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Use of *in vitro* gas production technique in the evaluation of fungal treated maize husk

Akinfemi, A[1]. and Ayoade J.A[2]

[1]Yaba College of Technology, Department of Agric. Technology, Epe Campus, Lagos
[2]University of Agriculture, Markurdi, College of Ruminant Management and Production
Corresponding author e-mail address: akinfemiabayomi@gmail.com

Target audience: Livestock farmers, Animal Scientists, Ruminant Nutritionists

Abstract

Maize husk is potential feed resources for ruminants if properly harnessed. Its uses is however limited by high fibre content and low digestibility which can be enhanced by fungal treatment. Maize Husk was degraded for 21 days using three different fungi: Pleurotus tuber-regium, Pleurotus ostreatus and Pleurotus pulmonarius. The resulting substrates were analyzed for changes in the chemica composition. The result obtained showed an increase in the crude protein (CP) from 6.62% for the control (untreated maize husk) to 9.25% for the Pleurotus tuber-regium treated maize husk (PTMH), 8.06% for the Pleurotus pulmonarius treated (PPMH) and 7.87% for the Pleurotus ostreatus treated (POMHA) maize husk. Contrarily, the crude fibre (CF) decreased significantly (P<0.05) from 33.19% (UNMH) for the control to 15.62% (PTMH). The CF fractions (NDF, ADF and ADL) also decreased significantly. The gas rate production (c) constant obtained in all the substrates under study were not significant. Gas volumes at 24h highest in PPMH (30ml) with the least recorded in UNMH (15ml). The fermentation of the insoluble, but degradable fraction (b, ml) range from 13 (control) to 27.33 (PPMH). The estimated organic matter digestibility also increased from 33.22% in the control to 46.99% in PPMH treated samples. The highest values in short chain fatty acid (SCFA) 0.657mol and metabolizable energy (ME) 8.19 MJ/Kg DM was also estimated for PPMH. The result obtained in this study showed improvement in the CP, and in vitro digestibility after fungal treatment suggesting the possibility of recycling maize husk into value added ruminant feed.

Keywords: Maize husk, ruminants, *in vitro* digestibility, fungi, maize husk

Description of problem

Ruminant livestock especially small ruminant (sheep and goat) play an important role in the economy of the people living in all parts of Nigeria. They are important source of income through the sales of products that originate from them, such as milk, meat, hide and skin,

and can be rightly regarded as source of wealth because it can be sold to obtain cash in difficult time. Furthermore, large numbers of small ruminant, especially goats are transported to various markets from north central to southern part of Nigeria for sale. Report (1) indicated that live animals serves as a store of wealth that can be called upon during difficult times or when there is a need for large sums of cash for school fees, funeral or wedding expenses. However, their production is limited by short supply of forages during the dry season and hence most farmers sustain their flock on crop agricultural wastes. In the cropping seasons (raining season) small ruminants rely solely on consumption of crop residues, by-products, natural pastures and forages for nutrient. But the prohibitive cost of concentrate diets in this part of the country necessitates the search for readily available nutritive feedstuffs. The problem with the crop residues is its high fiber and lignin, and low vitamins and mineral contents, more so the residues are burnt adding to the problems of ozone layer and also causing air pollution. Crop residues are in abundant in all parts of Nigeria. Among the common one is maize husk. Maize farmers in Nigeria often leave maize husk on the field to rot and sometimes, burn them after clearing the farms. In response to the shortage of ruminant in Nigeria, there is therefore the need to think of alternative feed resources such as fungal treated wastes. Maize husk may likely to serve as that alternative. Fungal treated maize husk will help to recycle the waste into rich livestock feed. The possibility of improving these residues for livestock feeding has been explored and three major treatments have also been advocated, namely: physical, chemical and biological treatments. Literature abounds on physical and chemical treatments with paucity of information on biological. Fungal treatment will serve as an alternative to all the already existing methods of treatment. The advent of biotechnology, with its inexpensive mode of application, has been used as a tool for the effective conversion of these wastes into useful products (2). Fungi, apart from increasing the protein contents of lignocelluloses can also reduce the fiber content, an effect traceable to the extracellular enzymes produced by white-rot fungi. Previous study indicated (3) a decrease in the crude fiber content of fungal treated sorghum stover.

The objective of this study was to investigate the fungal treatment of maize husk and the resulting impact on the chemical composition and *in vitro* digestibility.

Materials and methods

Preparation of experimental samples

Dried samples of maize husk were collected from the Teaching and Research Farm, Nasarawa State University, Shabu-Lafia, Nigeria. The materials were milled and oven-treated at 65^0C to constant weight for dry matter determination.

