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PHYSICAL CHARACTERISTICS AND CHEMICAL COMPOSITION OF *Panicum maximum* ENSILED WITH BREWERS' SPENT GRAINS FOR DIFFERENT PERIODS

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ABSTRACT

The effects of ensiling Panicum maximum (PM) with different proportions of brewers' spent grain (BSG) on the physical characteristics, chemical composition and fermentation pathways were investigated in this study. Four silage-based experimental treatments consisting of PM and BSG (0, 10, 20, 30%) were formulated into and ensiled inside 4-litre plastic silos. Each treatment was replicated 18 times. Samples were obtained and analyzed on Day 0, 7, 14, 21, 28 and 35 for pH, physical characteristics (colour, odour/aroma, texture and yeast growth) and chemical composition. The pH values were significantly ($p<0.05$) affected by duration of ensiling. The pH of the silages tended to reduce with time and higher proportions of BSG in the mixtures. The colour of silage reflected more of the green colour of the grass and got slightly brownish with time and higher proportions of BSG. All silages had pleasant aroma, which improved with higher proportion of BSG. Texture of the silages varied from slightly firm to firm. Yeast growth tended to reduce with higher proportion of BSG in the silage mixture. Dry matter recovered (60.01 – 87.69%) increased with increasing BSG in the silage. At Day 28, crude protein content (CP) of the silages varied from 8.05 to 13.65% and increased with increasing BSG in the silage. Energy value of the silages at Day 28 varied from 400.46 to 412.02Mcal/kg and increased with BSG in the silage. Addition of BSG to Panicum maximum improved the physical characteristics and chemical composition of the grass silage.

Keywords: Silage, Physical and Chemical properties, Fermentation pathways, Ruminant.
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INTRODUCTION

Guinea grass (*Panicum maximum*) is native to tropical Africa, where it grows in abundance. *Panicum maximum* (PM) is mostly utilized as pasture or for grazing by livestock, as cut and carry fodder and for preservation as hay and silage. Due to the low crude protein and nutritional composition of guinea grass (Tona *et al.* 2014; Ogunbosoye, 2013), it is preferable to supplement it with sources of fermentable carbohydrates and protein to improve feed and silage quality (Ifut *et al.*, 2015; Jolaosho *et al*., 2013).

Brewers' spent grain (BSG) are the solid residues left after the processing of germinated and dried cereal grains (malt) for the production of beer and other malt products (malt extracts and malt vinegar). Barley is the main grain used for brewing. Other grains used are wheat, maize, rice and sorghum. The chemical composition and nutritional value of BSG varies depending on

the type of grain used and industrial processing method. Brewers' spent grain is rich in protein and fermentable carbohydrates (Ekanem *et al.*, 2017).

Ensiling is one of the most efficient, cheapest, and safest ways for conserving forage and wet agro-industrial by-products such as BSG. Factors affecting the ensiling (fermentation) process include forage composition (dry matter, fermentable carbohydrates, buffering capacity, number and type of bacteria), ensiling technique to expel air (forage chop length, silo packing speed, silo packing density) and silo management during storage and feed out (Ekanem *et al.*, 2017; Sucu *et al*., 2016; Kung and Shaver, 2001; Kung, 2001). Fermentation analyses have long been used to ascertain the type of fermentation and silage quality (Ekanem *et al.*, 2017; Falola *et al.*, 2013; Kung and Shaver, 2001). Thus, the aim of this experiment was to evaluate the effect of ensiling duration on the physical characteristics and chemical composition of *Panicum maximum* supplemented and ensiled with brewers' spent grain.

MATERIALS AND METHODS

The study was carried out at the Goat Unit of the Department of Animal Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria. Uyo is located between latitudes 4˚59′ and 5˚04′ N and longitudes 7˚52′ and 8˚00′ E in the tropical rainforest zone which characterizes the South South agro-ecological zone of Nigeria. Rainfall is bi-modal with the first peak occurring around July, followed by a short dry spell referred to as "August break"; and the second peak occurs around September. The annual rainfall ranges from 800 – 3,200 mm per annum with mean annual rainfall of 2,190 mm and mean relative humidity of 81%. Annual temperature varies between 23 and 28^{o}C (Ifut *et al.*, 2015; Ifut and Mbaba, 2014).

