In Vitro Gas Production of Ensiled Guinea Grass-*Albizia saman* Pods Silage

Kenneth-Obosi, O.[1]*, Igbekoyi,A. J .[2], Binuomote, R. T,[3] and Babayemi, O.J.[2]

[1]*National Horticultural Research Institute Ibadan, Nigeria,*
[2]*Department of Animal science, University of Ibadan, Nigeria,*
[3]*Department of Animal production ,LadokeAkintola University, Ogbomoso, Nigeria.*
**Corresponding author: oyewonu@yahoo.com*

Abstract
Meeting nutritional requirement of ruminants during dry season is challenged due to low quality forage. This necessitates use of unconventional feedstuff. Horticultural waste, rich in nutrients ensiled with grass species provides efficient rumen environment for microbes to thrive and ferment feeds thereby increasing productivity of animals. Pods from Albizia saman tree; an ornamental plant used for landscaping is usually wasted in large volumes in Southwestern Nigeria. Thus, dry Albizia saman pods and Guinea grass cut at six weeks old were assigned into treatments in ratio (10:90), 20:80, 30:70 and 40:60 and ensiled using standard method. Gas production was recorded at 3, 6, 9, 12, 15, 18, 21, 24, 36 and 48 hr of incubation. In vitro gas characteristic and in vitro gas parameter; Organic matter Digestibility OMD), Short Chain Fatty Acid (SCFA) Metabolizable Energy (ME) and Gas Volume (GV) were determined for each silage using in vitro gas technique. Data were analysed using descriptive statistics and ANOVA at $p<_{0.05}$. There was no significant difference in GV, ME, OMD and SCFA. In vitro gas characteristic followed the same trend at 24 hr of incubation. At 36hr of incubation 40% Albizia saman pod inclusion silage had least value of gas produced(13.67 mL), insoluble but degradable fraction (28.10mL^3) and rate of gas production 'c' (0.027 mLh^{-1}). The 'c' was highest in 20% at 48 hr of incubation. All Albizia saman pod silages present quality feed. Thus, Albizia saman pod should be put to productive use; as silage for ruminant feed.
***Keywords**: ruminant, feed conservation, horticultural waste, forage, dry season feeding, rumen fermentation*

INTRODUCTION

Livestock feeding is of great concern to livestock farmers especially in the critical dry season when all forage are dried, lignified and thequality of forage mass available for animal consumption is depleted (Jetana*et al.,* 2010). Supplementation of this forage with legume feed source help the animals to achieve optimal performance of the expected metabolic activitiestowards increasedproductivity.*Albizia saman,*a leguminous tree also known as rain tree is grown as an ornamental shade tree whichbesides the aesthetic value, at the University of Ibadan Nigeria, more than two hundred well grown trees are planted to cast shade along the walk way. Also, in villages where elder sit under it for relaxation in the evening. It also yield large volume dark brown and leathery pods, the pods are valuable supplements to goats and other ruminants(Stewart and Dunsdon, 2000; Jetana*et al.*, 2010) who cherish it, as they naturally pick it up after dropping from the tree (Jolaosho*et al.*, 2006). Ruminants relish the pods that drop from the tree at the University of Ibadan, Nigeria (Otukoya, 2007). She also, reported that the seeds in thepods are hard and indigestible when eaten raw by ruminantshowever, the hard coat can be broken through anaerobic fermentation toallow animals access the nutrients.In Nigeria therearefodder trees, seeds and podswith valuable nutrient composition (Aribido, 2010) that can replace conventional feedthereby eliminating competition between humans and animals. With increasing demand for livestock products as a result of rapid growth in the world economies and shrinking land area future hopes of feeding ruminant animals is a challenge. Safeguarding their food security will depend on the better utilization of unconventional feed resources which do not compete with human food**.**This therefore necessitates research into available and affordable but, unconventional feedstuffs the first step of which is determining their nutrient qualities. *In vitro* gas production gives a quick, predictable response of feed with little financial implicationin assessing the feed quality. (Babayemi and Bamikole, 2006). Therefore the study was conducted to assessthe quality of ensiled Guinea grass-*Albizia saman*pods using *in vitro* gas production techniques.

