Nig. J. Anim. Prod. 2019, 46(1):216 - 224
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In vitro evaluation of mixed silage of *Pennisetum purpureum* and orange fleshed sweet potato vines

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Abstract

The study was aimed at determining the in vitro nutritive value of mixed silages of pennisetum purpureum and orange-fleshed sweet potato vines and evaluating their potential of being a new feed resource for ruminants during the dry season. Pennisetum purpureum (Pp) and orange-fleshed sweet potato vines (OFPV) were ensiled in experimental silos bottles after wilting for 24 hours in the following proportions viz:100%Pp (T1), 75%Pp +25%OFPV (T2), 50%Pp +50%OFPV (T3), 25% Pp +75% OFPV (T4) and 100% OFPV (T5). Each treatment was replicated ten (10) times and was kept for 14 and 28 days in the laboratory at a room temperature (20 – 25°C). The chemical composition of the silage at 14 and 28 days was determined. In vitro gas production of the silages was carried out for 48 hours. In vitro fermentation kinetics and gas production parameters of the silages were also estimated. Results revealed that ensiling periods had significant ($p<0.05$) effect on the dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents of the silages. Silage of 28 days had highest CP (13.45%) and lowest NDF and ADF contents (40.10 and 25.48% respectively). Silage containing 100% OFPV recorded highest ($p<0.05$) crude protein (15.95%), ash (15.81%) and lowest NDF (32.25%) and ADF (22.50%) contents. Gas production was lowest ($p<0.05$) in T1 at all incubation periods, and it increases with inclusion of OFPV, T5 had significant ($p<0.05$) highest gas production. Least gas production was observed in T1 at 42 and 48 hours incubation period (15.17 and 17.17 mL/200mg DM respectively). Silage of 28 days had the best potential gas production (37.51 mL). Fractional rate of gas production was higher in 28 days silage (0.06mL/hr), T3 (0.05mL/hr), T4 (0.05mL/hr) and T5 (0.06mL/hr) while lag phase was lowest in these silages. In vitro dry matter digestibility (IVDMD), metabolisable energy (ME) and short chain fatty acids (SCFA) were greater in silage of 28 days (63.73%, 5.01MJ/Kg DM and 0.02mL) with least percentage methane production (33.34%). Also, IVDMD, in vitro organic matter digestibility (OMD), ME, and SCFA increased as level of OFPV in the silages increases with T5 having the highest values for these parameters. Percentage methane production was least ($p < 0.05$) in T5 (26.55%). It can therefore be concluded that Pennisetum purpureum be supplemented with OFPV up to 75% and ensiled for 28 days or beyond to produce better quality feed resource for ruminants during the dry season. Ensiling 100% OFPV is also highly recommended as it exhibited the best nutritive potential.

Keywords: *Pennisetum purpureum*, potato vines, silage, nutritive value, ruminant nutrition

Introduction

In a bid to continuously searching for alternative feed resources that could solve the problem of feed shortage during the dry season for ruminant production, crop residues are still crucial and their contribution towards increase productivity of ruminant cannot be overemphasised. This is because new varieties of existing crops are developed and adopted faster by the farmers due to the values added to these crops. Sweet potato (*Ipomea batatas*) is one

of the priority research crops for sub-Saharan Africa because of its high yield of both tubers and vines. An (2004) reported that 30% of sweet potato production in the developing countries is used for animal feed. After potato and cassava, sweet potato ranks third in the world root and tuber crop production and the world production has been estimated at 110 million tonnes per annum (FAOstat,2008). Orange-fleshed sweet potato is a new variety that is now being propagated by the Institute of roots and tubers in Africa and in the Eastern and Western part of Nigeria. This is done to enhance the vitamin A status of individuals as this new variety is considered as excellent source of natural health-promoting compound (β-carotene) (Bovell-Benjamin, 2007). The acreage and consumption of orange-fleshed sweet potato has increased and substantial land area in Africa is being occupied with it (Tumwegamire *et al*., 2004). This increase has offer greater opportunities to harness the residues (vines) for livestock feeding. Increase milk production by dairy cows fed sweet potato vines has been reported by Peter (2008). Sweet potato vines have been reported to have a potential as a source of protein for livestock (Phuc,2000; Dung, 2000) with relatively high content of crude protein and low fibre constituents. Meanwhile, *Pennisetum purpureum* (Elephant grass) is one of the common grasses used in feeding ruminant in this part of the world. Elephant grass if not harvested at an early stage (less than 6-8 weeks) has low protein and energy content levels. Sweet potato vines can be fed to ruminant in combination with elephant grass. Such combination may have higher nutritive value than feeding elephant grass alone. Sweet potato vines and elephant grass can be preserved by means of ensiling for feeding ruminants (Nguyen, 2006). There is scarce scientific information on the nutritive value of new variety of sweet potato (orange-fleshed sweet potato) forage. This study is therefore designed to investigate the nutritive value of mixed silage of orange-fleshed sweet potato vines and *pennisetum purpureum* using *in vitro* method of evaluation.

