Nigerian J. Anim. Sci. 2016 (1):230 - 241

Fermentative characteristics and *in vitro* gas production of *Pennisetum purpureum* hybrid grass silage as influenced by manure type and age at harvest

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Target Audience: Forage Scientists, Ruminant Nutritionists

Abstract

The nutritive quality of Pennisetum purpureum hybrid grass silage as influenced by manure types and age at harvest were evaluated by their fermentative characteristics and in vitro gas production. The study was a factorial arrangement in a randomized complete block design which comprised four manure types (cattle, poultry, swine, goat) and no manure (control) and two harvesting times (4 and 8 weeks after cutback (WAC)) with three replicates. Results showed that the mean pH (4.70) of silage made from grass fertilized with cattle manure was significantly ($p<0.05$) different from the value of 5.20 in silage of unfertilized grass at 4WAC. Lactic acid contents of the silage ranged from 4.32 % to 6.99 %, with silage made from swine manure fertilized grass being highest ($p < 0.05$). Crude protein (CP) contents of the silage ranged from 6.30 % in silage made from unfertilized grass at 8WAC to 9.10 % in silage from grasses that were fertilized with goat and poultry manures at 4WAC. Neutral detergent fibre (NDF) contents ranged from 54 % to 66 % in silage made from grass that was harvested at 8WAC having the highest ($p < 0.05$) NDF contents. The highest in vitro gas production of 43.33 ml/200mg DM was recorded in silage from grass that was harvested at 4 WAC and fertilized with cattle manure. The silage with highest in vitro dry matter digestibility (56.67 %) was from poultry manure fertilized grass harvested at 4WAC with lowest value (49.67 %) in silage from unfertilized grass, harvested at 8WAC. The study showed that the silage of Pennisetum purpureum hybrid grass fertilized with swine manure and harvested at 4 WAC have higher quality above other manure types as well as unfertilized grass harvested at later stage.

Keywords: manures, digestibility, regrowth, *Pennisetum purpureum,* silage

Description of Problem

Majority of ruminants in the tropics, are mainly fed with grasses. However, these grasses grow rapidly during the wet season, becoming fibrous, coarse and low in nutrients as the season advances towards the dry season (1). These eventually leads to a reduction in nutrient intake by animals and thereby hamper their growth and productivity. The constraints posed by dry season on continual availability of pasture to

livestock in the tropics, has been a matter of concern in animal production. Silage production in the tropics is a sustainable means of supplementing feed for ruminants in the dry season (2). Ensiling is a potent general method for forage preservation and also a form of treatment to occasionally salvage the underutilized pastures for better acceptability and degradability (3).

There has been a renewed interest in the use of *Pennisetum purpureum* grass for ruminants feed in the tropics, since it has been identified as a high yielding forage species (4), and earlier reports had suggested that hybrids of Pennisetum are superior to their parents. (5) used *P. purpureum* as the female to produce an F_1 hybrid with *Pennisetum glaucum*, a late maturing millet which was superior to the parents. This hybrid had been found to combine the high yielding characteristics and perennial habit of elephant grass with the rapid early growth and the superior nutritive value of millet.

Researchers have shown that the application of fertilizer is needed to improve the fertility of the soil and consequently, the productivity and quality of the pasture grown on it (6, 7). Although, application of inorganic fertilizers boosts the performance and productivity of forages, its persistent use increase soil acidity and soil physical degradation which could destroys soil reaction and impedes the activities of soil micro organisms thereby reducing crop yield (8). Also, their unavailability and high cost borne by livestock producers have also been a major constraint. This has prompted the use of organic manures since they are cheap and readily available (9). Manure improves soil nutrients status thereby providing plants with better nutrient uptake that is eventually beneficial to animals. Such a profitable use will help in addressing the problem of manure management and disposal (10). Harvesting of forage species at the right stage of growth with proper management is among the strategies towards improving the nutritive values of forages (11).

