EVALUATION OF ENSILING QUALITIES OF SIGNAL GRASS (*Brachiaria mulato* II) AND COOK STYLO (*Stylosanthes guianensis)* AT DIFFERENT PROPORTIONS

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Abstract

The study evaluated the ensiling qualities of *Stylosanthes guianensis* (Cook), grown with *Brachiaria mulato* II (Signal grass) at different mixing ratios.. The treatments were the mixtures of *Stylosanthes guianensis* and *Brachiaria mulato* II at 100:0, 75:25, 50:50, 25:75 and 0:100% ratios laid in a completely randomized design (CRD) and replicated three thrice. Fresh *Brachiaria mulato* II and *Stylosanthes guianensis* were harvested, wilted for 24 hours and chopped to 2-3cm long for ease of compaction and combination for silage. The research materials in the silos were sealed tightly and allowed for 28 days fermentation period. At expiration, the resultant silages were scored for colour and aroma by three independent scorers. The results of silage showed that irrespective of different mixing proportions of *Stylosanthes guianensis – Brachiaria mulato* II, the colours of the ensiled mixtures were yellowish - green with pleasant and very sweet aroma. The pH recorded was slightly acidic in all the treatment combinations with a temperature range of 24.6^0 – 29.6^0C. Crude protein (CP) and ether extract (EE) were not significantly ($p>0.05$) affected by treatment, although numerically higher CP values were observed in the 50:50 mixture. Fibre fractions (ADF and NDF), dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV) varied significantly ($p<0.05$) among treatments. The 50% Mulato II and 50% Stylo mixture produced silage with improved crude protein and better feeding value indices, suggesting its suitability as high-quality ruminant feed during periods of forage scarcity. The study therefore recommends integrating forage legumes such as Stylosanthes guianensis with tropical grasses during silage production to improve nutritional quality and feed utilization.

Keywords: Cook stylo, signal grass, silage, nutrients

Introduction

The major constraint to livestock production in developing countries such as Nigeria is the scarcity and fluctuating quantity and quality of all year-round feed supply (Kallah, *et al*., 1997; Melesse *et al.,* 2022). Forages are essential for the successful operation and highly profitable animal production ventures. This is more relevant to ruminants which rely heavily on forages for their health and production in a cost effective and sustainable manner (Addo, 2019). Forage and fodder crops are essential in the development of national livestock industry as cultivated fodder crops and other feed resources partake heavily in the contemporary animal agriculture (Rusdy, 2016). About 50-80 % of Nigerians are involved in crop, livestock or crop-livestock agriculture (Adesogan *et al.,* 2020). The quality and quantity of feed resources are the major constraints of increasing ruminant productivity under tropical set up (Addo, 2019). Grasses due to their adaptations are advantageous over other plant species as they have surface rooting system and can respond effectively to management strategies (Lüscher *et al.,* 2022). Grasses generally are typically known to have low crude protein content that cannot solely sustain ruminants throughout the year, hence the need for intercropping with forage legumes. One way of increasing the grazing resources of natural pasture is to integrate forage legumes into the pastures, with the aim of diversifying the sources of forage and at the same time increasing the amount of protein available for the grazing animals as well as increasing the nitrogen uptake of associated forage grass (Rao *et al.,* 2021).

The importance of forage legumes in increasing herbage production from grasses and to enhance the quality of feed produced has been recognized (Muinga *et al*., 2007; Melesse *et al*., 2022). The seasonal variation in weather and climate results in feed inadequacy in quantity and quality in the tropical region. An alternative for alleviating this challenge is through ensiling the excess forages produced during the rainy season. It permits forage storage in amounts and quality to be used for animal feeding during the dry season, and serves as an alternative for roughage supplementation during dry season (Lüscher *et al.,* 2022). However, tropical forages are susceptible to losses during ensilage due to their low buffering capacity, humidity higher than 70 % and low water - soluble carbohydrate (WSC) content (Balsalobre *et al*., 2001; Rao *et al.,* 2021). Silage can be defined as any plant material that has undergone fermentation or “pickling” in a silo. Silo is any storage structure in which green, moist forage is preserved (Kung et al., 2022). The primary goal of making silage is to maximize the preservation of original nutrients in the forage crop for feeding livestock at a later date in livestock feeding programs (Kung *et al.,* 2022). The first essential objective is to achieve anaerobic conditions under which natural fermentation can take place. In practice this is achieved by consolidating and compacting the material and the sealing of the silo to prevent re-entry of air. Air that is trapped in the herbage is rapidly removed by respiratory enzymes (Muck *et al*., 2020). Where oxygen is in contact with herbage for a period of time, aerobic microbial activity occurs and yeast would grow. This causes the material to decay to a useless, inedible and frequently toxic product (Muck *et al.*, 2020). Finer chopping of plant material results in improved compaction and fermentation of silage. This then improves palatability and intake of silage (Meeske, 2005).