MATERIALS AND METHODS

Four bunker silos each were used to prepare the ensiled feedstuffs with different graded level grass and *Albizia saman* pod in the dairy farm of the Teaching and research Farm, University of Ibadan, Nigeria. *Albizia saman* pods (ASP) were gathered and sun dried. Guinea grass (GG) was harvested at 6 weeks old when the nutritive value was high and witted for 12 hours. The Guinea grass was chopped into 25 cm length to ease parking and consolidation for ensiling.Ratio of*Albizia saman* pod to Guinea grass was weighed according to the graded level of four treatments as 10% ASP + 90% GG, 20% ASP + 80% GG, 30% ASP + 70% GG, 40% ASP + 60% GG. 0.2% salt was added in layers to improve palatability. The silage was compacted tightly at intervals such that finger cannot easily penetrate into it. The silo was covered quickly because the bucker was lined with thick polythene folded and tucked invery well to make it air tight. Heavy sand bags were put on each silo to ensure airtight and maximum compaction. The silage was made to ferment for 40 days under anaerobic condition

In vitro techniques

Rumen fluid was obtained with suction tube technique from three West African dwarf goatspreviously fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal) and 60% *Panicum maximum* at 5% of body weight as described by Babayemi and Bamikole, (2006).The rumen liquor was kept in pre-warmedthermo flask at 39ºC. Incubation procedure was carried out according to Menke and Steingass (1988) using 120 ml calibrated transparent plastic syringes with fitted silicon tube. 200mg of each sample in triplicate were carefully inserted into the syringes and 30 ml inoculums containing cheese cloth strained rumen liquor and buffer solution (g/liter) (9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_4.7H_2O$ + 0.16 $CaCl_2.2H_2O$) (1:4 v/v) under continuous flushing with CO_2 and dispensed with 50 ml plastic calibrated syringe. Complete elimination of air in the inoculum was done by tapping and pushing upward the piston followed bytightening the silicon tube using metal clip to prevent the escape of gas. Incubation was carried out at 39 ± 1ºC and the volume of gas production was measured at 6 hour intervals: 0, 6, 12, 18, 24, 36 and 48 h. Post incubation4 ml of NaOH (10 M) was introduced to estimate methane production (Fievez*et al*., 2005). Then parameters such as Metabolisable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty acids (SCFA) were estimated at 24 h post gas incubation (Menke and Steingass, 1988). The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

The volume of gas produced at intervals was plotted against the incubation time, and from the graph, the gas production characteristics were estimated using the equation $y = a + b(1 - e^{-ct})$ as described by Ørskov and McDonald (1979). Where y = volume of gas produced at time 't', a = intercept (gas produced from the soluble fraction), b = gas production from the insoluble fraction

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c = gas production rate constant for the insoluble fraction (b), t = incubation time.

ME (MJ/Kg DM) = 2.20 + 0.136 GV + 0.057 CP + 0.0029 CF (Menke and Steingass, 1988).

OMD (%) = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA (Menke and Steingass, 1988).

(SCFA) (µmol) = 0.0239 GV – 0.0601 (Getachew*et al*., 1999). Where GV, CP, CF and XA are total gas volume at 24hr, crude protein, crude fibre and ash respectively. Data were analysed usingdescriptive analysis and analysis of variance at $p<0.05$ (SAS, 1999).

