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In vitro gas production and dry matter degradability of cassava top and maize stover mixture ensiled with *Albizia saman* pods

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Abstract

In vitro gas production is an indication of microbial degradability of feed samples. Thus, varying levels of mixture of cassava top (CT) and maize stover (MS) ensiled with Albizia saman pods (ASP) were examined. The feedstuff were dried and milled for gas determination in a completely randomized design. Samples were incubated using in vitro gas production technique. Gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h post incubation to estimate total gas volume (TGV), methane (CH_4), metabolisable energy (ME; MJ/Kg DM), organic matter digestibility (OMD; %) and short chain fatty acids (SCFA; µmol/200 mg DM). dry matter (DM, %) increased significantly ($p<0.05$) as inclusion of Albizia saman pods increases across the treatments. The crude protein (CP, %) contents were similar across the treatments. The total gas volume (TGV mL) produced by the diets were similar across the treatments. The values ranged from 4.81 to 5.26 for ME, 35.16 to 36.32 for OMD, 0.13 to 0.21 for SCFA and 5.33 to 7.33 for CH_4 production with no significant difference. The result showed that in vitro fermentation of the mixture of cassava top and maize stover ensiled with Albizia saman pods at 0:40:60% increased the dry matter degradability by 15%, enhanced metabolisable energy, organic matter digestibility and short chain fatty acids.

Keywords: Maize stover, *Albizia saman* pods, cassava top, total gas volume, methane

Introduction

In developing countries, livestock are fed mainly on crop residues and agro-industrial by-products containing a larger proportion of ligno-cellulosic feeds like cereal straws, stovers and sugarcane by-products (Babayemi and Bamikole, 2004). These feeds are poor in protein, energy, minerals and vitamins (Babayemi and Bamikole, 2006). Addition of foliage from tree or supplementation with seed meals, ensiling or addition of urea can improve the utilization of low quality roughages mainly through the supply of nitrogen to rumen microbes. The use of simple techniques for evaluation of the nutritional quality of these feed resources will contribute to their efficient utilization (Menke and Steingass, 1988; France and Siddon, 1993; Kahal *et al.*, 1995 and Getachew *et al.*, 1998). Growth and milk yield of ruminants are largely limited by forage quality which is mainly reflected in low voluntary intake and digestibility (Babayemi, 2009). The importance of these parameters in animal nutrition has long been recognized. *In vivo* determination of intake and digestibility of feedstuffs is time consuming, laborious, expensive, requires large quantities of feed and unsuitable for large scale feed evaluation (Coles *et al.*, 2005). Therefore many attempts have been made to predict intake and digestibility using laboratory techniques. Gas production reflect all fermented nutrients, soluble as well as insoluble; and fractions that are not fermentable which do not contribute to gas production (Babayemi *et al.*, 2004 and Fievez *et al.*, 2005). Furthermore, the kinetics of fermentation can be obtained from a single incubation, allowing the rate of fermentation to be calculated. Gas measurement is a direct measurement of microbial activity and can be a better index of forage metabolizable energy (ME) content than an indirect *in vivo* measured based on nutrients (Makkar, 2004).

The *in vitro* gas production method is quick, less expensive and accurate to predict feed intake, digestibility, microbial nitrogen supply and animal performance (Blummel and Orskov, 1993; Khazaal *et al.*, 1995; Babayemi *et al.*, 2004; Babayemi and Bamikole, 2006). Similarly, the technique can be used determine the amount of short chain fatty acids, carbon dioxide and metabolisable energy of feed for ruminants (Blummel and Becker, 1997; Getachew *et al.*, 1999). Total gas production can predict methane, Volatile Fatty Acids (VFA) and the individual VFA (Fievez *et al.*, 2005). Methane (CH_4) which is an important gas produced by ruminants during fermentation has been reported as a source of energy loss and it contributes to the destruction of ozone layer (Babayemi and Bamikole, 2006). This study was designed to assess the *in vitro* gas production and dry matter degradability of cassava top and maize stover mixture ensiled with *Albizia saman* pods.