The preservation of excess pasture during surplus in the form of silage is indispensable and this allows mitigating the feed deficit that could be encountered during the dry season. Ensiling is a potent general method for forage preservation and also a form of treatment to occasionally salvage the under-utilized pastures for better acceptability and degradability. The quality of silage is however, dependent on the quality of the crop at ensiling, type of fermentation, rate of pH decrease, moisture content of the crop and anaerobic conditions (Kung *et al.*, 2022). Silage making in the tropics is paramount if there will be an all year-round availability of forages for livestock. Fodder conservation is promoted with the main objective of ensuring feed availability during periods of feed limitation (Rao *et al.*, 2021). Silage quality depends on many factors such as plant species, implemented agro-technical measures and practices, preparation time, applied inoculants, especially stages of plant development in which it is used for the preparation of silage. McEniry *et al*. (2013) stated that the two most important pre-ensiling factors responsible for the quality of silage are plant species and stage of plant development at the time of harvest. This study therefore evaluated the ensiling qualities of *Stylosanthes guianensis* (Cook), grown with *Brachiaria mulato* II (Signal grass) at different mixing ratios.

Materials and methods

Experimental location, design and treatment

The experiment was conducted at the Animal Science Laboratory of the Faculty of Agriculture, Bayero University, Kano. The experimental area is located on latitude 11.97095^{o}N of the equator and longitude 8.42511^{o}E of the Greenwich meridien (GPS Coordinate, 2019). The treatments were mixtures of *Stylosanthes guianensis* and *Brachiaria mulato* II at 100:0, 75:25, 50:50, 25:75 and 0:100% ratios as T1, T2, T3, T4 and T5, respectively and laid in a completely randomized design (CRD) and replicated three times.

Silage making

Silage was made in a laboratory silo (500 gram capacity bottle). Fresh *Brachiaria mulato* II and *Stylosanthes guianensis* were harvested from the field and allowed wilted for 24 hours in order to reduce the moisture content after which were chopped to 2-3cm long for ease of compaction and combination for silage. The materials in the silos were sealed tightly and allowed for 28 days fermentation period. At expiration, the resultant silages were scored for colour and aroma by three independent scorers on a scale of 1 - 4 as presented in Table 1. Thereafter pH and temperature were determined using the digital pH meter and thermometer, respectively.

Table 1: Description of colour and aroma rating used as indices of silage quality

Scale	Colour	Aroma
1	Dark/Deep brown	Putrid or rancid
2	Light brown	Pleasant
3	Pale yellow	Sweet
4	Yellowish green	Very sweet

Source: Muhammad *et al*. (2009).

Chemical analysis

Silage samples collected were oven dried according to the treatments and milled to pass through 2 mm sieve and stored in a laboratory container. The milled samples were subjected to proximate analysis to determine crude protein (CP), Ash, crude fibre, ether extract (EE or fat) and nitrogen free extract (NFE) according to procedures described by AOAC (2019). Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) were determined using the method of Van Soest *et al*. (1991). The feeding value indices were calculated as follows:

Digestible Dry Matter (DDM)

$DDM (\%) = 88.9 – (0.779 \times ADF)$

Dry Matter Intake (DMI)

$DMI (\% \text{ of body weight}) = 120 / NDF$

Relative Feed Value (RFV)

$RFV = (DDM \times DMI) / 1.29$

These equations are commonly used for evaluating forage quality and predicting animal intake potential (Undersander *et al.*, 2020).

