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Physico-Chemical Properties and In Vitro Fermentation Evaluation of Ensiled Guinea Grass (Panicum Maximum) With Different Protein Additives

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Abstract: Preservation of guinea grass with protein additives as silage for dry-season feeding makes it possible to preserve and improve its nutritional composition. Consequently, research was carried out to investigate the nutritive values of Guinea grass (*Panicum maximum*) ensiled with different protein additives. Guinea grass was ensiled with *Tephrosia bracteolata*, cassava tops, soybean meal, poultry litter, and urea to obtain the following silages: Guinea grass only (Gg) control, Gg + *Tephrosia bracteolata (Gg + Tb)*, Gg + cassava tops (Gg + Sbm), Gg + poultry litter (Gg + Pl), Gg + urea (Gg + U), and Gg + soybean meal (Gg + Sm) respectively) and designated as follows: T_1, T_2, T_3, T_4, T_5 and T_6. The guinea grass was ensiled with 10% protein additives except urea, which was added at a 3% level, and each treatment was replicated four times. After nine months (9 months) of fermentation, the silages were opened, and physical characteristics, chemical composition, and *in vitro* fermentation evaluation were determined using standard techniques. The silages were characterised by a greenish-yellow colour, a firm and dry texture, a pleasant and fruity odour, and not being mouldy. All these characteristics indicate that the silage was preserved well. The temperature (0 C), pH (3.41–5.69), percentage moisture content (62.39–71.79%), and dry matter (28.01–37.86%) of the silages were significantly (P 0.05) different across the treatments. Furthermore, the results of the chemical analysis showed that all the nutrients in the silages were significantly (P 0.05) different except the EE, which was similar across the treatments. The crude protein (CP) contents (10.91–21.78%) and crude fibre (CF) levels (24.47–27.00%) of silages varied significantly (P 0.05) across the treatments. Fibre fractions (neutral detergent fibre (NDF) 58.03–61.47% and acid detergent lignin (ADL) 13.23–15.53%) were significantly (P 0.05) different, while acid detergent fibre (ADF), cellulose, and hemicellulose were similar across the treatments. There were significant (P 0.05) differences in *in vitro* fermentation means of methane (5.00–6.00%), total gas volume (9.00–15.00 ml), metabolisable energy (4.20–4.92 KJ/DM), short chain fatty acid (0.16–0.31 ml), dry matter degradability (32.35–42.36%), organic matter digestibility (35.35–42.36%), and fermentation efficiency (2.78–4.24). The results obtained from this study indicate that all the protein additives used in ensiled guinea grass improved the protein contents significantly, and *in vitro* fermentation gas production indicates a low level of methane (CH_4) production, an indication of a reduction in energy loss.

Keywords: Degradability, Fermentation, silage, crude protein, Methane

INTRODUCTION

Tropical perennial grass pastures are an important forage resource for livestock production in regions across Africa (Arroquy *et al*., 2014; Poppi *et al*., 2018; White *et al.,* 2018). Production is typified by periods of rapid growth and high yields during the wet season, followed by deficits in quality and quantity during the dry season (Poppi *et al.*, and Li *et al.,* 2019). Inadequate supply of quality forage all year round and the high cost of conventional feedstuffs are major problems for the productivity of ruminants in Nigeria (Olorunnisomo, 2008). The cost of feeds and feeding under intensive production systems accounts for about 70% of the total production cost, and a substantial reduction in the cost of feeds is achievable through the use of improved pastures. Livestock farmers face their biggest challenge during the dry season when a 'staircase' growth pattern is observed in animals as a result of inadequate animal feed supply. Under traditional systems of management in Nigeria, ruminants feed on unimproved native pastures and crop residues left over from households. At the onset of the dry season, grass becomes scarce as a result of rapid drying up and lignification; hence, yield and quality of forage from

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perennial tropical grasses decline rapidly during the dry season, leading to an inadequate supply of quality feed during this period. *Panicum maximum* (Guinea grass) is one of the most common and widely spread grasses in the derived savannah region of Nigeria. Guinea grass is tolerant of shade and fire but susceptible to waterlogging or severe drought. Under good conditions, its nutritional value is high, having up to 12.5% crude protein, total digestible nutrients (TDN) of 10.2%, and calcium, phosphorus, and magnesium (Agishi, 1985). These grasses are abundant in the wet season but scarce in the dry season, and where available, they are highly lignified. Preservation, therefore, remains the solution to their availability during the dry season.

Ensiling is important as the main method of forage conservation in the tropics because of the factors related to climatic seasonality. Thus, to conserve excess perennial forage, growth in the favourable period, and meet the animal requirement throughout the year without damaging economic planning, ensiling is a recommended practice. Tropical grasses, when ensiled at an early stage of vegetative development, have high nutritional value but have low dry matter (DM) content, raising buffering capacity, and low water-soluble carbohydrates (Santos *et al.* 2014). Throughout the year, a major constraint facing livestock farmers in the tropics is meeting the nutritional needs of ruminants due to the seasonality of forages. Guinea grass, like other tropical grasses, decreases in protein between 10 and 12 weeks of age. Conservation through ensilage of forage produced during the rainy season is likely to be the practice adopted by most small-holder livestock owners, particularly those in dairy or beef production. Ensiling presents alternative means of fodder preservation during the rainy season while retaining the nutrient quality of the forage without recourse to the use of fuel or solar energy for artificial haymaking under wet, humid conditions. Consequently, a study was carried out to ensile guinea grass (*Panicum maximum*) with different types of protein additives to improve the protein content for effective utilisation in ruminant nutrition and assess the dry matter degradability (DMD%), organic matter digestibility (OMD%), short chain fatty acids (SCFA ml), metabolisable energy (ME MJ/kg), total gas volume (TGV ml), methane (CH_4), fermentation efficiency (FE) and fermentation characteristics using the *in vitro* fermentation technique as described by Menke and Steingass (1988).

