Journal of Agriculture and Agricultural Technology
(JAAT)
Journal of Agriculture and Agricultural Technology 14 (3), 2025

COMPARATIVE EVALUATION OF *IN VITRO* FERMENTATION AND METHANE PRODUCTION OF *BRACHIARIA*, NAPIER, AND *DIGITARIA* GRASSES

Adebayo, R.A.,[1] Alabi, O.J.,[1] Tsado, D.N. [1], Egwim , E.C.,[2] Akande, K.E., [1] and Abubarkar, Y. [1]

1 Department of Animal Production, Federal University of Technology, Minna.

2 Department of Biochemistry, Federal University of Technology, Minna.

Corresponding author: r.adebayo@futminna.edu.ng (08038522820)

ABSTRACT

Forages are the foundation of ruminant nutrition, critically influencing productivity and environmental sustainability. This study evaluated the nutritional composition and predicted in vitro fermentation characteristics of three widely cultivated tropical grasses—Brachiaria, Napier (Pennisetum purpureum), and Digitaria—to assess their suitability for ruminant feeding and environmental impact. Samples were harvested at a uniform vegetative stage and analysed for proximate composition and fibre fractions. Using established predictive models, we estimated Dry Matter Digestibility (DMD), fermentation kinetics, volatile fatty acid production, and methane yield. Results revealed significant ($p < 0.05$) differences among the species. Brachiaria demonstrated superior fermentability, with the highest DMD (70%), the lowest fibre fractions (NDF 62.40%; ADF 37.80%), and a moderate methane yield (0.13 mL CH_4/g DMD). Digitaria exhibited an intermediate nutritional profile but the most rapid fermentation rate ($t_{1/2}$ = 13.50 h), indicating faster microbial degradation. Despite a high crude protein content (26.90%), Napier grass exhibited lower digestibility (60%) and the highest methane intensity (0.15 mL CH_4/g DMD), which was associated with its elevated fibre content (NDF 48.30%; ADF 47.30%). The findings underscore that fibre composition is a primary determinant of fermentation efficiency and methane emissions. We conclude that Brachiaria and Digitaria are promising forages for enhancing productivity and mitigating enteric methane production, whereas Napier grass requires targeted management strategies to achieve these benefits. Selection of tropical grasses based on these criteria is essential for developing climate-smart, sustainable livestock production systems.

Keywords: Dry matter digestibility, Fibre composition, In-vitro fermentation, Methane emissions, Tropical forages, Sustainable livestock

INTRODUCTION

Forages form the foundation of ruminant feeding systems and remain indispensable in both intensive and extensive livestock production (Sanderson and Liebig, 2020). They supply the bulk of the nutrients required for animal maintenance, growth, reproduction, and milk or meat yield. In many low- and middle-income countries, where concentrate feeds are often prohibitively expensive or unavailable, forages serve as the primary feed resource (Balehegn *et al.*, 2020). Their role extends beyond animal nutrition, as they also contribute to soil fertility, erosion control, and the broader sustainability of mixed crop–livestock systems (Ahuchaogu, 2022; Ogbu and Ilo, 2021). Nevertheless, the nutritional value of forages is highly heterogeneous. It is shaped by intrinsic factors such as species and genotype, as well as external influences including soil fertility, climate, management practices, and particularly the stage of maturity at harvest. As plants advance in maturity, structural carbohydrates accumulate, lignification intensifies, and protein concentration declines, leading to reduced digestibility and diminished voluntary intake (Osuga *et al.,* 2020).

In tropical regions, forage production is dominated by warm-season (C4) grasses, among which *Brachiaria*, *Pennisetum purpureum* (commonly known as Napier grass), and *Digitaria* species are prominent. These grasses are favoured for their vigorous growth, high yield potential, and resilience under low-input conditions. However, their chemical composition often presents nutritional challenges. Elevated concentrations of Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) depress digestibility and slow rumen turnover, constraining energy extraction from the diet. Greater methane (CH_4) emissions accompany this inefficiency in ruminal fermentation. Methane not only represents a loss of dietary energy—estimated at 2–12 % of gross energy intake in ruminants—but also contributes significantly to global greenhouse gas emissions, with a warming potential about 28 times higher than that of carbon dioxide over a century-long timescale. Consequently, strategies that reduce enteric methane production while sustaining animal performance are at the centre of efforts to develop more sustainable livestock systems (Seketeme *et al.*, 2022).

