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EFFECTS OF TREE LEGUME SEEDS ON SILAGE QUALITY OF *Panicum maximum* AT TWO STAGES OF MATURITY

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ABSTRACT

The present study evaluated the effects of tree legume seeds inclusion on the fermentation characteristics, chemical composition and *in vitro* gas production of *Panicum maximum* ensiled at two stages of maturity. The study was a 7 × 2 factorial arrangement consisting *P. maximum* at vegetative and reproductive stages ensiled with two tree seeds (*Albizia saman* and *Moringa oleifera*) at different proportions (100:0, 75:25, 50:50 and 25:75). The results showed significant ($p<0.05$) differences in all the parameters evaluated with *P. maximum* 75: *M. oleifera* 25 at reproductive stage having the highest value for lactic acid. The dry matter content of the silage increased as the proportions of the tree seeds increased. The CP contents of the silage ranged significantly ($p<0.05$) from 8.57 % in sole Panicum at the reproductive stage to 18.62% in *P. maximum* 25: *A. saman* 75 at the vegetative stage. The NDF contents which were below 70 % declined with higher proportions of the tree seeds. There was significant ($p>0.05$) increase in the volume of gas produced as the proportion of each seed increased in the silage at the two maturity stages. It can be concluded from this study that increasing levels of tree legume seeds in *P. maximum* at the two maturity stages had significant effect on the quality of the silage. This shows that silage made at the vegetative / reproductive stage of maturity of the grass and enriched with tree legume seeds would serve as an effective conservation strategy for dry season feeding.

Key words: Grass, *in vitro* gas production, Maturity stages, Silage, Tree legume seeds

INTRODUCTION

Panicum maximum is an important forage plant, distributed spatially across ecological niches in Nigeria, and plays a major role in intensive cattle production systems. However, obtaining higher yields of the grass is usually at the expense of decreasing quality, as the relationship between increasing dry matter yield as the plant matures, and quality traits - such as crude protein, has been reported to be negative (Ribeiro *et al*., 2014; Yoottasanong *et al*., 2015). This poses serious concerns, during silage production, where high amounts of grass biomass are needed for conservation, as if too fibrous grasses are conserved, the resulting silage would be low in quality, which would adversely affect intake, digestibility, and utilization by animals. This implies that the quality of the grasses must be increased so that good quality silage can be produced. Reports of increased forage quality when grasses and legumes are ensiled as mixtures have been reported (Wang *et al*., 2017). However, the availability of legumes in the natural pasture especially in the tropics is limited due to their susceptibility to trampling, and the aggressive nature of other species that outcompete them for resources (Ojo *et al*., 2016). Tree legume seeds are high in CP and as such, can be used as additives to enhance the quality of the grasses (Babayemi *et al*., 2004). This is especially since

browse tree seeds are abundantly available without any deliberate usefulness.

The successful ensiling of *P. maximum* grass with browse plant seeds, has the potential to improve DM, WSC content and ensure the success of the silage, as protein and energy would be provided by the respective browse seeds and grasses in the mixed silage. However, the stability of the fermentation process, must be taken into account, as successful ensiling is determined by the relationship between fermentation characteristics (organic acid concentrations which are succinctly used to define silage quality and the stability of fermentation process) and nutritive quality in silages. In addition, the digestibility of the resulting silage, can be evaluated through *in vitro* methods, which serves as an inexpensive and non-time consuming method for estimating forage digestibility (Makkar, 2002).

Studies on mixed silage have reported better fermentation process, and quality than is usually obtained with single species silage; however, none of these studies have dwelt on improving the quality of silage made from grasses harvested at advances maturity stages with tree legume seeds. Based on this, this study evaluated the nutritive quality of silage made from *Panicum maximum* at two maturity stages ensiled at different levels of some tree legume seeds (*Albizia saman* and *Moringa oleifera*).

MATERIALS AND METHODS

The experiment was carried out at the College of Animal Science and Livestock Production farm, Federal University of Agriculture, Abeokuta, (FUNAAB) Nigeria in 2018. This lies between the savannah agroecological zone of Western Nigeria (latitude 7.23^0 N, longitude 3.42^0 E, average annual rainfall of 1037mm).