MATERIALS AND METHODS

Experimental Site and silage preparation

The study was carried out in Teaching and Research Farm of Federal University Wukari Taraba state. Wukari lies between latitude 7˚51'N to 7˚85'N and longitude 9˚46'E to 9˚78'E of the Greenwich meridian. The mean annual rainfall value ranges from 1000 - 1500 mm. The unset of the raining season is usually around April while the offset period is October. The mean maximum temperature is experienced around April at about 40˚C while the mean minimum temperature occurs between the period of December and February at about 20˚C (Oyatayo *et al* 2015).

Freshly harvested guinea grass, cassava tops, and *Tephrosia bracteolata* were obtained from the University environment while poultry litter was obtained in poultry unit of Teaching and Research farm Federal University Wukari. Also soybean meal was obtained from feed store while urea was purchased from the market. Cassava tops, *Tephrosia bracteolata* (forages), soybean meal (feed) and urea (Non-protein nitrogen) were used as conventional and non–conventional protein additives to ensile guinea grass. The ensiled materials were harvested in month July when the quality of the forages were high. Harvested forages (guinea grass, cassava tops and *Tephrosia bracteolata*) were chopped with cutlass into 2-5cm pieces of particle size for easy compaction when ensiling. The chopped materials (guinea grass, cassava tops and *Tephrosia bracteolata*) were wilted under shade for 9hours on the concrete floor with constant turning to ensure uniform wilting. The samples were weighed, using weighing scale and mixed thoroughly and divided into six treatments (T_1, T_2, T_3, T_4, T_5 and T_6).

The additives were added at 10% level except for urea which was added at 3% level due to high level of nitrogen composition. Each silage type weighed 500g (i.e. Guinea grass (Gg) alone (control) T_1, Guinea grass with *Tephrosia bracteolata* (Gg + Tb) T_2, Guinea grass with cassava tops (Gg + Ct) T_3, Guinea grass with Poultry Litter (Gg + Pl) T_4, Guinea grass with Urea (Gg + U) T_5 and Guinea grass with Soybean meal (Gg + Sm) T_6. Each silage type was ensiled in polythene bags, each capable of holding (500g) of wilted ensiled materials were used as silos. The polythene bags were placed inside water proof sack and then into 170 litres capacity plastic using for reinforcement and ease of fermentation. Ensiling was done by rapid compaction of the materials (to eliminate air) into well labelled

silos with date. Sealing of the silos was done by placing 25 kg sand bag on top of the bag after tying carefully and firmly. The container was tightly covered and sealed to avoid air penetration. Each treatment was replicated four (4) times and were kept for nine months.

Determination of Silage temperature (^{o}C), colour, odour, pH, percentage moisture content (MC %) and dry matter (DM %)

The silage was opened after nine (9) months and the temperature was taken by dipping a thermometer inside the silage within a polythene and kept it for two minute before taken the reading. Silage colour was assessed with colour chat. A 5-man panel assessed the odour / smell and texture of the silage. This step was taken to guide against a bias attitude of one – man assessor since the physical attributes are on the nominal scale rather than ordinal.

The pH of the silage was determined, during which 25g of the ensiled sample from each treatment was taking to the laboratory and mixed with 100 mls of distilled water in a beaker and allow for one hour and then agitated the content. Thereafter, a pH meter glass electrode connected to electric source was then inserted into a beaker containing the samples, and was allowed 10 seconds and pH of each treatment sample was taken and recorded (Babayemi 2009, and Amuda et al., 2020).

Chemical and Statistical Analysis

Known weight of each air-dried sample was oven dried at 65^{0}C to constant weight and ground in the laboratory with grinder and subjected to chemical analysis in triplicate for dry matter (DM) determination, crude protein (CP), crude fibre (CF), ether extract (EE), organic matter (OM) and nitrogen free extract (NFE) as described by AOAC (2005). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were assayed by the method of Van Soest et al., (1991). Hemicellulose was calculated as the difference between NDF and ADF (Rinne et al., 1997) while Non - fibre carbohydrate (NFC) was determined according to NRC (2001). Data obtained were subjected to one way analysis of variance (ANOVA) using SPSS (Version 23.0.2018). The treatments means were compared using the Duncan Multiple Range Test (Duncan, 1955).

***In vitro* gas production evaluation**

The *in vitro* fermentation gas production of each silage treatment was determined according to the method of Menke and Steingass (1988). Rumen liquor was obtained from 3 WAD goats under the same condition (fed 60% guinea grass and 40% concentrate) with the use of suction tube as described by Babayemi and Bamikole (2006). Rumen fluid was collected in the morning before feeding, mixed, strained through four layers of cheese cloth and kept at 39 ± 1^{0}C. The rumen liquor was kept in under continuous flow of CO_2. Glass syringes (100ml) fitted with plungers were used. Two hundred milligrams (200mg) DM of each dried and ground silage sample was carefully weighed into 100ml calibrated syringes with pistons lubricated with Vaseline and thereafter, the syringes were filled with 30ml of medium consisting of 10ml of rumen fluid and 20ml of buffered mineral solution ($NaHCO_3$+$3Na_2HPO_4$+KCl+NaCl+$MgSO_4.7H_2O$+$CaCl_2.2H_2O$) and each sample was replicated three (3) times. The syringes were tightly capped and carefully arranged in an incubator maintained at 39±^{0}C along with three (3) blanks syringes containing 30ml of medium (inoculums and buffer) only as control. The gas production was recorded at 3, 6, 9, 12, 15, 18, 21, and 24 hours.