RESULTS AND DISCUSSION

Gas production parameters

Quality feed is of great importance in livestock production especially in dry season, it has implication on small ruminant subsector of agricultural sector in the nation economy (Ososanya *et al*., 2013).Gas produced during fermentation of nutrients in the rumen is a nutritionally wasteful product, but provides a useful basis from which Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty Acids (SCFA) could be estimated. 40% Inclusion of *Albizia saman* pod silage had the highest gas production (24.76 mL^3) this leads to high ME (6.60 MJ/KgDm), OMD (51.20%) and SCFA (0.49µmol). Rate of gas production in figure 1 is proportional to quantity of *Albizia saman* pod inclusion from 3 to 24 hours except for the treatment with 20% *Albizia saman* pod inclusion silage which produced gas lower than 10% inclusion of *Albizia saman* pods inclusion silage. The relatively low rate of gas production at 20% inclusion level is reflected in the low gas production parameters.At 24hrs, the level of degradability by the microbes is always a function of the *in-vitro* gas characteristics where 'b' the degradable fraction is least in 20% and highest degradable fraction is seen in 40% *Albizia saman* pod inclusion.The rate of degradation of substrate which is proportional to gas production in this study increasedwith *Albizia saman* pod inclusion except in 20% inclusion.

At 36hrs and 48hrs incubation, 30% inclusion produced highest gas volume from fig.2 this was followed by 10% inclusion. This might be due to depletion of degradable fraction of the substrate in the 40% *Albizia saman* pod inclusion overtime.At 36 and 48hrs of incubation the level of degradable fraction has been depleted while treatment with lower ASP inclusion and lower degradation are getting degraded with time.High production of gas from 40% inclusion of *Albizia saman*pod infers an increased proportion of acetate and butyrate but decrease in propionate production (Babayemi*et al.,* 2004). Acetate and butyrate are lipogenic, which leads to synthesis of butter fat in milk while propionate is glucogenic which leads to production of lean meat. Therefore, inclusion of *Albizia saman* in ruminant diet can be incorporated into silage for lean meat production

40% inclusion of *Albizia saman*pod silage had the highest gas volume hence the highest SCFA. The lowest gas volumes and SCFA were observed in 20% inclusion of *Albizia saman*. Also, gas production helps to measure digestion rate of soluble and insoluble fractions of feedstuff. Energy supplement produces higher gas compared to protein supplement and that protein fermentation does not lead to much gas production (Gatechew *et al.,* 1998). This could be responsible for lower gas production observed from the 20% inclusion of *Albizia saman*pod silage.

Gas production was directly proportional to SCFA (Babayemi and Bamikole, 2006), the higher the gas produced the higher the SCFA.SCFA level indicate the energy available to the animal, it contributes nearly 80% of animal daily energy requirement (Fellner, 2004). There is a direct relationship between SCFA

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andmetabolizable energy (ME). Hence, 40% inclusion of *Albizia saman*pod silage with highest gas production had highest SCFA and OMD. While the 20% inclusion of *Albizia saman*pods silage with the lowest gas volume had the lowest SCFA, ME and OMD.

SCFA measurement is very important for relating feed composition to production parameters and to net energy value of the silages, therefore production of SCFA from *in vitro* gas measurement will be increasingly important in a developing country like Nigeria. Nitiport and Sommart (2003); Kenneth-Obosi and Babayemi, (2013); Saliu and Ososanya (2017) all reported direct relationship between OMD and gas production. The quality of gas produced by ruminant during fermentation is a reflection of the amount of substrate degraded and the microbial metabolic activity (Kenneth-Obosi and Babayemi, 2013).

Table 3 shows the intercept value (a) for all silages ranged from 4.00mL^3 in 10% silage at 24 hours to 10.67mL^3 in 40% silage at 48 hours. The value of 'a' increases as the time of fermentation increases.It also increases as the level of inclusion of*Albizia saman* pod increases at each incubation period. Therefore, ensiling has softened the pod and seeds (Kenneth-Obosi *et al*., 2017), this increase the soluble fraction of the feed. The value for absolute 'a' used ideally reflects the fermentation of the soluble fraction in this study.The absolute gas production was highest for 40% silages at 48hr.This soluble fraction was found to be highest at this level (40% silage at 48hr).The soluble fraction makes it easily attachable by ruminal microorganism and leads to much gas production. Therefore, more ruminant microorganisms worked on the 40% *Albizia saman*pod inclusion and this leads to the highest gas production observed. The soluble fraction decreases as level of *Albizia saman* pod inclusion and incubation time increases.

