-A)	Moderate Olive Green (RHS 137-A)	Moderate Olive Green (RHS 137-A)	Dark Greyish Purple (RHS N186-C)	Moderate Olive Green (RHS 137-B)

Note. Different superscripts (a, b, c, d) in the same row indicate significant differences (P < 0.05). GU = Gama Umami, PC = Pakchong, MO = Mott, PU = Purple, LC = Local.

Moreover, an optimal leaf area is vital for increasing forage yield, as it allows for greater sunlight interception, which consequently promotes increased photosynthetic activity and higher biomass accumulation (Ojo et al., 2014).

Variations in leaf color among Napier grass cultivars are mainly caused by key pigments like chlorophyll, carotenoids, and flavonoids (Anić et al., 2024; Rafael et al., 2009). These pigments influence leaf color and also act as visible indicators of plant quality. The purple hue of the Purple cultivar indicates an accumulation of anthocyanins, pigments often linked to stress tolerance, UV protection, and specific genetic traits. Ho et al. (2023) noted that purple leaves in Napier grass are associated with high antioxidant capacity, mainly because of its high anthocyanin content. As a result, high anthocyanin levels not only help identify the cultivar but also offer insight into its potential physiological benefits and environmental adaptability. These traits provide an extra tool for identifying cultivars and serve as a reference for further study on how pigmentation relates to environmental adaptation and nutritional quality.

Optimal growth in this experiment was supported by the availability of soil nutrients, which play a critical role in plant physiological processes (Sharma et al., 2022). Overall, the variations in morphological traits among the five Napier grass cultivars reflect the combined influence of genetic factors and adaptive responses to the experimental environmental conditions.

Plant productivity

Table 3 shows the production data for the five Napier grass cultivars. Statistical analysis indicated significant differences (P < 0.05) in fresh biomass, leaf yield, stem yield, and the leaf-to-stem ratio among the cultivars. The 'Gama Umami' cultivar had the highest fresh biomass production (30.4 ± 0.13 ton/ha), followed by 'Pakchong' (24.6 ± 0.86 ton/ha), while the 'Mott' cultivar produced the lowest fresh biomass (12 ± 0.15 ton/ha). Similarly, 'Gama Umami' achieved the highest leaf yield (11 ± 0.07 ton/ha), compared to 'Local', which yielded only 4.8 ± 0.04 ton/ha. In terms of stem production, 'Gama Umami' again led with 19.4 ± 0.11 ton/ha, whereas 'Mott' had the lowest yield at 3.8 ± 0.08 ton/ha. The highest leaf-to-stem ratio was observed in the 'Mott' cultivar (68.5 ± 1.69%), indicating a greater proportion of leaves relative to stems, while 'Pakchong' had the lowest ratio (32.9 ± 2.33%). These results suggest that shorter cultivars like 'Mott' tend to have a higher proportion of leaves compared to taller ones. Overall, the findings show that 'Gama Umami' excels in total biomass production, while 'Mott' is better in the leaf-to-stem ratio.

The differences in productivity among Napier grass cultivars observed in this study reflect genetic variation that influences growth capacity and biomass accumulation. Notably, the 'Gama Umami' cultivar showed the highest total biomass production, supported by its morphological traits, including larger leaf area and optimal plant height. The increased leaf area, combined with wider leaves, improves photosynthetic efficiency by allowing more light interception, which in turn boosts solar energy absorption and promotes the synthesis of photoassimilates (Pinchi-Carbajal et al., 2024). Sathees and Santhiralingam (2022) reported that fresh biomass production is positively correlated with plant height and leaf area, a finding consistent with the results presented here.

Biomass productivity depends not only on morphological features but also on photosynthetic efficiency, nutrient absorption, and the ability to adapt to environmental conditions. Evitayani et al. (2022) stated that factors such as climate, soil fertility, and land management practices influence the harvest yield of napier grass. Additionally, water availability—primarily controlled by rainfall—plays a vital role in supporting plant growth and development. The 'Gama Umami' cultivar shows strong adaptability to the existing soil conditions and experimental environment, allowing it to reach its full growth potential. In contrast, cultivars like 'Local' and 'Purple' have lower productivity, likely due to genetic limitations in effectively allocating photoassimilates for optimal growth.

Cultivars with lower stem production, such as 'Mott', have a higher leaf-to-stem ratio. Halim et al. (2013) explained that the increased leaf-to-stem ratio in the 'Mott' cultivar is due to its shorter internodes. A higher leaf-to-stem ratio means more photoassimilates are directed toward leaf growth rather than stem growth, which helps improve forage quality. This is important because leaves—the parts of the plant that influence forage nutritional quality—contain more nutrients, especially crude protein and digestible fiber, compared to stems, which usually have higher crude fiber and lower overall dietary value (Fortes et al., 2014; Onjai-uea et al., 2023; Zailan and Jusoh, 2018).

However, smaller leaf areas tend to absorb less solar radiation, which lowers photosynthetic efficiency and total biomass production. This limited light interception explains the low biomass yield seen in the 'Mott', 'Local', and 'Purple' cultivars, despite their high leaf-to-stem ratios. In contrast, cultivars like 'Gama Umami' and 'Pakchong', with wider and longer leaves, can intercept light more effectively, supporting optimal photosynthesis and greater biomass accumulation.