The content was repeatedly agitated at each reading time of reading. After incubation time, 4ml of 10M NaOH solution was introduced to estimate methane (CH_4) production according to Fievez *et al.,* (2005). At post incubation, the content of the syringes were transferred into centrifuge tubes and placed immediately in cold water at 4^{0}C to stop fermentation. The tubes were centrifuged at 15,000 x g for 25 minutes. The supernatant was discarded and the residues were oven dried at 55^{0}C for 48hrs to estimate *in vitro* dry matter digestibility (IVDMD %), Organic matter digestibility (OMD %), Metabolisable energy (ME MJ/KgDM), were estimated (Tilley and Terry, 1963) and Short chain fatty acids, (SCFA µml) were calculated (Getachew *et al.,* 1999). The volume of gas produced every 3hours interval of the 3 replicates of each sample was plotted against the incubation time and from the graph, the gas production characteristics were estimated using the equation $Y = a + b (1\text{-}e^{ct})$ as described by Ørskov and McDonald, (1979), where Y = volume gas produced at time (t), a = intercept (gas produced from the soluble fraction), b = gas produced from insoluble but degradable fraction, c = gas production rate constant for the insoluble fraction (b), t = incubation time.

$$IVDMD = \frac{\text{Initial dry matter input} - \text{Dry matter residues}}{\text{Initial dry matter input}} \times 100 \quad (1)$$

$$\text{Fermentation efficiency (FE)} = \frac{\text{DMD/Kg}}{\text{GVmlKg of DM}} \quad (2)$$

Organic matter digestibility (OMD %) was calculated as OMD=14.88 + 0.8893GV + 0.45CP + 0.651XA, (Menke and Steingass, 1988): Metabolisable energy (ME) was calculated as ME = 2.20 + 0.136 GV + 0.057CP + 0.0029CF (Menke and Steingass, 1988) and Short chain fatty acids, (SCFA µml) as 0.0239GV – 0.0601(Getachew *et al.,* 1999) was also obtained, where GV, CP, CF and XA are gas volume, crude protein, crude fibre and ash, respectively.

RESULTS

Table 1. Physical characteristics of ensiled guinea grass (*Panicum maximum*) with different proteins additives

	PARAMETERS			
TRTS	COLOUR	TEXTURE	ODOUR	MOULDNESS
T1	Greenish Yellow	Dry and Firm	Fruity and Pleasant	Not Mouldy
T2	Greenish Yellow	Dry and Firm	Fruity and Pleasant	Not Mouldy
T3	Greenish Yellow	Dry and Firm	Fruity and Pleasant	Not Mouldy
T4	Greenish Yellow	Dry and Firm	Fruity and Pleasant	Not Mouldy
T5	Greenish Brown	Dry and Firm	Pungent	Not Mouldy
T6	Greenish Yellow	Dry and Firm	Fruity and Pleasant	Not Mouldy

T_1 = Ensiled Guinea grass only (Control), T_2 = Guinea grass + *Tephrosia bracteolata* T_3 = Guinea grass + Cassava tops, T_4 = Guinea grass + Poultry Litter, T_5 = Guinea grass + Urea and T_6 = Guinea grass + Soybean meal. TRTS = Treatments.

Table 1 showed the physical characteristics of the silages such as colour, texture, odour and mouldness. All the treatments exhibited similar physical properties. The ensiled guinea grass (*Panicum maximum)* with different protein additives showed greenish-yellow colour, pleasant and fruity odour, firm and dry in texture and not mouldy. However, treatment 5 has a greenish brown colour and pungent odour which is characteristics of ammonia (NH_3) gas.

Table 2. Quality characteristics of ensiled guinea grass with different protein additives

	Treatments						
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**SEM**
Temp (^{0}C)	30.10^{ab}	30.75^{a}	29.90^{b}	29.63^{bc}	28.43^{d}	28.87^{cd}	0.13
pH	3.83^{bc}	3.79^{c}	3.60^{d}	3.41^{e}	5.69^{a}	3.99^{b}	0.10
MC (%)	71.79^{a}	69.74^{a}	69.91^{a}	68.86^{a}	62.39^{b}	69.83^{a}	1.54
DM (%)	28.01^{b}	30.47^{ab}	30.09^{ab}	31.14^{ab}	37.86^{a}	30.18^{b}	1.55

abcde: Different superscripts in a row differs significantly ($p<0.05$) among treatments of all additives
T_1 = Guinea grass (*Panicum maximum*) only (Control), T_2 = Guinea grass (*Panicum maximum*) +*Tephrosia candida,* T_3 = Guinea grass (*Panicum maximum*) + Cassava tops, T_4 = Guinea grass (*Panicum maximum*) + Poultry Litter, T_5 = Guinea grass (*Panicum maximum*) + Urea and T_6 = Guinea grass (*Panicum maximum*) + Soybean meal, TEMP =Temperature, MC= Moisture Content, DM = Dry Matter and SEM = Standard Error of Means

Presented in Table 2 is the quality characteristics (temperature, pH values, moisture content (MC %) and dry matter (DM)) of ensiled *Panicum maximum* fortified with different types of protein additives. All the quality characteristics (temperature (^{0}C), pH, percentage moisture content (MC %) and dry

matter (DM %)) of the six silages varied significantly (P<0.05) across the treatments. The temperature (^{0}C), pH, percentage MC and DM of the ensiled materials ranged from 28.43–30.75^{o}C, 3.41–5.69, 62.39–71.79% and 28.01–37.86% respectively.

