

EVALUATION OF PHYSICO-CHEMICAL PROPERTIES AND FERMENTATIVE CHARACTERISTICS OF GRASS-LEGUME SILAGE FERMENTED WITH TWO STRAINS OF LACTIC ACID BACTERIA

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Abstract

This study aimed to evaluate the physico-chemical properties and fermentation quality of silage produced using two strains of lactic acid bacteria (LAB). The silage was formulated with 60% *Pennisetum pedicellatum*, 30% *Mucuna pruriens*, and 10% cassava peel. Four treatments were assessed: T₁ (control, no LAB), T₂ (inoculated with *Lactobacillus plantarum*), T₃ (inoculated with *Lactobacillus acidophilus*), and T₄ (inoculated with both LAB strains). Ensiling was done using standard procedures, and the silages were stored for 30 days under anaerobic conditions. Physical attributes including colour, odour, and texture were examined. Chemical analyses were conducted to determine pH, proximate composition, fibre fractions, and fermentative profiles. Crude protein content ranged from 11.86% to 17.51% across treatments, with no significant difference ($p > 0.05$). Crude fibre content was highest in T₁ (16.61%) and decreased progressively in T₂ (15.63%), T₃ (15.18%), and T₄ (14.91%). The highest acid detergent fibre (ADF) was recorded in T₁ (28.45%), while T₄ had the lowest (20.55%). All silages maintained pH values within the optimal range (4.40–5.10) for effective fermentation. Silage (T₂) showed the most favourable physical characteristics, potentially enhancing feed palatability and digestibility. Fermentation products differed significantly across treatments, with the exception of ammonia nitrogen (NH₃-N), which remained constant. The highest butyric acid content (0.45 mg/g) was observed in T₁, while T₄ recorded the highest levels of acetic acid (4.45 mg/g), propionic acid (0.29 mg/g), and total volatile fatty acids, reflecting superior fermentation quality with LAB inoculation.

Keywords: Fermentation quality, Lactic acid bacteria. *Mucuna pruriens*, *Pennisetum pedicellatum*. Silage

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Introduction

The increasing demand for sustainable livestock production in the tropics necessitates

the efficient utilization and preservation of locally available forages. Among the strategies for conserving seasonal forages is silage

production. Silage production has gained recognition due to its ability to maintain nutritional quality and prolong the usability of fresh forage beyond the harvest period. Silage making is an important tool for farmers in preservation of surplus feed during the wet seasoning ensuring all year round availability of feed (Ibhaze *et al.*, 2015). In the rainy season, there is an abundance of grass, while it becomes scarce in the dry season, and therefore, supplementing feed for ruminants in the dry scarcity periods is a necessity. The integration of legumes and grasses in silage production produce high quality silage with better physical and chemical quality. It improves the protein content and also balances the carbohydrate levels, creating a more complete and digestible feed for livestock (Hanum, 2021). The combination of leguminous and grass crops in forage systems has become an increasingly important practice to improve the nutritional quality of silage and enhance livestock productivity. Fermentation is a critical process in silage production, influencing the digestibility and palatability of the feed. The primary products of fermentation are lactic acid, acetic acid, and some ethanol. The balance of these acids affects the stability, taste, and nutritional quality of the silage (Muck, 2023). The production of organic acids, primarily lactic acid, lowers the pH and enhances both the palatability and digestibility of the silage. The objective of this study is to evaluate the nutritional potential of Lactic Acid Bacteria (LAB) strains on silage quality produced from grass-legume mixture. The physico-chemical attributes of silage such as pH, dry matter, crude protein, fiber content, and organic acid concentration are crucial indicators of fermentation success and feed value. Likewise, the profiles of fermentative products including lactic, acetic, and butyric acids directly influence the nutritive quality, aerobic stability, and acceptability of silage in ruminants (Kung and Shaver, 2001; Danner *et al.*, 2003). Therefore, evaluating these parameters provides a comprehensive

understanding of how microbial inoculants and forage composition affect silage quality. This study was conducted to assess the physico-chemical characteristics and fermentative profiles of silage produced from a combination of tropical grass and legume, fermented with two strains of lactic acid bacteria (*L. plantarum* and *L. acidophilus*). The objective was to determine the impact of microbial inoculation on fermentation dynamics and nutrient preservation in mixed silages, with implications for improving feed availability and quality in ruminant production systems.