Optimizing the ratio of functional organs—specifically leaves and stems—is crucial for maximizing biomass production in forage grasses. Larger leaves significantly improve light capture, CO_2 intake, and the production of photoassimilates needed for growth (Pinchi-Carbajal et al., 2024), while stems act as nutrient reserves that help regenerate new leaves after harvesting. Therefore, dwarf cultivars like 'Mott' are more appropriate for high-quality livestock feed because of their higher leaf-to-stem ratio. Conversely, taller cultivars such as 'Gama Umami' and 'Pakchong' perform better in total biomass output under intensive production systems.

Chemical composition, chlorophyll content, and the correlation between leaf area and chlorophyll content

Table 4 shows the chemical composition and chlorophyll content data for five Napier grass cultivars. Statistical analysis found significant differences ($P < 0.05$) in dry matter (DM), organic matter (OM), crude protein (CP), and chlorophyll content among the cultivars. The 'Gama Umami' cultivar had the highest DM in both stem (19.47 ± 0.04%) and leaves (19.50 ± 0.01%), followed by 'Pakchong' with slightly lower values, while the 'Local' cultivar recorded the lowest DM in both stem (15.11 ± 0.01%) and leaves (15.19 ± 0.08%).

OM content varied among the cultivars. The 'Local' cultivar showed the highest OM content in the stem (87.71 ± 0.39%), while the 'Mott' cultivar had the highest OM content in the leaves (83.84 ± 4.55%). Conversely, 'Pakchong' had the lowest OM content in the leaves (76.13 ± 0.33%). Regarding CP content, the 'Mott' cultivar reached the highest in the stem (9.13 ± 0.42%), and the 'Gama Umami' cultivar had the highest CP in the leaves (11.44 ± 0.16%). Additionally, the 'Pakchong' cultivar showed the lowest CP in the stem (3.80 ± 0.39%), while the 'Purple' cultivar had the lowest CP in the leaves (10.06 ± 0.72%).

Regarding chlorophyll, the 'Purple' cultivar achieved the highest total chlorophyll content (1.036 ± 0.11 mg/g), followed by 'Gama Umami' (0.879 ± 0.13 mg/g). In contrast, the 'Local' cultivar had the lowest total chlorophyll content (0.648 ± 0.25 mg/g). Additionally, the 'Purple' cultivar showed the highest levels of chlorophyll a (0.682 ± 0.45 mg/g) and chlorophyll b (0.353 ± 0.69 mg/g), indicating an improved photosynthetic capacity compared to the other cultivars.

Fig 2 shows the relationship between leaf area and chlorophyll content in five Napier grass cultivars. The scatter plot, with a nearly flat regression line, suggests that there is no significant trend between these two variables. The spread of data points indicates that other factors—such as genetic differences among cultivars and environmental conditions—might also affect chlorophyll content.

The differences in chemical composition and chlorophyll content among Napier grass cultivars are due to genetic variation and each cultivar's adaptive response to its environment. The 'Gama Umami' cultivar, known for its higher DM content, shows a greater ability to absorb and use soil nutrients, which helps increase biomass. Its higher DM also indicates it can maintain a strong cellular structure, essential for good growth. Lower DM in other cultivars is probably linked to an active growth phase, where higher water content lowers the proportion of dry matter in the plant tissues (Pacheco et al., 2024), reflecting differences in genetic and physiological adaptations. Halim et al. (2013) reported that taller Napier grass varieties tend to have higher DM content. This matches our finding of high DM levels in the 'Gama Umami' cultivar.

Conversely, the highest OM content in the stem was observed in the 'Local' cultivar, while the 'Mott' cultivar showed the highest OM content in the leaves. This variation likely indicates a greater proportion of organic compounds—such as fiber, carbohydrates, and other carbon-based molecules—that serve as metabolic energy sources for livestock. Pinchi-Carbajal et al. (2024) stated that higher OM content, especially in the leaves, reflects a plant's metabolic efficiency in producing complex compounds through photosynthesis. In contrast, lower OM levels in some cultivars may suggest a higher mineral content, which is also important for meeting livestock's essential nutritional needs. Therefore, each cultivar displays its specific potential based on the unique characteristics of its OM and mineral contents.

The CP content in the stem was highest in the 'Mott' cultivar, while the CP content in the leaves was highest in the 'Gama Umami' cultivar. These differences reflect genetic variation among cultivars in their efficiency of protein synthesis. Additionally, differences in growth rate, lignification, and the accumulation of structural carbohydrates among cultivars also influence CP content (Zailan and Jusoh, 2018). The high CP content observed in the 'Mott' cultivar may be linked to its elevated leaf-to-stem ratio, a factor that significantly affects forage quality (Xu et al., 2023). According to Rusdy (2016), the CP content in napier grass ranges from 4.4% to 20.4%, with an average of about 12%. These variations are mainly caused by genotype, harvest age, and environmental conditions.

A higher CP content in the leaves significantly enhances the nutritional quality of the forage compared to the stems, which mainly serve as supportive structures with higher crude fiber levels. Since leaves are the primary photosynthetic organ, they accumulate more nutrients vital for meeting protein needs during milk production and growth phases. Weber et al.

