Original Research Article

Fermentation characteristics and microbial counts of *Megathyrsus maximus* silage as influenced by *Moringa oleifera* seeds and ensiling period

Damilola Kola **Oyaniran**[1], Victoria Olubunmi **Ojo**[1], Ronke Yemisi **Aderinboye**[2], Temidayo Ayodeji **Adeyemi**[3], Jimoh Alao **Olanite**[1]

[1]*Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*
[2]*Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*
[3]*Agric-Food Business Management, the Estonian University of Life Science*

Correspondence to:
D. K. Oyaniran, Department of Pasture and Range Management, Federal University of Agriculture, P. M. B. 2240, Abeokuta, Nigeria. E-mail: oyanirandammy4real@yahoo.com, Tel.: +2348165082609

Abstract

The fermentation characteristics and microbial counts of *Megathyrsus maximus* ensiled at varying inclusions of *Moringa oleifera* seeds at different ensiling periods were investigated. Factorial experiment consisting of 3 varying proportions of grass and seeds (100:0, 75:25, and 50:50) and 4 ensiling duration (30, 60, 90, and 120 days) was carried out. The pH was significantly highest ($p < 0.05$) with the highest inclusion of seeds while pH decreased with increased ensiling duration. Colour and odour changed as the level of seed inclusion increased in the silage while the moisture content (9.10) was significantly higher ($p < 0.05$) at 30 days of ensiling. Ammonia nitrogen was highest at the highest level of seed inclusion while lactic acid was highest at 60 days of ensiling. Crude protein and tannin contents of the silage increased ($p < 0.05$) following the increased seed inclusion while there was a reduction as the ensiling duration increased. The total anaerobic bacteria count, total lactobacilli count, and total yeast count of the silage decreased following the increased inclusion of seeds in the silage. As the ensiling duration increased, a reduction in all the silage microbial counts was observed. Inclusion of 25% of the seeds to 75% of the grass for silage with ensiling up to 120 days supported improved fermentation characteristics, microbial and crude protein contents as well as a reduction in tannin content which implies that there will not be a detrimental effect on animals that feed on the silage.

Keywords: *Megathyrsus maximus*; *Moringa oleifera;* ammonia nitrogen; anaerobic bacteria; pH; temperature; volatile fatty acids

INTRODUCTION

In the tropics, most of the ruminant livestock production systems depend on natural pasture, of which grasses are the predominant species when compared with legumes due to their aggressiveness, high competition for nutrients, higher resilience under intensive grazing, and unfavourable climatic factors (CIAT, 2001).

During the dry season, animals are faced with problems with feed intake, poor digestibility, and performance due to the low quality of scarce available forages (Babayemi 2009). In order to alleviate the problem of feed scarcity, the conservation of forages into hay or silage when they are abundant and of high quality is important. However, most tropical grasses like *Megathyrsus maximus* also known as Guinea grass when matured contain a limited amount of protein content. High temperature peculiar to the tropics also causes the grass to grow more of the stem than of leaves leading to lignified and fibrous grasses. In order to provide quality feed to animals, the grasses can be augmented with legumes which contained a high source of protein. Mixtures of legumes with grasses will improve fermentable characteristics so that adequate quality of silage can be obtained (Bijelić et al., 2015). Legume forage seeds have been reported to be high in crude protein (Babayemi et al., 2004) and as such are suitable as protein concentrate and are nutritionally promising for the optimum performance of ruminants (Babayemi and Bamikole 2006). The seeds from browse trees in the tropics are abundant and do not have deliberate usage. *Moringa oleifera*is one of the most commonly cultivated browse plants in the tropics and subtropics which has the potential as alternative animal feed resources during dry season (Melesse, 2011). Previous research has shown that *M. oleifera* seeds generally have a 35.37% crude protein (CP), 4.98% ash, and 4.7% crude fibre (CF) (Compaoré et al., 2011). They also contain high content of fat, of which about 70% consists of oleic acid and 30–42% oil (Ogunsina et al., 2014; Zaku et al., 2015).

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 (Langston et al., 1962). Fermentation analysis reveals whether an excellent, average, or poor fermentation has occurred. Silage microflora plays an important role in the successful outcome of the conservation process. The dominance of homo-fermentative lactic acid bacteria (LAB) is preferable, as rapid acidification occurs, which decreases pH to inhibit the growth of undesirable microorganisms (clostridia, enterobacteria, and fungi) and reduces nutritional loss in the ensiling process (McDonald et al., 1991; Ridwan et al., 2015). During the ensilage process, LAB produced lactic acid to lower the pH and inhibited the growth of undesirable microorganisms, and produced good-quality silage (Ridwan et al., 2015).

