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In vitro gas fermentation kinetics of compounded ration containing graded levels of biodegraded corncob

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Abstract

Cob regarded as a waste product, constituting source of environmental pollution could be utilized as an energy source in feed formulation if properly processed and harnessed through fungal degradation. Hence, this study was designed to evaluate the nutritive value of biodegraded corncob meal based diet using in vitro gas production technique. Aspergillus niger was isolated and sub-cultured to obtain a pure culture. Degraded corn cob meals (DCCM) of four dietary treatments were prepared to include: T_1 (0% DCCM which served as the control), T_2 (15% DCCM), T_3 (30% DCCM) and T_4 (45% DCCM). Each diet sample (200mg) was incubated in buffered rumen liquor for 48 hours and gas volume was estimated using established in vitro gas production models. Amount of gas volume produced was determined every 3 hours for 48 hours of incubation in buffered rumen fluid. After 48 hours of incubation, methane gas produced was estimated and determined in triplicates. The proximate composition of Aspergillus niger biodegraded corn cob indicated that Aspergillus niger improved the nutritive value of the corn cob. Untreated corn cob meal was lower in nutritive values when compared with treated corncob meal with crude protein, crude fibre and ash values ranging from 6.88 to 9.78%, 32.68 to 26.37% and 2.87 to 2.88% respectively. The proximate composition of the dietary treatments showed that the crude protein varied from (11.67-12.67%), crude fibre (10.94-21.56%), ether extract (2.12-4.88%), ash (6.48-9.44%) and nitrogen free extract (58.17-62.99%). Results obtained for volume of gas (35.960-72.770mL/200mgDM) produced in time "t" denoted by (b) were significantly different ($P<0.05$) across the dietary treatments. However, rate of gas production (0.0297-0.0425mL/hr) and time between incubation and gas production (2.083-2.683hr) were not significantly different ($P>0.05$) across the dietary treatments. Cumulative gas volume production at 24 hours were significantly ($P<0.05$) influenced by different inclusion levels of DCCM with values obtained ranging from T_4 (15.33 ml/gDM) to T_3 (35.33 ml/gDM). There were significant differences ($P<0.05$) across the dietary treatments. The post estimated parameters ranging from metabolisable energy (4.96-7.74MJ/Kg DM), organic matter digestibility (37.98-58.03%), short chain fatty acids (0.31-0.78µmol), Methane estimate (3-8 ml/200mgDM) and Carbon dioxide estimate (10-30 ml/200mgDM). It can be concluded that 30% DCCM based diet had the potential of meeting the nutritional needs as small ruminant livestock feeds, if properly biodegraded and incorporated into feeds.

Keywords: Corn cob, *Aspergillus niger*, *in vitro* gas production, Biodegraded

Introduction

The major constraint of livestock production in Nigeria is the inadequate supply of good quality feed all year round due to incessant increase in population resulting to tight competition between man and livestock (Bawala and Akinsoyinu, 2006). However, food security solely depends on how efficient animals can better utilize non-conventional feed resources, which cannot be used as food for human (Makkar, 2000). Maize cob is a non-conventional feedstuff generated annually in the country as more people venture into the cultivation of maize. Although, this possess low feeding value simply because of their poor protein content, energy, minerals and vitamins but their conversion into useful protein rich products. The use of crop residue such as corncob in feeding animals especially ruminants had been reported with lots of limitations due to its high level of lignin (Preston and Leng, 1987) and low nutritive value and degradation rate (Sun and Cheng, 2002) thus preventing biodegradation in the rumen. Treatments employed to improve the nutritive value either by increasing its digestibility or enhancing palatability had been reportedly having their own limitations and few are environmental unfriendly (Silverstein *et al*., 2007). Recently, the biological treatments of crop residues improve the accessibility of cellulosic fractions, thus improving their digestibility and its feeding value thereby attracting extensive interests among researchers (Zhang *et al.,* 2007; Yu *et al.,* 2009). The potential of biological treatments has been explained by the ability of certain microbes to disrupt the plant cell wall by partially breaking down the lignin-carbohydrate complex (Keller *et al.,* 2003) thus improving their utilization in the rumen by increasing the availability of fermentable energy to ruminal microbes (Akin *et al.,* 1993). Thus, microbial conversion seems to be a practical and promising alternative for increasing the nutritional value of agro by-products by transforming them into animal feed and producing a value-added product (Villas-Boas *et al.,* 2002). The nutritive value of the poor quality nature of corncob can be improved by different methods of treatments and supplements. However, the key to improve the use of corncob for ruminants is to overcome the barriers in rumen microbial fermentation of lignocelluloses. The two well known factors limiting bacterial digestion of corncobs in the rumen are majorly because of its high level of lignifications and low contents of nitrogen, vitamins and minerals. Fungi (*Aspergillus niger* and *Penicillin spp*) have been reported to be the main source of enzymes such as cellulase, amylase, catalase, hemicellulase and xylanase which help to degrade the non-starch polysaccharide in the substrate into soluble sugar (Hamilyn, 1998). The *in vitro* gas production method of feed evaluation is a laboratory estimation of gas produced during fermentation of feed samples in buffered rumen fluid. The method is less expensive, fast and allows a large number of samples to be handled at a time such that research results could be obtained within a short period of time. Tona *et al.* (2013) reported that metabolizable energy, organic matter digestibility and short chain fatty acid values can be estimated from gas production parameters. The present study was therefore undertaken to determine the nutritive value of *Aspergillus niger* treated corn cob in formulating ruminant feed using the *in vitro* gas production technique.