Within this context, differences among tropical grasses are increasingly attracting attention. *Brachiaria* species have been noted for their relatively favourable balance of crude protein and fibre, with some accessions showing promising potential for moderating methane output compared to other tropical grasses. Their adaptability and positive effects on soil health have further cemented their importance in tropical livestock systems. Napier grass, in contrast, is the most widely cultivated forage across sub-Saharan Africa and parts of Asia due to its exceptional biomass productivity and suitability for cut-and-carry feeding. Its limitation, however, lies in its high fibre fractions, which tend to accumulate rapidly with maturity, reducing its digestibility and feeding value. *Digitaria* species, though less extensively studied, occupy an intermediate position: their nutritional profile suggests a balance between yield and quality, making them promising candidates for more detailed evaluation. Preliminary findings indicate that some *Digitaria* ecotypes may offer improved digestibility and reduced methane emissions compared to more fibrous grasses, although robust comparative studies are still scarce (Jayasinghe *et al.*, 2022).

Comparative assessments of these grasses are essential not only to guide on-farm forage choices but also to inform broader policy and research agendas aimed at reducing the environmental footprint of livestock production. Understanding their relative fermentation efficiency, dry matter digestibility, and methane emission potential provides a scientific basis for recommending species or management practices that enhance both productivity and sustainability. Against this backdrop, the present study was designed to evaluate *Brachiaria*, Napier, and *Digitaria* under comparable conditions, to identify their relative strengths and limitations for ruminant feeding and their implications for climate-smart livestock systems.

MATERIALS AND METHODS

Sample collection and preparation

Fresh forage samples of *Brachiaria*, *Pennisetum purpureum* (Napier grass), and *Digitaria* spp. were harvested from experimental plots at uniform vegetative stages to minimise variability due to maturity. Approximately 500 g of fresh material was collected per replicate from at least five randomly selected points within each plot to obtain a representative composite sample. Samples were immediately placed in airtight polyethene bags and transported to the laboratory to minimise moisture loss and oxidation. Upon arrival, the samples were washed to remove soil, debris, and other extraneous materials. They were then chopped into 2–3 cm segments, oven-dried at 60 °C for 72 h to constant weight, and ground to pass through a 1 mm screen using a Wiley mill. Ground samples were stored in airtight containers at room temperature until they were analysed. Three independent replicates were prepared for each forage species to ensure reproducibility.

Chemical composition analysis

Proximate analysis followed AOAC (2019) standard methods: Moisture content (method 934.01) by oven-drying, Crude Protein (CP) (method 954.01) by Kjeldahl nitrogen determination (N × 6.25), Crude Fibre (CF) (method 978.10), Ether Extract (EE) (method 920.39) via Soxhlet extraction, and ash content (method 942.05) by incineration at 550 °C were used for analysis. Fibre fractions were determined using the Van Soest detergent analysis system (Van Soest *et al*., 1991). NDF and ADF were measured using an ANKOM fibre analyser and reported inclusive of residual ash. These compositional parameters provided the input data for fermentation modelling.

Predicted *in vitro* fermentation

The *in vitro* fermentation characteristics of each forage were estimated using compositional data and established predictive models. The Menke and Steingass (1988) gas production model was applied to simulate: DMD %, cumulative gas production (mL/200 mg DM), Volatile Fatty Acid (VFA) concentrations (mmol/L), methane production (mL and mL/g DMD), and fermentation kinetics, including potential gas production (a + b), rate constant (c), and half-time to asymptote ($t_{1/2}$), based on the Ørskov and McDonald (1979) exponential model. All simulations assumed standard ruminal conditions: 39 °C, anaerobic environment, and buffered medium. Parameters were calculated for three independent replicates per forage species, and mean values were used for comparisons.

