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In Vitro Gas Production Assessment of *Panicum maximum* Incubated with *Leuceana leucocephala* at Varying Proportion to Predict the Nutritional Value for Ruminants

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Abstract

The study was carried out to determine the proximate composition, in vitro gas production characteristics and parameters of *Leucaena leucocephala* and *Panicum maximum* at varying proportions. Five diets were formulated such that *Panicum maximum* was substituted with *Leuceana leucocephala* at different ratio. T1 (100% *Panicum maximum*) , T2(100% *Leuceana leucocephala*), T3 (50% *P. maximum* + 50% *L. leucocephala*), T4 (75% *P. maximum* + 25% *L.leucocephala*) and T5(25% *P. maximum* + *75% L. leucocephala*). All data collected were subjected to analysis of variance. Dry matter values (34.43 – 35.95g/100g) decreased with the increased inclusion of *Leuceana leucocephala* in the diets, the crude fiber values (14.33 – 30.75g/100g) also follows the same trend while crude protein (CP) content (10.70 – 26.78g/100g) increased with the increased inclusion of *Leuceana leucocephala* in the diets. There were significant ($P < 0.05$) differences in the treatment means of organic matter digestibility (OMD 31.93 – 37.07%), Metabolisable energy (ME 3.62 – 4.33 MJ/kgDM), Short chain fatty acids (SCFA 0.04 – 0.16 µmol) and methane (ME 1.00 – 2.50 ml).The values(2.00 – 4.67 ml/200mgDM) obtained for immediate soluble 'a' was significantly ($P < 0.05$) different among the treatments. The extent of gas production (a+b) ranged from (2.00 – 4.67 ml/200mgDM), T1 (100% *Panicum maximum*) recorded the lowest while highest was observed in T2 (100% *Leuceana leucocephala*). The insoluble but degradable fraction 'b' ranged from 2.67 – 5.67ml/200mgDM, while the rate of gas production 'c' ranged from 0.04 – 0.14ml/hr. In conclusion, the enhanced values of crude protein, OMD, SCFA, and ME in the *Panicum maximum and Leuceana leucocephala* mixture indicated that the diets are able to meet the nutrients requirements of small ruminants in the tropics especially during the dry season.

Key words: In vitro, Gas production, Nutrients, *Leuceana leucocephala*, *Panicum maximum.*

Introduction

Browse species play a major role as feed for ruminants in arid and semi-arid regions, particularly during the dry season when poor quality forage and crop residues are common (Kibon and

Orskov, 1993). The inclusion of multipurpose trees like *Leuceana leucocephala, Gliricidia sepium* among others is one of the ways of improving the utilization of low quality forage. *Panicum maximum* is one of the common grasses in the derived savannah region of Nigeria. Under good conditions, its nutritional value is high having 9 – 12.5% Crude Protein, total digestible nutrients (TDN) of 10.2%, calcium of 0.05% and magnesium of 0.06% (McDonald *et al.,* 1995). However these values can be reduced during the dry season because of poor soil fertility and low rainfall which reduces the growth rate of the grass resulting into high cell wall contents and thereby lowering the quality of the forage. According to Andera and Pablo, (1999) a sustainable way of improving feeding value of poor quality pastures is through supplementation with browse plants. Browse species can be fed to ruminants at varying levels with *Panicum maximum* especially during the dry season as source of supplementary protein. This is because they survive long dry season by the possession of deep rooting system, low transpiration xerophytic foliage and often have good water harvesting architecture. *Leuceana leucocephala* is a promising feedstuff for goats when compared with other legumes such as alfalfa, *Lablab purpureus* and *Gliricidia sepium*. It is rich in nutrients, resulting in better dry matter intake, weight gain and reproductive performance (Kanani *et al*, 2006).The in vitro gas production technique is a laboratory estimation of rate and extent of nutrient disappearance in livestock feed sample for the purpose of assessing the potential nutritive value of the feed. It is also a method that is reproducible and parameters obtained correlate well with in vivo trials (Fajemisin, 2002). In vitro gas production technique has the advantage of not only being less expensive and less time consuming but it allows for more precision in experimental conditions than the in vivo method (Makkar, 2002). It is convenient, fast and allows a large number of samples to be handled at a time. It is based on the quantification of substrate degraded and of gas or short chain fatty acid produced in rumen fermentation system (McDonald, *et al*., 1995). In vitro gas production is a technique that involves volumetric measurement of gas production in phosphate and bicarbonate buffered in vitro incubation in syringes. Although gases produced during rumen fermentation are waste products and of no nutritive value to the ruminants, gas production tests are used routinely in feed research as gas volumes are related to both the extent and rate of substrate degradation (Blummel *et al.*, 1997a). Thus, the study was designed to evaluate the nutritional composition of *Panicum maximum* incubated with *Leuceana leucocephala*at different ratio by in vitro gas production technique.

