CHEMICAL COMPOSITION, *IN VITRO* TOTAL GAS AND METHANE PRODUCTION OF CONCENTRATE DIETS CONTAINING *Gliricidia sepium*

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ABSTRACT
The present study evaluated the *in vitro* gas production and post-incubation parameters of concentrate diets containing varying levels of *Gliricidia sepium* leaves (GS) (0%, 10%, 20% and 25%). The experiment was set up in a completely randomized design.Gas production was measured at 3, 6, 9, 12, 15, 18 and 24 hours of incubation and the methane was estimated at the end of the 24th hour of incubation, volatile fatty acid profile was measured and post incubation calculations was carried out. Data obtained were analysed using one way analysis of variance. The result obtained showed that the crude protein content of diets increased as the inclusion of GS increased ($P<0.05$) from 0 to 25% (10.90% to 14.71%) while NDF values reduced ($P<0.05$) as the reduced as the inclusion increased (49.94% to 42.69%). Total gas and methane production at 24 hours were not affected ($P>0.05$) by inclusion of GS. Also, DMD and OMD values were not affected ($P>0.05$) by the inclusion of GS in the diets. This study concludes that when *Gliricidia sepium* is used at inclusion level of 10% or more, it has potential to modify the rumen thereby reducing gas production during fermentation resulting in higher post-ruminal nutrient utilization.
Keywords: Gliricidia, *in vitro*, methane, digestibility, diets.

INTRODUCTION
Ruminant livestock raised in the tropics tend to exhibit cyclical variation in the quantity and quality of the available forages (Bamikole and Babayemi, 2008). This is so because, during the dry season, ruminant feeding systems depend on poor quality tropical foliage Poor nutrition results in low production performance, slow growth rate, loss of body condition and increased susceptibility to diseases and parasites. These poor quality forages are usually augmented with crop residues or agro-industrial by-products (Oni *et al*., 2012) and a number of browse plants worldwide serve as alternative feedstuffs for livestock (Rinehart, 2008; Fayemi *et al*., 2011). As a result, animals under semi-intensive and free-range systems have been observed feeding on them (Isah *et al.,* 2007). Proteinaceous legumes like *Gliricidia sepium* are abundantly found in the tropics and considered as high quality leguminous forage due to its high protein content with good amino acid profile. The incorporation of such resources into concentrate diets for ruminant feeding in combination with other crop residues and agro-industrial by-products will help combat both the challenge of dry season low feed quality and might also lead to a reduction in the cost of protein ingredients used in formulating ruminant diets.The *in vitro* method of feed evaluation for ruminants has gained wider acceptance due to its ease of adoption, repeatability, minimized use of animals and the decrease in funding of the in vitro evaluation of feeds (Getachew *et al.,* 2005).The objective of this study was to evaluate the chemical composition, *in vitro* total gas and methane production of concentrate diets containing *Gliricidia sepium* leaves.

Materials and Methods
The experiment was carried out at Animal Nutrition Department Laboratory, Federal University of Agriculture Abeokuta, Ogun state, Nigeria. The experimental design was a completely randomized design (CRD) with four concentrate diets formulated through the use of *Gliricidia sepium* leaves (GS) at varying levels (0%, 10%, 20% and 25%) along with cassava peel, cassava leaves, palm kernel cake, rice bran, wheat offal, salt, oyster shell and sulphur. Rumen fluid was collected using the method of collection described by Babayemi *et al* . (2006). Rumen liquor was collected from cattle into warm insulated flasks and used as the source of inoculums 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 24 hours of incubation at 3-hour intervals. The volume of methane gas from each sample was determined by dispensing 2mls of 0.4 sodium hydroxide (NaOH) in to the sample at the end of 24 hours of incubation (Fievez *et al* ., 2005). Analysis of chemical composition was done according to the methods of AOAC (2000).

Data obtained from this experiment were analysed using one-way analysis of variance option of the SPSS (IBM SPSS Statistics 23) software. Treatment means were statistically compared using Duncan's Multiple Range Test to identify differences between means and significant differences were declared if $p<0.05$.

Results and Discussion

Chemical composition of concentrate diets containing varying levels of *Gliricidia sepium* leaves is presented in Table 1. The result shows that there were significant ($P<0.05$) variations across the various levels of inclusion. Dry matter (91.19%), NDF (49.94%) and ether extract (4.39%) and metabolizable energy (8.19 MJ/kg) values were higher ($P<0.05$) in the control diet (0% GS) while the highest ($P<0.05$) crude protein value (14.71%) in the diet containing 25% GS. It is observed that the dry matter values reduced with increase in the level of GS while the NDF values also followed the same trend. The lowest ($P<0.05$) DM and NDF values were obtained in diets containing 25% GS (89.15% and 42.69%).