The study was a 2 × 7 factorial experiment laid out in a randomized complete block design. The factors were the: 2 maturity stages of *P. maximum* (vegetative – before inflorescence, and reproductive – at inflorescence), and proportions of the grasses and the tree legume seeds (75 grass:25 seed, 50 grass:50 seed and 25 grass:75 seed) (Table 1).

Matured pods of *A. saman* and *M. oleifera* were collected from the Department of Pasture and Range Management legume trees Farm of FUNAAB. The seeds were removed from the pods by threshing, sun-dried for 3 days, milled using a hammer mill, and allowed to pass through a 2mm sieve screen. The *P. maximum* grass was harvested from a previously established plot, by cutting them from 15 cm above ground level at each of the maturity stages (first at the vegetative stage and at the reproductive stage). The harvested forages were first wilted under an open shaded area for 2 hours, following which they were chopped to about 2 cm lengths. The grasses and milled seeds were ensiled using the treatment structure outlined in Table 1, and the mixtures were ensiled based on dry matter weight of the grass and tree legume seeds. The treatment silage were compressed into 960 ml laboratory bottle silos, and the lid of silage was sealed with duct tape to prevent air entry as much as possible. The sealed silos were stored at ambient temperature for 60 days.

At the end of the ensiling period, the silos were opened and samples were taken from the center of each silos for fermentation characteristics and chemical composition of the silages. The pH of the silage was measured with the use of pH meter (Hanna instruments, pH 211, microprocessor). Fermentation characteristics was carried out for determination of ammonia and organic acids using AOAC (1990) and Samuel *et al*. (1997) procedures, respectively.

Subsample of 500 g of fresh *P. maximum* grass, tree legume seeds and silage samples were oven dried at 65°C until constant weight was obtained and ground to pass through 1 mm sieve for proximate composition according to AOAC (2000) while neutral detergent fibre

(NDF) was determined according to Van Soest (1991). Non-fibre carbohydrate was calculated as NFC = 100 – (CP + Ash + EE + NDF). Tannin content was determined using the Vanillin-HCl method as described by Prince and Butler (1977).

In vitro gas production was carried out as described by Menke and Steingass, 1988 and gas production was recorded at 3, 6, 9, 12, 15, 18 and 24 hours using rumen liquor from cattle. Organic matter digestibility (OMD) were estimated as OMD= 14.88 + 0.889 GV+ 0.45 CP + 0.651 ash (Menke and Steingass, 1988). Short-chain fatty acids (SCFA) were estimated as SCFA = 0.0239 GV−0.0601 (Getachew *et al*., 2000). Metabolizable energy (ME) were calculated as ME= 2.20 + 0.1357 GV+ 0.0057 CP + 0.0002859 EE^2 (Menke and Steingass, 1988). Total gas volume (GV) was expressed as ml/200mgDM, CP and ash as percentage DM, ME as MJ/kgDM and SCFA as μmol/g DM. (Menke and Steingass, 1988). Total gas volume (GV) was expressed as ml/200mgDM, CP and ash as percentage DM, ME as MJ/kgDM and SCFA as μmol/g DM.

Table 1: Silage treatment structure

Maturity stages of *P. maximum*	**Proportions of the grasses and the tree legume seeds**
Vegetative stage	25 % *A. saman* seed + 75 % *P. maximum*
	50 % *A. saman* seed + 50 % *P. maximum*
	75 % *A. saman* seed + 25 % *P. maximum*
	25 % *Moringa oleifera* seed + 75 % *P. maximum*
	50 % *Moringa oleifera* seed + 50 % *P. maximum*
	75 % *Moringa oleifera* seed + 25 % *P. maximum*
	100 % *P. maximum*
Reproductive stage	25 % *A. saman* seed + 75 % *P. maximum*
	50 % *A. saman* seed + 50 % *P. maximum*
	75 % *A. saman* seed + 25 % *P. maximum*
	25 % *Moringa oleifera* seed + 75 % *P. maximum*
	50 % *Moringa oleifera* seed + 50 % *P. maximum*
	75 % *Moringa oleifera* seed + 25 % *P. maximum*
	100 % *P. maximum*

Statistical Analysis

Data collected were subjected to two - way analysis of variance. Treatment means were separated using Tukey's HSD test. All data were analyzed using the R Statistical Software (R Core Team, 2018).