The extent of gas production 'b' described the fermentation of the insoluble fraction. The gas volume of 20% inclusion of *Albizia saman*pod at 24hr (Table 1) had the lowest value of 18.00 mL^3 and was highest in the 10% *Albizia saman* pod inclusion *Albizia saman*at 48hr (Table 3). The value of 'b' increases as the incubation period increases.The 'b' predicts feed intake. Blummel and Orskov (1993) found that the 'b' value could account for 88% for thevariation in feed intake. The rate of gas production 'c' was lowest in 10% inclusion of *Albizia saman* pod silage at 48hr and highest in 40% inclusion of *Albizia saman* pod silage at 24hr (Table 1).

The rate of gas production increases as the time of incubation increases. The high value of the rate of gas production in 40% silage at 24hr(Table1) is possibly influenced by the carbohydrates fractions readily available to the microbial population. At 36hr(Table 2) and 48hr(Table 3) incubation period, the value of 'c' that had been increasing with level of inclusion of *Albizia saman* pod started decreasing at 30% inclusion of*Albizia saman* pod. This might be as a result carbohydrate fraction depletion which affected the kinetics of gas production. Kenneth-Obosi and Babayemi, (2015) indicated that the intake of feed is mostly explained by the rate of gas production which affects the rate of passage of the feed through the rumen.

High rate of gas production and extent of gas production were observed in 40% inclusion *Albizia saman* pod silage at 24 hours due to low fibre contents while low rate and extent of gas production were observed in 20% *Albizia saman* pod silageat 24hours due to high fibre contents.These findings were in agreement with the above reports.

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The soluble fraction 'a' increasedas the level of *Albizia saman pods*increases in the incubated silages in Tables 1, 2 & 3.Thisis an indication of the solubility of the*Albizia saman* pod compared to the high lignin content found in guinea grass (Chumpawadee and Pimpa, 2009). The structure of carbohydrate and protein in the diet determines the rate and extent of degradability. There was no significant difference in the degradability by the microbes in ('b') the degradable fraction of the silage.Microbial population in the rumen was supported by high crude protein content foundin the *Albizia saman* pod silage which determines the level of fermentation (Babayemi*et al*., 2010).

The Metabolizable Energy (ME), Organic Matter Digstibility (OMD) and Short Chain Fatty Acid (SCFA) values are higher (5.33, 40.42 and 0.36respectively.100% *Albizia saman* pod ensiled andfed to ruminant modified the rumen ecology, influence fermentation and increase animal performance contained secondary metabolites like tannins, saponin and essential oils (Babayemi *et al*., 2010; Gunun*et al*., 2018). The combination of Guinea grass and ASP produced higher gas at 24hr compared with gas production from *in vitro* fermentation of cassava top and maize stover ensiled with *Albizia saman* pod (Saliuand Ososanya, 2017). Hence, farmer can utilise abundant Guinea grass slashed off as weed and ensiled it with pods from *Albizia saman*trees found in different part of the country

The insoluble but degradable fraction 'b' and rate of degradation 'c' increased with increase in ASP in the silage at 24hr except at 20% ASP (Table 1) this agrees with the findings of Saliu and Ososanya, 2017. Although not significantly different but it can be inferred ensiling has softened the pod and the seed coat which makes degradation by the microbes possible compared favourably with silage with higher grass ratio. (Otukoya,2007). Rate of gas production which peaked at 36 hours and 48 hours in 20% inclusion maintained the same rate in 30% but declined in 40% inclusion of *Albizia saman* pod while at 48hours it starts to decline from 30% inclusion. Low (a+ b) recorded in 40% inclusion at 36hours was probably due to their high protein content, Khazaal*et al.,* 1995 reported that protein fermentation does not lead to much gas production. The peak rate of degradation 'c' for 40% ASP was achieved at 24hr and begins to decline as time progress this may be related to ability of the microbes to access the 'b' fraction in the pod compared to the treatment having higher percentage of Guinea grass

CONCLUSION

The result showed that 40% inclusion level of*Albizia saman* pod recorded highest gas volume, ME, OMD and SCFA which is a measure of digestion rate of soluble and insoluble fractions of the silages. Thus, more substrates are degraded at this inclusion level.10% inclusion level of *Albizia saman* pod had lowest gas volume, ME, ODM and SCFA which inferred that less substrate were degraded. Thus, *Ablizia saman* pod a waste that constitute nuisance to the environment can be put to use up to 40% inclusion guinea grass based silage for better performance animal and cleaner environment.

