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ORIGINAL RESEARCH

Interactive Effects of Nitrogen Fertilization and Harvest Age on the Nutritional Composition of *Brachiaria ruziziensis*

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ABSTRACT: High-quality forage is vital for livestock productivity, but farmers in Nigeria often face challenges due to inconsistent fertilizer use and poor harvesting practices. This study evaluates the effects of nitrogen fertilizer rates and harvest age on the nutritive value of *Brachiaria ruziziensis* to enhance forage quality and support sustainable livestock nutrition. A 3 × 2 factorial design was used, testing three fertilizer rates (0, 120, and 240 kg N ha^{-1} as NPK 15:15:15) and two harvest ages (8 and 10 weeks after planting), resulting in six treatments. Proximate composition, fiber fractions, forage quality indices, mineral content, in vitro gas production, and post-incubation parameters were analyzed using standard laboratory methods. Both fertilizer rate and harvest age significantly ($P < 0.05$) influenced dry matter (DM), crude fiber (CF), crude protein (CP), ether extract (EE), ash, non-fiber carbohydrates (NFC), and metabolizable energy (ME). Fiber components, including acid detergent fiber (ADF), hemicellulose, and cellulose, were also affected. Forage quality indices such as organic matter (OM), carbohydrate content (CHO), dry matter digestibility (DMD), dry matter intake (DMI), relative feed value (RFV), cell content (CC), total digestible nutrients (TDN), and net energies for intake, gain, maintenance, and digestible energy showed significant improvements with increased fertilizer rates and earlier harvest. Mineral contents—sodium (Na), calcium (Ca), phosphorus (P), potassium (K), manganese (Mn), iron (Fe), and copper (Cu)—varied significantly with treatments. In vitro gas production and post-incubation parameters also showed significant differences. The study concludes that applying NPK 15:15:15 at 120 kg N ha^{1} and harvesting at 8 weeks after planting optimizes biomass yield and improves proximate, fiber, mineral content, and forage quality, making it a cost-effective and sustainable approach for pasture management in Nigeria.

KEYWORDS: *Brachiaria ruziziensis*, in-vitro gas production, fertilizer rates, post-incubation parameters, spacing, weeks after planting.

1. Introduction

As the global population steadily approaches 9.6 billion by the year 2050, the pressure on agriculture to meet rising food demands continues to intensify; as such, achieving a 70% increase in food production is not only necessary to ensure food security but must also be balanced with ecological sustainability and resource conservation (Baye et al., 2024). In this context, the role of forage crops in supporting livestock productivity becomes increasingly critical, especially in tropical and subtropical regions where ruminants rely heavily on forages as their primary source of nutrition (Akinyode et al., 2021). Among the various forage species, *Brachiaria ruziziensis* has gained attention for its adaptability and potential to enhance ruminant feeding systems. However, maximizing its yield and nutritional quality remains a key challenge (Njoku et al., 2023).. Fertilizer application, particularly those containing nitrogen, has long been recognized as a fundamental agronomic tool to boost plant growth, improve leaf development, and increase forage biomass (Njoku et al., 2023). Likewise, determining the appropriate age at harvest plays a vital role in ensuring optimal forage digestibility and nutritional content (Sobande et al., 2024). These two factors, fertilizer management and harvest timing, are increasingly viewed as crucial levers in sustainable forage production.

Recent studies have explored how different fertilizer regimes and harvest intervals influence the productivity and quality of forage crops. For instance, balanced fertilizer formulas like N:P:K 15:15:15 have been shown to supply essential nutrients that contribute to plant vigor and stress resilience (Akinyode et al., 2021). However, much of the existing literature has either prioritized biomass yield over nutritional value or been conducted under controlled rather than field conditions. In addition, there is limited research examining how these factors interact under practical pasture systems, particularly within Nigeria's agroecological settings, where *Brachiaria ruziziensis* is increasingly cultivated.

To address these gaps, the present study investigates the effects of varying fertilizer application rates and harvest ages on the forage quality of *Brachiaria ruziziensis*. Thus, by focusing on essential nutritional indicators such as proximate composition, fiber fractions, digestibility, and energy content, this research aims to generate practical insights that support efficient and sustainable pasture management for ruminant production in tropical systems. This study determined which interaction effect of N:P:K 15:15:15 fertilizer application rate and age at harvest (8 and 10 weeks) promotes the best nutritive variables (proximate, fiber fractions, digestibility, mineral, and energy contents) in *Brachiaria ruziziensis*.

2. Materials and methods

2.1. Experimental site

The study was conducted at the Pasture unit of the Directorate of University Farms (DUFARMS) and the laboratory of the Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria; latitude 7.2235° N, 3.4403° E and 76 meters above sea level. The area located within the derived savannah vegetation zone of the Southwestern region has a humid climate with a mean annual rainfall of 1037mm and a temperature of about 34.7 ˚C (Figure 1A). The area has an annual average humidity percentage of 81.0% with July being the most humid and February the least humid (Figure 1B & C) (Weather and Climate, 2021).

2.2. Experimental design

The study was a 3 × 2 factorial arrangement laid out in a split-split plot design. Nitrogen (N) fertilizer (NPK 15-15-15) application rate per hectare was 0, 120, and 240 N kg ha^{-1} assigned to the main plot, and age at harvest (8 and 10) weeks after planting (WAP) was assigned to the subplot.