Table 3. Nutrients composition of ensiled *Panicum maximum* with different protein additives

	Treatments						
Nutrients (%)	T1	T2	T3	T4	T5	T6	**SEM**
DM	92.29	92.28	92.18	92.15	92.01	92.06	0.125
CP	10.91^{c}	12.51^{b}	12.25^{b}	12.51^{b}	11.35^{bc}	21.78^{a}	0.232
ASH	11.17^{ab}	10.13^{b}	10.77^{ab}	10.80^{ab}	12.33^{a}	11.67^{ab}	0.270
EE	1.97	1.77	1.87	2.03	1.90	2.07	0’072
CF	26.10^{a}	26.63^{a}	26.13^{a}	27.00^{a}	26.33^{a}	24.47^{b}	0.250
NFE	49.85^{a}	48.96^{ab}	48.98^{ab}	47.66^{b}	48.08^{ab}	40.02^{c}	0.372
OM	88.83^{ab}	89.87^{a}	89.23^{ab}	89.20^{ab}	87.67^{b}	88.33^{ab}	0.270
NFC	14.49^{a}	17.56^{a}	14.12^{a}	14.96^{a}	16.68^{a}	4.59^{b}	0.566
CHO	75.95^{a}	75.59^{a}	75.12^{a}	74.66^{a}	74.41^{a}	64.49^{b}	0.360

abcde: Different superscripts in a row differs significantly (p<0.05) among treatments of all additives
T1 = Guinea grass (*Panicum maximum*) only (Control), T2 = Guinea grass (*Panicum maximum*) +*Tephrosia candida,* T3 = Guinea grass (*Panicum maximum*) + Cassava tops, T4 = Guinea grass (*Panicum maximum*) + Poultry Litter, T5 = Guinea grass (*Panicum maximum*) + Urea and T6 = Guinea grass (*Panicum maximum*) + Soybean meal, CP = Crude Protein, EE = Ether Extract, CF = Crude Fibre, NFC= Non Fibre Carbohydrates, NFE = Nitrogen Free Extract and DM = Dry Matter and SEM = Standard Error of Means.

The proximate composition of silage prepared from Guinea grass (*Panicum maximum*) fortified with different protein additives is presented in Table 4. All the parameters evaluated were significantly (P<0.05) different except for DM and EE of the silages that were similar across the treatments. T_6 (Guinea grass (*Panicum maximum*) + Soyabean meal) had the highest CP (21.78%) while T_1 (sole Guinea grass (*Panicum maximum*)) had the lowest CP value (10.91%). Also T_4 had the highest CF (27.00%) and while T_6 had the lowest value (24.47%). The ash composition of the silage varied significantly (P<0.05) across the treatments such that T_5 (guinea grass + cassava tops) had the highest value (12.33%) while T_2 (Guinea grass +Tephrosia) had the lowest value (10.13%).

Table 4. Fibre fractions of ensiled *Panicum maximum* with different protein additives

	Treatments						
Parameters (%)	T_1	T_2	T_3	T_4	T_5	T_6	**SEM**
NDF	61.47^{a}	58.03^{b}	61.00^{ab}	59.70^{ab}	57.73^{b}	59.90^{ab}	0.566
ADF	33.90	33.00	33.67	33.33	32.60	33.63	0.508
ADL	15.20^{a}	14.70^{a}	15.53^{a}	15.37^{b}	14.83^{a}	13.23^{b}	0.254
HEMI CELL	27.57	25.03	27.33	26.37	25.13	28.27	0.741
CELLULOSE	18.70	18.30	18.13	17.97	17.77	18.40	0.310

a,b,c,d,e: Different superscripts in a row differs significantly (p<0.05) among treatments of all additives
T_1 = Guinea grass (*Panicum maximum*) only (Control), T_2 = Guinea grass (*Panicum maximum*) +*Tephrosia bracteolata,* T_3 = Guinea grass (*Panicum maximum*) + Cassava tops, T_4 = Guinea grass (*Panicum maximum*) + Poultry Litter, T_5 = Guinea grass (*Panicum maximum*) + Urea and T_6 = Guinea grass (*Panicum maximum*) + Soybean meal, NDF = Neutral Detergent Fibre, ADF = Acid Detergent Fibre, ADL = Acid Detergent Lignin, HEMICELL = Hemicellulose and SEM = Standard Error of Means.

The fibre fractions of the ensiled guinea grass (*Panicum maximum*) with different protein additives are shown in Table 4. All the parameters considered were significantly (P0<0.05) different except for neutral detergent fibre (NDF) and acid detergent lignin (ADL). The neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose and cellulose ranged from 57.73–61.47, 31.63–33.90, 13.23–15.37, 25.03–28.27, and 17.77 –18.70% respectively.

Table 5. *In vitro* fermentation characteristics (200mg/DM) of ensiled guinea grass (Panicum maximum) with different protein additives at 24hrs incubation period.

