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Use of an *In Vitro* Gas Production Technique to Evaluate Some Nigerian Feedstuffs

[1]A. Akinfemi, [2]Adu O. Adesanya and [3]V.E. Aya

[1]Department of Animal Science, Faculty of Agriculture,
Nasarawa State University, Keffi, Shabu-Lafia, Nigeria
[2]Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria
[3]Department of Animal Science, College of Agriculture, Shabu-Lafia, Nigeria

Abstract: *In vitro* gas production techniques stimulate the rumen fermentation process and they have been used to evaluate the potential of feedstuffs to supply nutrients to ruminants. Thus, five agricultural wastes: cassava peels (CPS), maize cobs (MCB), orange pulps (OPL), guinea corn threshed tops (GTH) and yam peels (YPS) were evaluated using an *in vitro* gas production technique. The gas production was continuously measured by incubating samples in buffered rumen from goats for 96hr. Cumulative gas production was recorded at 3,6,9,12,24,36,48,72 and 96 hr of incubation periods and the organic matter digestibility (OMD), short chain fatty acid (SCFA) and metabolisable energy (ME) were estimated. The chemical analysis of these wastes showed that MCB contained less crude protein (CP) (3.89%) but CP content in YPS was very high (11.14%). GTH had the highest crude fiber (CF) value compared with other feedstuffs. The NDF, ADF and ADL were significantly ($p<0.05$) different among the different agricultural wastes used in this study. MCB showed the highest level of NDF and ADL. Cumulative gas production at 24, 48 and 72hr was highest in YPS. The fractional rates (c) of gas production were highest in MCB and OPL. There were significant ($p<0.05$) differences among feedstuffs in terms of OMD, SCFA and ME. The ME ranged from 6.63 to 10.32 MJ/Kg DM while SCFA and OMD ranged from 0.66 to 1.24Mm and 48.32 to 72.10% respectively. YPS recorded the highest values for the estimated parameters. All the agricultural wastes under study revealed that these agricultural wastes could be valuable alternative animal feed sources in ruminant feeding.

Key words: Agricultural wastes · Fermentation · Metabolisable energy · Ruminants · Short chain fatty acid

INTRODUCTION

The Nigerian ruminant Industries is faced with the problems of meeting the nutritional requirement of the animals. This is primarily because the available grains and food crops are in high demands and the feasible options are the use of unconventional feedstuffs. Agricultural wastes are in abundance in Nigeria especially in the Northern Guinea Savanna where the practice of cereals and tuber crops farming is in large scale. In recent times, attempts to include these items in sufficient quantity in the feeds of the ruminant have not yielded much fruit because they are non conventional feedstuffs with little or no information on their nutritive value. [1] reported that the knowledge about their potential feeding values is insufficient.

The nutrient composition of feeds using chemical analysis is well documented in literature. But this does not provide enough information on the feeds nutritive value [1]. Furthermore, [2] and [3] reported that the determination of intake and digestibility of feedstuffs *in vitro* is time consuming, laborious, expensive, requires large quantities of feed and is unsuitable for large scale feed evaluation. In the developing countries like Nigeria where there is abundance of agricultural wastes, the use of *in vitro* becomes relevant. The *in vitro* method of feed evaluation is less expensive and less time consuming compared with *in vivo* methods.

The *in vitro* gas production system helps to better quantity the nutrient utilization and its accuracy in describing digestibility in animal has been validated in numerous experiments [1]. Although, gases produced during rumen fermentation are colossal waste products and of no nutritive value to the ruminant, but gas production test are used routinely in feed research as gas volumes are related to both the extent and rate of substrates degradation [4].

Correspoding Author: A. Akinfemi, Nasarawa State University, Keffi, Faculty of Agriculture,
Department of Animal Science, Shabu-Lafia, Nigeria

The present study was conducted to evaluate the nutritive value of some energy feedstuff using *in vitro* gas production method.

