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Research Article

NUTRITIONAL AND MICROBIAL CONTENTS OF VARIED COMBINATION OF ENSILED *PANICUM MAXIMUM* AND *VETIVERIA NIGRITANA* GRASS

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ABSTRACT
The microbial and nutritional value of varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana* was investigated. The experiment laid out in a completely randomized design which consist of the grasses mixed to make the following treatments: 100% *Vetiveria nigritana*, 100% *Panicum maximum*, 75% *Panicum maximum* + 25% *Vetiveria nigritana*, 50% *Panicum maximum* + 50% *Vetiveria nigritana* and 25% *Panicum maximum* + 75%*Vetiveria nigritana*. The grass mixtures were subjected to silage processing for 45 days. Chemical composition, quality, and microbial content proliferation rate of the silage were assessed. The pH range from 4.6-4.17, pleasant smell and firm textures of the silages were observed irrespective of the combination ratios except for pungent smell observed in 100% *Vertiveria nigritana* grass silage. Crude protein content and pH value for 25% *Panicum maximum* + 75%*Vertiveria nigritana* was 8.96% and 4.21 respectively. The mineral contents for 25% *Panicum maximum* + 75% *Vertiveria nigritana* was also higher than that of all other combinations. High fungi and coliform counts 2.97×10^5 and 5.6×10^4 respectively were recorded in 50% *Panicum maximum* + 50% *Vetiveria nigritana* silage. Results showed statistical differences ($P<0.05$) in the treatment combinations however it could be better improved if the grasses were better wilted to delay filling.

Keywords*: *Panicum maximum, Vetiveria nigritana, Nutrition, Grass

INTRODUCTION
An all year round pasture is not always guaranteed in the tropics especially in Nigeria as half of the period is dry season and successful ruminant keeping is thus limited by the availability of grazing resources in terms of quantity and quality in meeting the nutrient requirement of animals (Aderinola *et al*., 2011). In order to avoid the pasture scarcity during the off season, silage making is paramount. 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, (Babayemi, 2009). Ensiling could also help to increase the nutritional quality of forages when being ensiled with readily fermentable carbohydrates. *Vetiveria nigritana* (Vetiver) as reported by FAO, (2006) and Truong, and Hart (2001) is a native to India, Sri Lanka, Burma and Southeast Asia such as South China and Thailand and subtropical Northern Australia (Queensland). It is also found throughout the Region from Mauritania to North and South Nigeria, and in North-East, East, South tropical Africa and differently in Ceylon, Thailand, Malaysia and the Philippines
Vetiver grass (*Vetiveria nigritana*) had been discovered to play an important role in soil erosion and sediment control, as well as highly tolerant to extreme soil conditions (Truong and Baker, 1998). Kantawanichkul (1999) and Vetiver Network (2005) described *Vetiveria nigritana* as a perennial, persistent and fast growing plant showing year-round growth in tropical climates and a non-invasive species which grows up to 1.5 -2 m and it is commonly found in a wet locality, temporarily swampy, even slightly brackish environment. Vetiver grass was also reported by Liu and Cheng, (2002) to be edible herbage of high quality for cattle and goats particularly in the growing stage. Vetiver grass is also a promising feed resource because of its various advantages including high quality, fast growth rate and easy adaptation to the environment and can bear repetitive mowing without occupying farming land.

Guinea grass (*Panicum maximum*) is one of the grass species that belongs to the tribe Paniceae under the Gramineae family; it is well relish by ruminant animals (McDonald *et al.,* 2002). It has been introduced from Africa to various parts of the world with similar climatic conditions as could be found in South-East Asia, Pacific Islands, Sri-Lanka and India among others. Humphreys and Partridge, (1995) described Guinea grass (*Panicum maximum*) as a tall vigorous perennial grass with stems up to 3.5 m tall that varies widely in growth habit. They also observed that it grows in tropical and subtropical areas with more than 900 mm of rainfall on a wide range of soils. The deep, dense and fibrous root system allows guinea grass to survive quite long drought periods, but it performs best on well drained soils of good fertility in high rainfall regions.

In addition to its being classified among the best forage grass due to its high nutritive value, its roles in phytoremediation and control of soil erosion has been reported (Merki *et al*., 2004).

Cassava peel is a cheap and alternative source of energy which is readily available in peri-urban and village settlements. Due to its high starch content, cassava peel can serve as a source of readily fermentable carbohydrate and also improve energy concentration in tropical grass silage (Onua and Okeke, 1999). Varying degrees of success had been reported in the use of cassava peel as a livestock feed (Oke *et al.,* 2009; Ekwe *et al.,* 2011).