The chemical and microbiological characteristics of normal silage include high lactic acid levels relative to the levels of acetic and butyric acids, low pH, low content of ammonia and volatile nitrogen, and low numbers of spore forming anaerobes (12). Fermentation analysis reveals whether an excellent, average, or poor fermentation has occurred.

The use of *in vitro* gas production in evaluating forage digestibility is a quick and less expensive means of determining the nutritive value of feeds for ruminants (13). Total gas production can predict methane (CH_4) and volatile fatty acids (VFA) production (14). The present study was designed to evaluate the nutritive value of ensiled Pennisetum hybrid grass as influenced by manure and age at harvest.

Materials and methods

Experimental site

The study was carried out at the Teaching and Research Farm and Pasture and Range Management laboratory, Federal University of Agriculture, Abeokuta, Nigeria. The site is situated in the derived savanna agro-ecological zone of South Western Nigeria (latitude: 7°N, longitude 3.5°E, average annual rainfall: 1037 mm) Mean

monthly temperature ranges between 25.70 ^{0}C in July and 30.20ºC in February (15).

Land preparation, manure collection, analysis, application and planting of grass

The land used for the experiment was divided into three replicates with each replicate subdivided into plots of 3 x 4 m (12 m^2). The land have been previously cleared, ploughed and harrowed. After land preparation and before planting, soil samples were randomly collected from the plots at the depth of 0-15 cm using soil auger to determine the pre-planting nutrient status of the soil. Analysis of the soil from the site indicated that it was sandy silt with pH of 7.03; organic carbon 1.29% and available phosphorus 53.87mg/kg, potassium 0.20cmol/kg, calcium 2.77cmol/kg, magnesium 2.72cmol/kg, sodium 0.80cmol/kg and total nitrogen 0.11%. The four animal manure types used for this study were analysed for N content; swine (16.9 g/kg), cattle (15.6 g/kg), poultry (30.2 g/kg) and goat (15.3 g/kg) . Manure was applied by broadcasting to individual plots according to treatment.

After the application of manures, the plots were left for two weeks to rest before planting of the grass at 1 x 1m intervals. Eight weeks after planting, the grasses were cut back to 10cm above ground level to allow for uniform regrowth.

Experimental design

The study was a 5×2 factorial arrangement using randomized complete block design which comprised four manure types (cattle, poultry, swine, goat) and no-manure (control) and two harvesting times (4 and 8 weeks after cutback). The experiment was replicated three times.

Processing of *Pennisetum purpureum* hybrid grass into silage

Pennisetum purpureum hybrid grasses that were fertilized with different animal manure types as well as unfertilized grasses were harvested **at 4 and 8 weeks after cutback (WAC)** 15cm above ground level**.** The harvested grass samples were chopped into pieces of 2cm in length, wilted for 4 hours to reduce their moisture contents before ensiling. The forages were carefully packed into laboratory (960 ml) bottle silos after mixing thoroughly following the method described by (16) and were ensiled for a period of 6 weeks at an ambient temperature of 26 ^{0}C. At the expiration of the ensiling, the bottle silos were opened, the fermentative characteristics, chemical and *in vitro* analyses of the silages were determined.

Chemical analysis

Twenty five (25) g of silage was taken and mixed in distilled water. The supernatants from the mixtures was decanted into separate plastic bottles and sub-fractioned into two sets for determination of ammonia and volatile fatty acids (VFA) analyses using (17) and (18) procedures, respectively. The pH of the silage was measured with the use of pH meter (Hanna instruments, pH 211, microprocessor) immediately after the opening of the silos. Also, samples of 300g were taken from each silo and oven-dried to a constant weight at 65 °C. The dried foliage samples were milled through a 1mm sieve and the proximate, phosphorus (P) and calcium (Ca) composition of the silage were determined according to the standard methods of (19). Neutral detergent fibre

content was determined according to (20). *In vitro* gas production was determined according to procedure of (21).

Statistical analysis

Data collected were subjected to a two-way Analysis of Variance and treatment means were separated using Tukey at 5% level of significance (22).