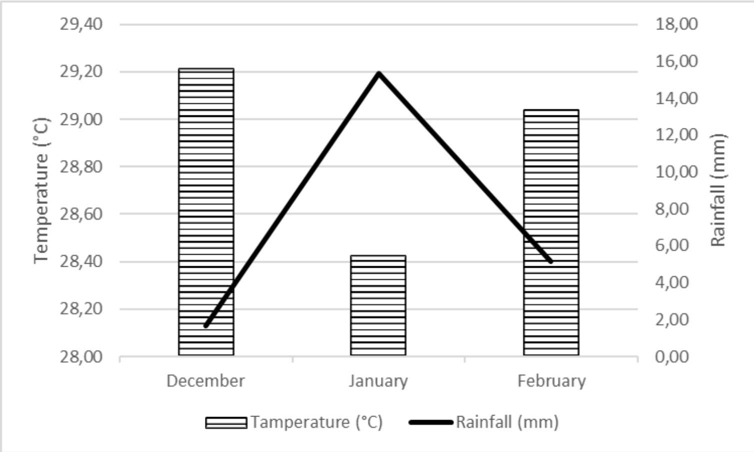

Fig 1. Average Temperature (ºC) and Rainfall (mm) at the research site during the study.

Fig 2. Variation in leaf color based on the RHS Color Chart. GU = Gama Umami, PC = Pakchong, MO = Mott, PU = Purple, LC = Local.

(2008) observed that CP content correlates with the plant's ability to absorb nitrogen—a key component in protein synthesis. Additionally, the variation in CP content among cultivars shows genetic differences in nitrogen uptake efficiency and the capacity for protein synthesis. Dwarf cultivars like 'Mott' tend to be more compact and shorter than standard cultivars, yet they have higher nutrient levels, making them a superior choice for high-quality livestock feed (Umami et al., 2016).

One of the main factors affecting plant growth is the amount of chlorophyll in leaves. Chlorophyll is crucial for photosynthesis—the process by which organic compounds are created from inorganic substances (Ettbeb et al., 2020). The light energy absorbed by chlorophyll is transformed into chemical energy through photosynthetic electron transport, chlorophyll fluorescence, and heat dissipation; these processes work together within the photosynthetic system (Wen et al., 2024). Additionally, the ability of leaves to capture light effectively is heavily influenced by their shape and size, such as leaf area, length, and width. Ernawati et al. (2023) highlighted that leaves are the main photosynthetic organs and that a plant's photosynthetic capacity largely depends on both the number and size of its leaves. Moreover, wider leaves can absorb more sunlight, providing increased energy for photosynthesis.

The findings of this study show that cultivars with larger leaf areas—such as 'Gama Umami' and 'Purple'—have higher total chlorophyll content. This matches what Rafael et al. (2009) observed. The study noted that genetic differences and environmental factors, like climate, affect levels of chlorophyll a and b. Chlorophyll a is directly involved in the photosynthesis process, while chlorophyll b absorbs light at different wavelengths and passes energy to chlorophyll a. Together, these pigments reflect higher photosynthetic efficiency (Souto et al., 2015).

Additionally, chlorophyll content can act as an indirect marker of a plant's nutritional health. Plants with high levels of chlorophyll typically have ample nitrogen because nitrogen is essential for chlorophyll production (Sharma et al., 2022). In this study, the 'Purple' cultivar showed the highest total chlorophyll content, indicating its superior photosynthetic ability. The purple color of this cultivar, due to its high anthocyanin levels, probably helps protect chlorophyll from damage caused by excessive light. The interaction between anthocyanins and chlorophyll allows the plant to use light more efficiently—anthocyanins absorb light at specific wavelengths and shield the photosynthetic machinery from oxidative stress. These protective strategies support optimal photosynthesis and help the plant adapt to different environmental conditions.

The relationship among DM, OM, CP, and total chlorophyll content shows that cultivars with high photosynthetic capacity tend to have better nutritional quality. Although stems—with their higher crude fiber and lower nutrient content compared to leaves—play an important role in providing structural support and energy reserves, the differences in nutrient composition between leaves and stems highlight genetic variations among cultivars. Overall, these results demonstrate that both genetic factors and the specific adaptive traits of each cultivar are key elements that determine forage quality. Therefore, cultivar selection should be customized to meet particular objectives—whether to increase total biomass, nutrient content, or photosynthetic efficiency—to optimize forage production for livestock feeding systems.

An analysis of the relationship between leaf area and chlorophyll content revealed a very weak positive correlation. This finding suggests that although leaf area slightly influences chlorophyll levels, the overall relationship is not statistically significant. Other factors, such as genetic variation, photosynthetic efficiency, and nutrient availability, seem to play a more dominant role. For example, the 'Purple' cultivar, which showed the highest total chlorophyll content in the current study, does not rely solely on leaf area to determine its chlorophyll levels. Instead, its higher chlorophyll content is likely due to an inherent genetic ability to optimize resource use, including nitrogen efficiency, which is vital for chlorophyll synthesis (Sharma et al., 2022). Additionally, the high anthocyanin content in the 'Purple' cultivar may provide extra protection against chlorophyll degradation caused by excessive light stress, thus supporting higher photosynthetic efficiency, as noted by Cheaib et al. (2023).

Variation among cultivars, as shown in the accompanying graph, highlights the importance of genetic factors in determining chlorophyll content. Although the 'Gama Umami' cultivar has a large leaf area, its chlorophyll level is lower than that of the 'Purple' cultivar. This supports the idea that the relationship between physical traits, like leaf area, and chlorophyll content is not straightforward or universal but is shaped by complex interactions between genetics and environmental factors. These findings indicate that leaf area alone cannot be the only measure for assessing chlorophyll content and photosynthetic efficiency. Future research should continue to explore how morphological traits, genetics, and environment interact to influence chlorophyll accumulation.