RESULTS AND DISCUSSION

Table 2 lists the chemical composition of the *P. maximum* grass and the seeds before ensiling.

Table 3 shows the effects of ensiling *P. maximum* with tree seeds on fermentative characteristics. All the silage fermentative parameters investigated were significantly different ($p < 0.05$). The pH values were within the range of 4.5 – 5.5 classified to be the range for good silage by Meneses *et al*. (2007). This pH reflects the adequacy of fermentation to resist the growth of undesirable microorganisms (Arriola 2011). Moreover, McDonald *et al*. (1991) further described that the characteristics of good silage should have NH_3-N contents less than 100 g kg^{-1} DM. Lactic acid contents of silage in this study fall within the range of 2 to 10 % reported by Kung and Shaver (2002) for various silages. From this study, mixing of grasses and tree seeds proved beneficial since the silages results showed that they were well preserved. This is in line with the report of Steurm *et al*. (2007) that the combination of grasses and legumes is very effective as high quality diet in ruminant feed.

Effects of ensiling *P. maximum* with tree seeds on the chemical composition were shown in Table 4. As the level of seeds increased in the mixtures, the CP contents in the silage were enhanced ($p < 0.05$). The CP contents of the silage with different tree seeds and at different proportions were greater ($p< 0.05$) compared to sole *P. maximum* at the two maturity stages. The CP levels in grass and seed mixtures in the current study were within or greater than the range of 110 - 130 g kg^{-1} DM which was considered adequate for maintenance and growth of small ruminants (NRC, 1985). This shows that ensiling the grasses with tree seeds tend to be more nutrient dense than grass alone. This implied that the seeds of the tree legumes can be used as supplement to poor quality grasses as silage in total mixed ration during the dry season. Although CP content was expected to decline with increasing maturity of the grass (Adane and Berhan, 2005), the addition of the seeds increased the CP contents. Ash contents were greater when grass was ensiled with seeds at different proportions than in sole grass. This is expected since the amount of structural carbohydrates increased as the plant matured.

The NDF content declined with higher proportions of tree seeds which suggest an improvement in the digestibility of the mixtures. The contents were higher with the sole grass than in mixtures with the tree seeds. In general, the NDF contents of the silage in this study were at level that can be easily degradable that is below 70 % (Turgut, 2008) if fed to animals.

Table 2: Chemical composition of fresh materials before ensiling

Parameters (%)	***Albizia saman* seed**	***Moringa oleifera* seed**	***P. maximum* Vegetative**	***P. maximum* Reproductive**
Dry matter	83.50	85.67	94.55	96.40
Crude protein	18.79	17.77	8.61	8.36
Ether extract	6.83	16.67	10.39	6.95
Ash	4.33	5.50	9.00	4.00
Neutral detergent fibre	51.33	52.00	65.00	67.00
Tannin (g kg^{-1})	90.86	108.25	ND	ND

Table 3: Effect of ensiling *Panicum maximum* at two maturity stages with *Albizia saman* and *Moringa oleifera* seeds on fermentative characteristics