Results and Discussion

Table 1 shows the effects of animal manures and age at harvest on the pH and fermentative quality of Pennisetum hybrid grass silage. There was significant difference in all the fermentative parameters of the silage considered as influenced by harvesting time and manure types. Silage from swine manure fertilized grass at the two harvesting times, recorded significantly higher ($P<0.05$) lactic acid contents than in silage made from grass fertilized with other animal manures. Meanwhile, buffering capacity of silage from unfertilized grass was highest ($P<0.05$) while the least was in silage from swine manure fertilized grass. The indicators of fermentation process are content of fermentation carboxyl acids and active acidity (pH) (23). The pH values recorded for most of the silages were within the range of 4.50-5.50 reported and classified as good silage by (24) with silage made from unfertilized grasses being highest. This fact suggests that activities of undesirable microorganism were slowed down by low pH. Silages made from 8WAC had lower pH than 4WAC. This agreed with (25), who found that silage made from forage that had been subjected to wilting was stable under a lower pH value.

The content of desirable lactic acid in this study falls within 23.7 g/kg to 58.9 g/kg dry matter for maize silage hybrids. The high lactic acid concentration of the silages is a clear indication of good preservation, which invariably results in the lowest loss of dry matter and energy during storage (26). These contents fall within the interval proposed by (27), from 3 to 13% of lactic acid in the silage juice.

The acetic acid concentration of the silage in this study was within the range classified as normal for grass silage (0.5-3.0 %DM) (26) and also falls in line with 0.87-2.26 %DM for sugarcane silage (26), which is within the range classified as normal for grass silage. The acetic acid concentration of the silage in this study is also within the range of 3.4-16.1 g/kg DM reported by (29) for different maize silage hybrids. All the silages in this study fulfilled condition of acetic acid content to classified as first quality class (content of acetic acid < 20 g/kg of dry matter) (29). (26) reported that high acetic acid concentration could suppress dry matter intake of silage when fed to ruminants.

The butyric acid concentration is an important indicator of proteolytic activity in the ensiled materials. The least values as observed in this study was in plant fertilized with swine manure and harvested at 4WAP (0.29%) while the highest content was in silage with unfertilized grass, harvested at 8WAP (0.51%). From a standpoint of negative influence to animal health, silages with high content of butyric acid are undesirable. The butyric acid concentrations in *P. purpureum* hybrid silage in this study exceeded the limit for first quality class (2.5 $g.kg^{-1}$ of dry

matter) (29) but can still be tolerated by animals without negative effects.

The buffer capacity (BC) of materials destined for ensiling is one of the characteristics that determine the passage from an initial butyric fermentation to the quick establishment of lactic fermentation organisms, which confer desirable characteristics to the silage (30). The lowest BC obtained was in silage that was from grasses that was fertilized with swine manure and harvested at 8WAP (1855 mmol kg^{-1} DM) while the highest content was from unfertilized plant harvested at 4WAP (2025 mmol kg^{-1} DM). Low BC allowed for a fast decline in pH in the ensiled mass, inhibiting acetic and butyric fermentation which is necessary for good preservation (31). The values are very similar to those observed for maize plants (32).

Ammonia nitrogen (NH_3-N) works as an important indicator of proteolytic activity during the fermentation process. According to (33), the ammonia concentrations must not be higher than 12 % of total nitrogen in well preserved silages. The silages from this study as influenced by age at harvest and manure application falls within the requirement

Table 1: Effect of animal manures and age a t harvest on the pH and fermentative quality of Pennisetum hybrid grass silage.