The study has 3 replicates, making a total of 12 treatment combinations per replicate, indicating a total of 36 plots measuring 5m × 5m used for the chemical analysis.

Table 1. Physico-chemical characteristics of the soil samples taken at 0–15 cm depth of the experimental plot.

Physico-chemical characteristics	Value
pH	6.50
Total nitrogen (%)	0.10
Organic carbon (%)	1.55
Available P (mg kg^{-1})	4.11
Acidity (cmol kg^{-1})	0.00
Exchangeable cations (cmol kg^{-1})	
Sodium (Na)	0.08
Potassium (K)	1.75
Calcium (Ca)	11.52
Magnesium (Mg)	1.31
Micronutrient (mg kg^{-1})	
Zinc (Zn)	77.47
Copper (Cu)	10.51
Manganese (Mn)	43.08
Iron (Fe)	43.08
Particle size (%)	
Sand (%)	79.00
Silt (%)	10.00
Clay (%)	11.00

2.3. Sampling, measurement and analysis

2.3.1. Sampling

Forage was harvested using a 0.5 × 0.5 m quadrat 10 cm above ground level using a sickle. The fresh weight of the harvested forage was recorded and then oven dried at 65°C until a constant weight was obtained. This was used to determine the dry matter percentage. The oven-dried forage was milled using a hammer mill and sieved using a 1 mm sieve screen. The 1 mm sieved samples were used for chemical analysis to determine the crude protein, fiber fractions (ADF, NDF), ash, and mineral content using standardized laboratory protocols. However, to promote quality and randomization, plot locations were randomized to reduce environmental bias, and sampling time was standardized to reduce diurnal variation.

2.3.2. Determination of proximate composition

The dry matter (DM), crude protein (CP), ether extract (EE), and ash of the hay samples were determined according to AOAC (2000), while non-fiber carbohydrates (NFC) components and estimated as NFC = 100 – (CP + EE + NDF + ash).

2.3.3. Determination of fibre fractions

The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of the samples were determined according to the procedure by Van Soest *et al.* (1991). ADF Cellulose was calculated as the difference between ADF and ADL, while hemicellulose was calculated as the difference between NDF and ADF.

2.3.4. Determination of mineral composition

Harvested forage samples were dried in a forced draught oven at 60 °C for 24 hours, followed by wet washing using nitric acid and hydrochloric acid at a ratio of 3:1. After that, samples were analyzed for Calcium (Ca), Phosphorus (P), Potassium (K), Magnesium (Mg), and Sodium (Na).

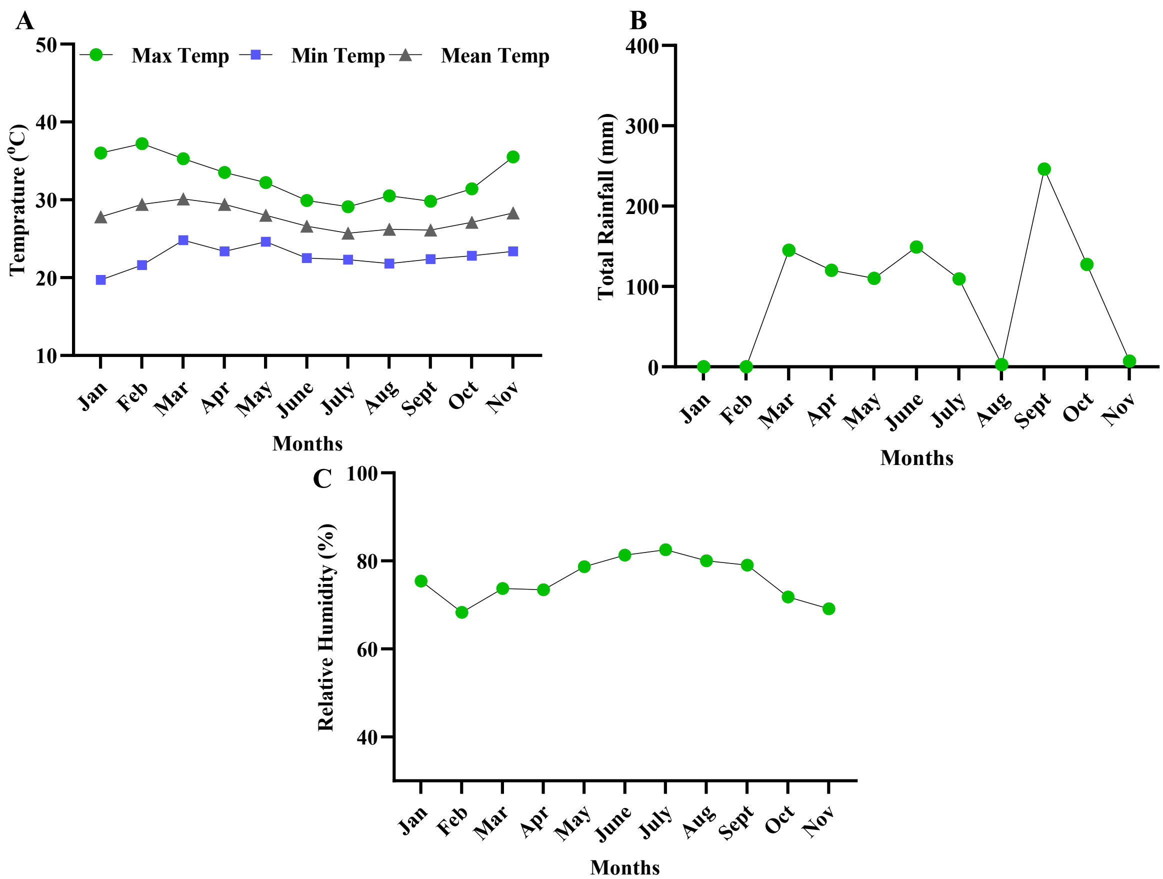

Figure 1. Temperature (A), total rainfall (B) and relative humidity (C) of the experimental location during the period of the study.