Stage	Proportion	NH_3-N	Lactic	Acetic	Propionic	Butyric	pH	Silage DM
		←		%		→		%
Vegetative	PM 75 : MO 25	6.66^{bcde}	6.80^{abcde}	1.20^{abcde}	0.80^{abcde}	1.20^{abcde}	4.74	63.41^{d}
	PM 50 : MO 50	7.99^{abc}	6.89^{abc}	1.26^{abc}	0.84^{abc}	1.26^{abc}	4.86	61.42^{ef}
	PM 25 : MO 75	6.72^{abcd}	6.46^{de}	0.97^{de}	0.65^{de}	0.97^{de}	4.87	76.06^{b}
	PM 75 : AS 25	6.43^{bcde}	6.68^{abcde}	1.12^{abcde}	0.75^{abcde}	1.12^{abcde}	4.82	46.59^{h}
	PM 50 : AS 50	6.43^{bcde}	6.84^{abcd}	1.23^{abcd}	0.82^{abcd}	1.23^{abcd}	4.78	61.73^{e}
	PM 25 : AS 75	5.85^{de}	6.52^{cde}	1.01^{cde}	0.68^{cde}	1.01^{cde}	4.63	78.79^{a}
	PM 100 : Seed 0	5.54^{e}	6.42^{e}	0.95^{e}	0.63^{e}	0.95^{e}	4.98	32.78^{k}
Reproductive	PM 75 : MO 25	7.76^{abcd}	6.99^{a}	1.33^{a}	0.88^{a}	1.33^{a}	4.41	40.96^{i}
	PM 50 : MO 50	6.85^{abcde}	6.97^{ab}	1.32^{ab}	0.88^{ab}	1.32^{ab}	4.80	56.66^{g}
	PM 25 : MO 75	7.47^{ab}	6.58^{bcde}	1.05^{bcde}	0.70^{abcde}	1.05^{bcde}	4.83	75.21^{c}
	PM 75 : AS 25	6.00^{abc}	6.57^{bcde}	1.04^{cde}	0.70^{abcde}	1.04^{cde}	4.90	38.94^{j}
	PM 50 : AS 50	7.97^{a}	6.66^{abcde}	1.11^{abcde}	0.74^{abcde}	1.11^{abcde}	4.70	60.91^{f}
	PM 25 : AS 75	7.30^{cde}	6.60^{abcde}	1.06^{abcde}	0.71^{abcde}	1.06^{abcde}	4.84	74.82^{c}
	PM 100 : Seed 0	6.54^{bcde}	6.57^{bcde}	1.05^{bcde}	0.70^{bcde}	1.05^{bcde}	4.82	23.93^{l}
SEM		0.41	0.08	0.05	0.04	0.05	0.41	0.09

$^{abc-k:\cdots}$Means in the same column with different superscripts differ significantly ($p<0.05$)

SEM: Standard error of mean, PM: *Panicum maximum*, AS*: Albizia saman*, MO: *Moringa oleifera*

Table 4: Effect of ensiling *Panicum maximum* at two maturity stages with *Albizia saman* and *Moringa oleifera* seeds on chemical composition

		DM	EE	Ash	CP	NFC	NDF	Tannin
Stage	**Proportion**	←			**%**		→	**mg kg^{-1}**
Vegetative	PM 75 : MO 25	94.30^{ab}	7.69^{e}	7.50^{b}	12.44^{e}	10.37^{a}	62.00^{d}	0.34^{d}
	PM 50 : MO 50	96.97^{ab}	11.01^{b}	8.00^{a}	14.44^{c}	6.55^{e}	60.00^{d}	0.41^{b}
	PM 25 : MO 75	95.50^{ab}	12.64^{a}	9.00^{a}	16.67^{b}	4.69^{f}	57.00^{e}	0.44^{a}
	PM 75 : AS 25	93.87^{ab}	8.48^{d}	8.00^{a}	13.02^{d}	8.75^{d}	61.00^{d}	0.18^{g}
	PM 50 : AS 50	94.16^{ab}	8.60^{d}	5.00^{c}	14.65^{c}	11.75^{b}	60.00^{d}	0.24^{f}
	PM 25 : AS 75	93.86^{ab}	9.23^{c}	4.50^{d}	18.62^{a}	10.40^{c}	58.00^{e}	0.26^{e}
	PM 100 : Seed 0	92.86^{b}	5.48^{h}	4.00^{d}	9.43^{h}	12.59^{a}	68.50^{b}	ND
Reproductive	PM 75 : MO 25	97.67^{a}	6.61^{f}	5.00^{c}	10.04^{g}	11.35^{b}	67.00^{b}	0.34^{d}
	PM 50 : MO 50	95.72^{ab}	7.38^{e}	5.50^{c}	11.33^{d}	11.79^{ab}	64.00^{c}	0.36^{c}
	PM 25 : MO 75	97.30^{a}	7.78^{e}	5.00^{c}	13.03^{d}	13.19^{a}	61.00^{d}	0.40^{b}
	PM 75 : AS 25	98.06^{a}	6.36^{g}	5.00^{c}	10.74	11.19^{b}	66.00^{b}	0.18^{g}
	PM 50 : AS 50	95.39^{ab}	7.06^{ef}	5.43^{c}	12.22^{e}	12.29^{a}	63.00^{c}	0.19^{g}
	PM 25 : AS 75	96.39^{ab}	7.46^{e}	9.50^{a}	17.33^{a}	8.71^{d}	57.00^{e}	0.26^{ef}
	PM 100 : Seed 0	95.74^{ab}	6.20^{g}	4.50^{d}	8.57^{h}	10.73^{c}	70.00^{a}	ND
SEM		0.66	0.93	0.78	0.99	2.20	1.46	0.01