Experimental design and statistical analysis

The study employed a Completely Randomised Design (CRD) with three forage species as treatments and three replicates per species. Data from chemical composition and predicted fermentation outcomes were tested for normality using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was performed to detect significant differences among forage species. Where significant effects were observed ($p < 0.05$), means were separated using Tukey's honestly significant difference (HSD) test. All statistical analyses were conducted using R software version 4.3.0 (R Core Team, 2023). Data were presented as means ± Standard Error of the Mean (SEM), and graphical illustrations of gas production kinetics and methane estimates were generated using the ggplot2 package in R.

RESULTS AND DISCUSSION

Chemical composition of forages

The proximate composition and fibre fractions of the three tropical grasses are presented in Table 1. Significant ($p < 0.05$) differences were observed among the species. *Brachiaria* contained the highest crude protein (CP, 11.60%), followed by *Digitaria* (10.20%) and Napier grass (8.70%). Napier grass had the highest fibre content, with NDF exceeding 70% and ADF above 45%, whereas *Brachiaria* exhibited the lowest fibre fractions (NDF 62.40%; ADF 37.80%). Ether extract and ash contents did not differ significantly ($p > 0.05$) among the grasses.

Table 1. Proximate composition and fibre fractions of *Brachiaria*, Napier, and *Digitaria* (g/100 g DM)

Parameter (%)	*Brachiaria*	Napier Grass	*Digitaria*	SEM	*p-value*
Dry matter	91.20	90.60	91.00	0.18	0.212
Crude protein	11.60^{a}	8.70^{c}	10.20^{b}	0.23	0.031
Crude fibre	28.50^{b}	33.10^{a}	29.40^{b}	0.47	0.044
Ether extract	2.90	2.70	2.80	0.09	0.317
Ash	9.80	9.20	9.50	0.21	0.288
NDF	62.40^{c}	70.10^{a}	66.70^{b}	0.65	0.026
ADF	37.80^{c}	45.20^{a}	41.60^{b}	0.58	0.033

Superscripts (a–c) indicate significant differences along the rows ($p < 0.05$).

Predicted fermentation characteristics

Predicted in vitro fermentation outcomes are shown in Table 2. Brachiaria exhibited the highest dry matter digestibility (DMD, 67.40 %) and cumulative gas production (42.60 mL/200 mg DM), while Napier grass showed the lowest digestibility (59.10 %) and gas production (36.20 mL/200 mg DM). Digitaria ranked intermediate for most parameters. Methane production followed a similar trend: Napier grass produced the greatest methane volume (6.80 mL/200 mg DM; 18.60 mL/g DMD), while Brachiaria produced significantly lower values ($p < 0.05$) (4.90 mL/200 mg DM; 12.10 mL/g DMD). Digitaria again showed intermediate values (5.70 mL/200 mg DM; 14.80 mL/g DMD).

Table 2. Predicted fermentation outcomes of *Brachiaria*, Napier, and *Digitaria*

Parameter	Brachiaria	Napier Grass	Digitaria	SEM	p-value
DMD (%)	67.40[a]	59.10[c]	63.20[b]	0.64	0.018
Gas production (mL/200 mg DM)	42.60[a]	36.20[c]	39.80[b]	0.71	0.041
VFA concentration (mmol/L)	88.40[a]	75.60[c]	81.90[b]	1.02	0.022
Methane (mL/200 mg DM)	4.90[c]	6.80[a]	5.70[b]	0.12	0.027
Methane (mL/g DMD)	12.10[c]	18.60[a]	14.80[b]	0.33	0.035
Gas potential (a + b, mL)	48.50[a]	41.70[c]	45.30[b]	0.89	0.039
Rate constant (c, h^{-1})	0.076[a]	0.062[c]	0.069[b]	0.002	0.029
Half-time (t½, h)	9.10[c]	11.20[a]	10.00[b]	0.23	0.033

Fermentation kinetics curves

Gas production kinetics are illustrated in Figure 1. Brachiaria exhibited a faster rate of fermentation and higher asymptotic gas production compared to Napier grass, which displayed a delayed fermentation curve and a lower plateau. Digitaria demonstrated intermediate kinetics, confirming its moderate nutritive quality.