Fiber composition

Table 5 shows the fiber composition data for five Napier grass cultivars. The neutral detergent fiber (NDF) content ranged from 60.71% to 66.36%, with the 'Gama Umami' cultivar showing the highest NDF value (66.36 ± 0.63%) and the Local cultivar the lowest (60.71 ± 2.1%). Acid detergent fiber (ADF) values ranged from 34.70 ± 1.93% to 38.04 ± 0.20%, with the 'Gama Umami' cultivar also having the highest ADF content (38.04 ± 0.20%) and the Local cultivar the lowest (34.70 ± 1.93%). Lignin content ranged from 3.77% to 4.68%, but differences among cultivars were not statistically significant ($P > 0.05$).

NDF represents the fiber fraction that is insoluble in a neutral detergent solution and makes up the most significant part of the plant cell wall. It includes cellulose, hemicellulose, lignin, silica, and fibrous proteins. In contrast, ADF is the fiber fraction remaining after extraction with an acid detergent solution and mainly consists of cellulose and lignin—components that are more resistant to digestion by rumen microbes (Umami et al., 2020). Animasaun et al. (2018) reported that most grasses harvested during the early growth phase (around 10 to 12 weeks) tend to have higher crude protein content; however, as the plant matures, an increase in structural components like NDF and ADF may decrease dry matter digestibility in ruminants (Keba et al., 2013).

The NDF content indicates the total amount of cellulose, hemicellulose, and lignin in the plant, which in turn affects the digestibility and forage intake by livestock. A lower NDF content suggests a higher potential for digestibility compared to cultivars with higher NDF levels. Conversely, ADF mainly includes cellulose and lignin—fiber components that are harder for ruminants to digest. Therefore, differences in ADF content reflect variations in fiber structure that ultimately influence forage quality and digestibility, with higher ADF values associated with lower digestibility. This negative relationship is further supported by Budiman et al. (2012), who reported that increases in ADF content are linked to reduced feed digestibility.

Lignin is a major factor limiting forage digestibility because it binds to cellulose and hemicellulose, thus inhibiting fiber breakdown by rumen microbes. According to Delastra et al. (2021), lignin content in a plant depends on the interaction between cultivar and plant age, increasing with each harvest as the plant matures. Raffrenato et al. (2017) stated that high lignin levels decrease nutritional value by blocking fiber digestibility. Similarly, Du et al. (2016) found that an increase in lignin—a non-degradable component of the cell wall—significantly hampers feed digestibility. Digestibility is a key factor in assessing the nutritional value of forage because the chemical makeup of the feed closely relates to its digestibility and the performance of the ruminants that consume it (Salama, 2019). The results of this study suggest that differences in fiber composition among napier grass cultivars can greatly influence forage utilization in livestock feeding systems.

In vitro digestibility

Table 6 shows the results of in vitro dry matter digestibility (IVDM) and in vitro organic matter digestibility (IVOM) analyses for five Napier grass cultivars. The IVDM values ranged from 64.80% to 81.74%, with the 'Mott' cultivar having the highest value (81.74 ± 5.20%) and 'Gama Umami' following at 79.51 ± 2.78%. In contrast, the 'Purple' cultivar recorded the lowest IVDM value (64.80 ± 3.86%). Similarly, IVOM values ranged from 70.58% to 81.95%, with the 'Mott' cultivar again showing the highest value (81.95 ± 1.45%) and the 'Local' cultivar the lowest (70.58 ± 1.35%). Statistical analysis indicated significant differences among the cultivars ($P < 0.05$), demonstrating that the ruminal degradation of dry matter and organic matter depends heavily on the specific cultivar.

IVDM refers to the proportion of a feed's dry matter that livestock can digest at a given level of consumption. In contrast, IVOM reflects how much of the organic components—such as protein, carbohydrates, fats, and vitamins—are digested during the animal's digestive process (Umami et al., 2022). High IVDM values indicate that a significant portion of the feed's dry matter is available for digestion by rumen microbes, supporting better ruminant performance. Similarly, high IVOM values suggest that most of the organic components can be efficiently fermented by rumen microorganisms. Variations in IVOM further imply that the chemical makeup and cell wall structure of the plant—particularly the levels of lignin and crude fiber—play a key role in determining the extent of organic matter digestibility.

Table 3. Production of five Napier grass cultivars.

Variables	Cultivars				
	GU	PC	MO	PU	LC
Fresh Production (tons/ha)	30.4±0.13^{a}	24.6±0.86^{b}	12±0.15^{c}	16.8±0.16^{c}	13.8±0.2^{c}
Leaf Production (tons/ha)	11±0.07^{a}	8±0.14^{b}	8.2±0.13^{b}	6.2±0.13^{c}	4.8±0.04^{c}
Stem Production (tons/ha)	19.4±0.11^{a}	16.6±0.48^{a}	3.8±0.08^{c}	10.6±0.13^{b}	9±0.18^{b}
Leaf-Stem Ratio (%)	36.2±2.29^{b}	32.9±2.33^{b}	68.5±1.69^{a}	36.6±3.35^{b}	35.2±3.25^{b}

Note. Different superscripts (a, b, c, d) in the same row indicate significant differences (P < 0.05). GU = Gama Umami; PC = Pakchong; MO = Mott; PU = Purple; LC = Local.

Table 4. Chemical composition and chlorophyll content of five Napier grass cultivars.