Materials and methods

The process of biodegradation was carried out at the Microbiology Laboratory of Institute of Agricultural Research and Training, Moor plantation, Apata, Ibadan while the *in vitro* study was carried out at the Department of Animal Science Laboratory, University of Ibadan, Ibadan.

Isolation of microorganism

The isolation of *Aspergillus niger* was carried out on Potatoes Dextrose Agar (PDA) using the samples obtained from the decaying corncob. Samples of the fungi were suspended in 10ml of sterile distilled water and shaken vigorously for 10minutes. This was diluted with sterile water until a spore count of approximately 3×10^6per ml was obtained; spore count was monitored by using the Hawksley Haemocytometer. 1.0ml of the resulting liquid was spread on the surface of the PDA and incubated at 37^0C for 7 days. The fungal isolates formed were sub-cultured until a pure culture of *Aspergillus niger* was obtained, this was done according to a similar research carried out by Iyayi and Aderolu (2004).

Solid state fermentation

The corn cob used in this experiment was obtained from Gege market in Ibadan. The process of biodegradation which lasted for seven (7) days was carried out according to the method of Iyayi and Aderolu (2004) using the fungus *Aspergillus niger*. The corn cob was grinded and sterilized with an autoclave machine at 121^0C for 15 minutes. This was allowed to cool down and then inoculated with the pure fungus culture and moistened with distilled water at the rate of 300ml per kg of corncob. After a period of seven days the action of the fungus was stopped by oven drying the substrate at 80^0C for 24 hours.

Preparation of the experimental diet

The dried materials (degraded corncob) obtained from the solid state fermentation was then incorporated at varying levels of 0%, 15%, 30% and 45% with other ingredients to formulate four dietary treatments T_1, T_2, T_3 and T_4 respectively where T_1 served as the control.

Table 1: Gross composition of the experimental diets

	Inclusion levels of Degraded Corncob Meal			
Ingredients	T_1 (0%)	T_2 (15%)	T_3 (30%)	T_4 (45%)
Maize bran	60.00	45.00	30.00	15.00
DCCM	-	15.00	30.00	45.00
Wheat offal	24.25	24.25	24.25	24.25
Palm Kernel Cake	10.00	10.00	10.00	10.00
Premix	0.25	0.25	0.25	0.25
Limestone	5.00	5.00	5.00	5.00
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	10.00	100.00
Calculated Analysis (%)				
Crude protein	14.36	13.71	13.12	12.52
Crude fibre	11.50	13.13	14.77	16.40
ME(MJ/Kg)	9.00	8.28	7.67	6.85