Materials and Methods

The experiment was carried out at Pasture Demonstration Plot and Small Ruminant Section of Teaching and Research Farm of the Oyo State College of Agriculture and Technology, Igbo-ora. The region is on latitude 7°43' North and 3°29' East, South West, Nigeria. It is located at an elevation of 17meters above sea level (World atlas, 2015). The rainy season extends between April and November and the dry season from December to March.

Procurement of Lactic acid bacteria

Lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus plantarium*) were purchased from reputable commercial laboratory, sub-cultured and characterization was done at the Microbiology Laboratory of Oyo State College of Agriculture and Technology Igbo-ora.

Harvesting, processing of forages and silage production

The *Mucunapruriens* and *Pennisetum pedicellatum* forages were harvested from an established Pasture Demonstration Plot of Oyo State College of Agriculture and Technology, Igbo-ora. The forages were cut using knife at a height of 30cm above the ground level before flowering stage to allow for good re-growth. The

harvested forage was wilted for 4 hours. Harvested forages were chopped using cutlass and a wood log. The chopped forage was compressed in ratio 30% *Mucuna pruriens*, 60% *Pennisetum pedicellatum* and 10% cassava peel. Liquid inoculants of two strains of lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus plantarium*) of 10^6 CFU/g were inoculated at 10ml/kg weight of chopped forage as additives. The forage was compressed to ensure utmost anaerobic condition. The polythene bag was sealed with masking tape and later covered with big polythene nylon to provide warmth. After four (4) weeks the silage was removed from the silo for physical characteristic evaluation and P^H value determination.

Testing of silage quality

pH of the silage was determined using a pH meter. The smell and the texture of the silages was adjudged by four (4) different individuals while the colour of the silages was determined using a colour comparator device.

Data collection and laboratory analysis

Chemical analysis of the silage

The chemical composition analysis of the silage was carried out at the laboratory of the College of Animal Science and Livestock Production, Federal University of Agriculture Abeokuta (FUNAAB). The dry matter, crude protein, ether extract and ash contents of the milled silage samples was determined according to AOAC. (2000). Non-fibre carbohydrate (NFC) was calculated as $NFC = 100 - (CP + Ash + EE + NDF)$. The Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) of the milled silage was determined using the procedure described by Van Soest *et al.* (1991). Cellulose content was taken as the difference between ADF and ADL while hemicellulose content was calculated as the difference between NDF and ADF.

Experimental Diet Preparation

Four different silages were prepared with

- Mucuna pruriens*-*pennisetum pedicellatum* cure with cassava peel.
- T₁ – 60% *Pennisetum pedicellatum* forage, 30% *Mucuna pruriens* forage and 10% cassava peel without LAB (Negative control).
- T₂ – 60% *Pennisetum pedicellatum* forage, 30% *Mucuna pruriens* forage and 10% cassava peel fermented with *Lactobacillus plantarium* bacteria strain.
- T₃ – 60% *Pennisetum pedicellatum* forage, 30% *Mucuna pruriens* forage and 10% cassava peel with *Lactobacillus acidophilus* bacteria strain.
- T₄ – 60% *Pennisetum pedicellatum* forage, 30% *Mucuna pruriens* forage and 10% cassava peel fermented with cocktail of *Lactobacillus plantarium* bacteria strain and *Lactobacillus acidophilus* bacteria strain.

Results and discussion

Results

Table 1 shows the results of physical characteristics of *Pennisetum pedicellatum* and *Mucuna pruriens* silage fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarium* cured with cassava peel. The result indicates that colour of the silage range from light brown to brownish green. T₁ silage without Lactic Acid Bacteria (LAB) had a light brown colour with a slightly pleasant smell, T₂ silage inoculated with *Lactobacillus plantarium* as additive had a brownish green colour and a pleasant smell, T₃ silage that was inoculated with *Lactobacillus acidophilus* as additive and T₄ that was inoculated with cocktail of the two strains of LAB had pleasant odour with light brown and greenish brown colour respectively. Texture across all treatments was firm, with T₁ and T₂ being noticeably moist or wet. Specifically, T₁ was described as firm and wet, T₂ as firm and moist, while T₃ and T₄ were simply firm. Regarding moldiness, slight surface mould

was observed in T₁, whereas no mold was found in Treatments 2, 3, and 4.