$^{abc-k:\cdots}$Means in the same column with different superscripts differ significantly ($p<0.05$)
SEM: Standard error of mean, DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fibre, NFC: Non-fibre carbohydrate, PM: *Panicum maximum*, AS*: Albizia saman*, MO: *Moringa oleifera*

There was significant ($p>0.05$) increase in the volume of gas produced as the proportion of each seed increased in the silage at the two maturity stages in Table 5. However, the volume of gas produced tended to be higher in silage containing *A. saman* seeds, than those with *M. oleifera* seeds. The higher gas produced might be attributed to higher content of degradable carbohydrates in the seeds as reported by Tona *et al.* (2013). The higher gas production in silage containing *A. saman* seeds above that of *M. oleifera* might also as well be as a result of higher CP content in *A. saman* seeds. Ojo *et al*. (2018) reported high gas production from browse seeds which could be due to high CP which served as nutrients for rumen micro-organisms that helped in digestion of feeds. Higher content of tannin in the *M.*

oleifera seeds might have been responsible for its lower gas production. Digestibility has been reported to be related to *in vitro* gas production (Fievez *et al.*, 2005) so that the higher the volume of gas produced, the higher the digestibility. The gas produced in *in vitro* fermentation reflects the extent of feed fermentation and digestibility (Getachew *et al*., 1998). Higher NDF content in sole Panicum silage harvested at reproductive stage could have been responsible for its lowest gas production which connote least digestibility.

Table 5: Effect of ensiling *Panicum maximum* at two maturity stages with *Albizia saman* and *Moringa oleifera* seeds on *in vitro* gas production

		3	6	9	12	15	18	21	24
Stage	**Proportion**	←			**ml/200mg DM**				→
Vegetative	PM 75 : MO 25	0.00^{d}	2.79^{e}	3.72^{e}	5.58^{e}	8.37^{de}	8.37^{d}	11.16^{d}	11.16^{e}
	PM 50 : MO 50	1.86^{b}	4.65^{cd}	4.65^{d}	6.51^{e}	9.30^{d}	9.30^{c}	13.02^{d}	13.02^{d}
	PM 25 : MO 75	2.79^{a}	3.72^{d}	9.30^{b}	11.16^{c}	15.81^{b}	18.60^{b}	21.39^{b}	21.39^{bc}
	PM 75 : AS 25	0.00^{d}	0.93^{g}	4.65^{d}	5.58^{e}	9.30^{d}	10.23^{c}	12.09^{d}	12.09^{de}
	PM 50 : AS 50	0.93^{c}	2.79^{e}	11.16^{ab}	13.02^{b}	18.60^{b}	22.32^{ab}	23.25^{b}	26.04^{b}
	PM 25 : AS 75	3.41^{a}	10.85^{a}	16.43^{a}	19.53^{a}	24.18^{a}	28.83^{a}	32.55^{a}	32.55^{a}
	PM 100 : Seed 0	0.93^{c}	1.86^{f}	2.79^{e}	4.65^{d}	5.58^{f}	6.51^{e}	7.44^{f}	10.23^{e}
Reproductive	PM 75 : MO 25	1.86^{b}	1.86^{f}	4.65^{d}	4.65^{f}	7.44^{ef}	8.37^{d}	9.30^{ef}	11.16^{e}
	PM 50 : MO 50	0.93^{c}	3.72^{d}	5.58^{cd}	7.44^{de}	8.37^{de}	10.23^{c}	12.09^{d}	12.09^{de}
	PM 25 : MO 75	0.93^{c}	5.58^{c}	6.51^{c}	9.30^{d}	12.09^{c}	13.95^{b}	17.67^{c}	17.67^{c}
	PM 75 : AS 25	0.93^{c}	5.58^{c}	6.51^{c}	10.23	11.16^{cd}	13.95^{b}	17.67^{c}	17.67^{c}
	PM 50 : AS 50	2.79^{a}	8.37^{b}	9.30^{b}	14.88^{b}	16.74^{b}	17.67^{b}	21.39^{b}	21.39^{bc}
	PM 25 : AS 75	1.86^{b}	3.72^{d}	11.16^{ab}	13.02^{b}	19.53^{ab}	22.32^{ab}	26.97^{b}	30.69^{a}
	PM 100 : Seed 0	0.00^{d}	0.93^{g}	2.79^{e}	3.72^{f}	6.51^{ef}	7.44^{de}	8.37^{f}	8.37^{f}
SEM		0.12	0.32	1.28	1.73	1.77	1.73	1.77	1.84