Harvesting time	Manure	pH	Buffering Capacity (mmol kg^{-1} DM)	NH_3-N (%)	Lactic acid (%)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)
4WAC	Cattle	4.70^{d}	2016^{b}	8.11^{b}	5.57^{d}	1.53^{a}	0.50^{bc}	0.40^{a}
	Swine	4.84^{c}	1897^{e}	7.43^{c}	6.56^{a}	0.81^{e}	0.46^{c}	0.29^{b}
	Goat	4.88^{c}	1901^{d}	7.99^{b}	6.03^{b}	1.04^{c}	0.52^{bc}	0.40^{a}
	Poultry	5.00^{b}	2002^{c}	8.01^{b}	5.88^{c}	0.92^{d}	0.64^{a}	0.35^{ab}
	Control	5.20^{a}	2025^{a}	13.33^{a}	4.32^{e}	1.21^{b}	0.56^{ab}	0.39^{a}
SEM		0.07	0.07	0.12	0.15	0.11	0.10	0.10
P-value		<0.001	<0.001	<0.001	0.005	<0.001	<0.001	0.005
8WAC	Cattle	4.50^{d}	2011^{b}	8.42^{b}	5.71^{c}	1.22^{b}	0.82^{a}	0.40^{bc}
	Swine	4.82^{c}	1855^{e}	6.58^{e}	6.99^{a}	0.74^{d}	0.59^{c}	0.35^{cd}
	Goat	4.84^{c}	1912^{d}	7.62^{c}	6.41^{b}	0.87^{c}	0.65^{bc}	0.45^{ab}
	Poultry	4.99^{b}	1986^{c}	7.22^{d}	5.75^{c}	0.82^{cd}	0.71^{b}	0.27^{d}
	Control	5.13^{a}	2012^{a}	12.01^{a}	4.74^{d}	1.41^{a}	0.82^{a}	0.51^{a}
SEM		0.14	0.16	0.14	0.18	0.11	0.08	0.03
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Harvesting time x manure type		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

$^{a-e}$: Means in the same column with different superscripts are significantly ($p<0.05$) different.
SEM- Standard Error of Means; WAC: weeks after cutback

Effects of manure types and harvesting times on the chemical composition of silage produced from Pennisetum hybrid grass was shown in Table 2. All the parameters of the silage for chemical composition were all significantly different ($P < 0.05$). Silage made from goat and poultry manure fertilized grasses recorded significant ($P < 0.05$) higher CP content at 4WAC than the other silages. Greater dry matter (DM) content in the silages from Pennisetum harvested at 8WAC underscores the importance of maturity of grasses before ensiling. Forages that are advanced in age have less moisture compared with young plants. As such, the level of DM concentrates the water soluble carbohydrates and improves the effectiveness of the lactic acid bacteria

and the quality of silage.

The crude protein content with a range of 6.3 – 9.1 % recorded for the silages at different harvesting ages and manure types is well above the threshold of 60 g/kg required by rumen microbes to build their body protein for effective digestion of forages by ruminants (34). This type of silage will be adequate in providing high quality feed for livestock production during the dry season. The values recorded for ether extract and ash contents of the silages were high enough to supply animals with their energy and mineral requirements for maintenance and other productive functions (35).

The NDF contents of Pennisetum hybrid ensiled at 4 and 8 week after cutback in this study were within 56.4-63.8 % reported for wilted and unwilted Panicum and Pennisetum silage (35). Majority of the NDF values recorded in this study were below the 65% suggested as the level at which intake of tropical feeds by ruminants would be limited (36).

Ensiling at 4WAC resulted in an increase in the mineral contents of the silages produced above the one at 8WAC. Generally, silages produced from grasses that were fertilized had higher Phosphorus (P) contents than unfertilized grasses. This result shows that the silage produced from Pennisetum hybrid may have been fortified by animal manures application. The range of P in this study falls between the normal requirements for growing cattle (1.1-4.8g/kg) (37). The range of values recorded for Ca in the present study was above the critical level of 3 g/kg DM recommended for ruminants needs in the warm wet climates (38).

Table 2: Effect of animal manures and age at harvest on the chemical composition of Pennisetum hybrid silage.