Table 2 shows the results of chemical composition of four different silages. The pH values ranged from 4.40 to 5.10. The rapid decline in silage pH during fermentation is a hallmark of effective silage production. The pH values of the silages was highest in treatment 1(T₁) with Treatments 2 (T₂) and 3 (T₃) exhibiting the lowest pH (4.40), indicating better fermentation, though differences were not statistically significant (P>0.19). Dry matter content showed significant variation (P<0.00), with T₂ having the highest percentage (90.56%) and T₃ had the lowest (88.44%). Crude protein was significantly higher (P< 0.05) in T₄ (17.51%) and T₂ (15.74%) though with different numerical values compared to T₁ (11.86%) and T₃ (12.25%), indicating improved nutritional quality in the former treatments. Crude fibre content decreased significantly (P<0.05) across treatments, with T₁ showing the highest value (16.61%) and T₄ the lowest (14.91%). Ash content also varied significantly (P<0.02), highest in T₂ (8.36%) and lowest in T₁ (7.33%). Ether Extract values did not differ significantly (P>0.51), ranging from 10.17% to 11.61%. The non-fibre carbohydrate (NFC) was highest in T₂ (4.00%) and lowest in T₁ (1.66%), but this difference was not significant (P>0.40). Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) were significantly lower in T₄ (20.55% and 59.42%, respectively) compared to other treatments (P<0.00), indicating better digestibility. Acid detergent lignin (ADL) did

not differ statistically across treatments (P>0.25). Cellulose content was highest in T₂ (17.75%) and lowest in T₄ (10.25%), showing a significant difference (P<0.00). Hemicellulose content also varied significantly (P<0.06), with T₃ recording the highest value (40.84%) and T₂ the lowest (38.17%).

The results of fermentative products of silage fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarium* is shown in table 3. Ammonia nitrogen (NH₃N) levels was highest in T₁ (6.80 mg/dl) and lowest in T₂, T₃, and T₄ with the same value of 4.25 mg/dl, though these differences were not statistically significant (P > 0.33), suggesting similar protein degradation across treatments. Total volatile fatty acids (TVFA) increased significantly among treatments (P < 0.05), with T₄ recording the highest value (6.67 mg/g) and T₁ the lowest (4.55 mg/g), indicating enhanced fermentation quality with LAB treatments, particularly in T₄. Acetic acid concentrations ranged from 3.03 mg/g in T₁ to 4.45 mg/g in T₄, with significant differences observed (P < 0.05). Treatments T₃ and T₄ had higher levels, suggesting more efficient fermentation processes. Butyric acid levels decreases across treatments (P < 0.05). T₁ had the highest value (0.45 mg/g), while T₄ had the lowest (0.31 mg/g), indicating reduced undesirable fermentation in LAB-treated silages. Propionic acid also varied significantly (P< 0.05) across the treatment, increasing from 0.20 mg/g in T₁ to 0.29 mg/g in T₄, which further supports improved fermentative quality with LAB inoculation.

Table 1: Physical characteristic of *Mucun apruriens* +*Pennicetum pedisellatum* sillages fermented with two strains of LAB.

Parameters	T1	T2	T3	T4
Colour	Light brown	Brownish green	Light brown	Brownish green
Odour/Smell	Slightly Pleasant	Pleasant	Pleasant	Pleasant
Texture	Firm and wet	Firm and moist	Firm	Firm
Moldiness	Surface slight mouldy	No mould	No mould	No mould