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Table 1: ***In vitro* fermentation parameters of graded levels of *Albizia saman* pod silages Levels of *Albizia saman* pod (ASP) inclusion**

Parameters	Treatment				
	10% ASP + 90% GG	20% ASP + 80% GG	30% ASP + 70% GG	40% ASP + 60% GG	SEM
Metebolizable energy (MJ/Kg DM)	6.06	5.65	6.36	6.60	0.60
Organic Matter Digestibility (%)	50.50	47.60	50.78	51.20	4.02
Short Chain Fatty Acid(µmol)	0.47	0.37	0.49	0.53	0.11
Gas Volume (mL^3)	21.67	18.00	23.00	24.76	4.42

SEM: Standard Error of Mean, ASP: *Albizia saman* Pod; GG; Guinea Grass

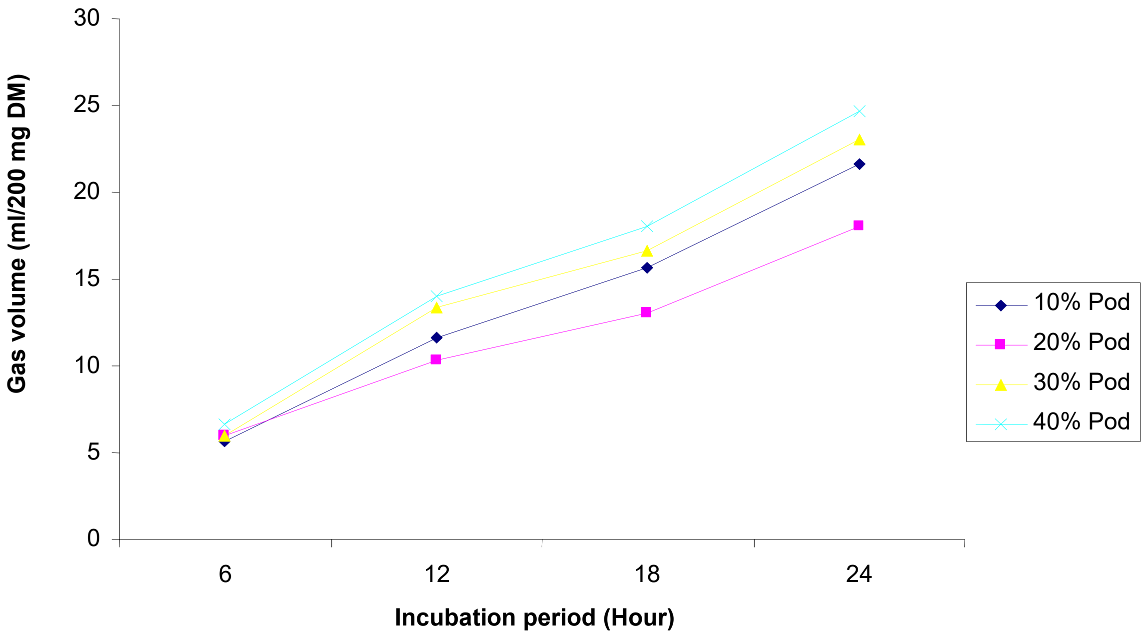

Figure 1. *In vitro* gas production of ensiled *Albizia saman* pod with Guinea grass

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Table 2: *In vitro* fermentation characteristics of graded level *Albizia saman* silages for 24 hours

