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Evaluation of nutritive values of tropical feed sources and by-products using *in vitro* gas production technique in ruminant animals

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Abstract

Nutritive value and fermentation characteristics of beans pods (BPS), dussa (fermented sorghum wastes) (DSS), groundnut shells (GNS) and maize offal (MZO) were evaluated by measuring the gas production in vitro for a period of 96 h. The crude protein (CP) contents were 12.24, 2.17, 7.39 and 2.54 % for BPS, DSS, GNS and MZO respectively. MZO showed the highest level of gas cumulative gas production all levels of incubation. The NDF, ADF and ADL were significantly different ($p<0.05$) among the agricultural wastes used in this study. The BPS, GNS and MZO showed the highest levels of NDF and ADF. The cumulative gas production for GNS was significantly ($p<0.05$) lower in comparison with other agricultural wastes. The fractional fermentation rate (c) at different times of incubation was high for DSS, BPS and MZO and lowest for GNS. Fermentation of the insoluble fraction (b) followed the same pattern. The short chain fatty acid (SCFA) ranged from 0.370 to 0.695 µm while organic matter digestibility (OMD) ranged from 39.27 to 49.63 %. Beans pod exhibited the greatest estimated Metabolisable Energy (ME), SCFA and OMD. This result suggests that the tropical feed sources under study are all potential sources of energy for ruminant animals.

Key words: In-vitro gas production, Nutritive value, Ruminant, Tropical feeds

Introduction

The use of crop residues and agricultural by-products in animal feeding is a very common practice in tropical countries especially Nigeria. Evaluating the nutritive value of these available feed resources are important as these could make an important contribution to the nutrition of livestock (Taphizadeh et al., 2008).

Fermentation characteristics of feedstuffs in buffered rumen fluid can be studied using *in vitro* techniques (Cone et al., 1997). The *in vitro* gas production system helps to better quantify nutrient utilization and its accuracy in describing digestibility in animals has been validated in numerous experiments (Taphizadeh et al., 2008).

In vitro gas production techniques stimulate the rumen fermentation process and they have been used to evaluate the potential of feed to supply nutrients to ruminants (Sandoval Castro et al., 2003).

There is therefore a need to develop, for use in tropical countries especially in Nigeria where simple and cheap techniques, can be used to screen rapidly agricultural wastes and by- products. In view of these, *in vitro* gas production readily comes to mind and could play an important role.

Materials and Methods

Sample collection

Dried samples of agricultural wastes (beans pods (BPS), dussa (fermented sorghum wastes) (DSS), and groundnut shells (GNS) and maize offal (MZO) were collected from the Teaching and Research Farm, Nasarawa State University, Shabu-Lafia, Nigeria. The samples were mill through a 1 mm screen and oven-treated at 65^{o}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 (AOAC, 1991) and the amount of crude protein was calculated (Nx6.25). Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and crude fibre (CF) were assessed using the methods proposed by Van Soest et al, (1991).

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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 Babayemi and Bamikole (2006a) 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^{o}C from the goats before they were offered the morning feed. Incubation procedure was as reported by Menke and Steingass (1988) using 120 ml calibrated transparent plastic syringes with fitted silicon tube. The sample weighing 200 mg (n=3) was carefully dropped into syringes and thereafter, 30 ml inoculums containing cheese cloth strained rumen liquor and buffer (g/litre) of 9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_3$ 7H2O + 16 Cacl2. $2H_2O$) (1:4 v/v) under continuous flushing with CO_2 was dispensed using another 50 ml 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^{o}C and the volume of gas production was measured at 3, 6, 9, 12, 15, 18, 21, 24, 48, 72 and 96 h, although the gas production is always critical between 21-96h. At post incubation period, 4 ml of NaOH (10M) was introduced to estimate the methane production as reported by Fievez et al., (2005). The post incubation parameters such as metabolisable energy, organic matter digestibility and short chain fatty acids were estimated at 24 h post gas collection according to Menke and Steingass (1988). 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 Orskov and McDonald (1979). 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 (Menke and Steingass, 1988), organic matter digestibility (OMD) (%) was assessed as OMD = 14.88 + 889Gv + 0.45CP + 0.651XA (Menke and Steingass, 1988). Short chain fatty acids (SCFA) as 0.0239 V – 0.0601 (Getachew et al.,1999) where Gv, CP CF and XA are total gas volume, crude protein, crude fibre and ash, respectively.

Statistical Analysis

Data obtained were subjected to analysis of variance of SAS, (1998). Where significant differences ($p<0.05$) occurred, the means were separated using Duncan's multiple range test.

Results and Discussion

The chemical composition of the evaluated agricultural wastes and by-products is presented in Table 1. The result shows that there were wide variations in crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) content. The CP content of beans pods (BPS) was higher than that of other feeds under study with the least CP obtained from dussa (fermented sorghum wastes) (DSS).