A flame photometer was used to estimate the concentration of K, while the concentration of Ca, P, and Mg was determined with atomic absorption spectrophotometry (Fritz and Schenk, 1979). The macro and microelement contents in the Hay were expressed in g kg^{-1}.

2.3.5. In vitro gas production and digestibility

The *in* vitro gas production of the harvested forage samples was determined following Menke and Steingass (1988). Milled samples weighing 200 ± 0.05mg were measured into 100 ml graduated glass syringes as the only substrate (n = 3). Also, triplicate bottles of incubation solution without substrate will be included as blanks to estimate the net gas production. Rumen contents were obtained from a slaughtered cow from a reputable abattoir very early morning, used as inoculum, and sieved with 4 layers of cheesecloth into a pre-warmed, insulated bottle in the laboratory. The mixture of macro and microelements, reduction, and buffer solution was mixed with distilled water. These solutions were mixed in a ratio of 2:1 with the primary fluid; 30ml of the inoculum was drawn into the syringe. The syringe was placed in a water bath with a shaker at a temperature of 390C °C; the gas produced in each syringe was observed and recorded at 24, 48, and 72 hours of incubation. Gas volume at each incubation time was expressed per incubated dry matter (DM) unit.

The kinetics of gas production were modeled using a non-linear regression equation 1.

$V = b(1 - e^{(-ct)})$ (Eq. 1),

Where V is the potential gas production at time t (ml/200 mg DM), b is the volume of gas evolved with time, and c is the rate constant of gas production.

Organic matter digestibility (OMD) was estimated using equation (2).

OMD = 14.88 + 0.889GV + 0.45CP + 0.651Ash (Eq. 2), as proposed by Menke and Steingass (1988).

Short-chain fatty acids (SCFA) were calculated according to equation (3).

SCFA = 0.0239GV – 0.0601 (Eq. 3),

based on the method of Getachew et al. (2004).

Metabolizable energy (ME) was determined using the equation (4).

ME = 2.20 + 0.1357GV + 0.057CP + $0.002859EE^2$ (Eq. 4),

as described by Menke and Steingass (1988).

In all equations, GV refers to total gas volume (ml/200 mg DM), CP and Ash are expressed as g/kg ODM, EE is ether extract, ME is expressed as MJ/kg DM, and SCFA as μmol/g DM.

2.4. Experimental model

The statistical model used for data analysis was as follows:

$Yijkl = \mu + Fi + Gj + (FG)ij + \sum ijkl$ (Eq. 5)

μ = Population mean

Fi = Effect of fertilizer treatments at different rates (0, 120, and 240kg N/ha-1) on harvested forage

Gj = Effect of age at harvest on growth, quality, and nutritive content of harvested forage

(FG)ij Interaction of fertilizer treatment and age at harvest on harvested forage

∑ijkl = Residual error estimates

3.4. Statistical analysis

The laboratory data collected were subjected to a 2-way analysis of variance using the SAS (1999) package with means separated using the Multiple Range Test at P<0.05 (Duncan, 1955). Corelation perason heatmap was generated by using R-studio (Metan Package).

3. Results

3.1 Proximate composition

Table 2 presents the proximate composition of *Brachiaria ruziziensis* as influenced by nitrogen fertilizer rates (0, 120, 240 kg N ha^{-1}) and harvest age (8 and 10 weeks after planting – WAP). Significant (*$P < 0.05$*) differences were observed in dry matter (DM), crude fibre (CF), crude protein (CP), ether extract (EE), ash, non-fibre carbohydrates (NFC), and metabolizable energy (ME) across treatments. The highest DM (92.90%), CF (16.94%), and NFC (12.19%) were recorded in plants fertilized with 120 kg N ha-1 at both 8 and 10 WAP. The control group (0 kg N ha^{-1}) had the highest EE (5.30%) and ME (18.12%), especially at 10 WAP. In contrast, the highest CP (10.29%) and ash content (14.75%) were obtained under 240 kg N ha^{-1} at 8 WAP. These results indicate that moderate nitrogen input (120 kg N ha^{-1}) enhances structural carbohydrate accumulation, while higher rates (240 kg N ha^{-1}) support protein and ash accumulation at early growth stages.

Table 2. Proximate composition of *Brachiaria ruziziensis* as influenced by fertilizer rate and age at harvest.