MATERIALS AND METHODS

Sample Collection: Dried samples of agricultural wastes (cassava peels, yam peels, orange pulp, maize cob and sorghum threshed tops) were collected from the Teaching and Research Farm, Nasarawa State University, Shabu-Lafia, Nigeria. The samples were mill through a 1mm screen and oven-treated at 65°C until a constant weight was obtained for dry matter determination.

Chemical Analysis: Nitrogen (N) content of the agricultural wastes was determined by the standard Kjeldhal method [5] and the amount of crude protein was calculated (N x 6.25). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) and Crude Fiber (CF) were assessed using the methods proposed by [6]. Concentrations of Ca, Mg and K of feedstuffs were determined by Atomic Absorptions spectrophotometer (GBC 908AA, GBA Australia).

***In Vitro* Gas Production Study:** Rumen fluid was obtained from three West African Dwarf female goats. The method of collection was as described by [7] using suction tube from goats previously 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% *Pannicum maximum* at 5% body weight. The rumen liquor was collected into the thermo flask that had been pre warmed to a temperature of 39°C from the goats before they were offered the morning feed. Incubation procedure was as reported by [8] using 120ml calibrated transparent plastic syringes with fitted silicon tube. The sample weighing 200mg (n=3) was carefully dropped into syringes and thereafter, 30ml inoculums containing cheese cloth strained rumen liquor and buffer (g/liter) of 9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_3$ $7H_2O$ + 16 $CaCl_2$. $2H_2O$) (1:4 v/v) under continuous flushing with CO_2 was dispensed using another 50ml plastic calibrated syringe. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The silicon tube in the syringe was then tightened by a metal clip so as to prevent escape of gas. Incubation was carried out at 39±1°C and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21, 24, 48, 72 and 96h. At post incubation period, 4ml of NaOH (10M) was introduced to estimate the methane production as reported by [9]. The post incubation parameters such as metabolisable energy, organic matter digestibility and short chain fatty acids were estimated at 24h post gas collection according to [8]. The average of the volume of gas produced from the blanks was deducted from the volume of gas produce per sample 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 [10].

where:
Y = volume of gas produced at time t,
c, = intercept (gas produced from the insoluble fraction (b),
t= incubation time.

Metabolisable energy (ME) was calculated as ME = 2.20 + 0.136Gv + 0.057CP + 0.0029 CF [7], organic matter digestibility (OMD) (%) was assessed as OMD = 14.88 + 889Gv + 0.45CP + 0.651XA [7], Short chain fatty acids (SCFA) as 0.0239 V-0.0601 [11] was obtained where Gv, CP CF and XA are total gas volume, Crude protein, crude fiber and ash, respectively. Data obtained were subjected to analysis of variance. Where significant differences occurred, the means were separated using Duncan Multiple range F-test of the [12] options.

RESULTS

The chemical composition of cassava peels, maize cobs, orange pulp, guinea corn threshed tops and yam peels are presented in Table 1. There were observed variations in the chemical composition of the different agricultural wastes, with CP ranging from 3.89 to 11.14, ash from 6.75 to 7.77, NDF from 68.48 to 70.63, ADF from 46.79 to 56.63 ADL from 13.88 to 15.89, cellulose from 32.91 to 40.74 and hemicellulose from 19.05 to 22.38. Yam peel recorded the highest CP (11.14%) and EE (6.37%). The crude fiber fractions (NDF, ADF and ADL differed significantly (P<0.05) among the different agricultural wastes under study.

Table 2: presents the mineral composition of five agricultural wastes. The calcium constituent ranged between 0.027% in CPS and 1.889% in YPS. Phosphorus content ranged from 0.005% in CPS to 0.078% in GTH. Sodium content was generally low and it ranged from 0.0031% in CPS to 0.0502% in OPL. Potassium content ranged from 0.0058% in GTH to 0.0925% in CPS. Magnesium content was lowest in MCB (0.084) and highest in GTH (0.366%).