Table 1. Chemical composition (g/100g DM) of different Agricultural waste.

Parameters	BPS	DSS	GNS	MZO	±SEM
Dry Matter	90.83^{b}	90.52^{b}	92.17^{a}	90.61b	0.10
Crude protein	12.25^{a}	2.17^{c}	7.39^{b}	2.54c	0.28
Ether extract	6.13	5.4	6.31	5.85	0.18
Ash	7.64^{a}	6.39^{b}	7.79^{a}	7.53a	0.11
Crude fibre	30.79^{a}	23.47^{d}	26.15^{c}	28.17b	0.21
Nitrogen Free Extract	50.79^{b}	53.00^{a}	44.53^{d}	46.18c	0.20
Neutral Detergent fibre	71.44^{a}	68.59^{c}	69.41^{b}	69.68b	0.13
Acid Detergent lignin	17.15^{a}	13.96^{c}	15.31^{b}	14.85b	0.13
Acid Detergent fibre	52.35^{a}	46.92^{c}	51.08^{b}	51.74b	0.12
Cellulose	35.13^{b}	33.15^{c}	35.77^{ab}	36.89^{a}	0.23
Hemicellulose	19.06^{b}	21.33^{a}	17.33^{d}	17.94^{a}	0.10

a,b,c,d means on the same column with different superscripts are significantly varied ($P < 0.05$), BPS = cowpea pod, DSS =dussa, GNS= groundnut shells, MZO= maize offal, SEM= standard error of the mean.

Table 2. Mineral composition (mg/Kg) of major minerals and trace minerals (ppm) of agricultural waste.

Minerals	BPS	DSS	GNS	MZO	±SEM
Major minerals					
Calcium	7.36[a]	3.55[d]	6.44[b]	4.16[c]	0.03
Phosphorus	0.91	0.727	1.343	0.72	0.12
Magnesium	5.345[a]	1.133[c]	2.53[b]	0.824[d]	0.07
Sodium	0.514[a]	0.054[c]	0.04[c]	0.268[b]	0.07
Potassium	0.346[c]	0.165[c]	7.82[a]	0.824[b]	0.01
Trace minerals					
Iron	4.98a	2.21b	0.129c	0.189c	0.12
Copper	0.0201	0.0274	0.0195	0.0107	0.00
Zinc	0.0586c	0.106c	0.0587c	0.0746b	0.00
Manganese	0.161b	0.033c	0.234a	0.083c	0.01

a,b,c,d means on the same column with different superscripts are significantly varied ($P < 0.05$), BPS = cowpea pod, DSS =dussa, GNS= groundnut shells, MZO= maize offal, SEM= standard error of the mean.

The CF content differed significantly ($p<0.05$) among the feedstuffs, whereas there were no significant difference ($p>0.05$) in the ether extract (EE) content. The NDF ranged from 69.41 to 71.44%, ADF from 46.92 to 52.35% and ADL from 13.96 to 17.15%. The beans pods (BPS), dussa (DSS), and maize offal (MZO) showed the lower DM contents while groundnut shell had the highest DM. All the obtained mineral content (Table 2) with the exception of phosphorus and copper differed significantly ($p<0.05$).

Wide variations were also observed in the gas volume production at different hours of incubation (Table 3). The result indicates that the cumulative gas volume after 24, 48, 72 and 96h of incubation was significantly different ($p< 0.05$). The gas volumes at 96 h ranked from the highest to the lowest: MZO, BPS and GNS respectively.

The fermentation of the insoluble fraction (b) of, BPS DSS and MZO were: 35.33, 41.33, 34.33 and 37.00mL respectively.

In vitro OMD, SCFA, ME and methane at 24h of incubation are shown in Table 4. The estimated ME, SCFA, OMD and CH_4 significantly differ ($p<0.05$) among the tested feedstuffs.

The variation observed in the chemical composition and mineral content of the different feedstuffs could be due to many factors such as stage of growth, maturity, species or variety (Von Keyserlingk et al., 1996; Agbagla-Dohanni et al., 2001; Promkot and Wanapat, 2004), drying method, growth environment (Mupangwa et al., 1997) and soil types (Thu and Preston, 1997). These listed factors may partially explain the differences in chemical composition (Chumpuwadee et al., 2007) between this study and others.

It can be seen that the fermentation of the insoluble fraction (b) of beans pod (BPS) and groundnut shell were low when compared to other feeds, probably a reflection of high level of lignin (Chumpuwadee et al., 2005). Additionally, cowpea pod (BPS) and groundnut shells (GNS) had high protein content. The protein fermentation does not lead to extensive gas production (Khazaal et al., 1995). The higher fermentation of the insoluble fraction were observed in dussa (DSS) and maize offal (MZO), possibly influenced by the carbohydrate fraction readily available to the microbial population (Chumpuwadee et al., 2007). Deaville and Given (2001) reported that kinetics of gas production could be affected by carbohydrate fraction.