$^{abc-g:\cdots}$Means in the same column with different superscripts differ significantly ($p<0.05$)
SEM: Standard error of mean; PM: *Panicum maximum*, AS*: Albizia saman*, MO: *Moringa oleifera*

At both maturity stages of *P. maximum*, OMD, SCFA and ME values for silage increased ($p<0.05$) as seeds content increased with higher values of OMD in vegetative above reproductive stage (Table 6).

The SCFA and ME values in this study indicated that the energy content in the silages will be readily utilized after digestion. The high metabolizable energy, organic matter digestibility and short chain fatty acid that were reported for legume seeds ensiled at different proportions with *P. maximum* at different maturity stages, could translate to higher dry matter intake in ruminants for an improved performance.

Increased post incubation parameters from the combination of *Panicum maximum* with seeds relative to sole *Panicum* substrate could be interpreted to mean increasing energy availability to the ruminant (Ojo *et al*., 2018).

Table 6: Effect of ensiling *Panicum maximum* at two maturity stages with *Albizia saman* and *Moringa oleifera* seeds on *in vitro* post incubation parameters

Stage	Proportion	Organic Matter Digestibility (%)	Short Chain Fatty Acid (µmol)	Metabolizable Energy MJ/kg
Vegetative	PM 75 : MO 25	33.03^{b}	0.15^{e}	3.27^{e}
	PM 50 : MO 50	34.85^{b}	0.21^{d}	3.69^{d}
	PM 25 : MO 75	40.94^{a}	0.46^{b}	5.04^{b}
	PM 75 : AS 25	34.43^{b}	0.23^{d}	3.79^{d}
	PM 50 : AS 50	44.21^{a}	0.57^{ab}	5.68^{ab}
	PM 25 : AS 75	47.02^{a}	0.68^{a}	6.33^{a}
	PM 100 : Seed 0	31.39^{c}	0.19^{de}	3.51^{d}
Reproductive	PM 75 : MO 25	33.69^{b}	0.23^{d}	3.79^{d}
	PM 50 : MO 50	34.00^{b}	0.36^{c}	4.53^{c}
	PM 25 : MO 75	38.58^{ab}	0.21^{d}	3.68^{d}
	PM 75 : AS 25	40.01^{a}	0.36^{c}	4.54^{c}
	PM 50 : AS 50	43.17^{a}	0.46^{b}	5.06^{b}
	PM 25 : AS 75	45.95^{a}	0.73^{a}	6.56^{a}
	PM 100 : Seed 0	30.90^{c}	0.25^{d}	3.87^{d}
SEM		1.39	0.05	0.25

$^{abc\text{-}e:\cdots}$Means in the same column with different superscripts differ significantly ($p<0.05$)
SEM: Standard error of mean; PM: *Panicum maximum*, AS*: Albizia saman*, MO: *Moringa oleifera*

CONCLUSION

The inclusion of the tree legume seeds improved the fermentation characteristics and quality of the silage. The improvement in quality was evident at both vegetative and reproductive stages of the grass and increased as the proportion of tree legume seeds in the silage increased. This shows that silage made at the vegetative / reproductive stage of maturity of the grass and enriched with tree legume seeds would serve as an effective conservation strategy for dry season feeding.

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