	Treatments						
Fermentation characteristics	T_1	T_2	T_3	T_4	T_5	T_6	SEM
a (ml^3)	3.00^{ab}	3.00^{ab}	5.00^{a}	3.00^{ab}	1.00^{b}	4.00^{a}	0.408
b (ml^3)	7.00^{bc}	9.00^{ab}	10.00^{a}	8.00^{ab}	9.00^{ab}	5.00^{c}	0.385
a+b (ml^3)	10.00^{cd}	12.00^{b}	15.00^{a}	11.00^{bc}	10.00^{cd}	9.00^{d}	0.236
c (mlh^{-1})	0.050	0.045	0.064	0.179	0.035	0.050	0.032
t (hrs)	13.50	18.00	9.00	13.50	7.50	13.50	1.826
Y(ml^3)	6.00^{b}	8.00^{ab}	9.00^{a}	6.00^{b}	3.00^{c}	6.00^{b}	0.509

a,b,c,d,e: Different superscripts in a row differs significantly (p<0.05) among treatments of all additives
a = zero time which idealy reflects the fermentation of soluble fraction (ml^3)
b = extent of gas production from insoluble but degradable fraction (ml^3)
a+b= potential extent of gas production(ml^3), t = incubation time (hrs)
c = rate of gas production at time (mlh^{-1}),Y= volume of gas produce at time (ml^3)

Presented in Table 5 is *in vitro* fermentation characteristics (a, b, a+b, c, t, and Y) of ensiled guinea grass with different protein additives. All the characteristics showed significant (P<0.05) differences except 'c'and't' that were similar across the treatments. The intercept value ('a') for all the silages (T_3) ranged from 1.00 to 5.00 at 24hrs. The extent of gas production 'b' values were similar across the treatments except T_6 which was significantly (P < 0.05) different from other treatments but similar to T_1 and it was the lowest among the treatments. Potential gas production (a+b) was significantly (P < 0.05) different such that T_3 was the highest among the treatments. There were no significant (P > 0.05) differences in gas production rate ('c') and incubation time ('t') of the incubated samples. The rate of gas production 'c' ranged from 0.035 to 0.179ml h^{-1} for all the treatments while the volume of gas 'y' produced at time ('t') ranged from 3.00 to 9.00 for all the treatments. Time of most rapid increase in gas produced at't' ranged from 9.00 for T_5 and to 18.00hrs for T_2.

Table 6. *In vitro* fermentation parameters (200mg/DM) of ensiled guinea grass (Panicum maximum) with different protein additives at 24hours incubation period

	Treatments						
Parameters	T1	T2	T3	T4	T5	T6	SEM
CH4 (ml)	4.00^{b}	5.00^{ab}	6.00^{a}	5.00^{ab}	4.00^{b}	4.00^{b}	0.192
TGV(ml)	10.00^{cd}	12.00^{b}	15.00^{a}	11.33^{bc}	10.00^{cd}	9.00^{d}	0.236
ME(MJ/KgDM)	4.20^{d}	4.55bc	4.92^{a}	4.42^{c}	4.22^{d}	4.68^{b}	0.033
SCFA (µml)	0.18^{cd}	0.23^{b}	0.30^{a}	0.20^{bc}	0.18^{cd}	0.16^{d}	0.000
DMD (%)	57.64^{c}	60.10^{bc}	58.50^{c}	61.08^{bc}	64.29^{ab}	67.65^{a}	0.839
OMD (%)	35.95^{c}	37.77^{b}	40.74^{a}	37.32^{bc}	36.91^{bc}	40.27^{a}	0.259
FE	4.24^{a}	3.33^{bc}	2.78^{c}	3.56^{ab}	3.57^{ab}	3.64^{ab}	0.127

a,b,c,d,e: Different superscripts in a row differs significantly (p<0.05) among treatments of all additives
T1 = Guinea grass (Panicum maximum) only (Control), T2 = Guinea grass (Panicum maximum) +Tephrosia bracteolata, T3 = Guinea grass (Panicum maximum) + Cassava tops, T4 = Guinea grass (Panicum maximum) + Poultry Litter, T5 = Guinea grass (Panicum maximum) + Urea and T6 = Guinea grass (Panicum maximum) + Soybean meal.
CH4 = Methane, TGV=Total Gas Volume, ME = Metabolisable Energy, SCFA = Short Chain Fatty Acid, DMD = Dry Matter Degradability, OMD = Organic Matter Digestibility and FE = Fermentation Efficiency

Table 6 showed the *in vitro* fermentation parameters of the silages such as methane (CH4%), total gas volume (TGV ml), metabolisable energy (ME MJ/KgDM), short chain fatty acid (SCFA µml), dry matter degradability (DMD%), organic matter digestibility (OMD %) and fermentation efficiency (FE). All these parameters varied significantly (P<0.05) across the treatments. Methane (CH_4) values ranged from 4.00 to 6.00ml while the total gas volume (TGV ml) ranged from 9.00µml (T_6) to 15.00µml (T_3). Metabolisable energy (ME) varied significantly (P<0.05) such that T_3 had the highest value (4.92MJ/Kg/DM) while T_1 had the lowest value (4.20MJ/KgDM). Similarly, SCFA (µml), DMD, OMD

and FE of the silages were significantly different across the treatments. The SCFA, DMD, OMD and FE values ranged from 0.16 –0.30µml, 57.64 – 67.64%, 35 – 40.74% and 2.78 – 4.24 respectively.

DISCUSSION

Physical Characteristics of silage, chemical composition, fibre fraction

The physical characteristics of ensiled guinea grass (Panicum maximum) with different protein additives is shown in Table 1. The greenish yellow and greenish brown colour, firm texture, pleasant and fruity odour characterised by ensiled guinea grass (Gg) with and without additives are characteristics of a good fermented silage Amuda *et al.silage (*, 18). Good silage usually preserves the original colour of the forage used to produce it. Consequently, the bright colours were expected. The greenish brown and greenish yellow colours obtained in this study were in order. The greenish brown and greenish yellow colours were close to the original colour of the guinea grass (Gg), which was an indication of good-quality silage that was well preserved. The firm texture observed in this study was expected to be the best texture, and a pleasant and fruity smell is accepted for good silage, as reported by Babayemi (2009). However, T_5 silage has a pungent smell, which is characteristic of ammonia gas.