Materials and methods

Experimental site

The experiment was carried out at the small ruminant unit of the Department of Animal Science, University of Ibadan, Nigeria. It is situated in the derived savanna vegetation belt (Latitude 7°27'N and 3°45'E) at an altitude between 200m and 300m above sea level; mean temperature of 25 – 29°C with an average annual rainfall of about 1250 mm. The soils are much drained and belong to the alfisol (Rhodic kandiustalf) (Babayemi *et al.*, 2003).

Preparation of samples

The fresh cassava tops were harvested from a farm in Ajegunle village, Oyo. Fresh Maize Stover was harvested from the maize farm in University of Ibadan while the pods of *Albizia saman* were handpicked from trees within University of Ibadan campus and were sun dried for 7 days. The fresh cassava tops and maize stover were chopped separately into 3-5 cm size and wilted for 24 hours. These chopped materials were mixed together with sundried *Albizia saman* pods, filled into the mini silos of four liter capacity plastic containers to form five treatments as shown in Table 1. The ensiled materials were well compressed, made air tight by keeping sand bags weighing 50 kg on top and kept under shade for 30 days. All samples were ground in a laboratory mill to pass through a 1 mm screen.

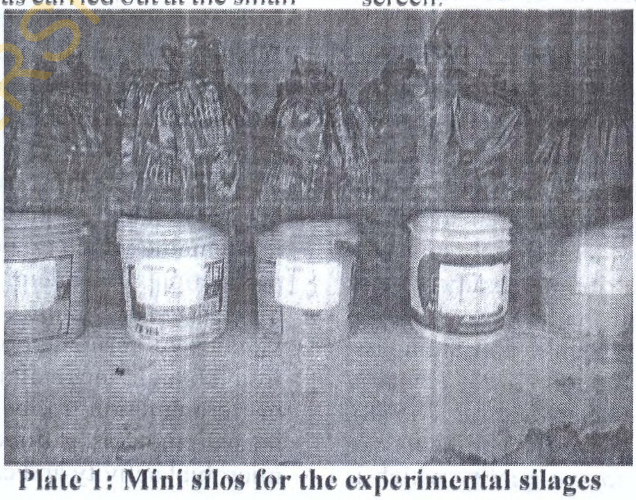

Plate 1: Mini silos for the experimental silages

Table 1: Percentage composition and calculated nutrients of the experimental diets

Ingredients (%)	Treatments T1	T2	T3	T4	T5
Cassava top	40	30	20	10	0
Albizia saman pods	0	10	20	30	40
Maize stover	60	60	60	60	60
Total	100	100	100	100	100
Calculated nutrients					
Dry matter	36.38	39.41	42.65	44.23	45.84
Crude protein	13.04	13.04	13.04	13.04	13.04
Crude fiber	19.56	19.41	21.76	19.11	19.96
Ash	6.38	6.26	6.14	6.02	5.90
Ether extract	9.00	8.00	10.60	11.40	12.20
Nitrogen free extract	41.32	33.33	42.66	38.33	44.00

T1-- 40% CT + 0% ASP +60% MS; T2 -- 30% CT + 10% ASP +60% MS; T3-- 20% CT + 20% ASP +60% MS; T4 -- 10% CT + 30% ASP +60% MS; T5-- 0% CT + 40% ASP +60% MS.

Chemical composition of cassava top and maize stover mixture ensiled with Albizia saman pods

Dry matter (DM) was determined by drying the samples at 65°C until constant weight was observed while ash was determined by igniting the samples in muffle furnace at 525°C for 8 h and nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1995). Crude protein (CP) was calculated as N × 6.25. Ether extract (EE) was determined according to the procedure outlined by AOAC (1995).