Table 1: Chemical composition of concentrate diets containing varying levels of *Gliricidia sepium* leaves

Parameters	0%	10%	20%	25%	SEM	P-value
Dry matter (%)	91.19^{a}	89.74^{b}	89.38^{c}	89.15^{c}	0.243	0.00
Crude protein	11.25^{d}	10.90^{c}	13.70^{b}	14.71^{a}	0.487	0.00
NDF	49.94^{a}	47.47^{b}	43.46^{c}	42.69^{d}	0.897	0.00
ADF	28.77^{c}	35.53^{a}	30.89^{b}	31.14^{b}	0.746	0.00
ADL	5.19^{c}	6.00^{b}	6.22^{b}	6.91^{a}	0.191	0.00
Ash	12.40^{c}	16.24^{a}	14.22^{b}	13.29^{c}	0.436	0.00
Ether Extract	4.39^{a}	2.55^{b}	3.22^{b}	3.53^{c}	0.204	0.00
ME (MJ/Kg)	8.19^{a}	7.53^{c}	7.69^{b}	7.72^{b}	0.075	0.00

The effect of inclusion of *Gliricidia sepium* at various levels on in vitro gas volume is presented in Table 2. Volume of gas produced at 6th and 12th incubation hour is significantly different ($P<0.05$) with 0% Gliricidia having the highest value in both period. At other time of incubation, volume of gas produce is not significantly ($P>0.05$) affected by the inclusion levels of *Gliricidia sepium*. The highest gas volume at 24th hour of incubation is (20.67ml) obtained from the control diet.

Table 2: Effects of inclusion of *Gliricidia sepium* on *in vitro* gas production

Incubation time	0%	10%	20%	25%	SEM	P-value
3	0	0	0	0	0.000	-
6	1.33^{a}	0.67^{ab}	0.00^{b}	0.00^{b}	0.195	0.01
12	10.67^{a}	8.33^{ab}	5.67^{b}	5.33^{b}	0.830	0.04
18	18.00^{a}	14.00^{ab}	12.00^{ab}	11.67^{b}	1.076	0.03
24	20.67	17.00	16.33	15.33	1.150	0.43

The effect of *Gliricidia sepium* on in vitro post incubation parameters is shown in Table 3. It shows that there is no significant difference (P>0.05) among the organic matter digestibility (OMD), CH4, %CH4 and dry mater digestibility (DMD).

Table 3: Effects of inclusion of varying *Gliricidia sepium* on *in vitro* post incubation parameters

	Levels of Gliricidia inclusion					
Parameters	0%	10%	20%	25%	SEM	P-value
CH4	6.67	5.00	4.00	4.33	0.492	0.24
%CH4	32.23	30.45	24.79	27.25	1.969	0.61
DMD (%)	81.67	83.33	81.67	95.00	2.784	0.28
OMD (%)	39.12	35.96	36.49	36.00	0.961	0.67

SEM: Standard error of mean

Dry matter (DM) values obtained in this study are similar to the range of 85.70 to 95.86 % reported by (Asaolu*et al.,* 2012) for fodder, with the lowest at 25% Gliricidia. The CP content range between 10.9 and 4.71% which is above the minimum level for maintenance of 7.70 % for goats (NRC, 1981), with the highest obtained at 25% Gliricidia (14.71%) and low at 10% Gliricidia (10.9). The CP is in agreement with the minimum of 8 % CP observed by (Norton 1994) to provide the ammonia levels required by rumen microbes for optimum activity. The CP (10.90-14.71%) and the in vitro digestibility (81.67-91.00%) is not in agreement with the range of (18-30 %CP) and (60-65% DMD) reported by Cook *et al*. (2005). Organic matter digestibility value was observed to be the highest at 0% Gliricidia. Feedstuffs that are inherent with certain anti-nutritive factor had been reported to be low in organic matter digestibility (Aregheore and Abdulrazak, 2005).

This study result is in accordance with report given by Gillespie (1998) that NDF is inversely related to the plants' digestibility. The higher the NDF (49.94) the lower the plant's digestibility (81.67) and vice versa. The values obtained for the various Gliricidia levels may imply a moderately high cell wall contents with all having different effects on the various level of Gliricidia and expressed on Dry matter (DM) basis.

The inclusion of *Gliricidia sepium* at various level does not affect the gas production with the lowest value of methane at 20% inclusion of *Gliricidia sepium*. Changes in methane production with increasing inclusion levels of *Gliricidia sepium* can be attributed to the tannin content which was also reported by Jayanegara *et al.* (2011). It has also been observed that a decrease in CH_4 production occurs with the inclusion of *Gliricidia sepium* in ruminant diets (Sebata *et al*., 2011). In addition, it was reported that with an increase in tannin concentration in browse species, a reduction effect on methane production was found (Sultan *et al*., 2012). The impact of tannins on methane production varies with tannin chemical structure (plant origin) as well as with its concentration.

CONCLUSION AND RECOMMENDATIONS

From the results of this study, it can be concluded that the chemical composition of diets were improved by inclusion of *Gliricidia sepium* leaves. It was also noted that gas production reduced as the inclusion increased. It is recommended that *Gliricidia sepium* leaves can be included in concentrate diets for ruminants at values above 10% of the total feed. Further studies are therefore recommended to investigate the response of animals in a feeding trial to these diets.

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