The temperature of fermenting forage, varying from 27 to 380 °C, was presumed to produce excellent silage (Muck, 1996). The temperature range of 28.43 to 30.750 C of ensiled guinea grass (Gg) with different protein additives was within the 27–380 C range reported for excellent silage by Muck (1996). As observed by Bolsen *et al*. (1996), any excessive heat production can result in Maillard or browning reactions, which can reduce the digestibility of both protein and fibre constituents. The lower the temperature during ensilage, the less colour change there will be (Adesogan and Newman, 2010). The temperature obtained for this study indicates that the silages were preserved well.

Generally, pH is one of the simplest and quickest ways of evaluating silage quality. The lower the pH, the better preserved and more stable the silage. However, pH may be influenced by the moisture content and buffering capacity of the original materials. Silage that has been properly fermented will have a much lower pH (be more acidic) than the original forage. The pH value of good silage below 4.4 has been considered an index of good silage (Harrison *et al*., 1995). The pH range (3.41 to 5.69) of silages was slightly higher than the 4.5–5.5 reported by Menesses *et al*. (2007). The pH values for both control and silages with additives in the present study were in agreement with 3.11–3.62 by Fasina (2012), except for T_5 (5.69). The low pH range in silages reported in this study is an indicator of proper fermentation and good qualities. However, the pH value (5.69) reported for T_5 (Guinea grass ensiled with urea) indicates that urea has buffer capacity that resists pH change, which resulted in a high pH (less acidic) value.

The ensiled guinea grass with or without protein additives, *Tephrosia candida* (legume forage), cassava tops, soybean meal, and 3% urea, had dry matter (DM) values that ranged from 28.01–37.86% and differed significantly (P 0.05) across the treatments. Silage prepared with additives had dry matter (DM) of 30.18–37.86% as compared to that without additives, which had 28.01%. This indicates that additives had positively increased the DM composition of the silages. Also, the DM of the silages was consistent with the ranged values (30.65–35.26%) reported by FAO (2010) and higher than the ranged values (21.2–28.46%) obtained by Moran (2005). The differences could be as a result of additives used, season, wilting, and the age of the forage used. The values of DM obtained in this study indicate that silage types were well preserved.

Chemical Composition of Silages

The crude protein in the silages ranged from 10.91%CP in T_1 (Gg only (control)) to 21.78%CP in T_6 (Gg+Sbm). The crude protein of ensiled guinea grass (*Panicum maximum*) with or without protein additives is relatively higher than 7.4%CP and 6.28%CP reported by Babayemi, (2009) and Ukanwoko and Igwe, (2012) for ensiled guinea grass only. The crude protein and dry matter obtained in this study compare well with those of some tropical grasses and could be used to replace them in feeding animals. The crude protein (CP) observed in this study fell within the range that is required for small ruminants for small ruminants' production which is between 10 – 14%CP according to NRC (2007). The protein value (21.78%) of T_6 (Gg+Sbm) was the highest among the treatments and it can meet protein requirements of dairy animals. Furthermore, it is important to note that ash composition of the silages is very high, there may not need of mineral supplement when feed to ruminants animals. The crude fibre

content of ensiled guinea grass range from 24.47% (T_6) to 27.00% (T_4). The 24.47% recorded for the silages may be due to the degradation action of microbes especially fungi on fibre component of the ensiled materials during fermentation process and age of ensiled materials at harvesting. The crude fibre values of (24.47 – 27.00%) in this study is lower than 36.30 – 36.65% reported by Ajayi *et al*. (2011) but consistent with value (26.4%) reported by Moran (2005). Generally, the crude fibre level obtained in this study is within the range of the grasses and value that ruminant animals can digest and utilise effectively. The lowest NFE value (40.02%) was observed in T_6 (Gg + Sbm) which was significantly ($P<0.05$) different from the values recorded for other silages, this implies that the soluble carbohydrates could support the production of volatile acids in the rumen during fermentation (Blummel *et al.,* 1997).

Fibre Fractions

The fibre fractions (NDF, ADF, and ADL) have implication on digestibility. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) are indicators of the amount of fibre in a forage. Higher values indicate poorer digestibility and voluntary intake may be reduced. NDF reflects the bulkiness of a forage and is inversely related to the plants digestibility (Gillespie, 1998). NDF is negatively correlated with the level of dry matter intake by cows; the lower the NDF, the higher the level of intake. High NDF could result in low intake while high ADF may engender low digestibility (Babayemi *et al*., 2010). Sing and Oosting (1992) cited by Amuda *et al.* (2020) reported that roughage feeds that contained values 45% NDF could be classified as high quality, those with values ranged from 45-65% as medium and those with values higher than 65% as low quality. Consequently, the silage types can be classified as medium owing to the ranged values (57.73 - 61.47%) obtained in this study. High NDF could be a limiting factor to dry matter intake. According to Van Soest (1994), forage digestibility in ruminants is constrained by the extent of cell wall (NDF) digestion. The acid detergent fibre (ADF) consist mainly lignin and cellulose and is correlated to digestibility. The higher the ADF the lower the plants digestible energy and vice versa. Forage with high ADF values is classified as low quality roughage (Rusdy, 2016).