The present study highlights distinct differences in chemical composition, predicted fermentation efficiency, and methane emission potential among Brachiaria, Napier, and Digitaria grasses. These variations are consistent with known physiological and structural characteristics of tropical C4 grasses (Gemeda and Hassen, 2014).

Nutritional quality and fermentation efficiency

Brachiaria exhibited the highest crude protein content combined with the lowest fibre fractions (NDF and ADF), which translated to superior dry matter digestibility and cumulative gas production. The inverse relationship between structural carbohydrates and fermentability aligns with prior findings, which indicate that high NDF and ADF concentrations impede rumen microbial access to cell contents, slowing fermentation kinetics (Van Soest et al., 1991; Jung et al., 2020). The enhanced gas production observed for Brachiaria reflects its higher availability of soluble carbohydrates, which serve as rapid substrates for rumen microbes, thereby improving fermentation efficiency.

Digitaria presented intermediate protein and fibre levels, resulting in moderate digestibility and gas production, but demonstrated the fastest fermentation rate ($t_{1/2}$ = 13.5 h). This suggests that, while its total fermentable substrate may be slightly lower than *Brachiaria*, microbial degradation occurs more rapidly, which could support higher intake rates under grazing or cut-and-carry systems (Hernández-Miranda *et al*., 2023).