Although Lactic acid bacteria are involved in the fermentation of silage and lowering the pH of the grass to about 4, Stiles (1996) observed that other unwanted bacteria such as Clostridia or Enterobacteria when present in silage causes an increase in the pH value, with the acetic acid and butyric acid in silage and hence producing ammonia-N, amines, keto acids and fatty acids and decreasing its nutritional value (McDonald, 1991).

The microflora in the silage environment can basically be divided into two groups, namely the desirable and the undesirable microorganisms. The desirable microorganisms are Lactic Acid Bacteria. The undesirable ones are the organisms that can cause anaerobic spoilage (e.g., Clostridia and Enterobacteria) or aerobic spoilage (e.g., Yeasts, Bacilli, Listeria and Moulds). Many of these spoilage organisms not only decrease the feed value of the silage, but also have a detrimental effect on animal health or milk quality.

This study thus aimed at determining the nutritional and microbial content of varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana* in the derived savannah zone of Nigeria.

MATERIALS AND METHODS

The experiment was carried out at the Cattle, Sheep and Goat unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso and at the Biology Laboratory Department of the University. Two experimental fields measuring 11m by 21.6m each with established *Panicum maximum and Vetiveria nigritana* grass was cut back to a height of 10cm. This was allowed to grow till 8weeks old at which they were cut for silage preparation. No fertilizer was applied and the silage was prepared using varied combination of *Panicum maximum and Vetiveria nigritana.*

The harvested *Panicum maximum and Vetiveria nigritana* at 8weeks of regrowth were chopped into a compactible length of about 4cm for ease of compaction and consolidation; it was then allowed to wilt for 12 hours in order to reduce the moisture content. Using a completely randomised design the grasses were ensiled as follows: 100% *Vetiveria nigritana*; 100% *Panicum maximum*; 75% *Panicum maximum* and 25% *Vetiveria nigritana;* 50% *Panicum maximum* and 50% *Vetiveria nigritana;* 25% *Panicum maximum* and 75% *Vetiveria nigritana* to serve as treatment 1,2,3,4 and 5 respectively. Each treatment was replicated thrice.

Dried cassava peels collected from a cassava processing plant was added as additive at the rate of 100g/1kg of compacted forage at an interval of 1kg of compaction in order to improve its quality by providing fermentable carbohydrate for the silage. The grass weighing 5kg per replicates for the different treatments was filled into a 15kg capacity plastic which was lined internally with polythene bags, and each layer of the grass was compacted manually to displace the air until containers were filled and the final compaction made. The polythene bags were wrapped over the material. Sand bag of 20kg was rolled on the filled material and left for 45 days for fermentation.

Data collection

Samples of silage were taken at after 45 days of fermentation for physical characteristics, pH and proximate composition according to AOAC (2005). Odour of the silage was judged on a scale of 1 - 5, using fermented cassava (which is relished by goats) as standard. Scale 1 represents a poor smell; 2, almost pleasant; 3, fairly pleasant; 4, pleasant; 5, very pleasant. The assessment was done by ten different people.

After opening the silage, sub-samples from different points and depths were taken by the use of gloved hands and these were mixed together for microbial content determination, dry matter and chemical composition determination. Colour assessment was done with visual observation. The pH of each sub-sample was determined using a pH meter. The texture of the silage was determined by visual observation based on how compact or loosed it was in terms of its appearance.

Chemical analysis

Collected silage samples were analysed for the proximate composition according to (A.O.A.C, 2005). The method of Van Soest *et al.,* (1991) was used to determine the NDF, ADF and ADL components of the samples.

Microbial load determination

Collected samples were analysed for bacteria, fungi and enterobacteria load using McConkey agar (MC), Yeast extract agar (Y.E.A) and Potato Dextrose agar (P.D.A) respectively. This is as described by Taylor *et al.,* (1997). All analysis was carried out using the aseptic technique by using sterilised equipments and solutions to prevent contamination; moreover this was done in duplicate. The microbes were counted and their Colony Forming Unit (g/ml) calculated using

$$C.F.U = N \times Wt \times D$$

Where;
C.F.U = Colony Forming Unit (g/ml)
N = Number of colony
Wt = Weight of sample (g)
D = Dilution factor (ml)

Statistical Analysis

Data generated were analyzed using analysis of variance by following the procedure of SAS (2002) while Duncan's multiple range test of the same package was used to separate means at a probability of 5%.