Materials and methods

Experimental location and silage preparation

The experiment was conducted at the Laboratory of Animal Nutrition Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The orange-fleshed sweet potato vines (OFPV) were collected immediately after harvesting of the tubers at the crop farm of the University.Old, yellow, dried and rotted vines were removed and the green ones were chopped into small pieces of about 2-4cm length and were allowed to wilt for 48hr under a well-ventilated shed. Wilting was done for 48 hours to reduce the moisture content of the vines. During wilting, the materials were turned regularly to ensure rapid and uniform wilting. *Pennisetum purpureum*(Pp) was also harvested around the University premises. This was equally chopped and wilted. The wilted OFPV and *P. purpureum* were weighed and thoroughly mixed together by hand in different proportions on a clean concrete floor as follows: 100%Pp (T1), 75%Pp +25%OFPV (T2), 50%Pp +50%OFPV (T3), 25%Pp +75% OFPV and 100%OFPV. Each treatment was rapidly filled and strongly pressed at interval (to release air from the ensiling materials) into the respective experimental silos (well labelled with date). The silos were then tightly covered and sealed to avoid air penetration. Each treatment was replicated 10 times and was kept for 14 and 28 days in the laboratory at a room temperature (20 – 25°C).

Chemical analysis and in vitro gas production experiment

Five silos per treatment were randomly picked and opened at day 14 and 28. Samples were taken from each silos at different points and depths, mixed together, weighed and oven dried separately at 60°C until constant weight was obtained to determine the dry matter. The dried samples were then ground and made to pass through 1mm screen. Samples were analysed for crude protein, ether extract and ash contents according to AOAC (2005). Fibre fractions (NDF and ADF) were also analysed using the procedure of VanSoest *et al*. (1991)

The *in vitro* gas production of each silage treatment was determined according to the method of Menke and Steingass (1988). Rumen fluid was collected from six WAD sheep (fed 40% concentrate feed and 60% *Panicum maximum* grass) with the use of suction tube as described by Babayemi and Bamikole (2006). The rumen fluid was collected in the morning before feeding, mixed, strained through four layers of cheese cloth and kept at 39±1ºC. The rumen fluid was kept under a continuous flow of carbon dioxide (CO_2). Glass syringes (100 mL) fitted with plungers were used. Two hundred (200) mg DM of each silage sample was carefully put into the syringes and thereafter the syringes were filled with 30 mL of medium consisting of 10 mL of rumen fluid and 20 mL of buffer solution ($NaHCO_3$+$3Na_2HPO_4$+KCl+NaCl+$MgSO_4$.$7H_20$+$CaCl_2$.$2H_20$) and each sample was measured five times. The syringes were tightly capped and carefully arranged in an incubator maintained at 39±1 ºC along with three blank syringes containing 30 mL of medium (inoculums and buffer) only as control. The gas production was recorded at 6, 12, 18, 24, 30, 36, 42 and 48 hours. The content was repeatedly agitated at each time of reading. Total gas values were corrected for blank incubation by deducting the mean gas volume produced from blank syringes, from volume of gas produced from the samples. At post incubation period, 4mL of sodium hydroxide (NaOH) was introduced to estimate methane production following the method of Fievez *et al.* (2005). Cumulative gas production data were fitted to the non-linear regression model of France *et al.* (2002). At the end of incubation, the content of the syringes were transferred into centrifuge tubes and placed immediately in cold water at 4ºC to stop fermentation. The tubes were thereafter centrifuged at 15,000 x g for 25 mins. The supernatant was discarded and the residue was oven dried at 55°C for 48 h to estimate i*n vitro*dry matter digestibility (IVDMD). Organic matter digestibility (OMD%), Metabolizable energy (ME MJ/KgDM) were estimated (Tilley and Terry, 1963) and short chain fatty acids, (SCFA mL) were calculated (Getachew *et al*., 1998) after 24 hours gas production according to the following formulas:

$A = b(1 - e^{-c(t-L)})$ (France *et al.,*2002)