The *Panicum maximum* (PM) was harvested from the University of Uyo Teaching and Research Farm, Use Offot, Uyo, which is on an elevation of about 60.96 m above sea level. The harvested grass was wilted for 24 hours, chopped into uniform length of 2 – 3 cm and mixed with Brewers' Spent Grain (BSG). The *Panicum maximum* contained (AOAC, 1990) Dry Matter (DM), 33.58%; Crude Protein (CP), 7.58%; Crude Fibre (CF), 30.50%; Ether Extract (EE), 3.56%; ash, 8.77% and Nitrogen Free Extract (NFE), 49.59%.The BSG was sourced from the Akwa Ibom State Champion Breweries PLC. The wet BSG was pressed mechanically to reduce the moisture (Ifut *et al.*, 2015). The PM and BSG were mixed in different proportions to form the following silage mixtures which correspond to the treatments:

Silage 1 = 100% *Panicum maximum* (PM)
Silage 2 = 90% PM + 10% Brewer's Spent Grains (BSG)
Silage 3 = 80% PM + 20% BSG
Silage 4 = 70% PM + 30% BSG

The ensiling procedure was as described by Ifut *et al.* (2015). Accurately weighed quantities of the silage components were mixed, filled and compressed into 4-litre plastic silos lined with polythene sheets. Each treatment had 15 replicates. Sand bags were placed on top of each silo to further expel air and prevent further re-entry of air. Samples of the silage mixtures were taken for pH determination, physical characteristics (odour, colour, and texture), dry matter (DM) and chemical composition at Days 0, 7, 14, 21, 28 and 35. At each day of sampling, three silos were opened per treatment for evaluation.

Samples of the silages were taken to the laboratory and oven-dried at 60^{0}C for 48 hours and subsequently ground using an electric blender. Dried and ground samples of

the silage were used for chemical analyses. To determine pH, 25g of the wet silage sample was soaked inside 100 ml of distilled water inside a beaker and stirred for 10 minutes. The pH of the supernatant was read using a pocket-size pH meter (S399590). Crude protein, ether extract, crude fibre and ash in the silages were determined according to the method of AOAC (1990). Nitrogen free extract (NFE) was estimated as: NFE = 100 - (CP + CF + EE + ash). The fibre fractions - neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined following the procedures of Van Soest *et al*. (1991). The gross energy of the silages was calculated using the Atwater general factor system (Atwater and Woods, 1896) and the fermentation pathways (type of fermentation, end products, theoretical dry matter and energy recovered) were arrived at based on the range of parameters postulated by Kung (2001). The theoretical dry matter and energy recovery rate were calculated as the final dry matter contents divided by the initial dry matter contents multiplied by 100 (Ekanem *et al.*, 2017; Kung, 2001). Data obtained were subjected to analysis of variance using SAS (2000) Statistical software. Means were separated using Duncan Multiple Range Test of the same Statistical package.