RESULTS AND DISCUSSION

The percentage chemical composition of varied combination of ensiled *Panicum maximum and Vetiveria nigritana* grass is as shown in Table 1. The highest significant dry matter (DM%) value ($P<0.05$) was observed in 100% Vetiver silage (35.53%) followed by 100% *Panicum maximum* (34.36%) which was similar to values obtained in 75% *Panicum maximum* +25% *Vertiveria nigtitana* and 50% *Panicum maximum* +50% *Vertiveria nigritana* while the least value was observed in 25% *Panicum maximum+75%Vetiveria nigritana* (32.69%). The values were within the DM range reported by Jackson and Forbes; (1970) in an experiment of four silages differing in dry matter content ranged between 19-43.2%. They also falls within the range of 29.95-36.23 reported by Falola *et al.,* (2013) in an assessment of the silage quality of vetiver grass ensiled with cassava peels. There were significant differences ($P<0.05$) in the crude protein (CP) contents of the silage. The highest crude protein value (8.96%) was observed in 25% *Panicum maximum* + 75% *Vetiveria nigritana* while the least value (7.33%) was observed in 100% *Vetiveria nigritana* silage. It was observed that as the DM content of the treatments decreases, there was an increase in the CP content of the treatments. Except for the sole *Vetiveria nigritana* treatment, increasing *Vetiveria nigritana* in the combination led to increase in the CP content of

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the silage. This means that the combination effect was well pronounced in the ensiling process. The CP reported was similar to the CP requirement recommended for small ruminants (NRC, 1981) but higher than values obtained by Falola *et al.,* (2013). The increasing CP will aid the proliferation of rumen microbes thus making more protein available for the animals and increase the digestibility rate.

Percentage ether extract (EE%) was observed to have its highest value in 100%Vetiver (7.62%) followed by 100% *Panicum maximum* (7.55%) while the least value was observed for 25% *Panicum maximum+75% Vetiveria nigritana* (6.30%). The highest value for Crude fibre (CF%) was observed for 100%*Vetiver* silage (23.72%) and the least numerical value was observed for 25%*Panicum maximum*+75%*Vetiveria nigritana* (16.99%). Values obtained for ash were not significantly (P >0.05) different from each other, the highest value was however observed for 25% *Panicum maximum*+75% *Vetiveria nigritana* (9.48%) followed by 50%*Panicum maximum+50%Vetiveria nigritana* (9.31%) while the least value was observed for 100%*Vetiveria nigritana* (8.51%).

Table 1: Chemical composition (%) of varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana*

Treatments	DM (%)	CP (%)	EE (%)	CF (%)	Ash (%)	NDF (%)	ADF (%)	ADL (%)
100%*Vetiveria igritana*	35.53^{a}	7.33^{b}	7.62^{a}	23.72^{a}	8.51	62.39^{a}	54.34^{a}	11.94^{a}
100%*Panicum maximum*	34.36^{b}	7.81^{b}	6.55^{b}	21.02^{b}	8.78	61.60^{a}	52.27^{b}	11.62^{ab}
75%*Panicum maximum +25%Vetiveria igritana*	33.96^{b}	8.11^{ab}	6.50^{b}	19.08^{c}	9.07	60.43^{b}	51.05^{c}	11.15^{ab}
50%*Panicum maximum +50%Vetiveria igritana*	33.98^{b}	8.37^{ab}	6.44^{b}	17.62^{d}	9.31	59.46^{b}	48.72^{d}	10.64^{bc}
25%*Panicum maximum +75%Vetiveria igritana*	32.69^{c}	8.96^{a}	6.30^{b}	16.99^{d}	9.48	57.90^{c}	46.94^{e}	9.67^{c}
SEM	0.22	0.17	0.17	0.48	0.16	0.33	0.50	0.20

^{abcde} *Means within the same column with different superscript, differ significantly (P< 0.05)*

The Neutral detergent fibre (%NDF) was observed to have its highest values on 100% Vetiver silage (62.39%) though similar to the value observed in sole *Panicum maximum* silage (61.60%) while the least value was observed in 25% *Panicum maximum*+75%*Vetiveria nigritana* (57.90%). Acid detergent fibre (%ADF) and Acid detergent lignin (ADL %) also had the highest values in sole (100%) Vetiver silage (54.34%) and (11.94%) respectively while the least values were observed in 25%*Panicum maximum* ensiled with 75% *Vetiveria nigritana* (46.94%) and (9.67%) respectively. The NDF was similar to the values obtained by Odugunwa *et al.,* (2007) and Falola *et al.,* (2013) but the ADF values were higher than values reported by these researchers. Variations in NDF and ADF values could be due to the type of forage, age at harvest of the forage and the varied combinations of the forage used. Lower ADL values (9.67-11.94%) were obtained in this study than values recorded by Odugunwa *et al.,* (2007 (18.70-24.62%). Man and Wiktorsson (2001) obtained lower ADL contents in guinea grass than cassava tops.