Variables	Cultivars				
	GU	PC	MO	PU	LC
DM stem (%)	19.47±0.04^{a}	18.51±0.01^{b}	15.54±0.17^{d}	15.92±0.05^{c}	15.11±0.01^{e}
DM leaf (%)	19.50±0.01^{a}	18.53±0.02^{b}	15.67±0.05^{d}	16.16±0.07^{c}	15.19±0.08^{e}
OM stem (%)	83.31±2.97^{b}	81.97±1.63^{b}	83.63±0.10^{b}	82.44±0.47^{b}	87.71±0.39^{a}
OM leaf (%)	81.82±0.71^{ab}	76.13±0.33^{bc}	83.84±4.55^{a}	77.17±1.19^{bc}	73.19±1.53^{c}
CP stem (%)	4.84±0.79^{b}	3.80±0.39^{c}	9.13±0.42^{a}	4.78±0.07^{b}	5.18±0.40^{b}
CP leaf (%)	11.44±0.16^{a}	10.98±0.2^{ab}	10.54±0.16^{bc}	10.06±0.72^{c}	10.53±0.40^{bc}
Chl A (mg/g)	0.607±0.67^{ab}	0.497±0.11^{a}	0.508±0.52^{ab}	0.682±0.45^{ab}	0.422±0.23^{b}
Chl B (mg/g)	0.271±0.64^{ab}	0.188±0.62^{b}	0.177±0.28^{b}	0.353±0.69^{a}	0.226±0.87^{b}
Chl Total (mg/g)	0.879±0.13^{ab}	0.686±0.17^{b}	0.685±0.08^{b}	1.036±0.11^{a}	0.648±0.25^{b}

Note. Different superscripts (a, b, c, d, e) in the same row indicate significant differences (P < 0.05). GU = Gama Umami; PC = Pakchong; MO = Mott; PU = Purple; LC = Local; DM = Dry matter; OM = Organic matter; CP = Crude protein; Chl = Chlorophyll.

Table 5. Fiber composition of five Napier grass cultivars.

Variables	Cultivars				
	GU	PC	MO	PU	LC
NDF (%)	66.36±0.63^{a}	64.24±0.29^{a}	65.95±0.62^{a}	65.01±0.74^{a}	60.71±2.1^{b}
ADF (%)	38.04±0.20^{a}	37.65±0.55^{a}	37.13±0.31^{a}	37.29±0.02^{a}	34.70±1.93^{b}
Lignin (%)^{ns}	3.77±0.10	4.22±0.08	4.53±0.60	4.68±1.13	4.58±0.91

Note. Different superscripts (a, b) in the same row indicate significant differences (P < 0.05).
GU = Gama Umami; PC = Pakchong; MO = Mott; PU = Purple; LC = Local; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber.

Overall, the 'Mott' cultivar showed the best performance in in vitro digestibility for both dry matter and organic matter. Its superior digestibility is probably due to its lower lignin content and a fiber structure that is easier to break down, as indicated by the previously observed ADF and NDF parameters. Additionally, the differences in IVDM are linked to higher crude protein levels, which help promote the growth of rumen microbes. High crude protein content encourages the proliferation of rumen microorganisms, thereby boosting fermentation activity and improving the digestibility of dry matter (Suwignyo et al., 2016).

Overall, the digestibility of Napier grass depends on various factors, including cultivar choice and management practices like harvest age, cutting frequency, and cutting height (Lounglawan et al., 2014). Furthermore, Zailan et al. (2016) found that high ambient temperatures can decrease digestibility by increasing lignification in the plant cell walls. The higher complexity of carbohydrate structures in the cell walls reduces the availability of cellular components—such as crude protein, fat, and water-soluble carbohydrates—that are more easily digestible by livestock.

Mineral content

Table 7 displays the mineral content of five Napier grass cultivars, all showing significant variation in each of the analyzed minerals: calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn). The highest Ca content was found in the 'Mott' cultivar (2326.18 ± 0.25 mg/kg), which was significantly different (P < 0.05) from the other cultivars, while the 'Gama Umami' cultivar had the lowest Ca content (1558.93 ± 1.05 mg/kg). For Mg, the 'Pakchong' cultivar had the highest level (1519.71 ± 0.3 mg/kg), and the 'Local' cultivar had the lowest (362.86 ± 0.43 mg/kg). Fe content varied considerably, with the 'Mott' cultivar showing the highest level (116.75 ± 0.42 mg/kg) and the 'Pakchong' cultivar the lowest (23.3 ± 0.30 mg/kg). Regarding Zn, the 'Local' cultivar had the highest content (29.88 ± 0.17 mg/kg), which was significantly different from the other cultivars. In contrast, the 'Gama Umami' cultivar had the lowest Zn level (15.59 ± 0.54 mg/kg).

Onyeonagu et al. (2013) stated that climate conditions and soil properties influence a plant's mineral content; additionally, variations in nutrient absorption capacity among forage cultivars also affect their mineral profiles. Ca, an essential mineral for bone formation, muscle contraction, and enzymatic activities in livestock, plays a crucial role in animal health. Therefore,

Table 6. Digestibility In vitro of five Napier grass cultivars.

Variables	Cultivars				
	GU	PC	MO	PU	LC
IVDM (%)	79.51±2.78^{a}	70.83±6.05^{b}	81.74±5.20^{a}	64.80±3.86^{b}	71.05±3.47^{b}
IVOM (%)	76.25±0.79^{b}	76.05±3.83^{b}	81.95±1.45^{a}	72.76±1.66^{bc}	70.58±1.35^{a}

Note. Different superscripts (a, b, c, d) in the same row indicate significant differences (P < 0.05). GU = Gama Umami; PC = Pakchong; MO = Mott; PU = Purple; LC = Local; IVDM = In Vitro Dry matter; IVOM = In Vitro Organic matter.

Table 7. Mineral content of five Napier grass cultivars.