Factors		Dry matter	Crude fibre	Crude protein	Ether extract	Ash	Non-fibre carbohydrate	ME
Fertilizer rate (kg/ha)	Age at harvest (weeks)	(%)						
0	8	90.84b	15.88a	7.77c	5.30a	7.94c	12.18a	14.74ab
	10	90.60b	16.05a	7.46c	4.39c	6.75c	12.16a	18.12a
120	8	92.90a	16.54a	10.06a	4.38c	7.55c	12.19a	15.01ab
	10	92.06a	16.94a	8.49c	4.84b	7.37c	12.17a	12.80abc
240	8	90.87b	14.88b	10.29a	4.81b	14.75a	12.03b	6.65c
	10	90.64b	13.44c	9.57b	5.25a	12.51b	12.14a	10.49bc
Standard error of the mean		0.25	0.33	0.30	0.10	0.72	0.02	1.12
P-value		0.024	0.002	0.002	0.000	0.000	0.012	0.023

Note. Different letters within the same column are significant (*P<0.05*).

Table 3. Fibre fractions of *Brachiaria ruziziensis* as influenced by fertilizer rate and age at harvest.

Factors		NDF	ADF	ADL	HEMI	CELLULOSE
Fertilizer rate (kg/ha)	Age at harvest (weeks)	(%)				
0	8	64.26	46.89a	17.09	17.37a	29.80bc
	10	63.29	48.96ab	17.28	14.33b	31.69abc
120	8	63.01	46.89a	16.36	18.21a	28.45c
	10	66.51	47.48b	17.23	19.03a	30.26bc
240	8	63.51	52.48a	17.17	11.03c	35.31a
	10	62.19	48.82ab	15.58	13.38bc	33.24ab
Standard error of the mean		0.71	0.75	0.27	0.76	0.70
P-value		0.656	0.046	0.442	0.000	0.022

Note: SEM = Standard error of mean; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent fibre; HEMI: Hemicellulose.

3.2 Effect on fibre fractions

Table 3 displays the influence of fertilizer and harvest age on the fibre components: neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose (HEMI), and cellulose. Significant differences (P < 0.05) were observed in ADF, HEMI, and cellulose, whereas NDF and ADL remained unaffected. The highest ADF (52.48%) and cellulose (33.24%) values were recorded in plants treated with 240 kg N ha-1 at 8 and 10 WAP, respectively. Meanwhile, the highest hemicellulose (19.03%) and ADL (17.23%) values were observed under 120 kg N ha^{-1} at 10 and 8 WAP, respectively. These results suggest that higher nitrogen levels increase fibre accumulation, particularly cellulose, likely due to enhanced cell wall formation during biomass buildup in later growth stages.

3.3 Forage quality indices

Table 4 outlines the quality indices, including organic matter (OM), carbohydrate (CHO), dry matter digestibility (DDM), dry matter intake (DMI), relative feed value (RFV), cell content (CC), total digestible nutrients (TDN), net energy for intake (NEI), net energy for gain (Neg), net energy for maintenance (NEM), digestible energy (DE), and metabolizable energy (ME). Significant ($P < 0.05$) variations were recorded in all parameters except RFV and CC. The highest values of DDM (54.00%), RFV (79.74%), TDN (46.63%), NEI (1.13 MJ kg-1 DM), Neg (0.34 MJ kg-1 DM), and NEM (1.06 MJ kg-1 DM) were found under 120 kg N ha^{-1} at 8 WAP. Conversely, the highest OM (93.26%), CHO (81.41%), and DMI (1.91%) were observed in the control at 10 WAP. These findings indicate that a moderate nitrogen rate (120 kg N ha^{-1}) at early harvest improves forage digestibility and energy yield, whereas delayed harvest under no fertilization increases structural carbohydrate concentration.

3.4 Mineral composition

Table 5 presents the macro- and micro-mineral composition of *Brachiaria ruziziensis* as influenced by fertilizer rate and age at harvest. Statistically significant differences ($P < 0.05$) were observed for sodium (Na), calcium (Ca), phosphorus (P), potassium (K), manganese (Mn), copper (Cu), and iron (Fe), while zinc (Zn) remained unaffected. The highest K (74.97 g/kg DM), Zn (0.26 g/kg DM), and Mn (0.45 g/kg DM) were observed in the control group. Maximum values for P (8.72 g/kg DM) and Fe (0.14 g/kg DM) were recorded under 120 kg N ha^{-1} at 8 WAP, while the highest Na (6.74 g/kg DM) and Ca (11.75 g kg DM) were obtained at 240 kg N ha^{-1} and 8 WAP. These results demonstrate that both fertilizer input and plant maturity significantly influence mineral accumulation, with younger plants exhibiting higher nutrient concentrations, especially under moderate to high nitrogen fertilization.

3.5 In vitro gas production and post-incubation parameters

Table 6 shows the in vitro gas production at 24 and 48 hours and associated post-incubation parameters, including organic matter digestibility (OMD), short-chain fatty acids (SCFA), metabolizable energy (ME), and net energy for lactation (NEL). All parameters differed significantly ($P < 0.05$) across treatments.

The highest in vitro gas production (9.50 ml at 24 h and 21.50 ml at 48 h), OMD (35.77%), SCFA (0.17 μmol g^{-1} DM), ME (3.55 MJ kg^{-1} DM), and NEL (2.02 MJ kg^{-1} DM) were obtained in plants fertilized with 240 kg N ha^{-1} and harvested at 10 WAP.

Table 4. Forage quality indices of *Brachiaria ruziziensis* as influenced by fertilizer rate and age at harvest.

