

Impact of Guinea Grass and Agro-Industrial By-Products on Rumen Fermentation Profiles of Dwarf Sheep Using *In-Vitro* Gas Production Technique.

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Abstract: Organic matter (OM), neutral detergent fiber (NDF) digestibility and metabolizable energy content of guinea grass and cassava peel with rice husk used as ruminant feeds were estimated from in vitro gas production technique. Three dietary treatments which were prepared include : TA (Guinea grass and concentrate supplement in a ratio of 70:30 was served as the controlled group), TB (Cassava peel with rice husk and concentrate supplement in a ratio of 60:10:30) and TC (Cassava peel with rice husk and concentrate supplement in a ratio 55:15:30). The experiment was in a complete randomized design (CRB) with each treatment replicated three times. The dietary treatments crude protein content varied from 12.6 - 18.75%, ether extract (7.24 – 8.77%), crude fiber (9.00 – 11.06%), ash (16.28 – 19.40%), nitrogen free extract (43.81 – 52.81%), neutral detergent fiber (9.75 – 11.98%), acid detergent fiber (15.27 – 17.17%). The results obtained showed that organic matter digestibility (56.10%), metabolizable energy (8.31MJ/KgDM), short chain fatty acids (0.99UM), gas volume at ml /12h and ml/24h (13.00 – 22.50ml) and methane (13.00ml) were significantly ($P<0.05$) better with animal on TA. Animals on TB had the highest ($P<0.05$) values in terms of dry matter (41.67%), neutral detergent fiber (42.80%), digestibility gas produced from the insoluble fraction (42.49ml) and gas production in rate constant for insoluble fraction (0.08mlh^{-1}). Incubation time (11.56hr) was significantly ($P<0.05$) highest for animals on TC only. Finally, this investigation demonstrated that cassava peel with rice husk and concentrate supplement in a ratio of 60:10:30 (TB) had potential on rumen fermentation profiles on dwarf sheep using in vitro gas production technique.

Keywords: Guinea grass, cassava peel, rice husk, in-vitro gas production

INTRODUCTION

Sheep and goats commonly called small ruminant animals are the most important livestock kept by greater percentage of the country's farming populace. Recently, more attention has been paid to small ruminants in developing countries as their advantages are becoming more understood than ever before, particularly for their ability to produce meat, milk and skin. The Nigerian small ruminant industry is faced with the problem of meeting the nutritional requirement and feeds supply for animals. Even were fodder resources are abundant, seasonal fluctuation in nutritive value make sustainable gains in production from good management and disease controlled programs unrealistic[1].

Guinea grass (*Panicum maximum*) is a tall vigorous perennial grass with stems up to 3.5m tall that varies widely in growth habit [2]. It produces high yields of palatable fodder to ruminant livestock and responds well to maturing at early growth stage, but rapidly declines in nutritive value with age. It dies if continually grazed close to the ground in the dry season; hence it needs rest late in the growing season

and supplement with other feeds to provide balance nutrient for maintenance in ruminant production [3]. Reling *et al.* [4] also reported that increase in maturity of guinea grass and long dry season had negative effect on the nutritive value, indicating that it would be best utilized at younger stages of development and wet season. Thus, the provision of good quality forage all year round most especially during the dry season has been a major problem of ruminant livestock production in the tropics. This explains why most ruminant animals under extensive system of rearing loss weight and have low productivity with poor body condition at the peak of the dry season[5].

It attend to alleviate ruminant feeds supply problems and looking for potential feed resources particularly those which survive during the dry season call for the use of unconventional feedstuffs for the production of small ruminant animals. The use of agro-industrial by-products have been identified to play an important role in the nutrition of small ruminant livestock and ensure all year round availability of feed. Cassava peels and rice husk are classified among other agro-industrial by-products that have great potential in