Variables	Cultivars				
	GU	PC	MO	PU	LC
Ca (mg/kg)	1558.93±1.05^{e}	1942.34±0.83^{d}	2326.18±0.25^{a}	1686.16±0.66^{b}	1890.56±0.59^{c}
Mg (mg/kg)	974.60±0.62^{b}	1519.71±0.3^{a}	451.56±0.64^{d}	481.07±0.57^{c}	362.86±0.43^{e}
Fe (mg/kg)	24.81±0.55^{d}	23.33±0.30^{e}	116.75±0.42^{a}	61.04±0.09^{c}	72.92±0.22^{b}
Zn (mg/kg)	15.59±0.54^{e}	23.17±0.46^{c}	24.87±0.63^{b}	16.55±0.50^{d}	29.88±0.17^{a}

Note. Different superscripts (a, b, c, d, e) in the same row indicate significant differences (P < 0.05). GU = Gama Umami; PC = Pakchong; MO = Mott; PU = Purple; LC = Local; Ca = Calcium; Mg = Magnesium; Fe = Ferrum; Zn = Zinc.

Table 8. Anti-nutrient content of five Napier grass cultivars.

Variables	Cultivars				
	GU	PC	MO	PU	LC
Saponin (%)	2.09±0.01^{b}	1.99±.011^{d}	2.07±0.01^{c}	1.67±1.01^{e}	2.25±0.01^{a}
Tannin (%)	1.99±0.02^{d}	2.08±0.12^{c}	1.71±0.18^{e}	2.57±0.01^{a}	2.20±0.11^{b}
Oxalic acid (mg/kg)	3256.46±5.25^{d}	3054.73±0.81^{e}	3899.30±0.73^{b}	3464.23±1.06^{c}	3941.61±0.67^{a}

Note. Different superscripts (a, b, c, d, e) in the same row indicate significant differences (P 0.05). GU = Gama Umami; PC = Pakchong; MO = Mott; PU = Purple; LC = Local.

the high Ca content in the 'Mott' cultivar may be especially beneficial for fulfilling the mineral needs of ruminants. These findings support those of Idris et al. (2011), who highlighted that Ca is essential for livestock health by helping to build strong bones and teeth, aiding blood clotting, and ensuring proper function of the heart, nervous system, and muscles.

Mg is a vital nutrient involved in energy metabolism, neuromuscular function, and the activity of digestive enzymes. Variations in Mg content among napier grass cultivars reflect the combined influence of genetic factors and environmental conditions on mineral accumulation. Mg is essential for activating vitamin D, supporting parathyroid hormone function, fueling cellular respiration through ATP production, and aiding nutrient transport across cell membranes (Suleiman et al., 2020; Waziri et al., 2013).

Fe is essential for oxygen transport through hemoglobin and supports immune function. A high Fe content in forage indicates its potential as a rich source of micronutrients. Likewise, Zn is needed for enzymatic functions, tissue growth, and reproductive processes in livestock. The notably high Zn content found in the 'Local' cultivar improves its suitability as a functional feed to support livestock performance. Overall, the distinct mineral profiles among the cultivars highlight that each one has unique strengths based on its specific mineral contents. Choosing a particular cultivar should be tailored to meet the specific mineral needs of livestock feed formulations or to address certain micronutrient deficiencies in tropical regions. Additionally, the variations in mineral content are influenced not only by genetic factors but also by soil properties and farming practices.

Anti-nutrient content

Table 8 shows the anti-nutrient levels in five Napier grass cultivars, with notable differences in each measured anti-nutrient, specifically saponin, tannin, and oxalic acid. The 'Local' cultivar had the highest saponin content (2.25 ± 0.01%), which was significantly higher than the other cultivars (P < 0.05), while the 'Purple' cultivar had the lowest saponin level (1.67 ± 1.01%). Regarding tannins, the 'Purple' cultivar recorded the highest level (2.57 ± 0.01%), and the 'Mott' cultivar had the lowest tannin content (1.71 ± 0.18%), with significant differences among the cultivars (P < 0.05). Oxalic acid levels also differed significantly (P < 0.05); the 'Local' cultivar had the highest concentration (3941.61 ± 0.67 mg/kg), and the 'Pakchong' cultivar had the lowest (3256.46 ± 5.25 mg/kg).

Both environmental factors and the genetic makeup of each cultivar influence the concentrations of anti-nutrients in plants. Some anti-nutrients act as protective mechanisms against environmental stresses and pests. In the case of forages, high saponin levels can negatively affect livestock by decreasing feed intake and impairing the absorption of nutrients such as glucose and cholesterol (Suleiman et al., 2020). Consequently, saponins have been shown to have hypocholesterolemic effects (Bright et al., 2024), which may help reduce the metabolic load on the liver. Kumar (1992) noted that saponins from different plant species display varying biological effects, likely due to structural differences in their sapogenin fractions. Although saponins have a bitter taste and foaming properties, they also support environmental sustainability by lowering rumen bacteria populations and thereby reducing methane production.