Factors		OM	CHO	DDM	DMI	RFV	CC	RFV	TDN	NEI	Neg	NEM	DE	ME
Fertilizer rate (kg/ha)	Age at harvest (weeks)							(%)						
0	8	92.06a	78.99ab	52.38a	1.87a	76.01	35.75	76.00	43.93a	1.06a	0.26a	0.98a	1.94a	1.59a
	10	93.26a	81.41a	50.76ab	1.91a	75.35	36.72	75.33	41.22ab	1.00ab	0.19ab	0.91ab	1.81ab	1.49ab
120	8	92.46a	78.02b	54.00a	1.90a	79.74	36.99	79.73	46.63a	1.13a	0.34a	1.06a	2.06a	1.69a
	10	92.64a	79.31ab	51.91a	1.81a	72.83	33.49	72.81	43.15a	1.05a	0.24a	0.96a	1.90a	1.56a
240	8	85.25c	70.16c	48.02b	1.89a	70.36	36.49	70.34	36.64b	0.89b	0.05b	0.77b	1.62b	1.33b
	10	87.49b	72.68c	50.87ab	1.93a	76.10	37.81	76.08	41.41ab	1.00ab	0.19ab	0.91ab	1.83ab	1.50ab
	SEM	0.76	1.01	0.57	0.02	1.44	0.71	1.44	0.98	0.02	0.03	0.03	0.04	0.04
	P-value	0.000	0.000	0.046	0.682	0.591	0.656	0.591	0.046	0.046	0.046	0.046	0.046	0.046

[abcd] Means along the same column with different superscripts are significant (P<0.05). SEM: Standard error of mean; OM: Organic matter; CHO: Carbohydrate; DMD: Dry matter digestibility; DMI: Dry matter intake; RFV: Relative feed value; CC: Cell content

