IN VITRO GAS PRODUCTION AND DIGESTIBILITY OF DIETS CONTAINING VARYING LEVELS OF *Leucaena leucocephala*

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Abstract
Effect of cassava peel based diet containing different levels of *Leucaena leucocephala* was studied to determine its influence on *in-vitro* gas production and digestibility. The treatments were inclusion of *Leucaena* at 0%, 5%, 10%, and 15% of concentrate diet. The experiment was set up in a completely randomized design. Data obtained were analysed using one way analysis of variance. Results showed that inclusion of Leucaena leaves increased ($p<0.05$) gas production values from 28.67ml (0%) to 39.14ml (15%). Dry matter and crude protein digestibility values decreased ($p<0.05$) with the inclusion of Leucaena leaves while organic matter digestibility increased ($p<0.05$) from 49.00% at 0% to 56.13% at 15% level of inclusion. Short chain fatty acid values obtained were not affected ($p>0.05$) by Leucaena inclusion. The highest ($p<0.05$) metabolizable energy value was obtained at 15% level of inclusion (13.25 MJ/kg) while the lowest ($p<0.05$) was obtained at 0% (9.75 MJ/kgDM). It is conclusion that the inclusion of Leucaena leaves in ruminant concentrate diet increase gas production and higher metabolizable energy.
Keywords: Leucaena, *in vitro*, gas production, digestibility.

Introduction
A major constraint to livestock production in developing countries is the scarcity and fluctuating quantity and quality of the year-round feed supply. These countries experience serious shortages in animal feeds of the conventional type. In improving animal production in Nigeria, it would therefore be reasonable to intensify research effort towards identifying and testing alternative supplementary feedstuff to ameliorate this unfavourable feed scarcity during the dry season (Ojebiyi *et al.,* 2008). In Nigeria, ruminants slowly gain weight in the rainy season and rapidly loose it in the dry season, yet in the traditional animal husbandry, ruminants in the tropics especially in Nigeria, are mainly fed with grasses, and an improved livestock production is not likely attainable and sustainable by grasses forage alone (Babayemi and Bamikole, 2006). Babayemi *et al.* (2003) had earlier reported that the forages are unimproved and low in nutritive values during the wet season, while during the dry season proper, they are fibrous, lignified with low protein values and even in short supply. Ruminants do better when energy and protein rich diets are strategically combined for feeding (Adegbola and Asaolu, 1986). However, the expensive nature of conventional feed as a result of competition between man and livestock (Ogunbosoye and Babayemi, 2010), makes this combination difficult. Thus, there is the need to source for locally available supplements such as browse plants which are less expensive and viable. In Nigeria, *Leucaena leucocephala* is one such browse plants. This study was therefore conducted to evaluated the *in vitro* gas production and digestibility values of cassava peel based diet containing different levels of *Leucaena leucocephala*

Materials and Methods

The experiment was carried out at Animal Nutrition Department Laboratory, Federal University of Agriculture Abeokuta, Ogun state, Nigeria. The study was subjected to a completely randomized design (CRD) and The animals were fed grasses (*Panicum maximum*) along compounded feeds with *Leucaena leucocephala* included as a supplement at varying levels of 0%, 5%, 10%, and 15%. Rumen fluid was collected from three (3) cattle from Cattle Production Venture, FUNAAB by using suction tube prior to morning feeding. The method of collection was as described by Babayemi *et al.* (2006). The ground samples were stored in plastic bags until used for experimental procedures. Rumen liquor was collected from cattle into warm insulated flasks and used as the source of inoculums. The donor animal was maintained daily on ad libitum *Panicum maximum*. The *in vitro* gas production procedure by Menke and Steingass (1988) was employed for the experiment. A sensitive scale was used to measure 200mg of substrate, replicated five times and were placed into 50ml calibrated plastic syringes fitted with plungers. The rumen fluid (inoculum) was filtered through layers of cheesecloth and mixed with buffer at a ratio of 1:2 (v/v), and resazurin dye was added to sustain the activities of microbes. Fifteen millilitres of the buffered inoculums was then added to each syringe containing the milled samples and the gas released was read directly on the graduated syringe. The syringes were placed in an incubator kept at 40°C along with blank syringes containing only 15ml of the buffered inoculums. The syringes were shaken at regular intervals to simulate rumen motility. Gas production was recorded for 48 hours of incubation at 3-hour intervals. Organic matter digestibility (OMD) was estimated as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 ash (Menke and Steingass, 1988). Short-chain fatty acids (SCFA) was estimated as SCFA = 0.0239 GV − 0.0601 (Getachew et al., 2000). Metabolizable energy (ME) was calculated as ME = 2.20 + 0.136 GV + 0.057 CP + 0.029 CP^2 (Menke and Steingass, 1988). Where, GV was the net gas production at 24 hrs and CP was crude protein. Gas volume at 24 hrs was expressed as ml/0.2 g DM, CP and ash as g/kg DM, ME as MJ/kg DM, OMD as % and SCFA as µmol/g DM. Data obtained from this experiment were analysed using one-way analysis of variance option of the SPSS (IBM SPSS Statistics 20) software. Treatment means were statistically compared using Duncan's Multiple Range Test to identify differences between means and significant differences were declared at $p<0.05$.