Table 2: Chemical composition (g/100 g DM) of mixture of cassava top and maize stover ensiled with *Albizia saman* pods

Parameters (%)	Treatments T1	T2	T3	T4	T5	SEM
Dry matter	31.22^{c}	35.83^{bc}	38.78^{b}	39.78^{ab}	44.22^{a}	1.51
Crude protein	13.06	13.27	13.35	13.22	13.16	1.73
Ash	9.67^{a}	9.43^{a}	9.60^{a}	8.73^{b}	7.03^{c}	0.10
Ether extract	8.10	7.97	7.80	6.63	7.43	0.74
NDF	34.80^{ab}	35.70^{a}	32.30^{bc}	29.50^{c}	30.63^{c}	0.97
ADF	31.83^{b}	36.77^{a}	37.70^{a}	33.90^{ab}	33.97^{ab}	1.26
ADL	24.50	25.93	24.60	25.17	25.87	1.17

^{a,b,c} Means on the same column with different superscript, differ significantly (P<0.05)
T1-- 40% CT + 0% ASP + 60% MS; T2 -- 30% CT + 10% ASP +60% MS; T3 -- 20% CT + 20% ASP +60% MS; T4-- 10% CT + 30% ASP +60% MS; T5-- 0% CT + 40% ASP +60% MS; NDF – Neutral detergent fibre; ADL – Acid detergent fibre; ADL – Acid detergent lignin.

***In vitro* gas production of cassava top and maize stover mixture ensiled with *Albizia saman* pods**

Fermentation of mixture of cassava top and maize stover ensiled with *Albizia saman* pods was carried out with rumen fluid which was collected from five mature West African dwarf goats via stomach tube as described by Babayemi *et al.* (2007). The goats were fed a diet of *Panicum maximum* (60%) and concentrate (40%) twice daily for 7days. The samples were incubated in the rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass (1988). Gas production was measured as the volume of gas in the calibrated syringes and recorded before incubation at 3, 6, 9, 12, 15, 18, 21 and 24 h after incubation. All samples were incubated with three syringes containing only rumen fluid-buffer mixture as blank. The net gas production for silage samples were determined by subtracting the volume of gas produced in the blank. At the end of the incubation period, 4mL of 10M NaOH was introduced to the syringes to estimate the volume of methane (CH_4) gas produced per sample.

Graph of the volume gas produced at every 3-hour interval of each sample was plotted against the incubation time. From the graph, the degradation characteristics were estimated as defined in the equation:

$y = a + b\,(1 - e^{-ct})$.

Where Y = Volume of gas produced at time (t)

t = time of incubation

a = intercept (gas produced from the soluble fraction)

b = gas produced from insoluble fraction c = gas production rate for the insoluble fraction (b) at time (t),

a+b=potential extent of gas production

The parameters estimated were dry matter degradability as described by Ørskov and McDonald (1979), metabolisable energy as described (Menke and Steingass, 1988) and organic matter digestibility and short chain fatty acid as established by Getachew *et al.* (1998). Data collected were subjected to one-way analysis of variance using the procedure of SAS (2000).

Table 4: In vitro gas production characteristics of cassava top and maize stover ensiled with Albizia saman pods at 24hrs incubation period

	Treatments					
Parameters	T1	T2	T3	T4	T5	SEM
a (mL^3)	1.33	1.33	2.67	2.00	3.33	0.62
b (mL^3)	6.67	8.00	7.33	8.00	8.00	1.25
a+b (mL^3)	8.00	9.33	10.00	10.00	11.33	0.88
c(mLh^{-1})	0.07^{ab}	0.09^{ab}	0.08^{ab}	0.07^{b}	0.12^{a}	0.001
t(hrs)	13.00	8.00	9.00	7.00	9.00	1.41
y(mL^3)	4.67	5.33	6.33	4.67	8.67	0.72

ab Means on the same column with similar superscript are not significantly (P 0.05) different.

a = intercept (gas produced from the soluble fraction), b = Potential gas production (ml/g DM) from the insoluble but degradable fraction, a+b=potential extent of gas production, c = gas production rate constant (h -1) for the insoluble fraction (b) production at time (t), t = incubation time, y = volume of gas produced at time t'.