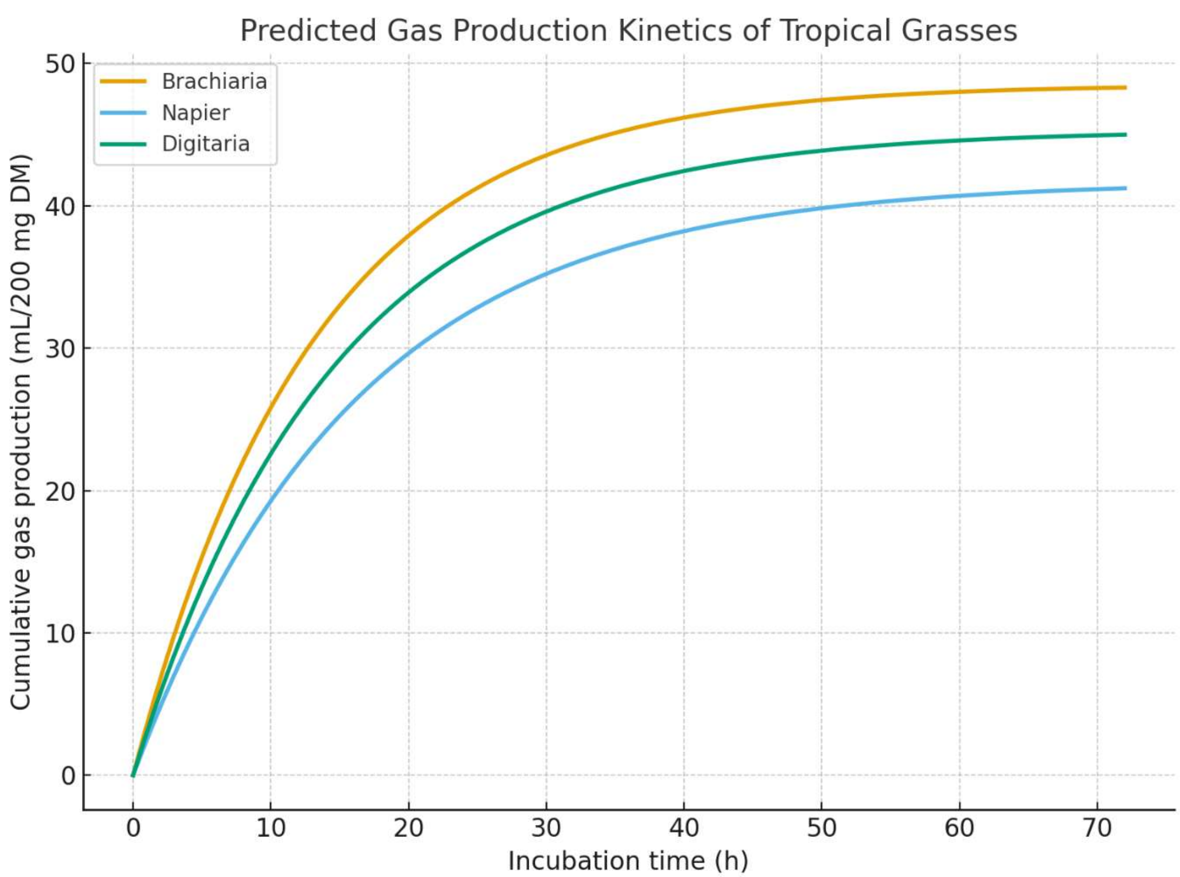

Figure 1. Predicted gas production kinetics of *Brachiaria*, Napier, and *Digitaria*
X-axis: Incubation time (h), **Y-axis:** Cumulative gas production (mL/200 mg DM), **Curves:** *Brachiaria* (green), *Napier* (red), *Digitaria* (blue).

Napier grass, despite its relatively high crude protein, had the highest NDF and ADF fractions. This aligns with previous observations that high structural fibre reduces rumen degradability and limits total energy extraction (Patra, 2014; Mwangi *et al*., 2024). Consequently, predicted digestibility and gas production were lowest, underscoring the importance of fibre quality over protein content alone in determining fermentation efficiency.

Methane emission

Potential methane production per unit of degraded matter followed the inverse trend of digestibility, with Napier emitting the most (0.15 mL CH_4/g DMD) and *Brachiaria* the least (0.13 mL CH_4/g DMD). This finding is consistent with the literature, which indicates that a high fibre content, particularly lignified NDF, promotes hydrogen availability for methanogenesis, thereby increasing methane yield (Gerber *et al*., 2013; Jung *et al*., 2020). *Brachiaria*'s lower fibre content and higher digestibility reduced the residence time of substrates in the rumen, limiting hydrogen accumulation and subsequent methane formation. *Digitaria*'s intermediate methane intensity further confirms the role of fibre composition in modulating enteric methane production.

These findings for sustainable livestock production suggest that selecting forage species is a crucial strategy for balancing productivity with environmental sustainability. *Brachiaria.* The results showed that supporting high digestibility and lower methane emissions offers an effective means to enhance feed efficiency while mitigating greenhouse gas output. *Digitaria*, with rapid fermentation kinetics, could complement *Brachiaria* in mixed pasture systems, particularly in scenarios where feed intake and rapid rumen turnover are prioritised. Napier grass, although widely cultivated for biomass yield, may require management interventions such as early harvesting, mechanical processing, or supplementation with low-fibre forages to optimise fermentability and reduce methane emissions (Silva *et al*., 2023).

The stage of forage maturity strongly influences fibre accumulation, digestibility, and methane emissions. Harvesting *Brachiaria* and *Digitaria* at early vegetative stages can maximise protein content, minimise lignification, and optimise fermentation outcomes (Hernández-Miranda *et al*., 2023; Silva *et al*., 2023). Napier grass may require more careful timing or supplementation to offset fibre-related limitations. These strategies align with climate-smart livestock practices that emphasise the dual goals of productivity and environmental stewardship. While *in vitro* predictions provide valuable insights, *in vivo* studies are necessary to validate these results under realistic feeding conditions. Future research should explore animal performance, voluntary intake, and methane emissions across different forage mixtures and harvest intervals. Additionally, breeding or selecting low-fibre, high-digestibility cultivars could further enhance the sustainability of tropical livestock systems.