Table 3. Gas volume and *in vitro* gas production characteristics.

Parameters	BPS	DSS	GNS	MZO	±SEM
Gas Production characteristics					
b (mL)	34.33[b]	41.33[a]	34.33d	37.00[b]	0.17
C (h^{-1})	0.0124[b]	0.0170[a]	0.0007	0.0120[b]	0.00
Gas Volume					
Gv 24h	27.33[a]	25.00[a]	18.00[b]	31.33[a]	1.16
Gv 48h	31.60[b]	33.00[a]	24.33[b]	38.67[a]	1.45
Gv 72h	34.33[ab]	37.67[ab]	30.00[b]	41.47[a]	1.65
Gv 92h	44.33[ab]	47.67[ab]	40.00[b]	51.67[a]	1.66

a,b, means on the same column with different superscripts are significantly varied ($P < 0.05$), b= fermentation of the insoluble but degradable fraction, c= gas production rate constant, SEM= standard error of the mean, GV = gas volume, BPS = cowpea pod, DSS =dussa, GNS= groundnut shells, MZO= maize offal, SEM= standard error of the mean.

Table 4. Metabolisable energy (ME) (MJ/kg DM), short chain fatty acid (SCFA) and organic matter digestibility (OMD).

Parameters	BPS	DSS	GNS	MZO	±SEM
ME (MJ/Kg DM)	6.69[a]	5.79[b]	3.14[c]	6.68[a]	0.06
SCFA (μM)	0.695[a]	0.687[a]	0.370[c]	0.537[b]	0.01
OMD (%)	49.63[a]	43.17[c]	39.27[d]	48.75[b]	0.07
CH_4 (mL	10.00[c]	17.00[a]	8.00[d]	13.00[b]	0.33

a,b,c, means on the same column with different superscripts are significantly varied ($P < 0.05$), ME = metabolisable energy, SEM= standard error of the mean, SCFA= short chain fatty acid, OMD= organic matter digestibility,CH4 = methane, BPS = cowpea pod, DSS =dussa, GNS= groundnut shells, MZO= maize offal.

The fast rate of gas produced (c) observed in dussa (DSS), beans pod (BPS) and maize offal (MZO) was probably influenced by the soluble carbohydrate fractions readily available to the microbial population. The relatively low content of fibre can facilitate the colonisation of the feed by the microbial rumen population, which in turn might induce higher fermentation rates, therefore improving digestibility (Van Soest, 1994). As the fermentation process is partially regulated by the fibrous content of the feeds, dussa ferments faster than groundnut shell (GNS), beans pod (BPS) and maize offal (MZO). Since gas production on incubation of feed is buffered rumen fluid is associated with feed fermentation and carbohydrate fraction (Sallam et al., 2008), so the higher gas production in maize offal (MZO) and dussa (DSS) could be related to fibre fraction content. This is in agreement with De Boever et al. (2005), who reported that gas production was negatively related with NDF content and positively with starch. The negative effect of cell wall content on gas production in groundnut shell could be due to reduction in the microbial activity through increasing the adverse environmental condition as incubation time progresses.

The reduction in gas and methane in groundnut shell (GNS) could be due to the conversion of CO_2 and H_2 to acetate instead of CH_4 (Miller, 1995). This is consistent with the findings of Sallam et al., (2008). The estimated ME in this study were consistent with those obtained for the concentrate feedstuff. (Chumpuwadee et al., 2007) and lower than those obtained for the different parts of *Enterelobium cyclocarpum* (Babayemi, 2006). There was a positive correlation between ME calculated and from the in vitro gas production together with CP and fat content with metabolisable energy value of conventional feed measured in vivo (Menke and Steingass, 1988). The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed (Getachew et al., 1998, 2002). The lowest SCFA predicted from gas production in groundnut shell (GNS), due to the lowest gas production, which was most evident during the first 24h of incubation. This is consistent with the findings of Blummel et al. (1990) who stated that different classes of feed incubated *in vitro* in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation but did not support observation on the GNS sample in this study. A high value of SCFA is an indication of energy availability to the animal. High digestibility of organic matter (OMD) obtained in beans pods (BPS) and maize offal (MZO) is because the major carbohydrate of their feedstuffs is starch, which is fermented by amylolytic bacteria and protozoa (Kotarski et al., 1992). This result implies that the microbes in the rumen and animal have high nutrient uptake.

Conclusion

The tropical agricultural waste and by-products showed a great variation in chemical composition and mineral content. The result of this study demonstrates that gas production characteristics of the feedstuff under study differed. Based on this study, High fermentation potentials of the different agricultural wastes and by-product ranked from the highest to the lowest were: dussa, beans pod, maize offal and groundnut shells respectively.

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