Table 1: Chemical composition (g/100g DM) of some Nigerian feedstuffs

Parameters	CPS	MCB	OPL	GTH	YPS	SEM
Crude protein	5.25^{c}	3.89^{d}	7.53^{b}	7.26^{b}	11.14^{a}	0.05
Crude fiber	23.62^{e}	29.69^{c}	24.65^{c}	31.77^{a}	12.78^{e}	0.03
Ether extract	6.37	5.68^{c}	5.64^{c}	6.28^{a}	5.87^{b}	0.02
Ash	7.77^{a}	7.67^{a}	6.75^{c}	7.17^{b}	6.75^{c}	0.02
NDF	68.48^{e}	70.63^{a}	69.17^{d}	70.28^{b}	70.17^{c}	0.02
ADF	47.41^{d}	51.58^{b}	46.79^{e}	48.62^{c}	56.63^{a}	0.02
ADL	14.22^{c}	16.83^{a}	13.88^{d}	14.94^{c}	15.89^{b}	0.02
Cellulose	33.19^{d}	34.75^{b}	32.91^{e}	33.68^{c}	40.74^{a}	0.02
Hemicellulose	21.07^{c}	19.05^{d}	22.38^{a}	21.66^{b}	13.54^{e}	0.03
NFE	56.99	53.07^{c}	55.43	47.52	43.46^{e}	0.08

a,b,c,d,e: means on the same row with different superscripts are significantly varied (P < 0.05), NDF = neutral detergent fiber. ADF = acid detergent fiber, ADL = acid detergent lignin, NDF= neutral detergent fiber, CPS=cassava peels, MCB= maize cobs, OPL= orange peels, GTH= guinea corn threshed top, YPS = yam peels SEM=Standard error of mean.

Table 2: Major mineral composition (mg/kg) and trace minerals (ppm) of some Nigerian feedstuffs.

Minerals	CPS	MCB	OPL	GTH	YPS	SEM
Major minerals						
Calcium	0.0273^{e}	0.4218^{d}	1.5135^{b}	0.5222^{c}	1.889^{a}	0.00
Phosphorus	0.005^{e}	0.026^{d}	0.048^{c}	0.078^{a}	0.064^{b}	0.00
Magnesium	0.1117^{d}	0.084^{e}	0.2712^{b}	0.366^{a}	0.204^{c}	0.00
Potassium	0.0925^{a}	0.0841^{c}	0.0915^{b}	0.0658^{e}	0.0072^{d}	0.00
Sodium	0.0031^{e}	0.0351^{b}	0.0502^{a}	0.00556^{c}	0.00515^{d}	0.00
Trace mineral						
Iron	0.0452^{c}	0.0356^{c}	0.124^{b}	0.753^{a}	0.130^{b}	0.00
Copper	0.00654^{d}	0.0078^{a}	0.00133^{d}	0.00216^{c}	0.002^{c}	0.00
Zinc	0.00227^{d}	0.00576^{b}	0.00554^{c}	0.01058^{a}	0.00165^{e}	0.00
Manganase	0.0045^{d}	0.0065^{c}	0.0178^{a}	0.0128^{b}	0.0018^{e}	0.00

a,b,c,d,e: means on the same row with different superscripts are significantly varied (P < 0.05), CPS=cassava peels, MCB= maize cobs, OPL= orange peels, GTH= guinea corn threshed top, YPS = yam peels SEM= standard error of mean,