Table 4: In vitro gas fermentation parameters of cassava top and maize stover ensiled with Albizia saman pods at 24hrs incubation period

	Treatments					
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
TGV(mL)	8.00	9.33	10.00	10.00	11.33	0.71
CH_4(mL/200mgDM)	5.33	4.67	6.00	7.33	6.67	0.62
OMD(%)	35.37	35.16	36.32	35.90	36.10	0.75
ME(MJ/Kg DM)	4.81	5.03	5.20	5.17	5.26	0.12
SCFA(mL)	0.13	0.16	0.18	0.18	0.21	0.02
DMD(%)	53.33	44.67	44.44	49.00	45.33	2.27

TGV-- Total gas volume (mL), CH_4—Methane (ml/200g DM), OMD -- Organic matter digestibility (%). ME -- Metabolizable energy (MJ/Kg DM), SCFA-- Short chain fatty acid (mL), DMD-- Dry matter degradability (%)

T1-- 40% CT + 0% ASP +60% MS; T2 -- 30% CT + 10% ASP +60% MS; T3 -- 20% CT + 20% ASP +60% MS; T4-- 10% CT + 30% ASP +60% MS; T5-- 0% CT + 40% ASP +60% MS

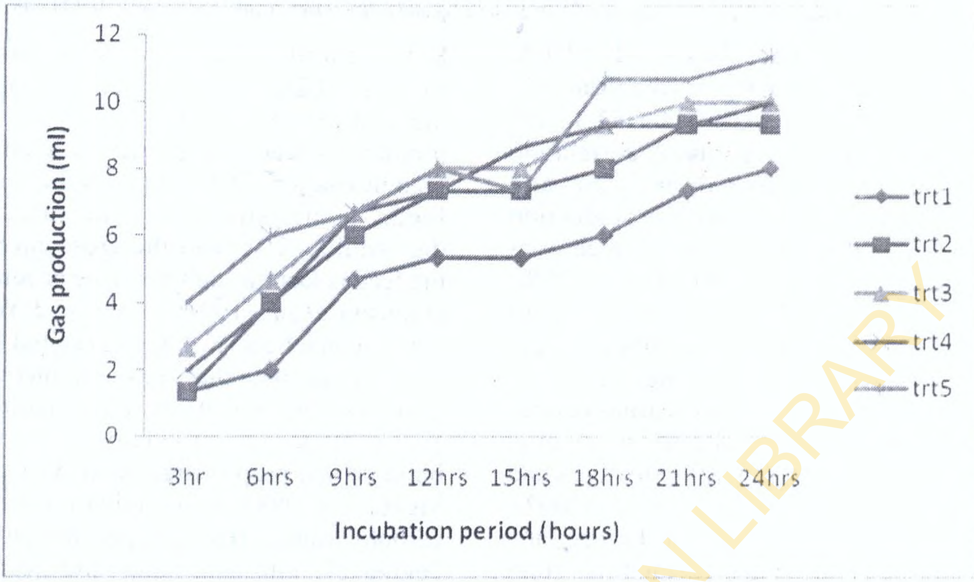

Fig l: In vitro gas production pattern of cassava top and maize stover ensiled with *Albizia saman* pods at 24hrs incubation period