RESULTS AND DISCUSSION

Table 1 shows the effect of time of ensiling on the physical characteristics (colour, odour, texture and yeast growth) of *Panicum maximum* ensiled with brewer's spent grain. The silages had the green (*Panicum maximum* leaves) and brown (BSG) colour of the components used in silage making. The colour of the silages (light green/greenish brown) was maintained throughout the ensiling duration. The preservation of the colour of the original ingredients contributing to the silages mixture corroborates the work of t' Mannetje (1999) and Ekanem *et al.* (2017) for properly fermented silage. The odour was slightly pleasant for silage 1 (100% PM) throughout the ensiling duration. The odour for diets 2 to 4 containing BSG was pleasant and this improved throughout the ensiling duration. Pleasant aroma in silages indicates the formation of organic acids in the mass which makes the silage to be readily accepted by ruminants. Pleasant aroma are accepted for good made silage (Ekanem *et al.*, 2017; Olorunnisomo and Fayomi, 2012; Kung and Shaver, 2001). The texture for silages 1 - 3 was slightly firm and remained such throughout the ensiling duration. For silage 4 with 30% BSG supplementation, the texture was slightly firm at days 0 – 27 of ensiling, but was firm from day 28 of ensiling till the end of the ensiling period. Firm texture has been reported to be the best texture of good quality silage (Ekanem *et al.*, 2017; Olorunnisomo and Fayomi, 2012; Kung and Shaver, 2001). Kung (2001) theorized that yeasts and not moulds are the primary microorganisms responsible for aerobic spoilage and heating in silages. There were no yeast growths in all the silages at day 0. Yeasts growths were observed in silage 1 on day 7 of ensiling. For silages 2 (10% BSG supplementation) and 3 (20% BSG supplementation), slight yeast growth were observed from day 21 of ensiling. However, for silage 4, no yeast growth was observed throughout the ensiling duration. The yeast growth observed in silage 1 at day 7 onward may be as a result of the high dry matter content or slightly firm nature of that silage which hindered it from being properly packed. Air pockets are difficult to expel in silage mixtures that is not well packed, thus leading to aerobic spoilage (Kung, 2001). Since silages 2 and 3 were well compacted, growth of yeasts was delayed until the 21st day of ensiling

Table 1: Effect of ensiling duration on the physical characteristics of *Panicum maximum* ensiled with brewers' spent grain

SILAGE	DAYS	COLOUR	ODOUR	TEXTURE	OTHER OBSERVATIONS
1	0	Light green	Slightly pleasant	Slightly firm	No yeast growth
	7	Greenish brown	Slightly pleasant		Yeast growth
	14	”	”	”	”
	21	”	”	”	”
	28	”	”	”	”
	35	”	”	”	”
2	0	Light green	Pleasant	Slightly firm	No yeast growth
	7	Greenish brown	Slightly pleasant	”	”
	14	”	”	”	”
	21	”	Pleasant	”	Slight yeast growth
	28	”	”	”	”
	35	”	”	”	”
3	0	Greenish brown	Pleasant	Slightly firm	No yeast growth
	7	”	”	”	”
	14	”	”	”	”
	21	”	”	”	Slight yeast growth
	28	”	”	”	”
	35	”	”	”	”
4	0	Greenish brown	Pleasant	Slightly firm	No yeast growth
	7	”	”	”	”
	14	”	”	”	”
	21	”	”	”	”
	28	”	”	Firm	”
	35	”	”	”	”

Silage 1 = 100% *Panicum maximum* (PM). Silage 2 = 90% PM + 10% Brewers' Spent Grains (BSG). Silage 3 = 80% PM + 20% BSG. Silage 4 = 70% PM + 30% BSG.

The effect of ensiling duration on the pH values of *Panicum maximum* and brewers' spent grain silage are shown in Table 2. There were significant ($p<0.05$) reduction in pH in all the silages as ensiling progressed. The pH reduced from 6.90 to a final value of 5.50, 6.00 to 5.00, 5.90 to 5.00 and 5.50 to 4.60 in silages 1, 2, 3 and 4, respectively. For silage 1, there was an increase in pH to 7.10 and 7.20 at days 7 and 14 respectively, before subsequent reduction to the final pH of 5.50. This was occasioned likely by the yeast growth observed. The yeast in the presence of air, metabolized lactic acid, caused a rise in pH, with subsequent proliferation of aerobic spoilage bacteria that were initially inhibited by reduced pH (Kung, 2001). The final pH of silage 1 (5.50) was higher than the reported values of 4.3 – 5.0 by Kung and Shaver (2001) for grass or legume silage, but lower than the range of 5.56 –5.66 reported by Falola *et al.* (2013) when they ensiled 100% vetiver grass. In contrast, the final pH recorded for silages 2 – 4 were 4.6 – 5.00 and was within the acceptable pH range for well-made grass-concentrate silage (Kung and Shaver, 2001). The pH of the silages reduced with increased BSG in the silage mixtures as more fermentable carbohydrates was available to favour effective fermentation

(Ekanem *et al.*, 2017; Olorunnisomo and Fayomi, 2012).