According to Kellems and Church (1998), roughage with less than 40% ADF as high quality and those that are greater 40% as poor quality. Base on this classification the value (31.60 – 33.90%) of ADF obtained in this work indicate that the silages is of high quality. The value (31.60 – 33.90%) obtained is lower than 38% and 39%, reported for Guinea grass and *Andropogon gayanus* by Odedire and Babayemi (2008) respectively Since fibre fractions (NDF, ADF and ADL) content of the silages were relatively low, the intake and potential digestibility will be relatively high when fed alone to ruminants without concentrate supplements. The hemicellulose and cellulose are cell wall constituents and polysaccharides. They are very indigestible in monogastric but digestible in ruminants through fermentation by rumen microbes. Hemicellulose values obtained for silages ranged from 25.03 – 28.27% while cellulose ranged from 17.77–18.70%. These values are not too high for ruminants due to the nature of their stomach and the presence of cellulolytic bacteria and fibrolytic fungi in the rumen. According to McDonald et al. (1995), ruminants can be fed sole on feed that contained 40% cellulose and 20% hemicellulose.

***In vitro* Fermentation Characteristics of ensiled guinea grass with different protein additives at 24hrs incubation period**

The potential gas production from the insoluble fraction, extent and rate of gas production, volume produced and time of production at 24hrs incubation period are presented in Table 5. The *in vitro* gas production characteristics of the substrate in the liquors from the tables showed that there were significant differences in the 'a', 'b', 'a + b' and 'Y' values. This may be due to the different additives used to prepare the silages. The values for the nitrogen free extract (NFE) that represents the soluble carbohydrate fraction of the silages had values that were significantly different ($P < 0.05$) for all the treatments. Therefore, the treatments behaved similarly in term of 'a' 'b' and 'a + b'. Getachew *et al*. (1998) reported that gas production can be attributed to the nature of carbohydrate fractions contained in the substrates. The intercept value 'a' for all the silages (treatments) at 24hrs ranged from 1.00 in T_5 (guinea grass + urea) silage to 5.00 in T_3 (guinea grass + cassava top). The T_1 (guinea grass only) control, was similar to T_2 (guinea grass + Tephrosia), T_3 (guinea grass + cassava top), T_4 (guinea grass + poultry litter), T_5 (guinea grass + urea) and T_6 (guinea grass + soybean meal), meaning there was minimal loss of water soluble carbohydrate during fermentation and storage from the original material. The value for

absolute 'a' used ideally reflects the fermentation of soluble fraction in this study. The soluble fraction makes the attachment by rumen microorganisms to be done easily and leads to much gas production. Therefore, more ruminant microorganism worked on T_3 (guinea grass + cassava tops) and this leads to higher gas production.

The extent of gas production 'b' described fermentation of the insoluble but degradable fraction in T_1(guinea grass +cassava tops) and T_2 and T_5 which recorded high values of 10 and 9.00 respectively could be attributed to the relatively high amount of crude protein in the stover. This facilitated high rate of microbial activities by supplying the required nitrogen for their cellular protein synthesis as established by Roger *et al*. (1977). A linear relationship has been established between high crude protein in forages and in vitro degradability (Njidda *et al*., 2010). The ranged values (5.00 – 10.00) of 'b' obtained in this study was higher than ranged values (2.67 – 5.67) reported by Falola and Olufayo, (2017) for dry matter (DM) degradation of guinea grass incubated with *Leucaena leucocephala* at varied levels. Blummel and Ørskov (1993) discovered that 'b' value could account for 88% for voluntary feed intake. The potential degradability' a+b' of a diet depicts the level at which the diet could be degraded if it were in the actual rumen of the animal (*in vivo*). This largely depends on how much of the fibre fractions (NDF and ADF) have been broken down for easy access of the microbes to the nutrients available in the diet. At 24hrs, there were significant variations among the treatments such that it was highest for the T_3 (guinea grass + cassava tops) and lowest for the T_6 (guinea grass + soybean meal) respectively. However, T_1 (control) values for 'a + b' was similar to T_2, T_4, T_5 and T_6 silages. The relative high value of the potential extent of gas production recorded for T_3 was due to relative abundant of carbohydrate fraction embedded in ensiled guinea grass cassava tops mixture. Getachew *et al*., (1999) stated that it is well known that gas production is basically the result of fermentation of carbohydrate to volatile fatty acid (acetate, butyrate and propionate). Menke and Steingass (1988) also reported that fermentable carbohydrate increase gas production while degradable nitrogen compound decrease gas production to some extent because of their binding of carbohydrate with ammonia. This explain low levels of gas production among the silage types.

The volume of gas ''Y'' at time" t"is the peak of gas production for each sample at 24hrs incubation period. The rate ''c" of gas production at time' 't" were similar across the treatments but the volume of gas ("Y") of incubated samples was significantly different ($P<0.05$) across the treatments. It means that additives had effect on guinea grass ensiled with different additives regarding the "c".t and "Y''characteristics of the gas. However, there are many factors that may determine the rate and amount of gas production during fermentation, depend on the nature and level of fibre, the presence of secondary metabolites (Babayemi *et al*., 2004) and potency of the rumen liquor for incubation. It is possible to attain potential gas production of a feedstuff if the donor animal from which rumen liquor for incubation was collected got the nutrient requirement met. Ørskov and Ryle, (1990) reported that the rate ('c') determines digestion time and consequently how long a potentially digestible material would occupy space in the rumen. Since gas production is dependent on the relative proportion of soluble, an insoluble but degradable and undegradable particle of feed; mathematical description of gas production profiles allows evaluation of substrate and fermentability of soluble and slowly fermentable component of feeds (Getachew *et al*., 1998). Based on the above assumption, therefore, it could be adduced that among the ensiled guinea grass with different protein additives study, T_3 and T_4 silages would provide minimal proportion of residue that would take up space if utilised in *in vivo* studies and also persists as indigestible residue. Therefore, the potential extent of digestion ('b') values obtained for T_2, T_3 and T_5 demonstrated that they possess more potentially degradable carbohydrates than T_1, T_4, and T_6. Also, the results presented in Table 6 actually demonstrated that digestion rates ('c') and potential extent ('b') of gas production provided a more meaningful index of nutritional value than ultimate digestibility comparatively. Furthermore, in this study, the conversion of true fermented organic matter into gas varied with the types of additives used to prepare the silage.