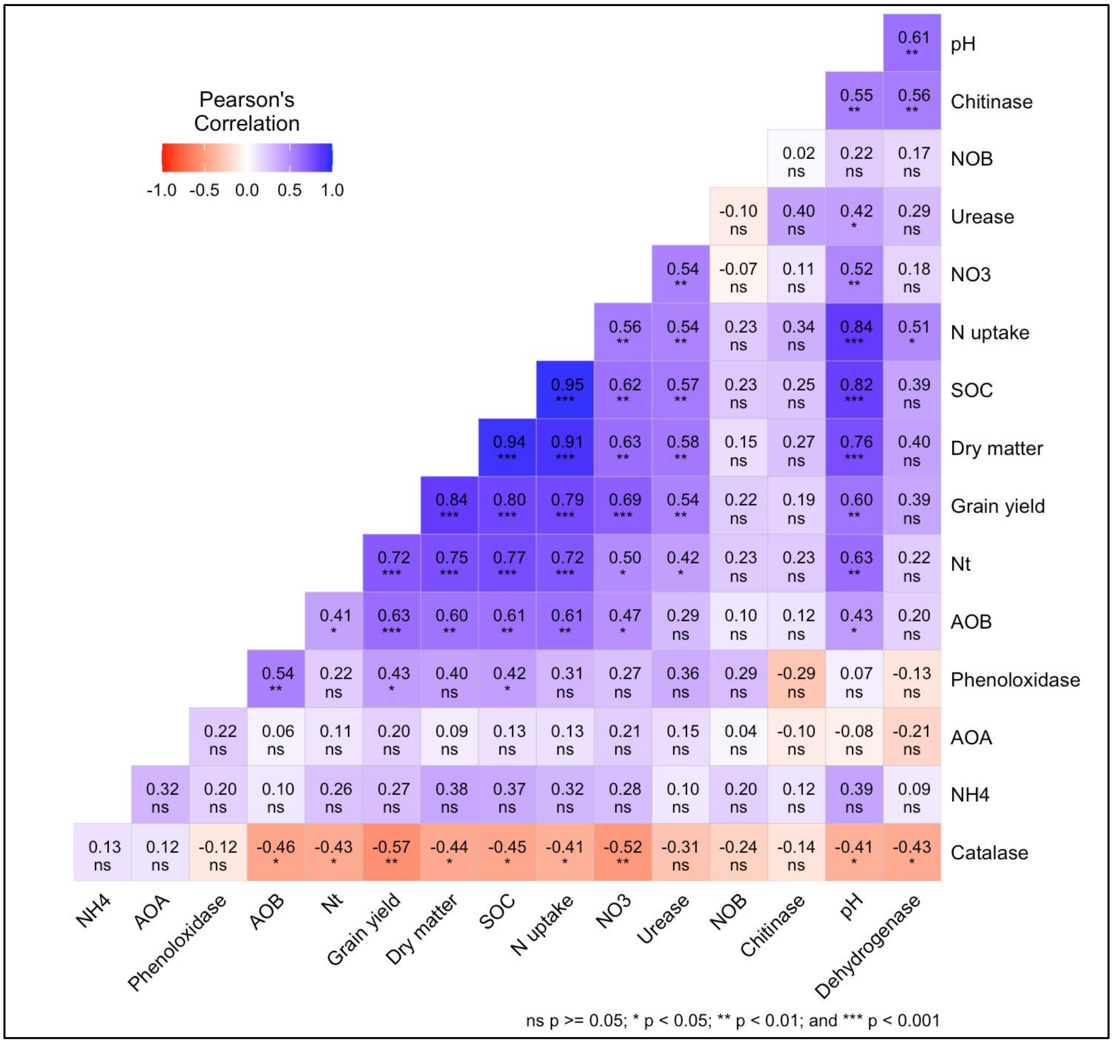

Figure 2. Pearson's correlation heatmap among soil biochemical properties, enzyme activities, and plant productivity parameters. The heatmap illustrates Pearson's correlation coefficients between key soil biochemical attributes (e.g., SOC, pH, NH4+ NO3-), enzyme activities (e.g., urease, catalase, phenoloxidase, chitinase), microbial functional groups (e.g., AOA, AOB, NOB), and plant performance indicators (e.g., grain yield, dry matter, N uptake, Nt). Positive correlations are shown in purple shades, while negative correlations appear in red shades. Asterisks indicate significance levels: *p < 0.05, **p < 0.01, ***p < 0.001, and "ns" denotes non-significant relationships. Strong positive correlations were observed among SOC, nitrogen uptake, and crop yield components, while catalase and phenoloxidase activities showed negative correlations with productivity metrics.

In contrast, the lowest values for most parameters were recorded in plants treated with 120 kg N ha^{-1} at 10 WAP. These findings suggest that high nitrogen input combined with later harvest enhances fermentation potential and energy content, possibly due to improved digestibility and microbial activity associated with younger, nutrient-rich tissues.

3.6 Correlations

The Pearson's correlation heatmap reveals several significant associations between soil biochemical properties, enzyme activities, and plant productivity indicators (Figure 2).

Table 5. Mineral composition of Brachiaria ruziziensis as influenced by fertilizer rate and age at harvest

Factors		Na	Ca	P	K	Mn	Zn	Fe	Cu
Fertilizer rate (kg/ha)	Age at harvest (weeks)	g/kg DM				mg/kg DM			
0	8	4.50^{b}	8.39^{c}	8.37^{ab}	74.97^{a}	0.34^{bc}	0.21	0.15^{a}	0.04^{b}
	10	4.43^{b}	11.52^{a}	7.17^{bc}	54.18^{b}	0.45^{b}	0.26	0.12^{b}	0.16^{a}
120	8	4.43^{b}	11.28^{ab}	8.72^{a}	53.81^{b}	0.01^{a}	0.24	0.14^{ab}	0.04^{b}
	10	5.18^{b}	9.77^{bc}	7.17^{bc}	66.48^{ab}	0.35^{bc}	0.23	0.13^{ab}	0.03^{b}
240	8	6.74^{a}	11.75^{a}	8.11^{ab}	73.74^{a}	0.32^{c}	0.24	0.13^{ab}	0.03^{b}
	10	6.06^{a}	11.52^{a}	6.49^{c}	64.68^{ab}	0.38^{bc}	0.23	0.13^{ab}	0.03^{b}
	SEM	0.23	0.35	0.24	2.60	0.01	0.01	0.00	0.01
	P-value	0.000	0.004	0.019	0.029	0.002	0.327	0.208	0.012

Note: Na-Sodium, Ca-Calcium, P-Phosphorus, K-Potassium, Mn-Manganese, Zn-Zinc, Fe-Iron, Cu-Copper.

Table 6. In vitro gas production (ml 200 mg-1) and post-incubation parameters of Brachiaria ruziziensis as influenced by fertilizer rate and age at harvest.

Factors		24 hr	48 hr	OMD (%)	SCFA (µmol g^{-1} DM)	ME (MJ kg^{-1} DM)	NEL (MJ kg^{-1} DM)
Fertilizer rate (kg/ha)	Age at harvest (weeks)						
0	8	6.50^{b}	11.50^{c}	29.32^{b}	0.10^{b}	3.13^{b}	1.64^{b}
	10	7.00^{b}	11.50^{c}	28.85^{b}	0.11^{b}	3.20^{b}	1.57^{b}
120	8	3.50^{c}	7.00^{d}	27.43^{b}	0.02^{c}	2.74^{c}	1.35^{c}
	10	3.50^{c}	6.50^{d}	26.62^{b}	0.02^{c}	2.73^{c}	1.32^{c}
240	8	7.00^{b}	16.00^{b}	35.34^{a}	0.11^{b}	3.22^{b}	1.76^{ab}
	10	9.50^{a}	21.50^{a}	35.77^{a}	0.17^{a}	3.55^{a}	2.02^{a}
	SEM	0.56	1.29	0.94	0.01	0.08	0.07
	P-value	0.000	0.000	0.000	0.000	0.000	0.001

SEM: Standard error of mean; OM- Organic matter; CHO- Carbohydrate; DMD-Dry matter digestibility; DMI-Dry matter intake; RFV- Relative feed value; CC- Cell content

Grain yield exhibited strong positive correlations with dry matter (r = 0.84***), soil organic carbon (SOC) (r = 0.80***), nitrogen uptake (r = 0.79***), and total nitrogen (Nt) (r = 0.72***), indicating that enhanced nitrogen availability and organic carbon content play critical roles in promoting crop yield. Similarly, dry matter also showed strong positive correlations with SOC (r = 0.69***) and nitrogen uptake (r = 0.72***), highlighting the interconnected influence of soil fertility parameters on biomass accumulation. SOC was further positively correlated with nitrogen uptake (r = 0.61***), AOB (r = 0.61**), and pH (r = 0.54**), suggesting that microbial activity and optimal pH conditions support nutrient cycling and availability

Conversely, negative correlations were observed between oxidative enzymes and yield components. Catalase activity negatively correlated with grain yield (r = –0.43**), dry matter (r = –0.57***), SOC (r = –0.45**), and nitrogen uptake (r = –0.44**), while phenoloxidase also showed significant negative associations with grain yield (r = –0.46**) and dry matter (r = –0.44**). These findings suggest that elevated levels of oxidative stress-related enzymes may reflect unfavorable soil microbial conditions or stress environments that impair plant growth.