Table 3: Gas volume and in vitro gas production characteristics

Gas Production	CPS	MCB	OPL	GTH	YPS	SEM
Characteristics						
b (mL)	44.33^{d}	37.33^{e}	57.66^{a}	51.66^{b}	50.67^{c}	0.04
c (h^{-1})	0.117^{d}	0.152^{c}	0.103^{e}	0.163^{b}	0.197^{a}	0.00
Gas volume						
Gv 24	43.67^{bc}	30.33^{d}	49.33^{ab}	35.67^{cd}	54.33^{a}	1.66
Gv 48	49.67^{b}	40.33^{c}	58^{a}	42.33^{c}	54.33^{ab}	1.20
Gv 72	57.67^{b}	45.33^{c}	66^{a}	54.33^{b}	63^{a}	0.73
Gv 96	62^{c}	49^{d}	69.33^{a}	61.33^{c}	66^{b}	0.53

a,b,c,d,e: means on the same row with different superscripts are significantly varied (P < 0.05), CPS=cassava peels, MCB= maize cobs, OPL= orange peels, GTH= guinea corn threshed top, YPS = yam peels SEM= standard error of mean,b= fermentation of the insoluble fraction, c= gas production rate constant.

Table 4: Estimated metabolisable energy (ME), Short chain fatty acid (SCFA) and organic matter digestibility (OMD)

Parameters	CPS	MCB	OPL	GTH	YPS	SEM
ME(MJ/kg DM)	8.51^{bc}	6.63^{d}	9.41^{ab}	7.56^{cd}	10.31^{a}	0.23
SCFA (μM)	0.98^{bc}	0.66^{d}	1.12^{ab}	0.79^{cd}	1.24^{a}	0.04
OMD (%)	60.73^{bc}	48.32^{d}	66.08^{ab}	5.20^{cd}	72.10^{a}	1.47

a,b,c,d: means on the same row with different superscripts are significantly varied (P < 0.05),SEM= standard error of mean, CPS=cassava peels, MCB= maize cobs, OPL= orange peels, GTH= guinea corn threshed top, YPS = yam peels SEM= standard error of mean SCFA= short chain fatty acid, OMD= organic matter digestibility, ME= metabolisable energy,MJ/Kg DM= mega joule per kilogram dry matter.

Cumulative gas volumes at 24, 48, 72 and 96h after incubation are shown in Table 3. The result showed that cumulative gas volume at 24, 48, 72 and 96h after incubation differed significantly ($P<0.05$). The gas volume ranked from the highest to the lowest was OPL, CPS, YPS, GTH and MCB.

The result obtained for the different agricultural wastes is presented in (Table 4). The predicted ME profile are similar in three agricultural wastes and varied widely being highest in yam peels (YPS). While CPS, OPL and GTH had lower values of ME. The result obtained showed that there were no significant ($p<0.05$) difference between CPS and OPL; and CPS and GTH.

DISCUSSION

There are many factors affecting chemical composition and mineral content of concentrate feedstuffs such as stage of growth, maturity, species or variety [13-15], drying method, growth environment [16] and soil types [17]. These factors interact to confer an influence on the nutritive value obtained for the agricultural wastes in this study.

Generally, apart from sodium and potassium, all the major minerals were within the range values previously reported [18]. The values were adequate to meet the requirement for growth, reproduction and milk in West African Dwarf sheep and goats [19]. The trace minerals in the present study were extremely deficient in all the agricultural wastes. This therefore means the ruminant feed may need fortification with minerals in form of either salt lick or diet inclusion.

For gas volume and *in vitro* gas production characteristics, [20] suggested that gas volume at 24h after incubation is an indirect relationship with metabolisable energy in feedstuffs. Gas production can be regarded as an indicator of carbohydrates degradation, [21,22] suggested that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate [23] and substantial changes in carbohydrates fractions were reflected by total gas produced [3]. Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while contribution of fat to gas production is negligible, [24]. Mathematical descriptions of gas production profiles allow analysis of data, evaluation of substrates and media related differences and fermentability of soluble and slowly fermentable components of feeds [1]. The rates of gas production (c) (Table 3) ranged from 0.103 to 0.197 h^{-1}.The fastest rate of gas production was observed in YPS, possibly influenced by the soluble carbohydrate fraction readily available to the microbial population. Slower rates were observed in CPS and OPL indicating that these residues were less readily available to the microbes in the rumen. [25] observed that the rate of gas production of cassava chip was higher than that of corn meal, broken rice and other industrial by-product. The fermentation of the insoluble but degradable fraction differed significantly ($p<0.05$) with the highest mean value observed in OPL followed by GTH. Although this is least expected because the higher lignin values compared with others, however, OPL and GTH had higher CP.