DCCM= Degraded Corncob Meal, ME= Metabolizable Energy

***In vitro* gas production study**

Rumen fluid was obtained with suction tube from four WAD goats that were previously fed 60% concentrate and 40% forages. The rumen liquor was collected from the goats before they were offered their morning feed, into a thermo flask that had been pre-warmed to a temperature of 39^0C. Incubation procedure was as reported by Menke and Steingass (1988) using 120 ml calibrated transparent plastic syringes with fitted silicon tube. The dietary treatments (T_1, T_2, T_3 and T_4) samples, in triplicates were carefully dropped into the syringes and then 30 ml inoculums containing cheese cloth strained rumen liquor and buffer (g/liter) of 9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57KCl + 0.47 NaCl + 2.16 $MgSO_4.7H_2O$ + 0.16 $CaCl_2.2H_2O$) (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 3, 6, 9, 12, 15, 18, 21 and 24 hours. At post incubation period, 4 ml of NaOH (10 M) was introduced to estimate methane production as reported by Fievez *et al.* (2005). The average of the volume of methane gas produced from the blanks was deducted from the volume of gas produced from the samples (The blanks contained only the inoculums and buffer). Metabolizable energy (ME) was calculated as ME=2.20+0.136GV+0.057CP+0.0029 CF (Menke and Steingass, 1988). Organic matter digestibility was assessed as OMD=14.88+0.889GV+0.45CP+0.651XA (Menke and Steingass, 1988). Short chain fatty acid (SCFA) as 0.0239GV-0.0601 (Getachew *et al.,* 1999) was also obtained. Where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively.

Determination of proximate composition

Samples of degraded corncob meal and the experimental diet containing *Aspergillus niger* biodegraded corncob were taken to the laboratory where proximate analysis were determined according to AOAC (2004).

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) while significant means were separated using Duncan multiple range test using SAS (2004).

Results and discussion

Table 2 shows the proximate composition of *Aspergillus niger* degraded corn cob and the experimental diets containing graded levels of *A. niger* degraded corn cob. The values obtained by Akinfemi *et al*. (2009) for the proximate composition of corn cob were lower than the values obtained in this study and this could be attributed to the degradation of the corn cob by *Aspergillus niger*. The increased values observed in the crude protein of biodegraded corn cob might probably due to the addition of fungi protein during solubilization and degradation (Belewu, 2005). This agreed with the findings of Zadrazil (1993); Belewu and Okhawere (1998) that the colonization of lignocellulosic waste by the fungi resulted in an increase in their nutritional value. The dry matter, ether extract and nitrogen free extract contents of the experimental diets decreased as the inclusion levels of DCCM increased across the dietary treatments while for the crude fibre, the values increased across the treatments and this might be attributed to increased soluble carbohydrate derived the from reduced lignin content of corn cob as a result of method employed. The crude protein (CP) values of the dietary treatments observed were above 10–12% CP recommended for moderate level in ruminant production (Gatemby, 2004). The ash values did not follow a particular trend but ranged from 6.48- 9.44%. There were also marked variations in nitrogen free extract (NFE) of the experimental diets containing 0% DCCM based diet having the highest values which could be an indication of high energy content in the diets. Okoruwa and Njidda (2012) reported that information on crude fibre (CF) of a diet is essential for the assessment of their fiber fraction contents. Nouala *et al.* (2006) also outlined that the environmental conditions of the rumen that are normally in favour of fibrolytic micro-organisms which always aid the degradation of high fibre diets.

Table 3 presents the gas production at various incubation hours of compounded ration containing graded levels of biodegraded corn cob. The results indicated that there were significant differences ($P < 0.05$) across the dietary treatments for the incubation hours of gas volume production except at 3 and 6 hours of incubation. It was observed that T_4 had the lowest values and T_3 had the highest values throughout the incubation hours. However, the cumulative gas volume produced at 24hours of incubation ranged from (15.33-35.33ml/gDM). Gas volume (GV) generally reflects the contents of fermentable carbohydrate or carbohydrate degradation and also probably the nitrogen and lipids (Aregheore and Abdulrazak, 2005). This was further explained by Akinfemi *et al*. (2009) that gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while contribution of fat to gas production is negligible.

Table 2: Proximate composition of diets containing graded levels of *Aspergillus niger* biodegraded corn cob and *Aspergillus niger* biodegraded corn cob meal

Parameters (%)	Inclusion levels of degraded corncob meal				
	DCCM	**T_1 (0%)**	**T_2 (15%)**	**T_3 (30%)**	T_4 (60%)
Dry matter	88.35	86.82	86.72	84.89	83.39
Crude Protein	9.78	11.75	12.23	12.67	11.67
Crude fibre	26.35	10.94	12.40	15.81	21.56
Ether extract	1.19	4.88	3.90	3.76	2.12
Ash	2.88	9.44	8.74	9.27	6.48
NFE	48.14	62.99	62.73	58.59	58.17

DCCM: Degraded Corncob Meal, T_1: 0% DCCM, T_2: 15% DCCM, T_3: 30% DCCM, T_4: 60% DCCM

Table 3: Gas production at various incubation hours of compounded ration containing graded levels of *Aspergillus niger* degraded corncob