In this study, we hypothesise that the inclusion of *M. oleifera* seeds at varying inclusions levels will enhance fermentable characteristics and microbial counts of *M. maximus* silage. In view of this, the present study is to evaluate the fermentation characteristics and microbial count of *M. maximus* silage with varying inclusions levels of *M. oleifera* seeds at different ensiling duration.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Directorate of University Farms, Federal University of Agriculture Abeokuta, Nigeria, West (7°15′N, 3°21′E, altitude 76 m). It has an average annual rainfall of 1,037 mm and a temperature of about 34.7 °C with an average relative humidity of 82% (Source: Agro meteorology Department, FUNAAB).

Collection of samples and procedure of silage making

The matured pods of *M. oleifera* were harvested using a tree-pruning pole attached to a sickle at the end. The collected pods were sun-cured for three days, and the seeds were extracted by manually opening the pods. The seeds were further sun-dried for three days and ground using a hammer mill to pass through a 3 mm sieve. *Megathyrsus maximus* which was planted at the spacing of 0.5 m × 0.5 m was harvested at 8 weeks of growth from 15 cm above ground level. The grass was chopped into 2 cm long, wilted for 2 hours, and mixed uniformly with the tree seeds at varying inclusions levels (100:0, 75:25, and 50:50). They were then packed according to the treatment into well-labelled laboratory glass jar bottle silos of 960 ml, compressed and compacted to ensure anaerobic conditions. They were ensiled for 30, 60, 90, and 120 days duration on laboratory tables. The treatments were replicated three times.

Experimental design

The study was a 3 × 4 factorial experiment. The factors were three varying inclusions levels of the grass and tree seeds (100:0, 75:25, and 50:50) and four ensiling durations (30, 60, 90, and 120 days).

Analysis of silage quality

At the end of each ensiling duration, the silos were opened according to treatment and immediately pH and temperature of the silage were determined using a portable pH meter (Hanna instrument, pH 211, microprocessor pH meter, K012818, Portugal).

The physical characteristics of the silage such as colour, odour, moistness, and mouldiness of the silage were determined (Appendix 1). Six trained silage experts were invited to assess all treatments using a scoring sheet (Ojo et al., 2020).

Twenty-five (25) grams of silage were taken and placed into separate plastic bottles and sub-fractioned into two sets for determination of ammonia and volatile fatty acids analyses using AOAC (1990) and Moisio and Heikonem (1989) procedures, respectively.

The second sub-portion was used for microbial (bacteria, fungi, and yeast) identification and counts according to the procedures of Hedges (2002).

Sub-samples of each silage at different storage duration were oven dried at 65 °C to a constant weight and milled to pass through a 1 mm sieve. The crude protein content of the silages was determined according to AOAC (2000) while tannin content was determined using the Vanillin-HCl method as described by Price and Butler (1977).

Statistical analysis

All data obtained were subjected to a three-way analysis of variance (ANOVA). Treatment means were separated using Turkey's HSD test. Data were analysed using the R Statistical Software (R Core Team 2020). Significance differences were tested at a 5% probability level.

RESULTS

Table 1 shows the effects of proportion and ensiling duration on the physical characteristics of *M. maximus* ensiled with *M. oleifera* seeds. The dry matter content (DM) increased, as the inclusions of the seeds in the silage increased with the highest ($p < 0.05$) value recorded for silage with an inclusion level of 50% *M. oleifera* seed (51.46 g/kg). The pH was significantly highest ($p < 0.05$) with the highest inclusion of seeds while the pH values ranged from 4.99 at 120 days of ensiling to 5.84 at 30 days of ensiling. Silage with 25% *M. oleifera* seed has the lowest pH value (5.30). Colour changed as the seed amount was increased in the

Table 1. Effects of ensiled proportion and storage duration on physical characteristics of *M. maximus* ensiled with *M. oleifera* seed