Where A = gas produced at time "t", b = potential gas production from the fermentable fraction (mL), c = fractional rate of gas production (mL/hr), t = incubation time (h), L = Lag time (hr)

$$IVDMD\ (\%) = \frac{\text{Initial dry matter input} - \text{Dry matter residues} \times 100}{\text{Initial dry matter input}}$$

$OMD\ (\%) = 14.88 + 0.8893GP + 0.448CP + 0.651A$

$ME\ (MJ/kg\ DM) = 2.20 + 0.136GP + 0.057CP + 0.002859EE^2$

$SCFA\ (mL) = 0.0239GP - 0.0601$

Where: GP = 24h net gas production, CP = crude protein (%), A = ash content (%), EE = ether extract (%)

Statistical analysis

All data collected were analysed in 5 x 2 factorial arrangement (five different treatments and two ensiling periods) using

the Analysis of Variance (ANOVA) and significant differences among means were compared using Duncan multiple Range F-test (SAS, 1999) with the following model:

$Y_{ijkl}=\mu+T_i+P_j+TP_{ij}+\Sigma_{ijkl}$

Where, Y_{ijkl} = observed value of the dependent variables, μ=Population mean, T_i=Main effect of different treatments, P_j=Main effect of ensiling periods, $(TP)_{ij}$ =Interaction effect of different treatments and periods of ensiling, $\square_{ijk}$ =Random residual error

Results and discussion

The chemical composition of the silages is summarised in Table 1. The effect of ensiling periods and treatments on gas production and fermentation characteristics was evident ($p < 0.05$) in Table 2. *In vitro* gas production technique is a frequently used method of assessing biological value of feeds based on their pattern of gas produced when incubated with rumen fluid under anaerobic condition. Silage of 28 days had highest ($p<0.05$) gas production throughout the incubation period, demonstrating greater degradation in the rumen. Getachew *et al*. (1998) had stated that gas produced in *in vitro* fermentation reflects the extent of feed fermentation and digestibility. The high gas production exhibited by silage of 28 days could be due to lower NDF and ADF recorded in this silage when compared with that of 14 days silage. Cell wall components have been observed to have negative correlation with gas production (Sallam *et al*., 2007). Gas production was lowest ($p<0.05$) in silage containing 100% *Pennisetum purpureum* (T1) at all incubation periods, and it increases with inclusion of orange-fleshed sweet potato vines (OFPV) with silage containing 100% OFPV (T5) having significant ($p<0.05$) highest gas production. In the early incubation period (up to 6 hours), 25%Pp75%OFPV (T4) and 50%OFPV50%Pp (T3) silages had similar ($p>0.05$) gas production (2.33 mL/200mgDM) that was higher than that of 75%Pp25%OFPV (T2) and 100%Pp (T1) silages. From 12 to 30 hours into the incubation, T1 to T4 had similar gas production ($p>0.05$). The gas production was least in T1 at 42 and 48 hours incubation period. This trend of gas production was inversely related to the fibre fractions of these silages as silage with higher NDF and ADF contents had lower gas production. Monção *et al*. (2014) had pointed out that the structural arrangement of the cell wall components influence the extent of degradation which implies that increase or decrease in gas production depends on chemical composition of the forage. In addition, the relatively higher gas production observed in silage of 28 days and that of T5 could be related to the high amount of crude protein content in these silages when compared with others as Hillman *et al*. (1993) submitted that gas production is positively related to microbial crude protein synthesis. In evaluating the nutritive value of feed, the rate at which a feed or its chemical components are digested in the rumen is as important as the extent of digestion. The kinetics of fermentation (patterns of feed fermentation) is one of the several factors influencing voluntary feed intake by ruminants. Silage of 28 days was observed to have the best potential gas production (b values) (37.51 mL). The potential gas production among the silages ranged from 24.85mL in T1 to 46.93mL in T5. However, this fell below the range reported by Noordar *et al*. (2017) when potato vine was processed and ensiled with different additives. The variation observed may be due to differences in the varieties of potato, the processing methods and the effect of additives used. Ensiling periods and