The physical characteristics of the *Mucuna pruriens* and *Pennisetum pedicellatum* silages fermented with two strains of lactic acid bacteria (LAB) showed slight but noteworthy differences across treatments. The variation in colour, light brown in T₁, T₃ and brownish-green in T₂ and T₄ suggests differences in fermentation quality, possibly linked to microbial activity and the specific LAB strains used. A brownish-green colour, as seen in T₂ and T₄, is often considered an indicator of optimal fermentation, indicating better preservation of the original forage material. This aligns with reports by Ogunbanwo *et al.* (2023), who noted that greenish coloration in silage implies minimal oxidative degradation and more efficient LAB fermentation. Brownish green colour (T₂ and T₄) may indicate better preservation of chlorophyll and less oxidation, which often correlates with enhanced lactic acid fermentation (Kung *et al.*, 2018). In terms of odour, all treatments except T₁ exhibited a pleasant smell, indicating successful fermentation. T₁ had only a slightly pleasant odour, coupled with the presence of surface mould, suggests suboptimal fermentation. According to Zhang *et al.* (2022), a pleasant silage smell often corresponds with dominance of *Lactobacillus plantarum* or *L. acidophilus*, which rapidly reduces pH and inhibits undesirable microbes.

The slightly pleasant odour in T₁ might reflect slower fermentation or less dominant LAB activity. The firm texture across all treatments indicates good structural integrity of the silages, but the moisture content varied. T₁ was described as firm and wet, while T₂ was firm and moist, potentially indicating higher water content, which might enhance fermentation but also raises the risk of spoilage if not properly managed. Firm and moist textures are desirable and signal that the ensiling process preserved the biomass without excessive decomposition or spoilage, which is consistent with findings by Muhammad *et al.* (2021), who emphasized the importance of adequate moisture and compaction for optimal LAB performance. Mould was only observed on the surface of T₁, while the other treatments remained mould-free. This further supports the idea that T₁ had inferior fermentation conditions, possibly due to higher moisture and non inoculation with LAB. These findings support the observations by Ogunade *et al.* (2016), who stated that effective LAB inoculation can significantly reduce mould formation by creating acidic environments that suppress spoilage organisms. As emphasized by Kim *et al.* (2020), mould development is minimized when rapid acidification (pH < 4.2) is achieved within the first few days of ensiling, a task effectively performed by homo-fermentative LAB like *L. plantarum*.

Table 2: Chemical composition and fibre fractions of *Mucuna pruriens*+*Pennisetum pedicellatum* silage cured with cassava peel fermented with two strains of LAB

Parameters	T1	T2	T3	T4	SEM	P-Value
Ph	5.10	4.40	4.40	4.50	0.31	0.19
Dry matter (%)	90.14 ^a	90.56 ^a	88.44 ^b	89.13 ^b	0.32	0.01
Crude protein (%)	11.86	15.74	12.25	17.51	1.11	0.15
Crude fibre (%)	16.61 ^a	15.63 ^{ab}	15.18 ^b	14.91 ^b	0.26	0.05
Ash (%)	7.33 ^b	8.36 ^a	7.79 ^{ab}	7.8 ^{ab}	0.15	0.03
EE	10.17	10.59	10.96	11.61	0.52	0.95
NFC	1.66	4.00	2.46	2.99	0.43	0.32
ADF	28.45 ^a	26.90 ^a	23.25 ^{ab}	20.55 ^b	1.20	0.00
NDF	64.84 ^a	64.57 ^a	64.32 ^a	60.10 ^b	0.75	0.04
ADL	10.45	8.95	10.70	10.30	0.44	0.25
Cellulose	17.02 ^a	17.75 ^a	12.55 ^b	10.25 ^b	1.26	0.02
Hemicellulose	36.35 ^b	38.17 ^{ab}	40.84 ^a	39.55 ^{ab}	0.70	0.06

^{a b c d} Means on the same row with different superscript are significantly different ($P < 0.05$). DM= Dry Matter, CP= Crude Protein, CF= Crude Fibre, EE= Ether Extract, NFE= Nitrogen Free Extract, NDF= Neutral detergent fibre, ADF= Acid detergent fibre, ADL= Acid detergent lignin, NFC= Non Fibre Carbohydrate.