The effect of ensiling duration on the percentage dry matter contents of *Panicum maximum* and brewers' spent grain silage is shown in Table 3. There were significant ($p<0.05$) reduction in the dry matter contents of all the silages especially at day 14 of ensilage. Reduction in silage dry matter was as a result of loss of water soluble carbohydrates during fermentation, corroborating other studies (Ekanem *et al.*, 2017; Ifut *et al.,* 2015; Jolaosho *et al.,* 2013; Falola *et al.*, 2013; Olorunnisomo and Fayomi, 2012; Akinola, 2008).

The Fermentation pathways of *Panicum maximum* and brewers' spent grain ensiled for 28 days are reported in Table 4. The type of fermentation for silage 1 was yeast (glucose) with ethanol and CO_2 as end products. Theoretical DM recovered was 60.01% while energy recovered was more than 100%. For silages 2 – 4, the fermentation type was heterolactic (glucose), with lactic acid, ethanol and CO_2 as end products. These fermentation type and end products were arrived at because of the 80.59 – 87.69% and 101.48 – 106.74% theoretical DM and energy recovered respectively (Kung, 2001). This was contrary to the homolactic (glucose) and heterolactic (fructose) fermentation type reported by Ekanem*et al.* (2017) when they ensiled cassava peels with BSG and/or poultry litter.

Table 2: Effect of ensiling duration on the pH values of *Panicum maximum* and brewers' spent grain silage

Days	SILAGE 1	SILAGE 2	SILAGE 3	SILAGE 4
0	6.90^{c}	6.00^{a}	5.90^{a}	5.50^{a}
7	7.10^{b}	5.50^{b}	5.70^{b}	5.20^{b}
14	7.20^{a}	5.40^{c}	5.90^{a}	5.10^{c}
21	5.30^{f}	5.00^{d}	4.90^{d}	4.70^{e}
28	5.80^{d}	5.00^{d}	5.00^{c}	4.80^{d}
35	5.50^{e}	5.00^{d}	5.00^{c}	4.60^{f}
SEM	0.19	0.09	0.11	0.08

$^{a-f}$ Means on the same column with different superscripts differ significantly ($p < 0.05$). Silage 1 = 100% *Panicum maximum* (PM). Silage 2 = 90% PM + 10% Brewers' Spent Grains (BSG). Silage3 = 80% PM + 20% BSG. Silage 4 = 70% PM + 30% BSG. SEM = Standard error of mean.

The chemical composition (%) of *Panicum maximum* and brewers' spent grain silage and BSG at Day 0 before ensiling and the chemical composition (%) of *Panicum maximum* supplemented and ensiled with brewers' spent grain at Day 28 are shown in Tables 5 and 6. Before ensiling (Day 0), the DM ranged from 26.23 – 38.33, CP ranged from 12.60 – 19.60, EE from 2.60 - 7.82, ash from 4.45 – 6.04, CF 3.38 – 4.08, NFE from 64.82 – 74.76, NDF from 62.16 – 64.86, ADF from 49.88 – 63.81 and ADL from 18.96 – 24.73%. Energy contents of the diets prior to ensiling ranged from 372.84 – 406.02Mcal/kg. After ensiling (Day 28), the chemical composition ranges of the silages