Statistical analysis

Data obtained in this study were analyzed using statistical analysis system (GENSTAT, 2014) Version 17.1. Significant means were separated using Least Significant Difference (LSD) at 5% level of probability.

Results and Discussion

Results

Silage characteristics

The results of pH, temperature, aroma and colour characteristics of silage made at different grass and legume mixing proportions are presented in Table 2. The mean values of pH obtained in Mulato II (75%) – Cook stylo (25%) (T4) were significantly different (p<0.05) from other treatment combinations. All the silage samples prepared were slightly acidic with pH value ranging from 5.2 - 5.8.

Table 2: Effect of grass-legume mixture at different proportion on silage characteristics

Treatment	pH	Temperature (^{0}C)	Aroma	Colour
Mulato II (0%) – Cook stylo (100%) (T1)	5.41^b	29.60^a	1	1
Mulato II (25%) – Cook stylo (75%) (T2)	5.21^b	28.57^a	2	4
Mulato II (50%) – Cook stylo (50%) (T3)	5.31^b	29.17^a	2	4
Mulato II (75%) – Cook stylo (25%) (T4)	5.83^a	26.07^b	4	4
Mulato II (100%) – Cook stylo (0%) (T5)	5.45^b	24.60^b	3	3
LSD	**0.3068**	**2.402**	-	-

Means within the same column with different superscripts differ significantly (p<0.05)

Effect of mixing proportion of *Brachiaria mulato* II with *Stylosanthes guianensis* on proximate composition and feeding value of silage

Effect of mixing proportion of *Brachiaria mulato* II with *Stylosanthes guianensis* on proximate composition and feeding value of silage is presented in Table 3. All the parameters evaluated were significantly different (p<0.05), except CP and EE. The numerically higher mean value of CP content (16.99 %) was obtained in T3 and decrease with the decrease in legume proportion. The relative feed value (RFV) in T3 (84.52%) was observed to be higher among the treatment combinations and absolutely revealed dry matter intake (DMI) that was not significantly different from the treatments with higher proportion of legume in grass-legume mixtures.

Table 3: Effect of mixing proportion of *Brachiaria mulato* II with *Stylosanthes guianensis* on proximate composition and other feed values of silage (%)

TRT	DM	ASH	CF	CP	EE	NFE	ADF	NDF	DMI	DDM	RFV
T1	93.4^{b}	9.10^{c}	15.98^{c}	15.94	4.00	61.39^{a}	40.18^{cd}	68.58^{b}	1.750^{a}	57.60^{ab}	84.00^{ab}
T2	93.50^{b}	11.35^{b}	16.22^{c}	16.50	4.80	60.32^{ab}	40.69^{c}	70.36^{b}	1.706^{a}	57.20^{b}	81.30^{b}
T3	94.20^{ab}	11.40^{b}	17.31^{c}	16.99	4.85	57.02^{bc}	38.86^{d}	69.37^{b}	1.730^{a}	58.63^{a}	84.52^{a}
T4	93.48^{b}	9.38^{c}	27.00^{a}	14.87	4.91	47.33^{d}	45.85^{a}	75.74^{a}	1.585^{b}	53.18^{d}	70.22^{d}
T5	94.58^{a}	12.53^{a}	22.43^{b}	14.77	3.40	54.22^{c}	43.86^{b}	73.92^{a}	1.624^{b}	54.73^{c}	70.05^{c}
P- value	0.048	0.003	0.001	0.644	0.033	0.001	<.001	0.002	0.002	<.001	<.001
SEM	0.2066	0.272	0.388	1.193	0.233	0.855	0.360	0.491	0.0111	0.280	0.787

Means within the same column with different superscripts differ significantly (p<0.05). DM=Dry matter, , CF= Crude fibre, CP= Crude protein, EE= Ether extract, NFE= Nitrogen free extract, ADF= Acid detergent fibre, NDF= Neutral detergent fibre, DMI= Dry matter intake, DDM= Digestible dry matter, RFV= relative feed value