The chemical composition of the silage reveals significant variations across treatments, highlighting the influence of microbial action on silage quality. pH values ranged from 4.40 to 5.10, with the lowest values observed in T₂ and T₃, indicating effective fermentation due to rapid acidification. The result aligned with the findings of Zhang *et al.* (2022), that optimal silage fermentation is characterized by pH values below 4.5, which inhibit the growth of spoilage organisms and preserve nutrients. Dry matter (DM) content ranged from **88.44% to 90.56%**, with T₂ having the highest DM. It shows that cassava peels enhanced DM retention. These improvements contributed to increased dry matter intake (DMI), enhanced nutrient retention, and better nitrogen utilization in the West African dwarf growing bucks. Cassava peel is a known absorbent and fermentable additive, improving silage dryness and sugar content (Ezeokeke *et al.*, 2022). From the results of this finding, Crude protein (CP) was significantly higher in T₂ (15.74%) and T₄ (17.51%) compared to T₁ and T₃, indicating that the combination of cassava peel and LAB enhanced nitrogen retention. Similar findings were reported by Yahaya *et al.* (2023), who observed improved DM content in cassava peel-cured silages inoculated with LAB. This also agrees with the findings of Ni *et al.* (2017), who reported increased CP in silages inoculated with *Lactobacillus plantarum*, due to reduced proteolysis and ammonia-N loss.

Crude fibre (CF) content decreased progressively from T₁ (16.61%) to T₄ (14.91%), showing significant differences ($P < 0.05$), a trend that reflects improved digestibility. Lower fibre values are desirable in ruminant diets as they enhance nutrient utilization. Reduced CF in T₃ and T₄ supporting the hypothesis that LAB

fermentation promotes **partial fibre degradation**. This is beneficial for ruminant digestibility. Lower CF values are desirable as they enhance energy availability (Muck *et al.*, 2018). Ash content recorded in this finding also suggests better mineral preservation or concentration, potentially due to lower leaching losses. LAB strains have been shown to improve fibre digestibility by promoting acid hydrolysis and enzymatic activities during ensiling (Kim *et al.*, 2020). The fibre fractions showed notable differences, especially in ADF and NDF, which were significantly lower. Lower fibre fractions are favorable for ruminant intake and energy availability, as highlighted by Khan *et al.* (2022), who emphasized the importance of reducing ADF and NDF in tropical forage silages to improve animal performance. Hemicellulose was highest in T₃ (40.84%), which could influence the slow-release energy potential of the feed. Treatments T₂ and T₄ displayed superior chemical compositions, particularly in CP and fibre digestibility indices, reflecting more efficient fermentation and nutrient preservation. These results affirm that silage quality is significantly enhanced by the combined use of cassava peel and LAB inoculants, echoing previous research findings by Chen *et al.* (2020), which stress the role of inoculants in improving silage nutritive value. Higher NFC (Non fibre carbohydrate) values provide fermentable energy to support microbial growth and fermentation. The use of cassava peels, rich in starch and sugars, may have contributed to this trend, as supported by Adesokan *et al.* (2023). The fermentative products of silage fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarium* demonstrated notable differences across treatments, indicating the influence of LAB strains on fermentation quality,

suggesting that protein breakdown was similar across treatments. This finding is consistent with the work by Zhang *et al.* (2024) found that the addition of *L. plantarium* significantly reduced ammonia nitrogen (NH₃-N) content and pH levels compared to the control group, indicating improved fermentation quality. These findings suggest that while *L. plantarium* can enhance fermentation, the extent of protein breakdown, as indicated by NH₃-N levels, may not always vary

significantly with different LAB strains, especially under conditions where protein degradation is minimal. Lower NH₃-N levels in this finding reflect better nitrogen preservation and reduced proteolysis, a desirable outcome of silage fermentation (Xu *et al.*, 2022). LAB inoculants are known to suppress clostridial activity and proteolytic bacteria, thereby reducing NH₃-N formation (Li *et al.*, 2023).