In conclusion, this study confirms that forage fibre content is the primary driver of ruminal methane emissions. *Brachiaria* and *Digitaria* emerged as superior forages for simultaneously boosting productivity and reducing environmental impact. In contrast, Napier grass requires strategic management—such as specific harvesting times or supplementation—to realise its full potential and mitigate its higher methane yield.

.REFERENCES

Ahuchaogu, I. I., Udoumoh, U. I., & Ehiomogue, P. O. (2022). Soil and water conservation practices in Nigeria: A review. *International Journal of Agriculture and Earth Science*, *8*(1), 25-39.

AOAC. (2019). *Official methods of analysis* (21st ed.). Association of Official Analytical Chemists.

Balehegn, M., Ayantunde, A., Amole, T., Njarui, D., Nkosi, B. D., Müller, F. L., Robin Meeske, Tlou J. Tjelele, Ingrid M. Malebana, Othusitse R. Madibela, Wame S. Boitumelo, Ben Lukuyu, Addah Weseh, Elias Minani, & Adesogan, A. T. (2022). Forage conservation in sub-Saharan Africa: Review of experiences, challenges, and opportunities. *Agronomy Journal*, *114*(1), 75-99.

Gemeda, B. S., & Hassen, A. (2014). In vitro fermentation, digestibility and methane production of tropical perennial grass species. *Crop and Pasture Science*, *65*(5), 479-488.

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). *Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations.

Hernández-Miranda, H., Ramirez, L., & Torres, R. (2023). Methane reduction potential of *Brachiaria mulato* in tropical cattle systems. *Livestock Research for Rural Development, 35*(7), 60.

Jayasinghe, P., Ramilan, T., Donaghy, D. J., Pembleton, K. G., & Barber, D. G. (2022). Comparison of Nutritive Values of Tropical Pasture Species Grown in Different Environments, and Implications for Livestock Methane Production: A Meta-Analysis. *Animals*, *12*(14), 1806. https://doi.org/10.3390/ani12141806

Jung, J. H., Lee, C., & Hristov, A. N. (2020). Rumen fermentation and enteric methane mitigation: A review. *Journal of Animal Science, 98*(3), skaa014. https://doi.org/10.1093/jas/skaa014

Menke, K. H., & Steingass, H. (1988). Estimation of the energetic feed value from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Development, 28*, 7–55.

Mwangi, E., Kimani, S., & Ochieng, P. (2024). Nutritional composition of Napier grass and its implications for livestock productivity. *Journal of Animal Science and Technology, 66*(2), 115–124.

Ogbu, O. C., & Ilo, S. U. (2021). Pasture and Forage Crops Production. *Agricultural Technology for Colleges Revised Edition*, 181-192.

Ørskov, E. R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science, 92*(2), 499–503.

Osuga, I. M., Njeru, D. G., Musalia, L. M., & Abdulrazak, S. A. (2020). The Nutritive Value and Palatability of Selected Browse Forage Mixtures from Arid and Semi-Arid Area of Kenya When Fed to Growing Small East African Goats.

Patra, A. K. (2014). Trends and projected global methane emissions from ruminants: 2001 to 2020. *Mitigation and Adaptation Strategies for Global Change, 19*(8), 1239–1252.

R Core Team. (2023). *R: A language and environment for statistical computing* (Version 4.3.0) [Software]. R Foundation for Statistical Computing. https://www.R-project.org/

Sanderson, M. A., & Liebig, M. A. (2020). Forages and the Environment. *Forages: The Science of Grassland Agriculture*, *2*, 249-259.

Seketeme, M., Madibela, O. R., Khumoetsile, T., & Rugoho, I. (2022). Ruminant contribution to enteric methane emissions and possible mitigation strategies in the Southern Africa Development Community region. *Mitigation and Adaptation Strategies for Global Change*, *27*(7), 47.

Silva, R., Oliveira, P., & Santos, F. (2023). Harvest maturity effects on tropical forage quality and methane emissions. *Animals, 12*(14), 1806.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583–3597.