this respect. Cassava (*Manihot esculenta*) peels in Nigeria are always discarded as waste and even constitute nuisance in waste disposal of these industries. This at present is posing a problem of health hazard to humans [6]. Cassava peels account for 10 to 13% of the tube by weight [7], this is in spite of the potential of the by-product as an animal feedstuff [8]. Several reports indicated that, among the root crops producing in Nigeria, cassava from which cassava peels are obtained constitute 20% total tubes produced annually [6, 9]. There is evidence in literature that cassava peels could serve as a cheap source of energy which can be utilized to a great extent in feeding of ruminant livestock [10]. However, the potential use of cassava peels to adequately fill the nutritional gap in ruminants are heavily constrained by intrinsic factors, which includes high crude fiber, low crude protein and the presence of toxic levels of hydrogen cyanide (HCN) that limits its utilization. Research results indicate that concerted effects had been made to reduce or totally remove these encumbrances militating against the use of cassava peels in livestock. The most viable option for circumventing these constraints is by fortifying with additional protein source and proper harnessing through biotechnological process [7].

Rice (*Oryza sativa*) husk is a by-product obtained in the processing of rice grains for human consumption. Rice husk is the hard protecting covering of grain of rice, it forms from hard materials which including praline silica and lignin. There is evidence in literature that rice husk constitute about 22% of the paddy during milling with 3.25% CP, 10% EE, 40.5% CF and 18.5% ash [11]. Presently, it is used as fuel for broilers, the ash can be used as fertilizer and the high silica content could be of use in steel industry. Rice husk has been used as ingredient in livestock feeds but the problem of low intake, digestibility, high silica content and abrasive characteristics are the limiting factors in its utilization. Drying, ensiling, fermentation and soaking in hot water are suggested methods of reducing the limiting factors in its utilization [12].

In Nigeria, little research has been done to characterize the combination of cassava peels and rice husk for nutrient content and evaluate them as potential feed resources in ruminant livestock. At present, attempts are therefore been made to include them in rations for ruminants. The *in vitro* gas production technique developed by Menke and Steingass [13] is a very useful tool for the rapid screening of feeds to assess their potential as energy sources and predict organic matter digestibility with metabolizable energy content of ruminant feeds with high accuracy. There is scant information on the use of *in vitro* techniques to determine organic matter and neutral detergent fiber digestibility's with metabolizable energy of cassava peels with rice husk. Hence, the objective of this study was to determine the impact of guinea grass and agro industrial by-products (cassava peels and rice husk) on

rumen fermentation profiles of dwarf sheep using *in vitro* gas production technique.

MATERIALS AND METHODS

Sources and Preparation of Experimental Samples

Guinea grass was obtained fresh from a pasture land within the Teaching and Research Farm of the Ambrose Alli University, Ekpoma. It was chopped manually to a length of about 6cm and sun dried. Cassava peels and rice husk were collected from their processing points located within Ekpoma metropolis and sun dried separately. Rice husk was soaked in hot water for about 2hrs before sun dried. Concentrate supplement feed that comprised 80% wheat offal, 18% brewery dried grain, 0.75 limestones, 0.5% dicalcium phosphate, 0.5% salt and 0.25% vitamin premix was purchased in Benin City.

Three experimental samples were prepared which were; A (guinea grass and concentrate supplement in a ratio of 70:30 which was served as the controlled group), B (cassava peels with rice husk and concentrate supplement in a ratio of 60:10:30) and C (cassava peels with rice husk and concentrate supplement in a proportion of 55:15:30). The concentrate supplement that was added to the three experimental samples was to provide sufficient nutrient for the rumen inoculums. The three samples were oven dried at 65°C to constant weight and residual weight recorded as the dry matter (DM) content of the samples. The dried samples were later crushed in a hammer mill fitted with a sieve size of 1mm and preserved in an air tight container for subsequent analysis.

Experimental Design and Proximate Analysis

The experimental samples prepared were randomly assigned to three (3) dietary treatments in a completely randomized design.

Proximate composition of the dietary samples was obtained according to AOAC [14]. Fiber fraction analysis (neutral and acid detergent fiber) was determined by the procedures of Van Soest *et al.* [15].

Nutritional Evaluation using In- Vitro Gas Method.