According to Omoniyi et al. (2013), tannin content in Napier grass ranges from 3.5% to 4%. The presence of tannins in forage raises concerns because they negatively affect feed intake and nutrient utilization efficiency in livestock. As anti-

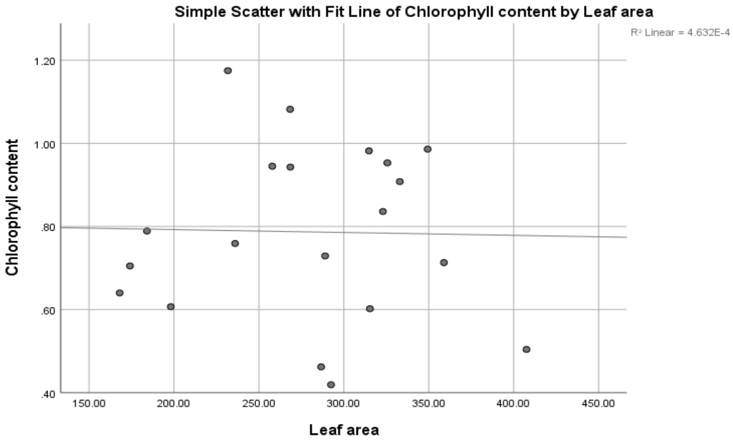

Fig 3. Relationship between leaf area and chlorophyll content in five cultivars of Napier grass.

nutrient compounds, tannins reduce feed palatability by imparting an astringent taste that decreases consumption and can hinder livestock growth. Additionally, tannins can bind with both external and internal proteins, including digestive enzymes like trypsin, chymotrypsin, amylase, and lipase, which ultimately lowers the effectiveness of protein utilization (Umar et al., 2007). Moreover, tannins can inhibit the absorption of essential minerals such as iron, potentially impacting livestock growth and health negatively (Nawab et al., 2020). Although low tannin levels may provide some protection against protein breakdown in the rumen, high levels should be avoided because they form complex cross-links with proteins and carbohydrates, making these nutrients unavailable for digestion (Piluzza et al., 2014). Onyeonagu et al. (2013) noted that tannin levels affecting digestibility in sheep and cattle range from 2% to 5%.

The presence of bioactive compounds like saponins and tannins in forage contributes to various physiological activities. Specifically, these compounds engage in multiple biochemical and biological functions, including anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic effects (Tava et al., 2022).

Oxalic acid, an anti-nutrient, is frequently found in tropical C4 forages and can decrease livestock productivity. The oxalic acid level in plants depends on the mineral content of plant tissues, especially inorganic ions that help maintain ionic balance within the plant. Rahman and Kawamura (2011) reported a positive correlation between oxalic acid and mineral contents in napier grass, indicating that changes in nutritional conditions can influence the levels of carboxylic acids, including oxalic acid.

High levels of oxalic acid can cause oral irritation and reduce the absorption of divalent minerals, especially calcium, by promoting the formation of insoluble salt compounds. These salts increase the risk of kidney stones, which in severe cases can lead to death in livestock, particularly ruminants (Suleiman et al., 2020). Additionally, plant maturity significantly influences oxalic acid accumulation; harvesting earlier results in higher oxalic acid content compared to a later, more optimal harvest time (Rahman et al., 2020). The presence of anti-nutrients in Napier grass can negatively impact livestock productivity. However, their effects can be either beneficial or harmful depending on factors like concentration, chemical structure, the specific plant part, the plant variety, and the livestock's physiological condition.

Materials and methods

Study site conditions and material preparation

A three-month field study was carried out from December 2023 to February 2024 at the Forage Laboratory trial site of the Faculty of Animal Husbandry, Gadjah Mada University (latitude: -7.769238, longitude: 110.386085) in Yogyakarta, Indonesia. Climate data collected during the study period were provided by the Meteorology, Climatology, and Geophysics Agency in Sleman, Yogyakarta, as shown in Fig. 1. The soil at the study site is classified as regosol (Hermanto et al., 2017). Before starting the study, soil samples were taken from depths of 0 to 20 cm at several sampling points and combined for chemical analysis. The findings of this analysis are shown in Table 1.

The current research employed five cultivars of napier grass: ‘Gama Umami’, ‘Pakchong’, ‘Mott’, ‘Purple’, and ‘Local’. Five Napier grass cultivars were chosen to represent both local and improved germplasm relevant to forage production in Indonesia. ‘Gama Umami’ represents a superior Indonesian cultivar, ‘Local’ reflects the traditional variety cultivated by farmers, while ‘Pakchong’, ‘Mott’, and ‘Purple’ are widely introduced cultivars with distinct agronomic and nutritional traits. Together, these cultivars provide a representative comparison of Napier grass diversity in Indonesia. Cutting materials for the Napier grass cultivars were obtained from the collection garden of the Laboratory for Forage and Pasture, Faculty of Animal Science, Universitas Gadjah Mada. The Napier grass seedlings were produced through stem cuttings from mother plants selected for their health and quality. Seedlings with a stem length of approximately 15–20 cm were chosen and prepared for planting in the designated trial area. Land preparation involved clearing the area of weeds and residual plant matter from previous crops. Subsequently, the soil was loosened using a hoe and plow to an optimal depth of approximately 20 cm, facilitating effective root growth.

Experimental design
A randomized group design was used to assign plant cultivars in the experimental area. The area, covering 140 m^2, was prepared by plowing and divided into 25 plots, each measuring 1 m by 1 m. The plots were arranged in five rows with five plots each, spaced about 1 m apart. In each plot, four plants of the same cultivar were planted, totaling 20 plants per cultivar. Planting was done using horizontally laid cuttings. Routine maintenance included watering twice daily (morning and evening) and weeding to remove unwanted plants.