DISCUSSION

The pH value is one of the simplest and quickest ways of evaluating silage quality; the lower the pH, the better the preserved and more stable is the silage. Silage that is properly fermented will have a much lower pH (more acidic to slightly acidic), than the original forage. The pH value of good silage below 4.1 has been considered as an index of good quality (Amuda *et al*., 2020). The pH values of all the silage samples in this study were within the range (5.5-6.1) of ensiled gamba grass fortified with tropical legumes (centrosema, lablab and groundnut leaves) (Muhammad *et al*. (2009). The decreasing pattern of pH value with the increase in grass proportion in the mixtures is in agreement with the previous report by Kung *et al*. (2022), who stated that, the highest pH values were in treatments with the most favourable seeding ratios for legumes, and this might be attributed to the increased buffering power of the leguminous ensiled biomass. Temperature is one of the essential factors affecting silage colour. The lower the temperature during ensiling, the less will be the colour change as excessive heat owing to high temperature do affect silage colour (Kung *et al.*, 2022). However, the range of temperature (24.6^0-29.6^0C) obtained during the fermentation period of 28 days was not in agreement with the temperature range (26.0^0-26.3^0C) reported by Amuda *et al*. (2017) for ensiled maize stover with and without additive.

Aroma is an important sensory parameter used to evaluate silage quality because it reflects the nature of the fermentation process and the types of organic acids produced during ensiling. Good quality silage is typically characterized by a pleasant, sweet or fruity smell, which indicates dominant lactic acid fermentation and minimal spoilage by undesirable microorganisms (Kung *et al.*, 2022). In this study, the aroma scores ranged from 1 to 4, corresponding to putrid/rancid to very sweet aroma, respectively. Treatments containing higher proportions of *Brachiaria mulato* II (75%) and moderate mixtures of grass and legume (50:50) produced silage with pleasant to very sweet aroma, indicating favourable fermentation conditions. This suggests that the combination of grass and legume improved the fermentation profile by providing a balanced substrate for lactic acid bacteria. The very sweet aroma recorded in T4 (75% Mulato II : 25% Stylo) suggests that the fermentation process was dominated by lactic acid production with minimal proteolysis or undesirable microbial activity. Sweet-smelling silage generally results from adequate fermentation of water-soluble carbohydrates which leads to lactic acid formation and stable preservation of nutrients (Muck *et al.*, 2020). The presence of a moderate proportion of legume in the mixture may have contributed additional nitrogen and buffering capacity that enhanced microbial activity during fermentation. Conversely, the lower aroma score observed in the treatment containing 100% *Stylosanthes guianensis* could be attributed to the higher buffering capacity and lower soluble carbohydrate content typical of legumes. Legumes generally ferment less efficiently during ensiling compared with grasses because they contain lower concentrations of fermentable sugars required for rapid lactic acid production

(Kung *et al.,* 2022). The pleasant aroma observed in most treatments in this study indicates that the silage fermentation process was generally successful. According to Muck *et al.* (2020), silages with pleasant or slightly sweet aroma are indicative of well-preserved forage and are usually highly acceptable to ruminant animals, thereby improving voluntary feed intake.