Rumen fluid was obtained from rumen of four (6) West African Dwarf (WAD) sheep with mean live-weight of 9.01kg that were previously fed with guinea grass and concentrate supplement at 5% of their body weight in the ratio of 70:30 respectively. The method of collection was as described by Babayemi and Bamikole [16] using suction tube. The rumen fluid was collected into the thermo flask that had been pre-warmed to a temperature of 39°C from the WAD sheep before they were offered the morning feeds. About 200mg weighed dry milled samples of the three standard feed substrates (A, B and C) were weighed into nylon bags, each sample was replicated three time (n=3) and dropped into 100ml graduated gas tight plastic syringes. Each of the syringes was injected with 30ml inoculums

containing cheese cloth strained rumen fluid that was mixed with buffer in a ratio of 1:4 (v/v) to avoid lowering the pH of the rumen fluid which may result to decrease in the microbial activities. Incubation was carried out at 39°C which lasted for 24hrs as described by Menke and Steingass[13] and gas volume was recorded.

At post incubation period, 4ml of NaOH (10M) was introduced to estimate methane production following the method described by Babayemi [17]. Data were obtained on gas volume (GV) and methane (CH₄) produced. The mean gas volumes obtained were fitted to the exponential equation $Y = a + b(1 - e^{-ct})$ as reported by Babayemi and Bamikole [16].

Where, $Y =$ volume of gas produced at time t
 $a =$ gas produced from the soluble fraction
 $b =$ gas produced from the insoluble fraction
 $c =$ gas production rate constant for the insoluble fraction (b)

Estimation of Organic Matter Digestibility, Metabolizable Energy Content and Short Chain Fatty Acids

Organic Matter Digestibility (OMD%) and Metabolizable Energy (ME, MJ/KgDM) were estimated using the method of Menke and Steingass [13] while Short Chain Fatty Acids (SCFA μ /M) were calculated as reported by Getachew *et al.* [18].

$$ME = 2.2 + 0.136*GV + 0.0057*CP + 0.0002865*EE^2$$

$$OMD = 14.88 + 0.889*GV + 0.0448*CP + 0.065*XA$$

$$SCFA = 0.0239*GV - 0.0601$$

Where GV, CP, EE and XA were net gas production (ml/200mgDM), crude protein, ether extract and ash of the incubated samples respectively. However, these equations have been standardized and validated as method to create the OMD, ME and SCFA prediction equations using data from 400 digestibility trials (*in vitro*) and the corresponding *in vitro* gas production tests [13, 18].

Apparent Dry Matter and Neutral Detergent Digestibilities

500mg samples were weighed into nylon bags and incubated (three nylon bags per sample per incubation time) in a buffered medium containing rumen fluid (400ml) for 24hrs. At post incubation time bags were hand washed in cold water to obtain zero time disappearance. Washed nylon bags were dried in a forced air oven at 50°C for 48hrs to determine apparent dry matter digestibility. Thereafter, the content from the bags were treated with neutral detergent solution to obtain neutral detergent fiber (NDF) digestibility. The

determination of apparent dry matter and neutral detergent fiber digestibility were according to the procedures of Van Soest and Robertson[19].

$$\text{Apparent Dry Matter Digestibility} = \frac{\text{Digested Substrate (DM)}}{\text{Weight of Sample DM taken for Incubation}} \times \frac{100}{1}$$

Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA). Significant differences between means were separated by Duncan Multiple Range Test [20].