Although gas production is a nutritionally wasteful products [26] but provides useful basis from which ME, OMD and SCFA may be predicted [19].

There was a positive correlation between metabolisable energy calculated from *in vitro* gas production together with CP and fat content with metabolisable energy value of conventional feeds measured *in vivo*, [8].

The organic matter digestibility (OMD) % was particularly highest in YPS ($P<0.05$). The OMD differed significantly among CPS, MCB and YPS compared with other agricultural wastes. Using the *in vitro* gas measurement and chemical composition in multiple regression equation [21,20,27,28] found a high precision in prediction of *in vivo* OMD. This group further used a correlative approach to predict the ME content of feed by *in vitro* gas volume measurement and chemical constituents and concluded that the prediction of ME is more accurate when based on gas and chemical constituents only [21]. Other workers [21,29-31] have also reported significant correlation between *in vitro* gas measurement and *in vivo* digestibility.

The SCFA estimated from gas production were 0.98, 0.66, 1.12, 0.79 and 1.24μM for CPS, MCB, OPL, GTH and YPS. There were significant differences in SCFA among the feedstuffs. The least SCFA estimated for MCB is due to a lower gas production which was evident in the first 24h of incubation (Fig. 1). [32] suggested that gas production from cereal straws and in different classes of feeds incubated *in vitro* in buffered rumen fluid was

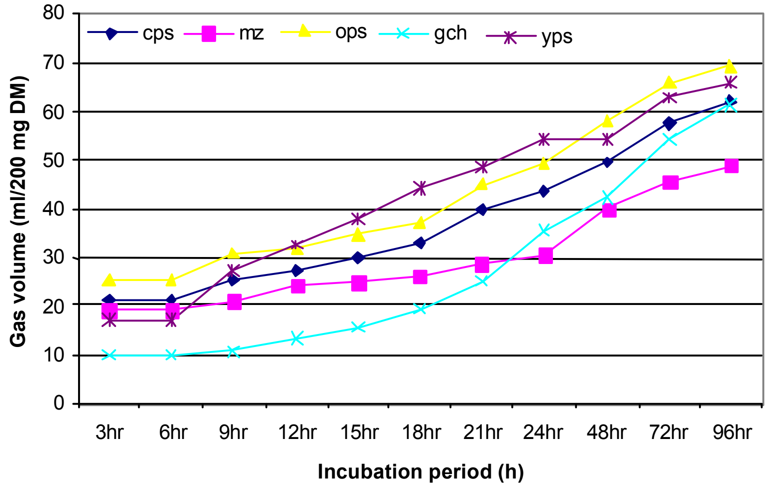

Fig. 1: *In vitro* gas production of cassava peels, maize cobs, orange peels, guinea corn threshed tops and yam peels

closely related to the production of SCFA which was based on carbohydrate fermentation. Since YPS had the highest estimated SCFA compared with other agricultural wastes, suggests a potential to make energy available to the ruminants. [33] reported a close association between SCFA and gas production *in vitro*.

CONCLUSION

The *in vitro* gas production techniques can be used to assess the nutritive value of tropical agricultural wastes and to differentiate between their potential digestibility and metabolisable energy contents. Chemical composition and *in vitro* digestibility are very useful in estimation of OMD, SCFA and ME. Yam peels (YPS), orange pulp (OPS) and cassava peels (CPS) revealed that these agricultural by-products possess the potentials of being included in the diet of ruminant animals.

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