	Inclusion levels of degraded corncob meal				
Time (hr)	T_1 (0%)	T_2 (15%)	T_3 (30%)	T_4 (45%)	SEM±
3	2.83	3.50	2.67	2.00	0.29
6	5.33	6.00	5.33	4.00	0.52
9	8.67^{ab}	11.00^{ab}	14.00^{a}	5.33^{b}	1.26
12	12.00^{b}	20.00^{a}	20.00^{a}	8.67^{b}	1.64
15	16.00^{b}	24.00^{a}	25.33^{a}	9.33^{c}	2.05
18	18.00^{b}	28.00^{a}	30.67^{a}	12.00^{b}	2.46
21	19.33^{b}	32.00^{a}	34.00^{a}	12.67^{c}	2.80
24	20.67^{b}	31.00^{a}	35.33^{a}	15.33^{b}	2.56

^{a,b,c} means along the same row with different superscripts are significantly different ($P<0.05$)

Table 4 shows the gas volume production parameters of the experimental diet. The results indicate that the volume of gas produced in time (b) were significantly different across the dietary treatments with T_1 having the lowest value and T_3 having the highest value which ranged from (35.960-72.770 ml/200mgDM). However, it was also observed that rate of gas production (c) and the time between incubation and gas production (Lag) were not significantly different across the dietary treatments. The cumulative gas volume (GV) of the degraded corn cob meal based diets were significantly different ($P< 0.05$) for each treatment with 45% DCCM bases diet having the lowest value (15.333 ml/gDM) and T_3 had the highest value (35.333 ml/gDM). In most cases, feedstuffs that show high capacity for gas production are also observed to be synonymous for high methane production. Methane production indicates an energy loss to ruminants and many tropical feedstuffs have been implicated to increase methanogenesis (Babayemi and Bamikole, 2006) as an integral part of carbohydrate metabolism (Demeyer and Van Nevel, 1975). It was also observed in this study that as the volume of gas increased, the time also increased. This was in agreement with results of Delgado *et al.* (2001).

Table 4: Gas volume production of compounded ration containing graded levels of *Aspergillus niger* degraded corncob

	Inclusion levels of degraded corncob meal				
Parameters	T_1(0%)	T_2(15%)	T_3(30%)	T_4(45%)	SEM±
b(mL/200mgDM)	35.960^c	55.330^b	72.770^a	38.230^c	5.00
c(mL/hr)	0.0410	0.0425	0.0375	0.0297	0.003
Lag(hr)	2.452	2.305	2.683	2.083	0.158
GV24hrs(mL/gDM)	20.667^b	31.000^a	35.333^a	15.333^b	2.563

^{a,b,c} means along the same row with different superscripts are significantly different (P<0.05)

b: Volume of gas produced in time ε . . c: Rate of gas production (h^{-1})

Lag: Time between incubation and gas production GV: Gas volume

Indicated in Table 5 are the post incubation parameters of diets containing graded levels of *Aspergillus niger* degraded corn cob. There were significant differences (p< 0.05) in the results obtained in the study. The metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acid (SCFA), gas volume (GV) and dry matter degradability (DMD) followed the same trend where values obtained increased across the treatments with the exception of T_4 (45% DCCM). The T_3 (30% DCCM) recorded the highest value in all the parameters (ME, OMD, SCFA, GV and DMD) with (7.74MJ/KG, 58.03%, 0.78μ mol, 35.33 ml/mgDM and 65.00%) while T_4 (45% DCCM) had the lowest value (4.96MJ/KG, 37.98%, 0.31μmol, 15.33 ml/mgDM and 33.00%) respectively. Though gas production is a nutritional wasteful product but it provides useful basis from which metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) may be predicted (Blummel and fi rskov, 1993). The ME content in this study was particularly high in 30% DCCM with (7.74MJ/KG) which is a good index for measuring the quality of feeds. Short chain fatty acids are simply referred to volatile fatty acids and their presence denote energy available in the feed stuff. The highest estimated SCFA in T_3 (30% DCCM) might be due to increase observed in the CP and CF content of the diet. This observation corroborated with the result reported by Hoffman *et al.* (2003) who stated that SCFA is an end product of carbohydrate fermentation and this therefore contributes and determines the amount of energy that can be supplied to the host animal. The gas production from different classes of feed (Blummel *et al.,* 1990) incubated in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation (Sallam *et al.,* 2007). Getachew *et al*. (2002) reported the association between SCFA productions from gas value, which is an indicator of energy available to animal. There is a relationship between SCFA and metabolisable energy i.e. the higher the SCFA produced from gas volume, the higher the metabolisable energy which is an indicator of energy in the feed (Getachew *et al.,* 2002). Also, Maheri-Sis and Mirzaei-Aghsaghali (2008) reported that a higher gas production can contribute significantly to energy supply via short chain fatty acid production. The OMD values obtained in the present study were higher than those reported for rice straw, linseed straw, date stone sugar bagasses (Sallam *et al.,* 2007), wild cocoyam, mixture of *Tephrosia candida* and guinea grass (Babayemi, 2006). The higher estimated OMD obtained in the 30% DCCM inclusion levels implied that microbes in the rumen of animal have increased nutrient uptake (Chumpawade *et al.,* 2007). Menke *et al.* (1979) suggested that gas volume after incubation is indirectly related to metabolisable energy in feed stuffs. Sommart *et al.* (2000) suggested that gas volume is a good parameter from which the qualities of diets predict in terms of digestibility, fermentation of products and microbial protein synthesis of the substrates by the rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and OMD were shown to have high correlation with gas volume (Nitipot and Sommart, 2003). Gas volume (GV) has also shown to have a close relationship with feed intake (Blumme land Becker, 1997) and growth rate (Blummel and fi rshov, 1993).