treatments had significant effect ($p < 0.05$) on the fractional rate of gas production (c) and lag phase (L) of the silages. The c values recorded in silage of 28 days and that of T3, T4 and T5 were similar to that reported by Getachew *et al*. (2004). The high fractional rate of gas production observed in silage of 28 days and that of T3, T4 and T5 could be as a result of lower ADF content of these silages and this corroborated observation made in previous studies (Sandoval-Castro, 2005; Bamikole and Babayemi, 2008). Also, better intake of these silages over others could be predicted in livestock based on their respective c-values, as this signal the potential ruminal load of the feed and it had been reported that the rate of degradation is an important parameter in the assessment of fermentation of crop residues in the rumen (Preston, 1986). The lag phase (L) was shorter in 28 days silage (2.77 hr) and in T3, T4, and T5 (2.64, 2.82, and 2.05 hr respectively) compared with silage of 14 days, T1 and T2. However, the range of lag phase observed in this current study (2.05 – 4.35 hr) was higher than those reported by Noordar *et al*. (2017) for potato vines treated with different additives. Lag phase as explains by Dehority (2003) is the period when feed particles are hydrated and colonised by rumen microorganism. Different factors stated to affect the length of the lag phase include nature of substrate incubated, the amount of inoculum used and the microbial species present in the inoculum (France *et al*., 2005).

Table 1: Chemical composition (%) of mixed silage of *Pennisetum purpureum* and orange-fleshed sweet potato vines

	Parameters						
Ensiling periods (days)	DM	CP	EE	Ash	NFC	NDF	ADF
14	35.14^{a}	12.10^{b}	3.30	12.97	30.47	41.55^{a}	27.47^{a}
28	33.74^{b}	13.45^{a}	3.13	13.27	29.64	40.10^{b}	25.48^{b}
SEM	1.36	0.55	0.38	0.46	1.59	2.28	0.78
Treatments							
T1	36.61^{a}	9.75^{d}	3.17	11.54^{d}	20.54^{d}	55.00^{a}	30.46^{a}
T2	31.98^{b}	12.15^{c}	3.08	11.96^{cd}	27.44^{c}	45.38^{b}	28.54^{b}
T3	31.68^{b}	12.65^{c}	2.67	12.33^{c}	34.61^{a}	37.75^{c}	26.25^{c}
T4	29.94^{c}	13.39^{b}	2.75	14.00^{b}	36.11^{a}	33.75^{d}	24.63^{d}
T5	26.99^{d}	15.95^{a}	4.42	15.81^{a}	31.58^{b}	32.25^{e}	22.50^{e}
SEM	1.46	0.41	0.55	0.26	1.10	0.67	0.57

^{a,b,c,d,e} Means on the same row having different superscripts are significantly different ($p< 0.05$), SEM – Standard error of mean, T1-100% Pp, T2-75%Pp25%OFPV, T3-50%Pp50%OFPV, T4-25%Pp75%OFPV, T5-100% OFPV, Pp-Pennisetum purpureum, OFPV-Orange-fleshed sweet potato vines

In vitro gas production parameters of mixed silage of orange-fleshed sweet potato vines and *Pennisetum purpureum* is significantly influenced ($p < 0.05$) by the ensiling periods and the treatments (Table 3). Silage of 28 days exhibited higher values for *in vitro* dry matter digestibility (IVDMD), metabolisable energy (ME) and short chain fatty acids (SCFA) (63.73%, 5.01MJ/Kg DM and 0.02mL and least in percentage methane production (33.34%). Also, IVDMD, ME, SCFA and *in vitro* organic matter digestibility (OMD) increase as level of OFPV in the silages increased with T5 having the highest values for these parameters. Percentage methane production was least ($p < 0.05$) inT5. The high IVDMD, OMD, and SCFA exhibited

by T5 could be attributed to the low NDF and ADF content of this silage when compared to others. However, the values recorded for these parameters in the current study were lower than those reported by Noordar *et al*. (2017). This might be as a result of additives used and differences in the variety of potato under study. It can be observed from this study that the IVDMD, OMD, ME and SCFA of the silages improved with addition of OFPV which demonstrated the potential of the vines to add value to the *Pennisetum purpureum* by supplying soluble components and highly degradable fibre. Percentage methane production decreases as level of OFPV increased in the silage with lowest value (26.55%) recorded in T5 and this silage happened to have highest gas production which was in contrast with the report of Babayemi (2007) that feedstuffs with high gas production capacity are observed to be synonymous for high methane production. This implies that OFPV has potential of reducing methane production which may be due to the presence of some secondary compounds that were not determined in this study.