The proximate composition of ensiled Mulato II, Stylo and their mixtures at different mixing proportions in all the parameters evaluated revealed significant differences except in CP and EE across the treatments. The least CP value was obtained in sole Mulato II (14.77 %) which was lower than the value reported by Amuda *et al*. (2020) with 36.09 %. The CP content of silages ranged from 9.77 – 16.99 % and higher than the range (9.44 - 14.88 %) recorded by Amuda *et al.* (2020) in gamba grass ensiled with different graded levels of *Centrosema pubescence* and was contrarily to the findings that, higher CP value are obtained from high levels of legume inclusion Kallah *et al*. (1997). Silage made with Mulato II Stylo at 50:50 (T3) revealed a higher CP content (16.99 %) and the result was in line with the fact that the use of Stylo and Centro improved the silage quality of grass (Muhammad *et al*., 2008). In contrast, the higher the level of legume inclusion; the higher the CP and conversely, the lower the CF content of the silage (Muhammad *et al*., 2009). The CP content observed in this study in all the treatments was within the NRC (2001) recommended percentage of 12- 16.50 % as CP required for young and lactating animals, respectively. However, the CP in silages increased after ensiling, and mixed silages had higher CP than sole silages. Although, according to an earlier study, CP decreases after ensiling because oxygen is consumed during respiration and proteolysis at the onset of ensiling (Muck *et al.,* 2020). Another recent study showed similar results with the findings of this study that the increase in CP is due to excess protein produced by the fermentative microbial communities (Lai *et al*., 2022). The higher CF content (27.00 %) in T4 (Stylo:Mulato II) (25:75) and lower in sole Stylo observed in this study was similar to the findings of Moran (2005) and Elkholy *et al*. (2009); but lower than the least values of 33.10 and 36.30 % reported by Ajayi *et al*. (2012) and Amuda *et al*. (2020), respectively. Ash content was not within the favourable ash content of grass and legume forages of 7 – 9%, but is significant to what was reported by Ajayi *et al*. (2012) and Amuda *et al*. (2020), respectively. Nitrogen free extract indicates the soluble carbohydrate of the forage plant. The range (47.33 – 61.39 %) obtained was higher than the range (35 – 53 %) in ensiled gamba grass with graded levels of centro and obtained in maize stover silages (Amuda *et al*., 2020). Sanderson; (2010) reported that the proportion of grasses is positively correlated with the fibre and digestibility of legume–grass mixtures (Sturludóttir *et al*., 2014). Sing and Oosting (1992) reported that roughage feeds containing values of less than 45 % fibre could be classified as high quality, those with values ranging from 45 – 65 % as medium and those with higher than 65 % as low quality. The RFV is used to predict the intake and energy content of the forages and the higher value, the better the nutritional value of the forage. The value obtained in this study was positively correlated with the CP content, suggesting that the treatment combination with higher value revealed a higher RFV and this might be attributed to the contribution of legume proportion.

The neutral detergent fibre (NDF) content observed in this study ranged from 68.58% to 75.74% across treatments. NDF represents the structural components of plant cell walls and is an important indicator of feed intake potential. Higher NDF levels generally reduce voluntary feed intake because they increase rumen fill and limit digestion rate (Mertens, 2021). The relatively higher NDF values observed in treatments with greater grass proportions may be attributed to the fibrous nature of Brachiaria species compared with legumes. Similarly, acid detergent fibre (ADF) values differed significantly among treatments, with the highest value recorded in the treatment containing higher proportion of Mulato II grass. ADF represents the less digestible components of the plant including cellulose and lignin. Increased ADF content generally results in reduced digestibility of forage. The lower ADF values observed in the 50:50 mixture suggest improved digestibility and better feeding quality. Dry matter intake (DMI) is an important parameter for predicting animal performance. In this study, DMI values ranged from 1.585 to 1.750% of body weight, indicating moderate intake potential of the silages produced. Treatments with lower NDF values exhibited higher DMI values, which agrees with previous reports that NDF is inversely related to voluntary feed intake in ruminants (Oba and Allen, 2021). Digestible dry matter (DDM) values ranged between 53.18% and 58.63%, indicating moderate digestibility of the silage mixtures. The highest DDM recorded in the 50:50 mixture suggests improved nutritional availability due to the complementary interaction between the grass and legume components. Relative feed value (RFV) integrates both intake and digestibility indices to provide an overall estimate of forage feeding quality. The RFV values obtained ranged from 70.05 to 84.52, indicating moderate forage quality according to standard forage classification systems. The higher RFV recorded in the 50:50 mixture indicates better feeding value and supports the use of balanced grass-legume combinations in silage production (Undersander *et al.*, 2020; Mertens, 2021).

Conclusion

This study has shown that mixture proportions have significant effect on the dry matter yield and nutritional quality of legume–grass mixtures rather than species diversity. It can be concluded that the use of legumes in preparation of grass silages has advantages to silage quality characteristics and nutritional composition. This study has revealed that the composite silage made from 50% Mulato II plus 50% stylo resulted in silage with higher CP and a better dry matter content.

Recommendations

It can be recommended that livestock farmers should adopt grass–legume mixtures, particularly the 50:50 ratio of *Brachiaria mulato* II and *Stylosanthes guianensis*, for improved silage quality and nutritional value. Future research should evaluate the effect of silage additives and microbial inoculants on fermentation quality of tropical forage mixtures. Further studies should also investigate animal performance responses (growth rate, milk yield and feed efficiency) when animals are fed silage produced from these forage mixtures.

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