Results and Discussion

In vitro gas production characteristics of mixture of cassava top and maize stover ensiled with *Albizia saman* pods are presented in Table 3. The *in vitro* gas production characteristics were not significantly (P > 0.05) different across treatments. The intercept value (a) for all the treatments ranged from 1.33 to 3.33 at 24 h. The extent of gas production 'b' values was similar across the treatments. Potential gas production (a+ b) and incubation time ('t') were not significantly (P >0.05) different across treatments. There were significant (P < 0.05) differences only in gas production rate ('c') of the incubated samples. The rate of gas production ranged from 0.07 to 0.12 ml h^{-1} for all the treatments while the volume of gas 'y' produced at time ('t') ranged from 4.67 to 8.67 for all the treatments. Time of rapid gas production ranged from 7.00 h to 13.00 h. Hillman *et al.* (1993) reported that gas production is positively related to microbial protein synthesis. Although, gas production is a nutritional wasteful product (Mauricio *et al.*, 1999) but it provides a useful basis for the prediction of metabolizable energy, organic matter digestibility and short chain fatty acids. More importantly, gas production helps to measure digestion rate of soluble and insoluble fractions of feedstuff (Menke and Steingass, 1988, Pell and Schofield, 1993).

In vitro gas production parameters of mixture of cassava top and maize stover ensiled with *Albizia saman* pods are presented in Table 4. The gas produced is directly proportional to the rate at which substrate are degraded (Doano *et al.*, 1997). Also, gas volumes have shown a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993). Metabolisable energy (ME), short chain fatty acid (SCFA) and dry matter degradability (DMD) production were not different significantly (P<0.05) for the five treatments. The values for the ME, SCFA, DMD and methane (CH_4) ranged from 4.81 to 5.26, 0.13 to 0.21 and 44.44 to 53.33 and 4.67 to 7.33% respectively. A correlation between ME measured *in vivo* and predicted from 24 h *in vitro* gas production and chemical composition of feed was reported by Menke and Steingass (1988). When feedstuffs are incubated with buffered rumen fluid (inoculums), gas production is basically the result of microbial degradation of carbohydrates under anaerobic condition to acetic, propionic and butyric acids {Steingass and Menke (1988), Getachew *et al.* (2002); Khazaal *et al.* (1995) and France and Siddon (1993)}. Gas production from protein fermentation is relatively small compared to carbohydrate fermentation. The contribution of fat to gas production is negligible. Beuvink and Spoelstra (1992) further stated that gas is produced mainly when carbohydrate feedstuff are fermented to acetate and butyrate with fermentation to propionate yielding gas only from buffering of the acid, therefore, forage which produce high amount of propionate should produce low gas volume. Gas production was directly proportional to SCFA (Beuvink and Spoelstra, 1992), the higher the gas produced, the higher the short chain fatty acids. Short chain fatty acid (SCFA) is directly proportional to ME (Menke *et al.*, 1979).

In most cases, feedstuff that showed high capacity for gas production was also observed to be synonymous for high methane production. Methane is a dietary energy loss and is an important greenhouse gas contributing to global warming (Johnson and Johnson, 1995) by trapping outgoing terrestrial impaired radiation 20 tons more effectively than CO_2. The IPCC (2001) reported domestic livestock as one of the largest single source of methane with 80 to 115 million tonnes per year equivalent to 0.15-0.20 of total anthropogenic methane. Therefore, reduction of methane production leads to greater efficiency in feed utilisation. Depending on the level of feed, composition of the diet and digestibility, 2.15% of the gross energy in the feed is lost through methane production (Johnson *et al.*, 1991; Holter and Young, 1992). Getachew *et al.* (2002) stated that it is well known that gas production is basically the result of fermentation of carbohydrate to volatile fatty acid (acetate, butyrate and propionate). Also, Menke and Steingass (1988) reported that fermentable carbohydrate increase gas production while degradable nitrogen compound decreases gas production to some extent due to binding of carbohydrate with ammonia.

Conclusion

The result showed that *in vitro* fermentation of the mixture of cassava top and maize stover ensiled with *Albizia saman* pods at 0:40:60% increased the dry matter degradability by 15 %, enhanced metabolisable energy, organic matter digestibility and short chain fatty acids.

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