Proportion	DM %	pH	Temp. °C	Moisture	Colour	Mouldiness	Odour
*M. maximus*100:*M. oleifera* 0	32.63^{c}	5.49^{b}	28.99^{a}	8.39^{b}	9.36^{a}	8.66^{c}	25.97^{a}
*M. maximus*75:*M. oleifera* 25	44.88^{b}	5.30^{c}	28.34^{b}	8.87^{a}	8.14^{b}	9.00^{b}	25.84^{a}
*M. maximus*50:*M. oleifera* 50	51.46^{a}	5.64^{a}	29.26^{a}	8.86^{a}	7.26^{c}	9.33^{a}	25.02^{b}
SEM	1.92	0.09	0.29	0.19	0.17	0.13	0.34
Duration							
30 days	46.87^{a}	5.84^{a}	28.76^{b}	9.10^{a}	8.05^{b}	9.41^{a}	26.95^{a}
60 days	45.22^{a}	5.65^{ab}	30.45^{a}	8.83^{b}	8.54^{a}	9.11^{a}	26.11^{b}
90 days	41.39^{b}	5.43^{ab}	29.17^{b}	8.83^{b}	8.54^{a}	9.17^{a}	25.78^{b}
120 days	38.47^{c}	4.99^{b}	27.08^{c}	8.06^{c}	7.89^{b}	8.29^{b}	23.62^{c}
SEM	2.89	0.08	0.19	0.22	0.28	0.12	0.28
P-value							
Proportion	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0051
Duration	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Proportion × Duration	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

a, b, c means on the same column with different superscripts are significantly different ($p < 0.05$), SEM = standard error of means. DM = Dry matter content

Table 2. Effects of ensiled proportion and storage duration on silage fermentative characteristics of *M. maximus* ensiled with *M. oleifera* seed (%)

Proportion	NH_3-N	VFA	Lactic	Acetic	Propionic	Butyric	CP	Tannin
M. maximus 100: *M. oleifera* 0	7.32^{b}	24.01^{a}	6.41^{a}	2.97^{a}	0.43	0.50	72.80^{c}	ND
M. maximus 75: *M. oleifera* 25	7.64^{b}	22.58^{b}	6.14^{b}	2.79^{b}	0.41	0.47	114.32^{b}	3.26^{b}
M. maximus 50: *M. oleifera* 50	8.00^{a}	23.91^{a}	6.41^{a}	2.92^{ab}	0.43	0.48	130.59^{a}	3.64^{a}
SEM	0.16	0.48	0.10	0.06	0.01	0.02	2.31	0.10
Duration								
30 days	7.71^{b}	23.57^{a}	6.34^{b}	3.02^{a}	0.45^{a}	0.50	113.23^{a}	4.09^{a}
60 days	8.20^{a}	25.11^{a}	6.75^{a}	2.92^{a}	0.44^{ab}	0.49	107.93^{b}	3.61^{b}
90 days	7.69^{b}	23.63^{a}	6.34^{b}	2.93^{a}	0.41^{bc}	0.49	104.86^{b}	3.47^{b}
120 days	7.01^{c}	21.70^{b}	5.84^{c}	2.71^{b}	0.39^{c}	0.45	97.59^{c}	2.62^{c}
SEM	0.18	0.60	0.11	0.07	0.01	0.02	6.41	0.08
P-value								
Proportion	0.0004	0.0514	0.0390	0.0699	0.3540	0.4738	<.0001	<.0001
Duration	<.0001	0.0003	<.0001	0.0076	0.0058	0.5538	<.0001	<.0001
Proportion × Duration	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

a, b, c means on the same column with different superscripts are significantly different ($p < 0.05$), CP = crude protein, VFA = volatile fatty acids, SEM = standard error of means

Table 3. Effects of ensiled proportion and storage duration on microbial counts (g kg^{-1} DM) of *M. maximus* ensiled with *M. oleifera* seed

Proportion	TBC (× 10^6 CFU/g)	TLC (× 10^6 CFU/g)	TFC (× 10^6 CFU/g)	TYC (× 10^6 CFU/g)
M. maximus 100: *M. oleifera* 0	2.75^a	1.70^a	1.67^{ab}	0.76^a
M. maximus 75: *M. oleifera* 25	1.79^b	1.45^b	1.61^b	0.23^b
M. maximus 50: *M. oleifera* 50	1.44^c	0.92^c	1.95^a	0.28^b
SEM	0.09	0.07	0.11	0.05
Duration (days)				
30	2.53^a	1.83^a	2.19^a	0.58^a
60	1.97^b	1.30^b	1.56^b	0.41^{ab}
90	1.81^{bc}	1.19^{bc}	1.67^b	0.37^b
120	1.66^c	1.09^c	1.54^b	0.34^b
SEM	0.15	0.09	0.13	0.09
P-value				
Proportion	<.0001	<.0001	0.0474	<.0001
Duration	<.0001	<.0001	0.0004	0.0368
Proportion × Duration	<.0001	<.0001	<.0001	<.0001