Production feature	Treatment				
	10% ASP + 90% GG	20% ASP + 80% GG	30% ASP + 70% GG	40% ASP + 60% GG	SEM
a (mL^3)	4.00	6.00	6.00	6.67	0.51
a+b (mL^3)	21.67	18.00	22.33	24.76	4.42
b (mL^3)	17.67	12.00	16.33	21.33	4.33
c(mLh^{-1})	0.053	0.050	0.060	0.067	0.006
y(hrs)	13.33	11.67	15.00	15.67	1.16
t(hrs)	14.00	14.00	14.00	14.00	.60

a = zero time intercept which ideally reflects the fermentation of soluble fraction; b = extent of gas production/insoluble but degradable fraction ;(a+b) =potential extent of gas production; C = rate of gas production; y= volume of gas produce at time (t).; t = Incubation time; SEM= standard error of means

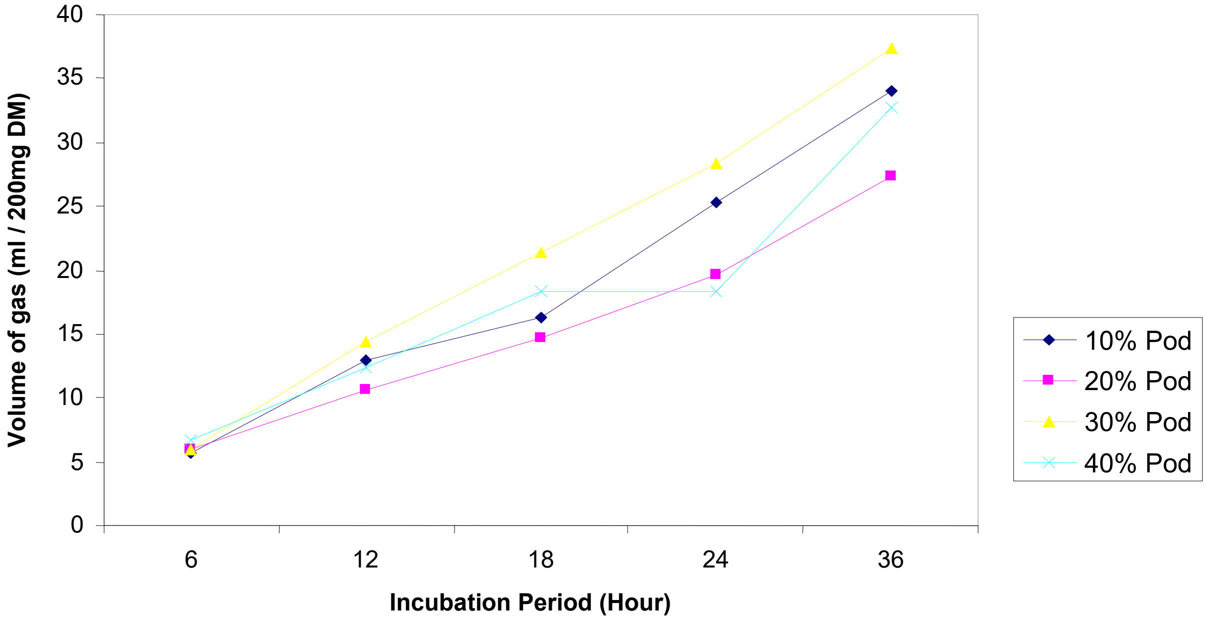

Figure 2. *In vitro* gas production of ensiled *Albizia saman* pod with Guinea grass for 36 Hours

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Table 3:*In vitro* fermentation characteristics of graded level *Albizia saman* pod silages (36 hours)

Production feature	Treatment				
	10% ASP + 90% GG	20% ASP + 80% GG	30% ASP + 70% GG	40% ASP + 60% GG	SEM
a (mL^3)	5.67	6.00	5.67	6.67	0.37
a+b (mL^3)	34.00	37.33	37.33	32.67	6.35
b (mL^3)	28.33	31.33	31.67	28.00	6.32
c(mLh^{-1})	0.033	0.037	0.037	0.027	0.004
y (hrs)	16.00	18.33	18.33	13.67	2.12
t (hrs)	16.00	16.00	16.00	16.00	1.53

a = zero time intercept which ideally reflects the fermentation of soluble fraction; b = extent of gas production/insoluble but degradable fraction ;(a+b) =potential extent of gas production; C = rate of gas production; y= volume of gas produce at time (t).; t = Incubation time; SEM= standard error of means