RESULTS AND DISCUSSION

Table 1. Presents the chemical composition of the experimental diets. The chemical composition of the experimental diets varied. Dry matter (DM) content varied from 90.00% in T_B to 90.48% in T_C, while CP contents ranged from 12.06% in T_B to 18.75% in T_A. The high dry matter values obtained in this study could be due to the fact that they were prepared from dried ingredients which were characteristically high in DM. The CP values observed in this study were above 10–12% CP recommends for moderate level in ruminant production[21]. Thus, these diets could probably provide adequate nitrogen requirement for rumen microbes to maximally digest the components of the dietary fiber. Ether extract (EE) and crude fiber (CF) contents were in ranged of 7.24–9.77% and 9.00 to 11.06% respectively. Ash values which were similar in values varied from 16.28 to 19.40% with T_A recorded the highest and T_B the lowest. There were also marked variations in nitrogen free extract (NFE) of the diets (43.81–52.61%) with T_B having the highest NFE values observed in the diets generally which was an indication of high energy content in the diets. Neutral and acid detergent fiber were similar in values ranged from 9.76 to 11.98% and 15.27 to 17.17% respectively with T_C recorded the highest followed by T_B and then T_A. The CF, NDF and ADF values increased in trends as cassava peels decreased with increased level of rice husk inclusion in the diets, indicating the high CF content in rice husk [11]. Okoruwa and Njidda reported that information on CF of a diet is essential for the assessment of their fiber fraction contents [5].

Presented in Table 2, are the apparent dry matter (DM), neutral detergent fiber (NDF) and organic matter (OM) digestibilities with metabolizable energy (ME) and Short Chain Fatty acids (SCFA) of the experimental diets. Apparent dry matter (DM) and neutral detergent fiber (NDF) digestibility's were significantly different ($P < 0.05$) among treatments. DM and NDF digestibilities ranged from 9.09 to 41.67% and 38.30 to 42.80% respectively. The values obtained for the dietary treatments in NDF digestibility were not in conformity with the ranged values (52.40 to 64.10%) reported for some agro industrial by products by

Aregheore and Abdulrazak [22]. Apparent DM digestibility values obtained in this study seems to portray the probably extent of NDF digestibility of the diets.

NDF digestibility varied among diets with increased in inclusion level of rice husk. This corroborates the assertion by Njidda and Binuomote [23] that dietary treatments influence NDF digestibility. The variation in the NDF digestibilities in which T_B (42.80%) was observed to have the highest value might have resulted from variation in the degree of lignifications in the diets.

Though gas production is a nutritional wasteful products but it provides useful basis from which ME, OMD and SCFA may be predicted [24]. OMD shared no significant different (P>0.05) between T_B (46.71%) and T_C (49.81%) but T_A (56.10%) was significantly higher (P<0.05) than T_B and T_C. Menk and Steingass [13] reported that, there is a positive correlation between metabolisable energy calculated from *in vitro* gas production together with CP and fat content as well as ME value of conventional feeds measured *in vivo*. The ME content in the study was particularly highest in T_A (8.31MJ/KgDM) compared with T_B (6.91MJ/KgDM) and T_C (7.35MJ/KgDM).

The low OMD and ME obtained for T_B and T_C might probably connected with the presence of high fiber and abrasive characteristics in rice husk[11]. The presence of these anti-nutritive factors could affect the bioavailability of nutrients, therefore making it impossible for microbes to utilize the T_B and T_C well for the production of useful volatile fatty acids as the major sources of energy to the ruminant livestock.

The SCFA estimated from gas production were 0.99, 0.76 and 0.83µm for T_A, T_B and T_C respectively. There were significant differences in SCFA among the diets (T_A, T_B and T_C). The low SCFA estimated for T_B and T_C were due to the lower gas production which was evident in the first 24hrs of incubation. The highest estimated SCFA in T_A compared to T_B and T_C, suggests a potential to make energy available to the ruminants. Blummel and Orskov [24] suggested that gas production from different classes of feeds incubated *in vitro* in buffered rumen fluid was closely related to the

production of SCFA which was based on carbohydrate fermentation.