Factors		DM	CP	EE	ASH	NDF	Ca	P
Harvesting time	Manure	(%)					(g/kg)	
4WAC	Cattle	94.40^{a}	8.17^{c}	8.00^{d}	13.00^{c}	56.00^{b}	6.90^{b}	2.27^{c}
	Swine	85.40^{e}	8.63^{b}	9.00^{c}	12.00^{d}	60.00^{a}	7.14^{a}	2.37^{b}
	Goat	93.40^{b}	9.10^{a}	16.33^{a}	13.00^{c}	56.00^{b}	6.60^{c}	2.32^{bc}
	Poultry	92.00^{c}	9.10^{a}	7.33^{e}	14.33^{a}	54.00^{c}	7.06^{a}	2.46^{a}
	Control	87.33^{d}	8.17^{c}	14.00^{b}	13.67^{b}	54.00^{c}	7.10^{a}	1.98^{d}
SEM		0.72	0.13	0.57	1.03	0.94	0.04	0.09
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
8WAC	Cattle	96.40^{c}	7.93^{a}	6.00^{e}	8.00^{b}	66.00^{a}	6.99^{a}	2.37^{c}
	Swine	97.20^{a}	7.47^{b}	8.00^{c}	8.00^{b}	66.00^{a}	6.84^{b}	2.28^{d}
	Goat	94.40^{d}	7.47^{b}	7.00^{d}	9.00^{a}	52.00^{b}	6.46^{d}	2.46^{b}
	Poultry	96.60^{b}	7.23^{c}	10.00^{a}	9.00^{a}	62.00^{c}	6.67^{c}	2.57^{a}
	Control	90.00^{e}	6.30^{d}	9.00^{b}	7.00^{c}	64.00^{b}	6.78^{b}	1.01^{e}
SEM		0.92	0.22	0.64	1.99	1.18	0.42	0.80
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Harvesting time x manure type		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a-e}$: Means in the same column with different superscripts are significantly ($p<0.05$) different.
SEM- Standard Error of Means. WAC: weeks after cutback; DM: Dry matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fibre; Ca: Calcium; P: Phosphorus

Table 3 shows the effects of animal manures and age at harvest on the *in vitro* gas production of Pennisetum hybrid silage. There was significantly (P<0.05) higher *in vitro* digestibility in silage made from grasses that were harvested 4WAC over the one harvested at 8WAC with cattle fertilized grasses having a significant (P<0.05) higer digestibility above other manure types and control. The *in vitro* gas production technique is a useful tool in determining the nutritional value of forages because the volume of gas produced by forage species reflects the end products of the fermentation of its substrate to volatile fatty acids (VFA), microbial biomass and neutralization of the VFA, thereby demonstrating the nutritional value of such forages (39). From this study, it reveals that Pennisetum hybrid silage that were fertilized and harvested at 4 weeks after cutback, produced higher gas than those of unfertilized, suggesting that they were of higher nutritional value than unfertilized grasses and it connotes high digestibility of the forages. Higher gas production at 4 over 8 weeks of harvest was in agreement with the reports of (40) which stated that maturity affects gas production and it decreases with advancement in age of the plant. This is because age at harvest is an important factor affecting nutritive value of forages.

Table 3: Effect of animal manures and age at harvest on the *in vitro* gas production of Pennisetum hybrid silage