Materials and method

Site of the experiment

The experiment was carried out at Animal Nutrition laboratory of the department of Animal science, University of Ibadan, Ibadan, Nigeria.

Collection of forage samples

The forage samples were harvested from the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The plants samples were air-dried at room temperature for

seven days and milled to pass through 1mm mesh and stored in different air-tight labeled container.

Experimental diets preparation

Five experimental diets were formulated such that *Panicum maximum* was substituted with *Leuceana leucocephala* at ratio *T1 = 100% Panicum maximum, T2= 100% Leuceana leucocephala, T3 = 75% P.maximum + 25% L. leucocephala, T4 = 50% P.maximum + 50% L. leucocephala, T5 = 25% P.maximum + 75% L. leucocephala.*

Chemical analysis

Known weight of each air-dried sample was oven dried at 65^0C to a constant weight for dry matter determination (DM). Proximate analysis; crude protein, crude fiber, ether extract and ash were analyzed according to the standard methods of (AOAC, 1995).

In vitro gas production and statistical analysis

Rumen fluid was obtained from three West African dwarf goats using stomach tube before the morning feed. The animals were fed with concentrate diets (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soya bean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal). The use of rumen liquor and buffer (9.8g $NaHCO_3$+2.77g Na_2HPO4+0.57g KCl+0.47g NaCl+0.12g $MgSO4.7H_2O$+0.16g/litre $CaCl_2.H_2O$) (1:4, v/v) under continuous flushing with CO_2 for incubation was as reported by Menke and Steingass, (1988). The gas production was measured at 3, 6,9,12,15,18,21 and 24 hour. After 24 hour post incubation 4ml of 10M NaOH solution was introduced as described (Fievez *et al*., 2005) to estimate methane. Metabolizable energy (ME,MJ/KgDM) and organic matter digestibility (OMD %) were estimated as established by (Menke and Steingass, 1988) and short chain fatty acids (SCFA) was calculated as reported by Getachew *et al*., (1998) using 24 h post incubation. ME = 2.20 +0.136* Gv+ 0.057* CP +0.0029* CF, OMD = 14.88+ 0.889Gv +0.45CP + 0.651XA; SCFA = 0.0239*Gv – 0.0601, where Gv, CP, CF,and XA are net gas production (ml/200mgDM), crude protein, crude fiber and ash of the incubated samples respectively. Data obtained were subjected to analysis of variance and means were compared where significant using Duncan Multiple Range F- test (SAS, 2002).