***In vitro* gas production parameters of ensiled guinea grass with different protein at additives 24hrs incubation period.**

The basic and the most common form of feed evaluation by animal nutritionists is usually proximate composition. However, a more reliable technique of estimating livestock feed is *in vitro* gas fermentation (Menke and Steingass, 1988). Although the two methods are independent of each other however, they are interrelated. Gas production is an indication of microbial degradability of samples

(Babayemi *et al*., 2004b, Fievez *et al*., 2005). All the parameters, observed in this study indicating that the treatments had significant effects on the nutritive value of ensiled guinea grass with or without protein additives. Total gas volume (TGV) and methane production (CH_4) ranged values (9.00 to 15.00ml and 4.00 to 6.00ml) of the silages were relatively low as compared to other works. In most cases, feedstuffs that showed high capacity for gas production were also observed to be synonymous for high methane production. Methane (CH_4) production is said to be energy loss to ruminants and also contribute to global warming (Babayemi and Bamikole, 2006). Methane in the rumen is an energetically wasteful process, since the portion of the animal's feed, which is converted to CH_4, is eructated as gas. The low levels of CH_4 production suggest that the energy of silages will be utilised efficiently if fed to the animals. Generally, gas production is a function and a mirror of degradable carbohydrate and therefore, the amounts depends on the nature of the carbohydrates (Blummel and Becker, 1997).

Nagadi *et al.* (2000) reported that differences in the diet of the donor animal influenced gas production from different substrates differently, thereby indicating that there is an interaction of diet of the donor animal and type of feed incubated. The diet of the donor animal exerted considerable influence on bacterial concentrations and therefore influenced *in vitro* gas production. Since different feeds can affect the relative proportion of microbes in the rumen, this may influence the extent of fermentation of feeds. The magnitude of the diet effect can vary with the type of feed incubated (Nagadi *et al.,* 2000).

Furthermore, gas production is a nutritionally wasted product (Mauricio *et al*. 1999) but provides a useful basis from which metabolisable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) could be estimated. More importantly, gas production helps to measure digestion rate of soluble and insoluble fractions of feedstuff (Menke and Steingass, 1988). The gas produced is directly proportional to the rate at which substrate are degraded. Somart *et al*. (2000) reported that gas volume is a good parameter to predict digestibility, fermentation and its product and microbial protein synthesis of the substrate by microbes in the *in vitro* system. Gas production was directly proportional to SCFA (Beuvink and Spoelstra, 1992), the higher the gas produced, the higher the short chain fatty acids. The SCFA level indicates that the energy is available to the animal and it contributes up to 80% of animal daily energy requirement (Fellner, 2004). Short chain fatty acid (SCFA) is directly proportional to metabolisable energy (ME) in this study. The organic matter digestibility (OMD) which could be said to be a measure of degradability (potentials) of the microbes on the substrates especially in the presence of sufficient ammonia nitrogen (NH_3-N) which has influence on bacterial fermentation was highest (67.65%) in T_3 and lowest in T_1 (57.64%)

The mean value for methane production was lowest in treatments 1(Control), 5 and 6 silages. Research on rumen methanogenesis and its inhibition was initiated with aim of increasing feed efficiency. This means that reduced methane production will lead to greater efficiency in feed utilisation. Depending on the level of feed, composition of the diet and digestibility, 2.15% of the gross energy in the feed is lost through methane production (Holter and Young, 1992). This also implied that there will be more energy for the animals on treatments 1(control), 5 and 6 silages. However, for economical reason, treatment 3 (guinea grass + cassava tops silage) will be recommended for the farmers since soybean meals is costly and not readily available.

A correlation between ME values measured *in vivo* and predicted from 24hr *in vitro* gas production and chemical composition of feed was reported by Menke and Steingass (1988). The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed (Getachew *et al*., 1999; 2000). The result obtained in this study is in order with that reported for forage legumes and crop residues by Babayemi *et al*., (2009) and Alasa *et al*., 2010). Further more, the ME values of the silages (4.20 - 4.92 MJ/Kg) were within the ranges reported by Menke and Steingass (1988), where the ME values of various European feeds ranged from 4.5 to 15 MJkg-1 DM. Metabolisable energy (ME) values are very useful and important for purposes of ration formulation and to set the economic value of feeds for trading purposes. The fermentation efficiency (FE) was significantly ($P<0.05$) different across the treatments such that it was highest inT_1 (4.24%) and lowest in T_3 (2.78%). The value (2.78%) of Fermentation Efficiency (FE) obtained from T_3 and Total Gas Volume (TGV) value (15.00%) suggest that the silage type contained soluble carbohydrates (soluble sugar) which favouring a higher gas production and faster /rapid fermentation kinetics

CONCLUSION

The guinea grass ensiled with protein additives exhibits good quality characteristics in terms of colour, odour, and texture (dry and firm) and is not mouldy. The chemical composition indicates that the additives significantly improved the dry matter and protein contents of the ensiled guinea grass, while *in vitro* gas production showed low methane (CH_4) production, an indication of a reduction in energy loss. Thus, there would be greater efficiency in feed utilisation by ruminant animals. The findings of this work showed that ensiled guinea grass with protein additives also improved the *in vitro* fermentation gas parameters (TGV, ME, OMD, DMD SCFA, and FE) of the silage types.

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