Table 2: *In vitro* gas production and gas production characteristics of mixed silage of *Pennisetum purpureum* and orange-fleshed sweet potato vines

	Ensiling periods			Treatments					
Hours of incubation	14 days	28 days	SEM	T1	T2	T3	T4	T5	SEM
6	1.87^{b}	2.47^{a}	0.27	1.33^{c}	1.17^{c}	2.33^{b}	2.33^{b}	3.67^{a}	0.26
12	4.27^{b}	6.27^{a}	0.75	3.00^{c}	3.00^{c}	4.67^{bc}	5.67^{b}	10.00^{a}	0.47
18	7.00^{b}	10.47^{a}	1.23	5.00^{c}	5.33^{c}	7.67^{bc}	9.00^{b}	16.67^{a}	1.03
24	9.87^{b}	14.93^{a}	1.61	7.00^{c}	8.67^{bc}	10.83^{bc}	13.17^{b}	22.33^{a}	1.50
30	13.33^{b}	18.93^{a}	1.85	9.83^{c}	12.50^{bc}	14.67^{bc}	16.83^{b}	26.83^{a}	1.90
36	16.13^{b}	22.07^{a}	1.89	12.67^{c}	16.00^{bc}	17.83^{bc}	19.50^{b}	29.50^{a}	2.11
42	18.53^{b}	24.80^{a}	1.94	15.17^{c}	18.67^{b}	21.00^{b}	21.83^{b}	31.67^{a}	2.29
48	20.60^{b}	26.73^{a}	1.93	17.17^{c}	21.00^{b}	23.33^{b}	23.67^{b}	33.17^{a}	2.36
Fermentation characteristics									
b (mL)	30.90^{b}	37.51^{a}	2.19	24.85^{e}	28.69^{d}	32.99^{c}	37.58^{b}	46.93^{a}	1.85
c (mL/hr)	0.04^{b}	0.06^{a}	0.01	0.02^{b}	0.03^{b}	0.05^{a}	0.05^{a}	0.06^{a}	0.01
Lag phase (hr)	3.67^{a}	2.77^{b}	0.28	4.35^{a}	4.24^{a}	2.64^{b}	2.82^{b}	2.05^{b}	0.43

^{a,b,c} Means on the same row having different superscripts are significantly different (p< 0.05), SEM– Standard error of mean, T1-100% Pp, T2-75%Pp25%OFPV, T3-50%Pp50%OFPV, T4-25%Pp75%OFPV, T5-100% OFPV, Pp-Pennisetum purpureum, OFPV-Orange-fleshed sweet potato vines

Table 3: *In vitro* gas production parameters of mixed silage of *Pennisetum purpureum* and orange-fleshed sweet potato vines

	Parameters					
Ensiling periods (days)	IVDMD (%)	OMD (%)	ME (MJ/KgDM)	SCFA (mL)	CH_4(mL/200 mgDM)	%CH_4
14	55.87^{b}	41.14	4.24^{b}	0.18^{b}	9.10	45.03^{a}
28	63.73^{a}	39.63	5.01^{a}	0.20^{a}	9.27	33.34^{b}
SEM	3.27	1.85	0.25	0.04	0.51	2.29
Treatments						
T1	53.33^{c}	34.17^{d}	3.72^{e}	0.11^{d}	9.42^{a}	41.73^{a}
T2	52.67^{c}	35.37^{d}	4.08^{d}	0.15^{d}	8.25^{b}	41.17^{a}
T3	54.67^{c}	38.35^{c}	4.40^{c}	0.20^{c}	10.00^{a}	42.94^{a}
T4	61.33^{b}	41.70^{b}	4.76^{b}	0.25^{b}	10.25^{a}	43.55^{a}
T5	77.00^{a}	52.33^{a}	6.16^{a}	0.47^{a}	8.00^{b}	26.55^{b}
SEM	4.02	1.33	0.22	0.04	0.65	3.47

^{a,b,c,d,e} Means on the same row having different superscripts are significantly different (p< 0.05), SEM– Standard error of mean, T1-100% Pp, T2-75%Pp25%OFPV, T3-50%Pp50%OFPV, T4-25%Pp75%OFPV, T5-100% OFPV, Pp-Pennisetum purpureum, OFPV-Orange-fleshed sweet potato vines, CH_4-methane

Conclusion

The study showed that ensiling *Pennisetum purpureum* with orange-fleshed sweet potato vines for 28 days improved the chemical composition in terms of crude protein, neutral detergent and acid detergent fibre. *In vitro* gas production, fermentation kinetics and *in vitro* gas production parameters of the silages were enhanced with inclusion of orange-fleshed sweet potato vines in the silage. *Pennisetum purpureum* can therefore be supplemented with OFPV up to 75% and ensiled for 28 days or beyond to produce better quality feed resource for ruminants during the dry season.

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Received: 19th September, 2018
Accepted: 10th February, 2019