Results and discussion

The result of proximate composition is presented in Table 1. It was observed that there were significant variations in the values of determined nutrients with the exception of dry matter (DM). The dry matter DM of the experimental diets ranged from 34.43 in T2 to 35.95g/100g in T1 while crude protein CP of the diets ranged from 10.70 to 26.78g/100g, it was observed that CP increased as the level of *Leucaena leucocephala* increased, it implied that the diets could be good sources of protein that would meet the requirement of small ruminants for growth and production. The improved crude protein CP values observed agreed with the findings of Ezenwa

and Aken'ova (1988) that the effect of legume components in a grass-legume mixture would increase the protein quality of the mixture. The crude fiber values observed in this study ranged from 14.33 (T2) – 30.75g/100g (T1) and these values were lower than the values (47.80 – 51.20%) reported for other forage leguminous plants (Ifut, 1987). T1 had the highest ash value (11.05g/100g) which was lower than the value reported by Fajemisin *et al*, (2015). The lowest NFE value (29.69g/100g) was observed in T2 (100% *L. leucocephala)*which was significantly ($P < 0.05$) different from the values recorded for other *P.maximum L. leucocephala* diets, this implied that the soluble carbohydrates could support the production of volatile fatty acids in the rumen during fermentation (Blummel *et al*, 1997b). The results of the OMD, ME, SCFA and Methane are presented in Table 2. Organic matter digestibility, (OMD) could be said to be a measure of degradability potentials of the microbes on the substrates especially in the presence of sufficient ammonia nitrogen (NH_3-N) which has influence on bacteria fermentation. The OMD observed was highest (37.07%) at T1 and lowest (31.93%) at T2. Short chain fatty acids SCFA (0.07-0.16µmol), observed in this study differed significantly ($P < 0.05$) among the diets treatments. Short Chain Fatty Acids (SCFA) or volatile fatty acid (VFA) is a reflection of energy availability in a feed stuff, its level indicates the energy available to the animal; it contributes up to 80% of animal daily energy requirement (Fellner, 2004). This is one of the end products of rumen fermentation. Short Chain Fatty Acid SCFA is very important for relating feed composition to production parameters and to net energy values of the forages, therefore production of short chain fatty acid (SCFA) from in vitro gas measurement will be increasingly important in a developing country. The metabolizable energy (ME) obtained from this study were significant ($P < 0.05$) the values ranged from 3.62-4.33MJkgDM.The in vitro gas production method has been widely used to evaluate the energy value of several classes of feed (Getachew *et al.,* 1998). The result obtained in this study were within the range (2.99 – 4.75) reported by Babayemi and Bamikole, (2006). Methane production observed in this study ranged from 1.50 – 2.00ml200mg/DM T1 and T4 recorded the highest while the lowest was observed in T5. Methane production is said to be energy loss to ruminants and also contribute to global warming (Babayemi and Bamikole, 2006). In most cases, feedstuffs that show high capacity for gas production are also observed to be synonymous for high methane production; also most tropical feedstuffs have been implicated to increase methanogenesis (Babayemi *et al*., 2004; Babayemi and Bamikole, 2006) as an integrated part of carbohydrate metabolism. Although gas production is a nutritionally wasteful product (Mauricio *et al.*, 1999) but provides a useful basis from which ME, SCFA and OMD may be predicted. Short chain fatty acid (SCFA) or volatile fatty acid (VFA) such as butyrate and acetate suggest a potential to make energy available to ruminants. Table 3 shows the in vitro gas production characteristics of *Panicum maximum* incubated with L*eucaena leucocephala* at different proportions. The intercept value 'a' at 24 hours ranged from 2.00 – 4.67ml200mg/DM. The value 'a' used ideally reflect the fermentation of soluble fraction. The values observed for 'a' were significant ($P < 0.05$) among the treatments. The soluble fraction makes the attachment by rumen micro-organism to be done easily and lead to much gas production. There were significant differences in the extent of gas production 'a+b'

which ranged from 4.67 to 9.34ml200mg/DM. The potential degradability a+b of a diet depicts the level at which the diets could be degraded if it were in actual rumen of the animal (*in vivo*). This largely depends on how much of the fiber fraction (NDF and ADF) have been broken down for easy access of the microbes to the nutrients available in the diets. The extent of gas production 'b' described the fermentation of insoluble but degradable fraction of the samples, result obtained in this study ranged from 2.67 – 5.67 ml200mg/DM. Blummel and Orskov (1993) found that 'b' value could account for 88% for voluntary feed intake. The rate of gas production 'c' ranged from 0.04 – 0.14ml/hr the values were significantly ($P < 0.05$) different among the treatments, T2 recorded the highest while T1 had the lowest. Babayemi *et al.,* (2004) reported that factors that determine the rate of gas production during fermentation depend on the nature and level of the fiber, the presence of secondary metabolite and the potency of rumen liqour for incubation. Orskov and Ryle, (1990) also reported that the rate 'c' determines digestion time and consequently how long a potentially digestible material would occupy space in the rumen.