$^{a, b, c}$ means on the same column with different superscripts are significantly different ($p < 0.05$) SEM = standard error of means, TBC = Total anaerobic bacteria count, TLC = Total lactobacillus count, TFC = Total fungi count, TYC = Total yeast count

silage and the odour was significantly lower ($p < 0.05$) in silage with an inclusion level of 50% *M. oleifera* seed (25.02). The mould content was similar in the silage at 30, 60, and 90 days of ensiling but significantly lower ($p < 0.05$) at 120 days of ensiling (8.29). The odour (26.95) and moisture content (9.10) were significantly higher ($p < 0.05$) at 30 days of ensiling with the least values recorded at 120 days of ensiling (23.62 and 8.06). The 100% *M. maximus* silage significantly ($p < 0.05$) had the lowest score of moisture (8.39) and mould (8.66) contents.

The effects of inclusion levels and ensiling duration on silage fermentative characteristics of *M. maximus* ensiled with *M. oleifera* seeds are presented in Table 2. Ammonia nitrogen was higher in 50% *M. oleifera* seed (8.00%) than in the others. Silage with the inclusion level of 25% *M. oleifera* seed had lower values of VFA (22.58%) and lactic acid (6.14%) compared to others. The VFA and lactic acid had the least values at 120 days of ensiling. There were no significant ($p > 0.05$) differences in the propionic and butyric acid as the level of seed inclusion in the silage increased. Crude protein and tannin contents of the silage increased ($p < 0.05$) following increased seed inclusion while there was a reduction as the ensiling duration increased. The crude protein (130.59 g/kg) and tannin content (3.64 g/kg) were significantly highest ($p < 0.05$) for 50% *M. oleifera* silage.

The lowest ammonia nitrogen (7.01%), VFA (21.70%), and lactic acid (5.84%) were obtained at 120 days of ensiling.

Effects of ensiled proportion and storage duration on microbial counts of *M. maximus* ensiled with *M. oleifera* seed are presented in Table 3. The TBC and TLC contents of the silage decreased following the increased inclusion of seeds in the silage. The highest value of TBC (2.75 × 10^6 CFU/g), TLC (1.70 × 10^6 CFU/g), and TYC (0.76 × 10^6 CFU/g) were recorded for 100% *M. maximus* silage. The TFC ranged from 1.61 × 10^6 CFU/g in 25% *M. oleifera* silage to 1.61 × 10^6 CFU/g in 50% *M. oleifera* silage. The highest ($p < 0.05$) values of TBC (2.53 × 10^6 CFU/g) and TLC (1.83 × 10^6 CFU/g) were obtained at 30 days of ensiling. The TFC was significantly higher ($p < 0.05$) at 30 days of ensiling (2.19 × 10^{6}CFU/g)

DISCUSSION

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 Meneses et al. (2007). This fact suggests that the activities of undesirable microorganisms were slowed down by low pH. As the ensiling duration increased, the pH continued to reduce which helped to keep the quality of the silage.

The colour of the sole *M. maximus* silage was greenish and classified as desirable (Bates 1998). However, scores for colour decreased with an increasing proportion of *M. oleifera* seeds in the silage producing greenish-brown, due to the colour of the seed which was brown. The colour of the silage was close to the original colour of the grass, which was an indication of good quality silage that was well preserved (Oduguwa et al., 2007).

More moisture content in the grass without seeds indicated the significance of absorption of any of the excess moisture in the silage which might lead to effluent loss. Slight or no mould was recorded for all the silages which show that they were well preserved. The odour fell within the range of 24–28 which was desirable and required for good silage (Bates 1998).

The fermentation characteristics of silages from this study showed that the inclusion of *M. oleifera* seeds improved the grass silage. This could be due to high CP content in the silage and proteolytic activities in the silage. The NH_3-N concentration of the silages fell within 5.0–15.0% of CP as reported by Kung and Shaver (2002) for normal grass and legume silages.