were: DM 23.00 – 26.91, CP 8.05 – 13.65, CF 3.18 – 3.55, ash 4.14 – 4.96, EE 6.61 – 8.90, NFE 70.73 – 77.19, NDF 60.31 – 62.82, ADF 49.20 – 51.06, ADL 23.36 – 25.27% and energy 400.46 – 412.02Mcal/kg. There was a reduction in DM and CP of the ensiled diets compared to the un-ensiled and this agreed with previous studies (Falola *et al.*, 2013; Babayemi, 2009). The reduced DM and CP were due to the fermentation of soluble carbohydrates and proteolysis respectively, which were retained as silage organic acid (Ifut *et al.,* 2015; Olorunnisomo and Fayomi, 2012). The silages CP (8.05 – 13.65%) contents was higher than the critical value of 7.00% recommended for small ruminants (NRC 1981), but within the minimum recommended 10 - 12% for ruminants (ARC, 1984). The fermentation of DM and CF (celluloses and hemicelluloses) to soluble carbohydrates and organic acid resulted in increases in NFE, EE and energy in the ensiled mass compared to the un-ensiled mass (Ekanem *et al.*, 2017; Ifut *et al.,* 2015; Ekanem, 2012). Ash contents of 4.14 – 4.96 % were recorded for all the silages on day 28.

Table 3: Effect of ensiling duration on the percentage dry matter contents of *Panicum maximum* and brewers' spent grain silage

Days	SILAGE 1	SILAGE 2	SILAGE 3	SILAGE 4
0	38.33[a]	33.39[a]	29.00[a]	26.23[b]
7	25.16[b]	27.48[c]	26.29[b]	26.57[a]
14	20.00[e]	20.14[f]	20.13[f]	24.09[c]
21	23.00[c]	24.09[e]	22.41[e]	24.00[d]
28	23.00[c]	26.91[d]	24.09[d]	23.00[e]
35	22.03[d]	28.00[b]	26.08[c]	23.00[e]
SEM	1.47	0.98	0.70	0.35

[a - f]Means on the same column with different superscripts differ significantly ($p<0.05$). Silage 1 = 100% *Panicum maximum* (PM). Silage 2 = 90% PM + 10% Brewers' Spent Grains (BSG). Silage 3 = 80% PM + 20% BSG. Silage 4 = 70% PM + 30% BSG. SEM = Standard error of mean.

Table 4: Fermentation pathways of *Panicum maximum* and brewers' spent grain ensiled for 28 days

Parameters	SILAGE 1	SILAGE 2	SILAGE 3	SILAGE 4
Type of fermentation	Yeast (glucose)	Heterolactic (glucose)	Heterolactic (glucose)	Heterolactic (glucose)
End products	Ethanol, CO_2	Lactic acid, ethanol, CO_2	Lactic acid, ethanol, CO_2	Lactic acid, ethanol, CO_2
Theoretical DM recovery (%)	60.01	80.59	83.07.	87.69
Theoretical E recovery (%)	107.41	106.74	101.48	102.33

Silage 1 = 100% *Panicum maximum* (PM). Silage 2 = 90% PM + 10% Brewers' Spent Grains (BSG). Silage 3 = 80% PM + 20% BSG. Silage 4 = 70% PM + 30% BSG. E = Energy

Undersander (2009) recommended a 10 % or less ash content of hay or silage made from forages. The values obtained in this research were within acceptable range for good quality silage.

Neutral detergent fibre (NDF) values for the silages at Day 28 were 62.82, 62.36, 63.57 and 60.31 % for silages 1, 2, 3 and 4 respectively. Compared with the values of the un-ensiled mixture (Table 5), NDF values were reduced for all the treatments at day 28 of ensiling. McDonald *et al*. (1991) suggested that concentrations of NDF in the silages that were lower than the original herbage reflect the breakdown of hemicelluloses during ensiling which provides additional substrate for fermentation. Ekanem (2012) also reported NDF values lower than the original herbage in silages consisting of cassava peals, brewer's dried grains and poultry litter at day 21. The reduction in the NDF contents of the silages with increase in ensiling duration was also in line with the findings of Jolaosho *et al.* (2013) and Akinola (2008).