Table 3: Fermentative products of silage fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarium*

Parameters	T1	T2	T3	T4	SEM	P-value
NH ₃ N(mg/dl)	6.80	4.25	4.25	4.25	1.69	0.33
TVFA(mg ⁻¹)	4.55 ^b	5.52 ^{ab}	5.98 ^a	6.67 ^a	0.32	0.05
ACET(mg ⁻¹)	3.03 ^b	3.68 ^{ab}	3.99 ^a	4.45 ^a	0.61	0.05
BUT(mg ⁻¹)	0.45 ^a	0.37 ^{ab}	0.39 ^a	0.31 ^b	0.06	0.05
PRO(mg ⁻¹)	0.20 ^b	0.24 ^{ab}	0.26 ^a	0.29 ^a	0.04	0.05

TVFA: Total Volatile Fatty Acid, ACET: Acetic Acid, BUT: Butyric Acid, PRO: Propionic Acid

Total volatile fatty acids (TVFA), which are a key indicator of fermentation quality, increased significantly across treatments ($P < 0.05$). T₄ recorded the highest TVFA value (6.67 mg/g), while T₁ had the lowest (4.55 mg/g), indicating enhanced fermentation efficiency with LAB inoculation, particularly in T₄. This aligns with the findings of who reported that LAB inoculation leads to a higher TVFA content, a reflection of improved fermentation. TVFA is a sign of active microbial metabolism, particularly from hetero-fermentative LAB, which enhances the preservation qualities of silage (Yang *et al.*, 2022). Acetic acid concentrations, which are a direct product of LAB fermentation, also varied significantly, ranging from 3.03 mg/g in T₁ to 4.45 mg/g in T₄. The higher acetic acid levels in T₃ and T₄ suggest more efficient fermentation, which is consistent with the results reported by Chen *et al.* (2020), where increased acetic acid production was associated with better fermentation quality and nutrient preservation. This is also in agreement with the findings of Chen *et al.*; (2023) who

reported that silages treated with *L. plantarium*, is known to increase acetic acid concentration. Butyric acid levels, a marker of undesirable fermentation, followed a decreasing trend across treatments, with the highest value recorded in T₁ (0.45 mg/g) and the lowest in T₄ (0.31 mg/g). The significant reduction in butyric acid levels in LAB-treated silages highlights the suppression of undesirable fermentation, aligning with the observations of effective reduction of butyric acid through LAB application that has been documented in several recent studies, confirming their role in improving silage safety (Zhang *et al.*, 2024). Propionic acid also varied significantly ($P < 0.05$), increasing from 0.20 mg/g in T₁ to 0.29 mg/g in T₄. This further supports the idea that LAB inoculation improves fermentation quality, as propionic acid is often associated with more stable and higher-quality silage (Kung *et al.*, 2003). The findings suggest that the use of LAB strains, particularly *Lactobacillus acidophilus* and *Lactobacillus plantarium*, significantly enhances the fermentation process, resulting in improved

silage quality. These results align with the findings of previous studies and highlight the importance of LAB in optimizing silage fermentation and improving its nutritional value. This is also in consistent with the findings of *Li et al.* (2023), who noted that combinations of LAB can shift fermentation pathways to favor beneficial acids

Conclusion

Silage fermented with LAB improved the physical, chemical, and nutritional quality of the feed. Treatments with incorporated LAB inoculants, enhanced superior fermentation characteristics, optimal pH levels, enhanced crude protein content, with moderate level of crude fibre, and better forage preservation. These improvements contributed to increased dry matter intake (DMI), enhanced nutrient retention, and better nitrogen utilization in the West African dwarf growing bucks. Silage fermentation also influenced rumen metabolites. LAB-treated silages, particularly goats fed diets T₃ and T₄, resulted in increased total volatile fatty acids and acetic acid levels, alongside reduced butyric acid highlighting better fermentation efficiency. Though rumen microbial populations and end-products such as ammonia nitrogen (NH₃-N) and total volatile fatty acids (TVFA) did not differ across treatments, the results of the study indicated that T₄ diet, with its higher crude protein and lower crude fiber contents, could provide superior nutritional benefits for ruminant animals. Improved silage quality can enhance feed intake, digestibility, and animal performance.

Recommendations

Future studies should explore the use of other lactic acid bacteria strains and their synergistic effects on silage fermentation and animal performance. Additionally, trials to evaluate the performance and digestibility of silage in animals should be conducted to assess its practical application.

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