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 ensiling duration (Kung and Shaver, 2002). Kung et al. (2018) and Niekerk et al. (2010) also reported that lactic acid is the major factor responsible for reducing the pH of ensiled forage and insufficient lactic acid bacteria in silage could result in a delay in the drop of pH by favouring the undesirable bacteria species fermentation. Lactic acid content in *M. maximus* silage in this study falls within 4.74 to 6.99% reported for *Pennisetum* silage as influenced by manure type and age at harvest by Ojo et al. (2016). The lactic acid also falls within the interval proposed by Catchpoole and Henzel (1971), from 3 to 13% of lactic acid in the silage juice. Acetic acid was above 2% DM which is desirable for good-quality silage (Ward, 2000; Schroeder, 2004). The ammonia nitrogen was lowest at 120 days of ensiling due to a lower amount of crude protein content.

The lowest VFA and lactic acid obtained at 120 days of ensiling could be attributed to lower total bacteria and lactobacillus counts which converted water-soluble carbohydrates to organic acids. Moreover, the decrease in volatile fatty and lactic acids with increased storage duration might be connected to a reduction in cell breakdown and release of plant juices as the storage increased which are much more available at early storage duration. Cell breakdown and release of plant juices are prerequisites for the production of significant amounts of volatile fatty and lactic acids during ensiling, and the complete exclusion of fresh air from the silage mass which is usually expected to result in cell breakdown early storage duration (Shao et al. 2005).

The increase in the CP contents of the silage, as the level of *M. oleifera* seed increased in this study, was in line with the findings of Babayemi and Igbekoyi (2008) who reported a similar trend where CP increased in silage with the increase in the inclusion of *Albizia saman* (Jacq.) Merr. pods in ensiled guinea grass mixture. In a similar trend, Ojo et al. (2018) reported that CP increased with the increase in the inclusion of treated *E. cyclocarpum* seeds which were ensiled with *P. purpureum*. Generally, there are usually improvements in the CP content of the silage made from the mixture of grass and browse seeds than that of sole grass silage (Feyissa et al., 2014). The CP contents in this study were generally above the threshold of 60g/kg required by rumen microbes to build their body protein for effective digestion of forages by ruminants (Van Soest (1994).

Heat production during the ensiling process is normal in relation to silage temperature even in a well-managed silo (Adesogan and Newman 2014), this could have been responsible for the reduction in tannin content as the ensiling increased. This shows that ensilage helps in the reduction of anti-nutritional contents forages.

The reduction of microbial counts in the silage as the level of *M. oleifera* seed increased could be attributed to an increase in dry matter content of the silage leading to a reduction in the growth of lactic acid bacteria which affects the rate of fermentation in the silage (Kung et al., 2018; Whiter and Kung 2001). The presence of optimum lactic acid in silage had been reported to improve animal performance (Nsereko et al., 2008). The highest microbial contents at 30 days of ensilage above 60 days could be due to the acidity of the fermenting medium in relation to the pH of the silage (Akintokun et al., 2007). Both the fungi and yeast levels of the silages in this study were low and therefore safe for animal consumption. Although the total lactobacillus counts in this study were more than 1×10^5 CF|U/g which is necessary for a successful fermentation (Haigh, 1990), the silages produced in this study were all desirable.

CONCLUSIONS

Inclusion of 25% of the seeds to 75% of the grass for silage with ensiling up to 120 days supported improved fermentation characteristics, microbial contents, crude protein contents as well as a reduction in tannin content which implies that there will not be a detrimental effect on animals that feed on the silage.

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CONFLICT OF INTEREST

The author declared no conflicts of interest with respect to the research, authorship, and publication of this article.

ETHICAL COMPLIANCE

The authors have followed ethical standards in conducting the research and preparing the manuscript.

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Appendix 1. Silage: the physical evaluation sheet

Factors	Description	Score range
Colour	Desirable: Green to yellowish-green	9–12
	Acceptable: Yellow to brownish	5–8
	Undesirable: Deep brown or black indicating excessive heating or putrefaction.	0–4
Odour	Desirable: Light, pleasant odour with no indication of putrefaction	24–28
	Acceptable: Fruity, yeasty, musty, which indicates a slightly improper fermentation. Slight burnt odour, sharp vinegar odour	11–23
	Undesirable: strong burnt odour indicating excessive heating. Putrid, indicating improper fermentation.	0–10
Moistness	No free water when squeezed in hand. Well Preserved	9–10
	Some moisture can be squeezed from silage or silage dry or musty.	5–8
	Silage wet, slimy, or soggy, water easily squeezed from the sample. Silage too dry with a strong burnt odour	0–4
Mouldiness	No mould	9–10
	Slightly mouldy	5–8
	Highly mouldy	0–4

Source: Ojo et al. (2020) adapted from Bates (1998)