The Percentage mineral composition of varied combination of ensiled *Panicum maximum and Vetiveria nigritana* grass is as shown in Table 2. The result showed significant differences (P<0.05) among the treatments with 25%*Panicum maximum* +75% *Vetiveria nigritana* having the highest value for all the mineral composition: Nitrogen (1.68%), Phosphorus (0.60%), Potassium (0.66%), Magnesium (0.41%), Iron (181.40mg/kg), Zinc (29.20mg/kg) and Copper (8.30mg/kg). An interaction effect could have led to the release of higher concentration of minerals in the mixture as varied combination of grass reported higher quantities of all the minerals. Odugunwa *et al.,* (2007) also reported higher mineral contents in combination of cassava tops and guinea grass silage than in single forage silage. However availability of these minerals in the forage is dependent upon their availability in the soil (Miles and Manson, 2000).

Table 2: Mineral composition (%) of varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana*

Treatments	%N	%P	%K	%Mg	%Fe	%Zn	%Cu
100%*Vetiveria nigritana*	1.22[d]	0.36[d]	0.49[d]	0.26[d]	167.00[d]	26.37[c]	6.20[c]
100%*Panicum maximum*	1.07[e]	0.28[e]	0.42[e]	0.19[e]	160.27[e]	25.27[d]	5.07[d]
75%*Panicummaximum+25%Vetiveria nigritana*	1.37[c]	0.43[c]	0.52[c]	0.30[c]	164.27[c]	27.27[b]	7.00[b]
50%*Panicummaximum+50%Vetiveria nigritana*	1.51[b]	0.51[b]	0.62[b]	0.35[b]	178.80[b]	27.17[b]	8.07[a]
25%*Panicummaximum+75%Vetiveria nigritana*	1.68[a]	0.60[a]	0.66[a]	0.41[a]	181.40[a]	29.20[a]	8.30[a]
SEM	0.03	0.02	0.14	0.12	1.30	0.21	0.18

[abcde] *Means within the same column with different superscript, differ significantly (P< 0.05)*

The physio- chemical properties of the varied combination of the ensiled *Panicum maximum –Vertiveria nigritana* is as shown in Table 3. Parameters considered include its colour, smell, texture and pH. Colour of the silages ranges from light yellow in sole vetiver silage to yellowish green in sole *Panicum maximum* silage and their mixtures. This agreed with the observation of Babayemi (2009) that good silage usually preserves well the original colour of the pasture or any forage. The yellowish –green colour was closed to the original colour of the grass, which was an indication of good quality silage (Oduguwa *et al.*, 2007) in terms of colour. Also the light yellow colour was in accordance with the report of Kung and Shaver (2002) who observed that when a green plant material that is ensiled produces yellow colour, it can be said to be well-made silage.

Table 3: Colour, Smell, Texture and pH of varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana*

Ensiled grass	Silage quality			
	Colour	Smell	Texture	pH
100%*Vetiveria nigritana*	Light–Yellow	Pugent	Moderately firm	4.60
100%*Panicum maximum*	Yellowish-Green	Very pleasant	Firm	4.17
75%*Panicum maximum+25%Vetiveria nigritana*	Yellowish-Green	Pleasant	Firm	4.28
50%*Panicum maximum+50%Vetiveria nigritana*	Yellowish-Green	Fairly pleasant	Firm	4.35
25%*Panicum maximum+75%Vetiveria nigritana*	Yellowish-Green	Almost pleasant	Moderately firm	4.21

Differences were observed in the odour of the treatments as sole Vetiver silage gave a pungent odour, 100% *Panicum maximum* silage had a very pleasant smell; fairly pleasant smell was observed for 50% *Panicum maximum*+50% *Vetiveria nigritana* while 75% *Panicum maximum+25% Vetiveria nigritana* has pleasant smell.