Results and Discussion

Table 1 shows the gas production of cassava peel based concentrate diet with varying levels of Leucaena. Gas production was increased ($p<0.05$) with the inclusion of Leucaena leaves as the values ranged from 28.67 at 0% to 39.14 ml at 15% level of inclusion. This increase was consistent across all periods of incubation. Increased gas production as recorded in this study can be attributed to additional protein provided by the Leucaena which would have increased as availability of ammonia for rumen micro flora, stimulating microbial growth and increasing rate of breakdown of the forage (Barros-Rodriguez *et al.*, 2013). Increase in the amount of protein available to microorganism is positively related gas production according to the report of Hilman *et al*. (1993) who asserted that gas production is positively related to microbial protein synthesis. These results were similar to the work of Wilson and Hatfield (1997) who reported that cell wall of legume usually does not undergo lignification as that occurs in the cell wall of grass. Thus the diet with high inclusion level of Leucaena leucocephala is easier to be degraded by rumen microorganisms compared to the diet without or with low inclusion.

Table 1: Gas production values of cassava peel based concentrate diet with varying levels of *L. leucocephala*

	Levels of *Leucaena* inclusion					
Incubation time	**0%**	**5%**	**10%**	**15%**	SEM	P value
3	1.56^{c}	3.38^{ab}	2.75^{bc}	4.29^{a}	0.289	0.00
6	8.22^{b}	12.38^{a}	12.75^{a}	14.14^{a}	0.611	0.00
12	17.78^{b}	24.25^{a}	23.25^{a}	26.00^{a}	0.989	0.01
18	23.11^{b}	31.25^{a}	32.00^{a}	33.43^{a}	1.183	0.00
24	28.67^{b}	36.00^{a}	38.00^{a}	39.14^{a}	1.295	0.01

^{ab} Means on the same row with different superscripts are significantly different (P<0.05)

Metabolizable energy (ME), organic matter digestibility (OMD), ash content, crude protein, gas production, dry matter digestibility, crude protein digestibility and short chain fatty acids (SCFA) levels as related to the varying inclusions of Leucaena were shown in Table 2. The values of ME, OMD and SCFA at 5%, 10% and 15% inclusion levels were all High in value and significant (P<0.05) as compared to the 0% inclusion level (control) and ranged between 10.75 and 13.25 MJ/kg DM; 53.13 and 56.13% and 0.88 and 1.00µmol, respectively. The values observed in the current study are higher than the ranges of 2.99 to 4.75 MJ/kg DM (ME) and 21.46 to 33.80 % (OMD) reported by Babayemi and Bamikole (2006) for *P. maximum* (grass) and *Tephrosia candida* (browse plant) mixtures and 4.36 to 6.53 MJ/kg DM; 31.23 to 49.09% and 0.08 to 0.54µmol as reported by Tona (2014). Short chain fatty acids are volatile fatty acids (VFA) and their presence denote energy is available in a feedstuff. These observations imply that the dietary treatments (5%, 10% and 15%) have potential to make energy available to ruminants. In another research (Ajayi and Babayemi, 2008), it was further explained that SCFA is one of the end products of rumen fermentation, and that a high volume of gas is produced when substrate is fermented to acetate and butyrate, however, relatively lower gas production is associated with propionic production. This is in agreement with the observation of Ngamsaeng *et al*. (2006) that in vitro fatty acid production has low correlation with volatile fatty acid production particularly that of propionate.