Additionally, chitinase showed positive correlations with SOC (r = 0.56**) and pH (r = 0.61**), indicating its potential role in promoting organic matter turnover and nutrient availability in neutral to slightly alkaline soils. Urease activity showed weak but positive associations with nitrogen uptake (r = 0.42*) and grain yield (r = 0.22 ns), implying a limited but potentially beneficial role in nitrogen mineralization. Overall, these correlations underscore the importance of maintaining soil organic matter, microbial health, and balanced enzymatic activity to support efficient nutrient cycling and maximize crop productivity under sustainable soil management practices

4. Discussion

The evaluation of proximate composition, fibre fractions, forage quality indices, mineral content, and in vitro gas production is crucial in understanding the nutritional value and digestibility potential of forage crops like *Brachiaria ruziziensis*. These indices play a fundamental role in determining the suitability of forages in ruminant diets, as they directly influence animal performance, feed intake, digestibility, and overall productivity. For instance, parameters like dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and metabolizable energy (ME) provide insights into the balance between energy, protein, and fibre necessary for efficient livestock nutrition.

In this study, the application of nitrogen fertilizer at varying rates and harvest age significantly influenced the nutritional and fermentative profiles of *Brachiaria ruziziensis* (Tables 3 and 4). The results revealed that fertilizer rate and age at harvest had pronounced effects on most proximate and fibre quality indices. Plants fertilized with 120 kg N ha^{-1} and harvested at 8 weeks after planting (WAP) exhibited higher dry matter (DM), crude fibre (CF), and non-fibre carbohydrate (NFC) content. These improvements may be attributed to the enhanced nitrogen availability that supports vegetative growth and cellular expansion, resulting in greater biomass accumulation

(Wang et al., 2025). Previous research by Akinyode et al. (2021) and Sobande et al. (2024) also demonstrated that moderate nitrogen fertilization and optimal harvest age significantly enhance biomass yield and dry matter content, corroborating the current findings.

The crude protein (CP) and ash content peaked under the 240 kg N ha^{-1} treatment harvested at 8 WAP. This is likely because higher nitrogen levels stimulate nitrogen assimilation and protein synthesis, especially in actively growing young tissues (Wang et al., 2025). Moreover, ash content, which reflects the inorganic mineral content of the forage, was significantly higher at early harvests under high nitrogen conditions. According to Aguiar et al. (2014), the uptake and accumulation of inorganic elements, influenced by nitrogen fertilization, directly contribute to increased ash content. This trend also aligns with the findings of Izydorczyk et al. (2024), who suggested that older plants accumulate structural compounds like cellulose and lignin, thereby increasing mineral deposition and ash levels in maturing tissues.

Interestingly, the ether extract (EE) and ME were higher in the unfertilized control, which might reflect a shift in carbon allocation due to nitrogen deficiency. Under low nitrogen conditions, plants redirect assimilated carbon toward the synthesis of energy-rich lipids and non-structural carbohydrates, as posited by the carbon–nutrient balance theory (Adamczyk et al., 2010). This theory suggests that in the absence of sufficient nitrogen, plants accumulate secondary metabolites and storage compounds rather than proteins. Similar observations were reported by Zayed et al. (2023), indicating that nitrogen scarcity restricts protein synthesis, thereby enhancing the accumulation of carbohydrates and lipids.

The fibre fractions also varied significantly, particularly in acid detergent fibre (ADF), hemicellulose (HEMI), and cellulose content. ADF and cellulose content increased under high nitrogen application (240 kg N ha^{-1}), supporting the notion that nitrogen promotes cell wall thickening and structural integrity. Liu et al. (2016) reported that balanced nitrogen fertilization enhances plant vigour, which accelerates cell division and strengthens cell walls via increased cellulose synthesis. This increase in cellulose is a compensatory mechanism to support taller and structurally denser plant tissues under rapid growth. As Hemati et al. (2022) observed, ageing tissues tend to lignify more, making cellulose and lignin cross-link, which further increases ADF and decreases digestibility.

Although RFV and cell content (CC) did not differ significantly across treatments, other forage quality indices such as dry matter digestibility (DDM), total digestible nutrients (TDN), and net energy parameters (NEI, Neg, and NEM) showed considerable variation. The highest feed value indices were observed under 120 kg N ha^{-1} at 8 WAP, indicating a balance between digestible nutrients and manageable fibre levels. Kamalak et al. (2010) similarly reported that RFV, DDM, and dry matter intake (DMI) decrease as cell wall components increase, especially lignin and ADF. Thus, optimizing harvest age at earlier stages and using moderate nitrogen enhances feed quality without excessively increasing structural carbohydrates.