Gas volume (GV) and *in vitro* gas production characteristics of the experimental diets are shown in Table 3. Gas volume at ml/12h and ml/24 production ranged from 8.50 – 13.00ml/12h and 14.50–22.50ml/24h respectively. Significant differences (P>0.05) were observed among dietary treatments in GV (ml/12h and ml/24h) with lowest and highest GV values obtained on T_A (13.00 and 22.50ml) and T_C (8.50 and 14.50ml) respectively. Gas volume (GV) generally reflects the contents of fermentable carbohydrate or carbohydrate degradation and also probably the nitrogen and lipids [22]. This was further explained by Akinfemi *et al.* [25] that gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while contribution of fat to gas production is negligible. The fermentation of the insoluble but degradable fraction (b) did not differ significantly (P>0.05) between T_A (35.69ml) and T_B (42.49ml) but significantly (P<0.05) higher when compared with T_C (30.50ml). The lower fermentation of the insoluble but degradable fraction (b) observed on T_C was expected because of the higher lignin values from the rice husk. The rate of gas production (c) ranged from 0.06 to 0.08mlh⁻¹. The fastest rate of gas production was observed in T_B, possibly influenced by the soluble carbohydrate fraction readily available to the microbial population. Slow rate was observed in T_C indicating that this residue was less readily available to the microbes in the rumen. Incubation time (t^{1/2}) was highest on T_C (11.56hr), followed by T_B (10.02hr) and finally T_A (8.96hr). The significant highest (P<0.05) incubation time was observed in T_C compared to T_A and T_B. This observation was further buttressed by the fact that more time was taken by microbes to ferment high lignin present in T_C. It is important to note that methane production was significantly (P<0.05) least on T_B (9.99ml) compared to T_C (11.00ml) and T_A (13.00ml). The low CH₄ observed on T_B reflected methanogenesis that was obviously suppressed and translated to a more efficient utilization of the diet. Babayemi reported that methane production in the rumen have a negative correlation with utilization of feeds in ruminants livestock[17].

Table 1: Chemical composition (%DM basis) of the experimental diets

Nutrients	Treatments		
	T _A	T _B	T _C
Dry matter	90.18	90.00	90.48
Crude protein	18.75	12.06	17.47
Ether extract	7.24	8.77	8.36
Crude fibre	9.00	10.08	11.06
Ash	19.40	16.28	19.30
Nitrogen free extract	45.61	52.81	43.81
Neutral detergent fibre	9.76	11.02	11.98
Acid detergent fibre	15.27	16.19	17.17

Table 2: Apparent Dry Matter (DM), Neutral Detergent Fibre (NDF) and Organic Matter (OM) Digestibilities with Metabolizable Energy (ME) and Short Chain Fatty Acids (SCFA) of experimental diets.

Parameters	Treatments			
	T _A	T _B	T _C	SEM ±
DM digestibility (%)	9.09 ^c	41.67 ^a	33.33 ^b	1.06
NDF digestibility (%)	38.30 ^b	42.80 ^a	40.10 ^a	1.02
OM digestibility (%)	56.10 ^a	46.71 ^b	49.81 ^b	0.87
ME (MJ/Kg/DM)	8.31 ^a	6.91 ^b	7.35 ^b	0.21
SCFA (µM)	0.99 ^a	0.76 ^b	0.83 ^b	0.16

^{a b c} Means on the same row with different superscript are significantly different (P<0.05)

Table 3: Gas Volume (GV) and *In Vitro* Gas Production Characteristics of the experimental diets.

Parameters	Treatments			
	T _A	T _B	T _C	SEM ±
GV (ml/12h)	13.00 ^a	9.01 ^b	8.50 ^b	0.08
GV (ml/24h)	22.50 ^a	15.50 ^b	14.50 ^b	1.06
b (ml)	35.69 ^a	42.49 ^a	30.50 ^b	2.05
c (ml h ⁻¹)	0.07 ^{a b}	0.08 ^a	0.06 ^b	0.08
t ^{1/2} (hr)	8.96 ^c	10.02 ^b	11.56 ^a	1.82
CH ₄ (ml)	13.00 ^a	9.99 ^b	11.00 ^b	1.02

^{a b c} Means on the same row with different superscript are significantly different (P<0.05)

CONCLUSION

It was concluded that agro industrial by-products have the potential of meeting the nutritional needs as small ruminant livestock feeds, if properly harnessed and combined as feeds. This was more pronounced without any adverse effect, when cassava peels with rice husk and concentrate supplement in a proportion of 60:10:30 was used to assess rumen fermentation profiles of dwarf sheep using *in-vitro* gas production technique .

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