The texture for the present silage was firm with exception to silage made from sole *Vetiveria nigritana* and 25%*Panicum maximum*+75%*Vetiveria nigritana* which were moderately firm. Kung and Shaver, (2002) reported that good silage was expected to be firm

The pH of the silage falls within the range of 4.6-4.17, the highest pH value was observed in sole vetiver grass silage while the least value was observed in silage from sole *Panicum maximum*. The pH value in the present result ranges from 4.6- 4.17 and falls within the range of 5.5-3.5 reported by Meneses *et al.*, (2007). Kung and Shaver, (2002) observed that good quality grass and legumes silage –pH values in the tropics ranges between 4.3-4.7. Properly fermented silages usually have lower pH. Meeske *et al.*, (2000) and Schroeder (2004), reported that the rate of pH decrease influences the quality of the silage and that the slower rate of decrease in pH will provide more time for the growth of Enterobacteria (coliform) which grow well at pH of 5 and higher. This could be responsible for the high bacteria and coliform count recorded in 100% *Vertiver nigritana* and 50% *Panicum maximum* + 50% *Vertieria nigritana* as shown in Table 4. The rate of decrease in pH is also determined by the epiphytic bacteria present in the crop before ensiling and the level of fermentable sugar (water soluble carbohydrates), which in turn depends on stage of maturity (Meeske *et al.*, 2000; Schroeder, 2004).

Table 4: Colony forming unit of microbes in varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana*

Treatment	Colony forming unit(g/ml)		
	Bacteria	Fungi	Coliform
100%*Vetiveria nigritana*	$3.43x10^{5\ a}$	$1.20x10^{5\ (b,c)}$	$4.5x10^{4\ (a,b)}$
100%*Panicum maximum*	$2.45x10^{5\ c}$	$1.74x10^{5\ b}$	$2.1x10^{4\ c}$
75%*Panicum maximum+25%Vetiveria nigritana*	$2.54x10^{5\ c}$	$9.3x10^{4\ c}$	$4.0x10^{4\ b}$
50%*Panicum maximum+50%Vetiveria nigritana*	$2.71x10^{5\ b}$	$2.97x10^{5\ a}$	$5.6x10^{4\ a}$
25%*Panicum maximum+75%Vetiveria nigritana*	$2.70x10^{5\ b}$	$2.53x10^{5\ a}$	$2.5x10^{4c}$
SEM	6.54	4.50	2.95

[abc] *Means within the same column with different superscript, differ significantly (P< 0.05)*

The colony forming unit (CFU) (g/ml) of microbes in varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana* is as shown in Table 4. There were significant differences (P<0.05) among the treatments. The Bacteria count was higher (P<0.05) on sole *Vetiver* (3.4 x10^5) followed by treatment 4 (50%*Panicum maximum+50%Vetiveria nigritana*) (2.71 x10^5). The least colony count of bacteria was observed in 100% (sole) *Panicum maximum*, though similar to (P>0.05) to 75% *Panicum maximum* + 25%Vertiver grass. The highest value (P<0.05) for Fungi was observed in 50%*Panicum maximum+ 50%*Vetiveria nigritana (2.97x10^5) while 75%*Panicum maximum*+25%*Vetiveria nigritana* was observed to have the least value (9.30x10^4). Silage combination of 50%*Panicum maximum+50%Vetiveria nigritana* was observed to have the highest value (P<0.05) value for Coliforms followed by sole vertiver grass silage and 75% *Panicum maximum* + 25%Vertiver nigritana which were similar (P>0.05) . The Colony forming unit (C.F.U) (g/ml) of microbes in varied combination of ensiled *Panicum maximum* and *Vetiveria nigritana* shows from the result obtained that Bacteria, Fungi and coliform were isolated and their CFU thus calculated with coliform being an indicator of contamination. The high values obtained for coliform might be have been due to a high moisture content in these silage mixtures as was observed by Limin (2001) who also observed that undesirable bacteria called clostridia or coliforms tend to thrive in very wet silages and can result in excessive protein degradation, DM loss, pH increase and production of toxins.

Conclusion and recommendation

The observation in the chemical composition (dry matter, crude protein, neutral detergent fibre and acid detergent lignin) and mineral contents of grass silage could be better improved by ensiling grass with legumes. The high coliforms (CFU) value obtained for 50%Panicum maximum+50%*Vetiveria nigritana* indicated a poor silage, but the combination could be better improved by taking precaution against factors that could reduce the silage quality such as the buffering content, delayed filling, increasing amount of water soluble carbohydrates and types and numbers of bacteria on the plant.

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