Mineral composition was also significantly affected by both nitrogen fertilization and harvest age, except for zinc (Table 6). Younger plants (8 WAP) generally had higher concentrations of macro and micro minerals, including phosphorus, calcium, potassium, and manganese. As plants mature, these nutrients are often redistributed toward reproductive structures or stored in roots, leading to decreased leaf concentrations, as reported by Tilahun et al. (2017). The result from this study aligns with Akinyode et al. (2021) and Sobande et al. (2024), who documented higher mineral content in younger *Brachiaria ruziziensis* plants. Moreover, Zhang et al. (2007) emphasized that NPK fertilizers enhance mineral uptake by promoting root activity and metabolic processes, thereby improving forage nutritive value.

In vitro gas production and post-incubation indices provided further insight into the fermentability and energy availability of *Brachiaria ruziziensis* forage. The highest values of gas production at 24 and 48 hours, as well as organic matter digestibility (OMD), short-chain fatty acids (SCFA), metabolizable energy (ME), and net energy for lactation (NEL), were obtained under 240 kg N ha^{-1} at 10 WAP. This suggests that higher nitrogen levels delay lignification and increase fermentable substrates, enhancing microbial fermentation in the rumen. Murthy et al. (2020) and Costa et al. (2024) found similar trends, where nitrogen-enriched and younger forages yielded higher digestibility and fermentation potential due to increased soluble cell contents and reduced fibre-lignin complexes.

However, the lowest fermentation parameters were noted under 120 kg N ha^{-1} at 10 WAP, indicating that intermediate nitrogen levels may not sufficiently enhance metabolic processes at later stages. According to Batista et al. (2014), nitrogen application improves digestibility by reducing lignin synthesis and increasing rumen-accessible nutrients. Sobande et al. (2024) also pointed out that standard nitrogen applications influence the structural–nonstructural carbohydrate ratio, affecting fermentation kinetics and microbial activity. Thus, optimizing both nitrogen input and harvest timing is crucial for maximizing forage fermentability and energy efficiency in livestock feeding systems.

Overall, the results of this study underscore the significant influence of nitrogen fertilizer rates and harvest timing on the nutritional, mineral, and fermentation profiles of *Brachiaria ruziziensis*. Appropriate nitrogen management, particularly at 120 to 240 kg N ha^{-1}, and timely harvesting at 8 to 10 weeks can substantially improve the overall feed value, making it a viable strategy to boost forage quality and animal productivity under tropical conditions.

Furthermore, the Pearson’s correlation heatmap analysis reinforces these findings by elucidating strong interrelationships among soil biochemical traits, enzyme activities, and plant productivity indicators (Figure 2). Positive correlations between grain yield, dry matter, soil organic carbon (SOC), and nitrogen uptake suggest that enhanced soil fertility directly translates into higher biomass and nutritional quality (Holub et al., 2020). Conversely, significant negative associations between oxidative enzymes (such as catalase and phenoloxidase) and key yield parameters indicate that elevated oxidative stress may suppress plant growth and nutrient assimilation (Hasanuzzaman et al., 2020). These correlations support the conclusion that maintaining microbial balance, soil organic matter, and enzymatic homeostasis is crucial for achieving optimal forage performance under varying nitrogen regimes and harvest schedules (Suvendran et al., 2024).

5. Conclusion

Brachiaria ruziziensis, a widely adapted tropical forage, plays a crucial role in livestock nutrition. However, optimizing its nutritive quality requires effective agronomic practices, particularly appropriate fertilizer application and harvest timing. This study evaluated the combined effects of NPK 15:15:15 at 120 and 240 kg N ha^{-1} and harvest ages of 8 and 10 weeks after planting (WAP) on its nutritional composition. Findings revealed that applying 120 kg N ha^{-1} and harvesting at 8 WAP significantly improved the forage's proximate composition, including dry matter (DM), crude fiber (CF), and non-fiber carbohydrates (NFC). Additionally, key forage quality indices, such as dry matter digestibility (DDM), total digestible nutrients (TDN), and net energy values (NEI, Neg, NEM), were enhanced. These improvements suggest increased feed value, digestibility, and nutrient density. The combination also supported better plant vigor and cell wall development, likely due to enhanced metabolic activity and structural reinforcement. Thus, moderate fertilization with timely harvest can sustainably improve the forage quality of *Brachiaria ruziziensis* for enhanced livestock performance.

Author Contribution

Conceptualization: Pro. Olanite Jimoh Alao, Formal Analysis: Sobande Olorunsogo Ariyo, Writing-Original Draft Preparation: Sobande Olorunsogo Ariyo, Writing-Review and Editing: Sobande Olorunsogo Ariyo, Adebowale Sadiat, & Odebode Tomisin Esther, Data Curation: Prof. Ojo Victoria Olubunmi Aderemi & Dr. Adeoye Samson Adewale, Investigation: Sobande Olorunsogo Ariyo, Adebowale Sadiat, & Odebode Tomisin Esther, Supervision: Prof. Olanite Jimoh Alao, Prof. Ojo Victoria Olubunmi Aderemi, & Dr. Amisu Ahmed Adeyemi, Project Administration: Sobande Olorunsogo Ariyo & Dr. Amisu Ahmed Adeyemi. Visualization: Sobande Olorunsogo Ariyo. All authors have read and agreed to the published version of the manuscript.

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