Conclusion

The enhanced values obtained in terms of in vitro gas production parameters and gas characteristic indicated that *Panicum maximum* - *Leuceana leucocephala* diets can meet the nutrients requirement of small ruminants particularly during the dry season.

Treatments	Dry matter	Crude protein	Crude fibre	Ash	Ether-extract	NFE
1	35.95	10.70^{d}	30.75^{a}	11.05^{a}	10.44^{c}	37.06^{a}
2	34.43	26.78^{a}	14.33^{d}	9.85^{b}	15.35^{a}	29.69^{c}
3	34.78	15.74^{c}	26.17^{b}	10.45^{a}	15.90^{a}	31.74^{b}
4	34.95	13.23^{cd}	30.08^{ab}	10.75^{a}	11.65^{c}	34.29^{b}
5	34.60	18.26^{b}	20.23^{c}	10.15^{a}	14.12^{b}	37.24^{a}
SEM	0.52	0.08	0.42	0.09	0.46	0.95

T1 = 100% Panicum maximum. T2= 100% Leuceana leucocephala, T3 = 75% P.maximum + 25% L. leucocephala, T4 = 50% P.maximum + 50% L. leucocephala, T5 = 25% P.maximum + 75% L. leucocephala.
SEM means standard of means,$^{a, b,c}$ means with different superscripts on the same row are significantly different $p > 0.05$

Table2: In vitro gas production characteristics (200mg/DM) of the forage mixtures

Treatments	OMD(%)	SCFA(µmol)	ME (Mj/kg/DM)	Methane(ml)
1	31.93	0.07	3.62	2.00
2	37.07	0.16	4.33	1.50
3	32.57	0.07	3.68	1.50
4	35.93	0.08	3.74	1.50
5	33.56	0.08	4.01	1.50
SEM	1.28	0.03	0.20	1.00

T1 = 100% Panicum maximum, T2= 100% Leuceana leucocephala, T3 = 75% P.maximum + 25% L. leucocephala
T4 = 50% P.maximum + 50% L. leucocephala, T5 = 25% P.maximum + 75% L. leucocephala
SEM means standard of means,$^{a, b,c}$ means with different superscripts on the same row are significantly different $p > 0.05$

Table3: In vitro parameters (200mg/DM) of *P.maximum* and *L. leucocephala* at different ratio.

Treatments	A	a + b	b	C
1	2.00^{c}	4.67^{c}	2.67^{c}	0.04^{c}
2	4.67^{a}	9.34^{a}	5.67^{a}	0.14^{a}
3	4.33^{b}	7.66^{b}	3.33^{b}	0.07^{b}
4	2.67^{c}	5.19^{c}	3.52^{a}	0.08^{b}
5	4.66^{a}	9.18$^{a\,b}$	4.52^{b}	0.12^{a}
SEM	0.87	0.03	0.79	1.44

T1 = 100% Panicum maximum, T2= 100% Leuceana leucocephala, T3 = 75% P.maximum + 25% L. leucocephala
*T4 = 50% P.maximum + 50% L. leucocephala, T5 = 25% P.maximum + 75% L. leucocephala*s
SEM means standard of means,$^{a,\,b,c}$ means with different superscripts on the same row are significantly different p > 0.05
a = gas produced from immediately soluble fraction,b= extent of gas production from insoluble but degradable fraction
a + b = potential extent of gas production,c = rate of gas production at time (t).

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