Table 5: Post incubation parameters of diets containing graded levels of *Aspergillus niger* biodegraded corn cob

Parameters	Inclusion levels of Degraded Corncob Meal				
	T_1 (0%)	T_2 (15%)	T_3 (30%)	T_4 (45%)	SEM±
ME (MJ/KG)	5.69^b	7.12^a	7.74^a	4.96^b	0.36
OMD (%)	44.69^b	53.63^a	58.03^a	37.98^c	2.48
SCFA (µ mol)	0.43^b	0.68^a	0.78^a	0.31^b	0.06
GV 24hr (ml/mgDM)	20.67^b	31.00^a	35.53^a	15.33^b	2.56
DMD (%)	56.00^c	57.00^b	65.00^a	33.00^d	3.60

^{a,b,c,d}, means along the same row with different superscripts are significantly different (P<0.05)

ME: Metabolizable Energy, OMD: Organic Matter Digestibility SCFA: Short Chain Fatty Acid, GV: Gas Volume, DMD: Dry Matter Degradability, DCCM: Degraded Corncob Meal

Presented in Figure 1 is the Methane gas production of diet containing graded levels of *Aspergillus niger* degraded corn cob meal. Methane (ml/200 mgDM) gas production observed in this study ranged from 5 to 8ml/200mg DM across the dietary treatments, the least and the highest being from T_2 (5% DCCM) to T_3 (30% DCCM) respectively. Methane production has negative effect on the animal in one hand as it is an energy loss to the animal and on the hand, when accumulated in the rumen; it results in bloat (Babayemi, 2006).

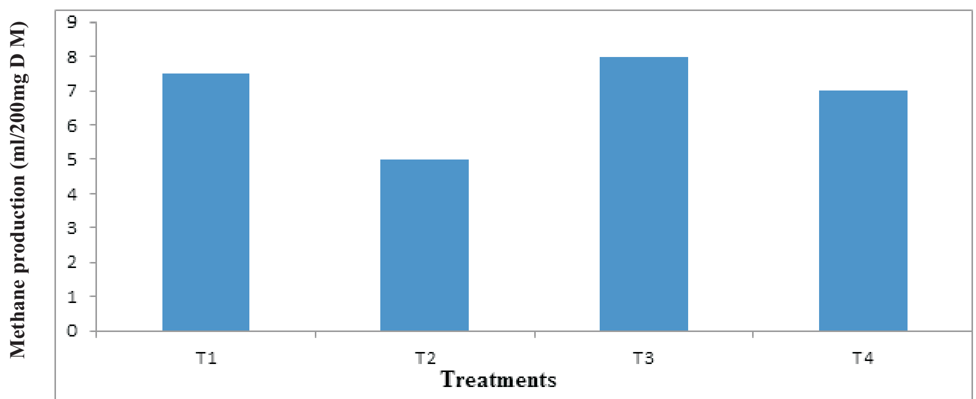

Fig. 1: Methane gas production of compounded ration containing graded levels of *Aspergillus niger* degraded corncob.

Conclusion

The study obtained indicated that the fungi treated and untreated corn cob meal differed in their chemical composition as the *Aspergillus niger* treatment employed helped in improving corn cobs nutritive values. More so, the inclusion of 30% DCCM into animal's diet gave the best results for all parameters evaluated except the methane which had the highest.

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