Harvesting time	Manure	3hr	6hr	9hr	12hr	24hr	36hr	48hr
4 WAC	Cattle	3.67^{a}	6.67^{a}	9.67^{a}	13.67^{a}	24.33^{a}	33.33^{a}	43.33^{a}
	Swine	2.67^{c}	4.67^{c}	7.67^{c}	11.00^{c}	21.33^{c}	33.33^{a}	41.33^{b}
	Goat	3.17^{b}	5.67^{b}	8.67^{b}	12.00^{b}	23.00^{b}	32.67^{a}	39.67^{c}
	Poultry	4.33^{a}	7.00^{a}	10.00^{a}	10.00^{c}	20.33^{c}	31.67^{a}	39.00^{c}
	Control	0.83^{d}	1.00^{d}	1.33^{d}	2.00^{d}	10.67^{d}	23.00^{b}	30.67^{d}
SEM		0.55	0.64	0.84	1.07	1.78	2.59	3.08
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
8 WAC	Cattle	2.00^{c}	2.67^{c}	3.67^{c}	4.67^{c}	11.00^{c}	17.00^{c}	23.33^{c}
	Swine	5.67^{b}	5.67^{b}	6.67^{b}	8.33^{b}	16.00^{b}	22.67^{b}	27.67^{b}
	Goat	6.67^{a}	8.00^{a}	9.33^{a}	11.67^{a}	21.00^{a}	27.33^{a}	34.33^{a}
	Poultry	0.67^{d}	2.00^{c}	4.00^{c}	5.33^{c}	12.00^{c}	20.67^{b}	26.67^{b}
	Control	2.10^{c}	2.52^{c}	3.38^{c}	4.91^{c}	10.01^{c}	16.77^{c}	23.00^{c}
SEM		0.37	0.89	0.59	0.89	1.19	2.38	3.05
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Harvesting time x manure type		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

$^{a-d}$: Means in the same column with different superscripts are significantly (p<0.05) different.
SEM- Standard Error of Means; WAC: weeks after cutback

Effects of animal manures and age at harvest on the *in vitro* dry matter digestibility of Pennisetum hybrid silage is shown on Figure 1. Silage that was made from poultry manure fertilized grass and harvested at 4WAC recorded highest dry matter digestibility above others. The *in vitro* dry matter digestibility (IVDMD) of silages made from fertilized grasses recorded higher digestibility than the unfertilized ones. Nutrient levels of grasses have been noted to have a positive effect on IVDMD (41). As the plants ages, the quality of the IVDMD of the silage decreases. (42) reported similar depressed IVDMD of the grass species harvested at relatively advanced ages,

and this reduction in digestibility has been associated with increased levels of lignin deposition in the cell wall with increasing maturity (43).

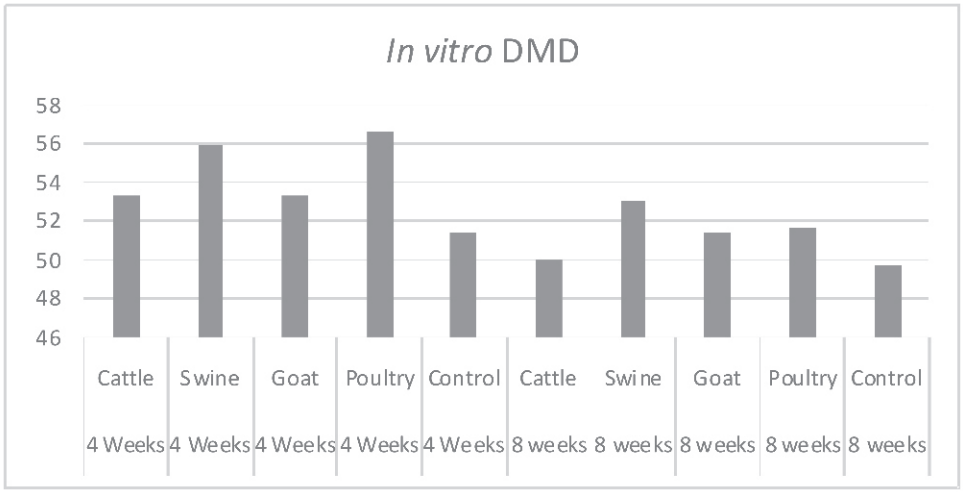

Figure 1: Effect of animal manures and age at harvest on the *in vitro* dry matter digestibility of Pennisetum hybrid silage

Conclusion and application

From this study, silage made from *Pennisetum purpureum* hybrid grass fertilized with swine manure and harvested at 4WAC recorded higher nutritive quality. For higher quality silage which can serve as supplements for grazing ruminants during the dry season, grasses should be fertilized with swine manure and harvested at 4 weeks after cutback.

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