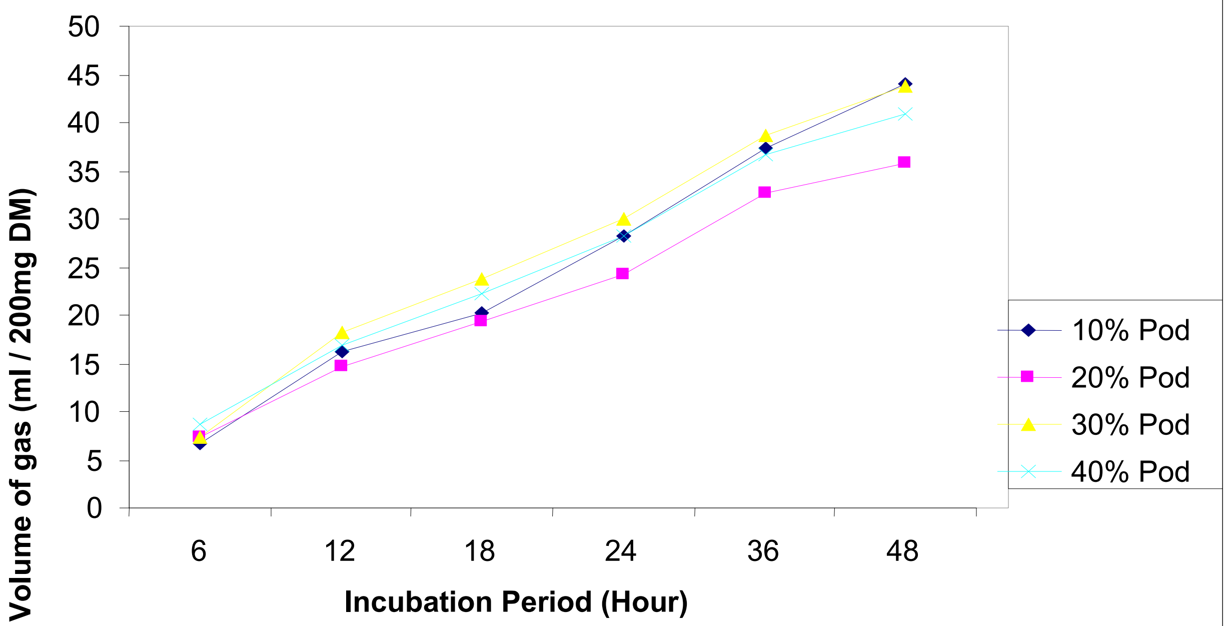

Fig. 3 *In vitro* gas production of ensiled *Albizia saman* pod with Guinea grass for 48 h

Table 4: *In vitro* fermentation characteristics of graded level *Albizia saman* silages for 48 h

Production feature	Treatment				
	10% ASP + 90% GG	20% ASP + 80% GG	30% ASP + 70% GG	40% ASP + 60% GG	SEM
a (mL^3)	6.67	7.33	7.33	10.67	0.79
a+b (mL^3)	44.00	35.67	43.67	41.00	6.57
b (mL^3)	37.33	28.33	35.33	30.33	6.91
c(mLh^{-1})	0.023	0.037	0.030	0.027	0.003
y(hrs)	16.33	18.67	18.33	21.00	1.93
t(hrs)	12.00	18.00	12.00	18.00	1.41

a = zero time intercept which ideally reflects the fermentation of soluble fraction; b = extent of gas production/insoluble but degradable fraction ;(a+b) =potential extent of gas production; C = rate of gas production; y= volume of gas produce at time (t).; t = Incubation time; SEM= standard error of means

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