EVALUATION OF PHYSIO-CHEMICAL PROPERTIES OF GRASS-LEGUME SILAGE FERMENTED WITH TWO STRAINS OF LACTIC ACID BACTERIA

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ABSTRACT
This study was carried out to investigate the physio-chemical composition of silage fermented with two strains of Lactic Acid Bacteria (LAB). The silage was prepared as 30% Mucuna pruriens,60% Pennisetum pedicellatum and 10% cassava peel. T_1 with LAB as additive, T_2 inoculated with Lactobacillus planetarium, T_3 inoculated with Lactobacillus acidophilus and T_4 inoculated with cocktail of the LAB. Silages were prepared using standard ensiling methods and stored for 30 days before conducting analyses of pH, proximate and fibre fraction contents composition, and physical characteristics like the colour, odour and texture. Results indicated no significant differences ($p>0.05$) in crude protein whose content ranges within 11.86 - 17.51%. In terms of fiber content, T_1 diet had the highest value of crude fibre (16.61%), with 15.63%, 15.18%, and 14.91% recoded in T_2, T_3 and T_4, respectively. Highest value (28.45%) of ADF was recorded in diet T_1 while the least value for ADF (20.55%) was noted in diet T_4 silage. The pH values of all treatments were within the acceptable range for successful fermentation (4.40–5.10). From this study, diet T_2 has the best physical characteristic that can aid feed intake and nutrient digestibility of the ruminant animals. However, other treatments also show acceptable physical characteristics that can aid feed consumption. The results of the study revealed that physio-chemical characteristics of compounded grass-legume silage enhance its physical characteristics and nutritional value of the silage as feed resources for tropical livestock production.
Keywords: Silage, Physio-chemical, *Mucuna pruriens, Pennisetum pedicellatum,* Additives

INTRODUCTION

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 Nigeria, the long dry season characterized by animal feed shortages (both in quantity and quality) and poor nutritional value of forages is a major factor limiting their sustainable growth and productivity. 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.

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 $7^0$43' North and $3^0$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*) was purchased from reputable commercial laboratory, sub- cultured and characterization was done at the micro biology laboratory of Oyo State College of Agriculture and Technology Igbo-ora

Harvesting, processing of forages and silage production

The *Mucuna pruriens* and *Penisetum pedicellatum* forages was 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 will be wilted for 4 hours. Harvested forages were chopped using cutlass and a wood log. The chopped forage was compressed in ratio 30*% Mucuna pruiens,60% Penisetum pedicellatum* and 10% cassava

peel. Liquid inoculants of two strains of Lactic acid bacteria (*Lactobacillus acidophilus* and *Lactobacillus plantarium*) of 10^6CFU/g was 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 a big polythene nylon to provide warmth. After 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 was analyzed at the laboratory of the College of Animal science, Federal University of Agriculture Abeokuta (FUNAB). The dry matter, crude protein, ether extract and ash contents of the milled silage samples was determined according to A.O.A.C. (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 silage was prepared with *Mucuna pruriens, penicetum pedicellatum* cure with cassava peel into four dietary treatments

T_1 – 60% *Penicetum pedicellatum* forage, 30% *Mucuna pruriens* forage and 10% cassava peel without LAB (Negative control)

T_2 – 60% *Penicetum pedicellatum* forage, 30% *Mucuna pruriens* forage and 10% cassava peel fermented with *Lactobacillus plantarium* bacteria strain

T_3 -60% *Penicetum pedicellatum* forage,30 *Mucuna pruriens* forage and 10% cassava peel with *Lactobacillus acidophilus* bacteria strain

T_4 -60% *Penicetum 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

Table 1: Physical characteristic of Mucuna *pruriens* +*Penicetum 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 mist	Firm	Firm
Moldiness	Surface slight mould	No mould	No mould	No mould

Table 2: Chemical composition and fibre fractions of *Mucuna pruriens*+*Penicetum pedisellatum* 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

RESULTS AND DISCUSSION

The result in table 1 indicated that color of the silage range from light brown to brownish green. T_1 silage without Lactic Acid Bacteria (LAB) has a light brown colour with a slightly pleasant smell, T_2 silage that has *Lactobacillus plantarum* as additive has a brownish green colour and have a pleasant smell, T_3 silage that has *Lactobacillus acidophilus* as additive and T_4 that has cocktail of the two strains of LAB has pleasant odour with light brown and greenish brown colour respectively. The texture of the silage in T_1 was firm and mist with slight mould on the surface while that of the T_2 was firm and wet with no mould and T_3 and T_4 silage was firm without mould. A study by Zhao *et al.* (2022) found that LAB fermentation helps retain the natural pigments in silage, such as chlorophyll, by reducing oxidative degradation. Dark brown or black silage is often associated with spoilage or overheating, conditions effectively minimized by the controlled fermentation observed in this study. This results aligns with findings by Guo *et al.* (2021), who noted that silages fermented with *Lactobacillus plantarum* exhibited a fresh, lactic aroma due to the dominance of lactic acid and the suppression of foul-smelling compounds like butyric acid and ammonia. The colour, smell and texture of silage apparently improved with inclusion of cassava peel in the mixture. This confirms earlier reports that cassava meal or peel had positive influence on silage characteristics of tropical forages (Olorunnisomo, 2011). Table 2 shows the results of chemical composition of four different silages. The pH values ranged from 4.40 to 5.10. T_1 that contains no LAB as additive recorded the highest pH (5.10) which was not significantly different from T_2, T_3 and T_4. The rapid decline in silage pH during fermentation is a hallmark of effective silage production. A pH below 5.0 as observed in this study for silage with LAB as additive is critical for inhibiting spoilage microorganisms (Zhao *et al.,* 2022). The presence of *Lactobacillus acidophilus* and *Lactobacillus plantarum* facilitates a faster drop in pH, which aligns with results by Guo *et al.* (2021), where LAB-fermented silages achieved superior preservation due to a controlled acidic environment. The crude protein content (CP), was not significantly different across the treatments, ranges from 11.86 - 17.51%. Diets T_2, T_3 and T_4 has higher protein content compare with the control diet T_1. This result is consistent with the findings of Oduguwa *et al*. (2007), where LAB fermentation was shown to enhance the protein bioavailability of legume-based silages. The breakdown of complex proteins into simpler peptides by LAB enzymes facilitates better nutrient digestibility. Crude fiber (CF) content value ranged from 14.19 to 16.61% was recorded in this current study. Reduced CF contributes to improved digestibility; as higher fiber content can limit nutrient availability in ruminants. The findings agree with research by Zhao *et al.* (2023), which demonstrated reduced CF in silages inoculated with specific LAB strains. NDF content is the best indicator to predict folder quality as it related to intake potential of folder. The Acid Detergent Fiber (ADF) recorded in this study ranged from 20.55 – 28.45%, silage with less than 40% ADF is classified as high quality feed for ruminants as reported by (Rusdy, 2016.).

CONCLUSION

The results of the study indicated that T4 diet, with its higher crude protein and crude fiber contents, could provide superior nutritional benefits for ruminant animal production.

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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