Table 5: Chemical composition (%) of *Panicum maximum* and brewers' spent grain silage and BSG at Day 0 before ensiling

Parameters	SILAGE 1	SILAGE 2	SILAGE 3	SILAGE 4	BSG	SEM
Dry matter	38.33^{b}	33.39^{c}	28.94^{d}	26.23^{e}	43.29^{a}	1.65
Crude protein	12.60^{e}	15.23^{d}	17.85^{c}	19.60^{b}	23.80^{a}	1.02
Ether extract	2.60^{e}	3.23^{d}	7.82^{b}	7.16^{c}	9.64^{a}	0.73
Ash	6.00^{a}	6.04^{a}	4.45^{c}	5.04^{b}	2.92^{d}	0.31
Crude fibre	4.04^{a}	4.08^{a}	3.82^{b}	3.38^{c}	2.00^{d}	0.21
Nitrogen free extract	74.76^{a}	71.42^{b}	66.06^{c}	64.82^{d}	61.64^{e}	0.26
Neutral detergent fibre	64.86^{b}	63.12^{d}	62.16^{e}	63.81^{c}	66.30^{a}	0.38
Acid detergent fibre	49.88^{e}	63.12^{c}	62.16^{d}	63.81^{b}	65.81^{a}	1.51
Acid detergent lignin	24.73^{b}	23.12^{c}	18.96^{e}	20.05^{d}	43.13^{a}	2.36
Energy (Mcal/kg)*	372.84^{e}	375.79^{d}	406.02^{b}	402.12^{c}	428.52^{a}	5.52

^{a,b,c,d,e} Means along the same row with different superscripts differ significantly (P<0.05). Silage 1 = 100% PM. Silage 2 = 90% PM + 10% BSG. Silage 3 = 80% PM + 20% BSG. Silage 4 = 70% PM + 30% BSG. SEM = Standard error of mean.*Calculated.

Table 6: Chemical composition (%) of *Panicum maximum* supplemented and ensiled with brewers' spent grain at Day 28

Parameters	SILAGE 1	SILAGE 2	SILAGE 3	SILAGE 4	SEM
Dry matter	23.00^{c}	26.91^{a}	24.09^{b}	23.00^{c}	0.49
Crude protein	8.05^{c}	10.85^{b}	10.50^{b}	13.65^{a}	0.60
Crude fibre	3.18^{b}	3.49^{a}	3.55^{a}	3.26^{b}	0.05
Ash	4.96^{a}	4.49^{b}	4.57^{b}	4.14^{c}	0.09
Ether extract	6.62^{c}	6.61^{c}	8.90^{a}	8.22^{b}	0.30
Nitrogen free extract	77.19^{a}	74.56^{b}	72.48^{c}	70.73^{c}	0.73
Neutral detergent fibre	62.82^{a}	62.36^{b}	60.57^{c}	60.31^{d}	0.33
Acid detergent fibre	49.85^{c}	51.06^{a}	49.20^{d}	50.69^{b}	0.22
Acid detergent lignin	24.09^{b}	25.27^{a}	23.36^{b}	24.50^{b}	0.40
Energy (Mcal/kg)*	400.46^{d}	401.13^{c}	412.02^{a}	411.50^{b}	1.66

^{a,b,c,d} Means along the same row with different superscripts differ significantly (P<0.05). Silage 1 = 100% PM. Silage 2 = 90% PM + 10% BSG. Silage 3 = 80% PM + 20% BSG. Silage 4 = 70% PM + 30% BSG. SEM = standard error of mean.*Calculated

CONCLUSIONS

- *Panicum maximum* should not be ensiled alone as silages made from *Panicum maximum* and brewers' spent grain had better physical and chemical characteristics compared to *Panicum maximum* ensiled alone.
- The terminal pH for all the silages was reached at day 21 of ensiling. Thus these silages could be fed out after 21 days of ensiling.
- Silage 4 (70% PM + 30% BSG) was adjudged the best feed for ruminants as it maintained the colour of the original ingredients, had the most pleasant aroma, firmest texture, no yeast growth, least pH, highest crude protein, highest dry matter and energy recovered and other chemical composition.

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