Table 2: In vitro digestibility and post incubation parameters of cassava peel based concentrate diet with varying levels of *L. leucocephala*

	Levels of *Leucaena* inclusion					
Treatment	**0%**	**5%**	**10%**	**15%**	**SEM**	**P-value**
Gas production	28.67^{b}	36.00^{a}	38.00^{a}	39.14^{a}	1.295	0.01
Crude protein	9.89^{d}	10.63^{c}	11.99^{b}	13.23^{a}	0.232	0.00
Ash	3.27	3.67	3.25	3.09	0.105	0.25
Crude protein digestibility	45.22^{a}	35.73^{ab}	36.67^{ab}	32.50^{b}	1.822	0.08
Dry matter digestibility	24.16^{a}	17.20^{b}	22.19^{ab}	19.50^{ab}	0.956	0.05
Organic matter digestibility	49.00^{b}	53.13^{ab}	54.75^{ab}	56.13^{a}	1.040	0.08

Short chain fatty acids (µmol/g DM)	0.88	1.00	1.00	0.88	0.043	0.58
Metabolizable energy (MJ/kgDM)	9.75[d]	10.75[c]	11.88[b]	13.25[a]	0.280	0.00

Conclusion and Recommendations

It can be concluded that supplementation of cassava peel based feed with *Leucaena leucocephala* leaves increased *in-vitro* gas production as level of inclusion increases gradually from 0% to 15%. Also adding *Leucaena* at 15% level of inclusion resulted in increased levels crude protein, metabolizable energy, short chain fatty acids and organic matter digestibility was recorded at this level of inclusion. *Leucaena leucephala* leaves could be included at a level of 15%, in the feed of a ruminant animal

References

Ojebiyi, O. O., Farinu, G.O, Togun, V.A., Akinlade, J.A., Aderinola, O.A., Olayemi, T.B. and Moronfolu, O.O. (2008). Studies on the growth and haemathological attributes of weaned rabbits fed graded levels of sun-dried cassava peel and blood meal mixture, Proceedings of 32nd Annual conference of Nigerian Animal Production. Cross River, Nigeria 18(9):213-216.

Babayemi, O. J. and Bamikole, M. A. (2006). Nutritive value of Tephrosia candida seed in West African Dwarf goats. Journal of Central European Agriculture 7(4): 731 – 738.

Babayemi, O.J.; Bamikole, M.A.; Daniel, I.O.; Ogungbesan, A.; and Babatunde, O. 2003.Growth Nutritive value and dry matter degradability of Tephrosia species. Nigeria journal of Animal production 30 (1); 62-70

Adegbola, A.A. and Asaolu, V.O. (1986). Preparation of cassava peels used in small ruminant production in Western Nigeria. In: Preston, T.R. and Nuwanyakapa, M.Y., (eds). Towards Optimal Feeding of Agricultural by-products to livestock in Africa. Proceedings of a workshop held at the University of Alexandria, Egypt. Oct. 1985 ILCA, Addis Ababa, Ethiopia. Pp. 109-115.

Ogunbosoye, D.O., Babayemi, O.J., (2010). Potential values of some non-leguminous browse plants as dry season feed for ruminants in Nigeria. *Afri. J. Biotech*., 9 (18): 2720-2726.

Babayemi, O. J., 2006. Antinutritional factors, nutritive value and in vitro gas production of foliage and fruit of *Enterolobium cyclocarpum*. *World J. Zool*., 1(2): 113-117.

Menke, K.H. and Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28: 7–55.

IBM SPSS 2011. Statistics Version 20. IBM Corporation.

Barros-Rodríguez M, Solorio-Sánchez J, Sandoval-Castro C, Klieve AV, Briceno-Poot EB, Ramirez-Aviles L, Rojas-Herrera R. 2013. Effect of two intake levels of Leucaena leucocephala on rumen function of sheep. *Tropical Grasslands-Forrajes Tropicales*, 1:55–57.

Wilson, J.R. and R.D. Hatfield. 1997. Structural and chemical changes of cell wall types during stem development: Consequences for fibre degradation by rumen microflora. *Aust. Journal of Animal Research*. 48: 165-180.

Tona, G.O., V.O. Asaolu, O.A. Amao and Akingbade, A.A. 2013. Evaluation of chemical composition and in vitro fermentation parameters of Moringa oleifera leaf meal based diets as feed for ruminants in Nigeria. *Agricultural Journal* 8 (4) 165-169 (Medwell Journals).

Ngamsaeng, A., M. Wanapat and Khampa, S. 2006. Evaluation of local Tropical plants by in vitro rumen fermentation and their effects on fermentation end-products. *Pak. J. Nutr*. 5 (5): 414-818.

Ajayi, F.T. and Babayemi, O.J. 2008. Comparative in vitro evaluation of mixtures of *Panicum maximum* cvNtchisi with stylo (*Stylosanthes guianensis*), Lablab (*Lablab purpureus*), Centro (*Centrosema pubescens*) and Histrix (*Aeschynome nehistrix*). *Livestock Research for Rural Development*.20 (6).