Data collection of morphological parameters and productivity
Morphological measurements were taken from 20 plants per cultivar at 70 days after planting. At that time, both the measurements and the cutting (i.e., harvesting of the plant material) were performed. The measured morphological parameters included plant height (in cm), leaf length (in cm), leaf width (in cm), stem diameter (in cm), number of leaves, and leaf area. Forage productivity was evaluated by determining the fresh weight of leaves and stems, the combined yield of these components, and the leaf-to-stem ratio (%). Fresh weight was measured by weighing either individual plant parts or the entire plant after defoliation.

Measurement of leaf area
Leaf length was measured from the base to the tip, and leaf width was measured at the widest point. Leaf area (cm^2) was calculated using the formula:
Leaf Area (cm^2) = L × W × c
L = Leaf length (cm)
W = Leaf width (cm)
c = Constant
A constant of 0.68 was used, based on the study by Payne et al. (1991), which employed *Pennisetum glaucum*. This constant was adopted because the leaf morphology of *Pennisetum glaucum* is similar to that of the plant under investigation, as both belong to the same genus, *Pennisetum*.

Evaluation of chemical components and in vitro digestibility
To compare nutrient content and evaluate the forage potential of the five Napier grass cultivars, samples were collected from all plant parts harvested 10 cm above the ground. These samples were separated into stems and leaves and then mechanically chopped into small pieces using a chopper machine. The chopped material was then wrapped in newspaper and immediately dried at 55°C for 72 hours. After drying, the samples were ground in a Wiley mill until they passed through a 1 mm sieve for proximate analysis. The chemical composition was determined according to AOAC (2005) standards, and dry matter (DM) and organic matter (OM) were measured using the method described by Kamal (1997). Crude protein (CP) was determined by analyzing the nitrogen concentration using the Kjeldahl method and calculated as CP = N × 6.25 (AOAC, 2005).
Forage fiber analysis—including Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and lignin—was performed using the detergent method developed by Van Soest et al. (1991). The analysis was carried out using a Fiber Analyzer that speeds up the filtration and extraction processes, or manually through gravimetric methods. Livestock experimental procedures, including the collection of rumen fluid for in vitro analysis, received approval from the Ethical Clearance Committee of the Laboratory for Integrated Research and Testing, Universitas Gadjah Mada (letter number 00004/II/UN1/LPPT/EC/2025), ensuring compliance with animal welfare and ethical research standards. In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were assessed using a modified version of the method described by Tilley & Terry (1963) to determine the extent of feed digestibility by rumen microbes.

Chlorophyll content analysis
Approximately 1 g of leaf sample was extracted using pre-cooled acetone. The leaves were first crushed in a mortar, then 10 mL of cold acetone was added. The extraction involved incubating the crushed material and solvent at room temperature for 12 hours to ensure complete solubilization of chlorophyll. After incubation, the mixture was centrifuged at 2000 rpm for 10 minutes to separate the liquid extract from the solid residue. The clear supernatant was then used for spectrophotometric analysis to determine the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll (Jiang et al., 2017).
Absorbance readings were taken at wavelengths of 663 nm and 645 nm using a spectrophotometer. The chlorophyll concentrations were calculated in milligrams per gram of fresh tissue based on these absorbance values. The formulas, as outlined by Gu et al. (2016), were used for the calculations.
Chlorophyll a (mg/g) = $(12.7 \times A_{663}) - (2.59 \times A_{645})$
Chlorophyll b (mg/g) = $(22.9 \times A_{645}) - (4.7 \times A_{663})$
Total Chlorophyll (mg/g) = $(8.2 \times A_{663}) + (20.2 \times A_{645})$
All readings were taken after calibrating the spectrophotometer to zero using a solvent mixture (acetone or ethanol) as the blank.

Evaluation of mineral content
Mineral content (Ca, Mg, Fe, and Zn) was measured using flame atomic absorption spectrophotometry following AOAC (2005) methods. Samples were first ashed in an electric furnace at 550°C until a grayish white ash was formed. The ash was

then dissolved in a 5% nitric acid (HNO_3) solution before measuring absorbance with a flame atomic absorption spectrophotometer. The mineral analysis results are expressed in mg/kg.

Evaluation of anti-nutrient content

Forage samples were dried in an oven at 55°C until a constant weight was reached, then ground to approximately 1 mm particle size. The ground samples were extracted with 70% ethanol at a material-to-solvent ratio of 1:10 (w/v). Total saponin and tannin contents were measured using UV-Vis spectrophotometry following procedures adapted from Price et al. (1987) and Makkar (2003). Saponin content is expressed as a percentage (% w/w) relative to Quillaja bark, while tannin content is expressed as a percentage (% w/w) relative to tannic acid. Oxalic acid was analyzed using high-performance liquid chromatography (HPLC), based on a modified method from Savage et al. (2000), with results reported in milligrams per kilogram (mg/kg).

Statistical analysis

The parameters of the five Napier grass cultivars were statistically analyzed using one-way analysis of variance (ANOVA) within a randomized complete block design (RCBD), where the plant cultivar served as the treatment. Post-hoc comparisons were performed with Duncan's New Multiple Range Test (DMRT) to identify significant differences among cultivars. Statistical analyses were carried out with SPSS version 26.0 (Steel et al., 1997). Additionally, correlations between leaf area and chlorophyll content across the cultivars were assessed using correlation coefficient analysis.

Conclusions

The study shows that the five Napier grass cultivars in Indonesia display significant differences in their morphology, production, nutrient and mineral content, digestibility, and anti-nutrient levels. Each cultivar provides unique benefits, indicating that selection can be customized to suit specific livestock system needs or development objectives. These results offer valuable insights for choosing Napier grass cultivars that promote sustainable livestock production in Indonesia.

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