The fungus

The sporophores of *Pleurotus tuber-ragium, Pleurotus pulmonarius* and *Pleurotus ostreatus* growing in the wild

were collected from University of Ibadan botanical garden. These were tissue cultured to obtain fungal mycelia (4). The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

Degradation of acha straw by *P. tuber-regium, P. pulmonariu* and *P. ostreatus*

Preparation of substrate

The jam bottles used for this study were thoroughly washed, dried for 10min. at 100^{0}C. 25.00g of the dried milled substrates were weighed separately into a jam bottle and 70ml distilled water were added. The bottle was immediately covered with aluminum foil and sterilized in the autoclave at 121^{0}C for 15 min. Each treatment was in triplicates.

Inoculation

Each bottle was inoculated at the center of the substrate with 2, 10.00mm mycelia disc and covered immediately (5). They were kept in the dark cupboard in the laboratory at 30^{0}C and 100% relative humidity (RH). At day 21 day of inoculation, the experimental bottles were autoclaved to terminate the mycelia growth. Samples of biodegradation were oven dried to constant weight for chemical analysis and *in vitro* digestibility.

Chemical analysis

Nitrogen (N) content of the agricultural wastes was determined by the standard Kjeldhal method (6) and the amount of crude protein was calculated (Nx6.25). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF) were assessed using standard methods (6).

***In vitro* gas production**

Rumen fluid was obtained from, three West African Dwarf female goats through suction tube via the esophagus before morning feed. The animals were fed with 40% concentrate (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fishmeal) and 60% Guinea grass. Incubation was carried out (8) in 120ml calibrated syringes in three batches at 39^{0}C. To 200mg sample in the syringe was added 30ml inoculums containing cheese cloth strained rumen liquor and buffer (9.8g $NaHCO_3$ + 2.77g Na_2HPO_4 + 0.57gKCL + 0.47gNaCl + 0.12g$MgSO_4.7H_2O$ + 0.16g$CaCl_2$. $2H_2O$ in a ratio (1:4 v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 hrs. After 24hr of incubation, 4ml of NaOH (10M) was introduced to estimate the amount of methane produced (9). The average volume of gas produced from the blanks was deducted from the total volume of gas produced. Fermentation characteristics were estimated using the equation $Y = a + b (1 – e^{ct})$ (10), where Y = volume of gas produced at time't', a = intercept (gas produced from the soluble fraction), b = gas production rate constant for the insoluble fraction, (a + b) = final gas produced, C = gas production rate constant for the insoluble fraction (b), t = incubation time. Metabolizable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) were estimated (8) and short chain fatty acids (SCFA) was calculated (11). ME MJ/kg DM = 2.20 + 0.136 *Gv

+ 0.057* CP + 0.0029*CF; OMD = 14.88 + 0.88Gv + 0.45CP; SCFA = 0.0239*Gv – 0.0601; Where Gv, CP, CF and XA are net gas production (ml/200mg DM), crude protein, crude fiber and ash of the incubated sample respectively.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) and where significant difference occurred means were separated by Duncan method (12) using Statistical Analysis System (SAS) package.

Result and Discussion

Shown in Table 1 are the results of chemical composition (g/100g DM) of maize husk treated with three strains of fungi. Treatment effect as affected by chemical composition was significant ($P<0.05$). The results showed an increase in CP from 6.62% (UNMH) to 9.25% (PTMH), EE from 1.94% (UNMN) to 3.64% (PTMH), while CF decreased from 33.19% (UNMH) to 15.62% (PTMH). The CP increase in the treated maize husk may be associated with the intricate network of fungal mycelium embedded in the treated substrate. It may also be the result of secretion of certain extracellular enzymes into the maize husk which are proteineous in nature during their breakdown and its subsequent metabolism (13). The decreasing CF and CF fractions (NDF, ADF and ADL) may be the result of the activities of the fungal used on the decomposition of the fiber contents, During fungal growth part of the cell wall is converted into soluble sugars to provide energy (14) a phenomena that could be responsible for decrease in major fiber (15).

Presented in Table 2 are the results of gas production over 24 h incubation periods. Gas volumes provide information on digestibility. The higher the gas volume the more the digestibility. From our present result, gas volume increased progressively in all the substrate under study, but was higher in the treated maize husk. This observation suggests

Table1: Chemical composition (g/100g DM) of fungal treated maize husk

Parameters	UNMH	PTMH	PPMH	POMH	SEM
DM	87.30^{a}	86.10^{a}	86.65^{a}	85.62^{a}	0.1
CP	6.62^{b}	9.25^{a}	8.06^{ab}	7.87^{ab}	0.30
CF	33.19^{a}	15.62^{c}	22.41^{b}	24.15^{b}	0.93
EE	1.94^{c}	3.62^{a}	2.73^{b}	2.85^{b}	0.93
ASH	3.12^{a}	2.66^{b}	2.78^{ab}	2.97^{ab}	0.71
CHO	55.13^{c}	68.83^{a}	64.02^{ab}	62.17^{b}	0.94
NDF	70.94^{b}	75.37^{a}	69.25^{d}	70.42^{c}	0.09
ADF	45.98^{a}	41.27^{c}	42.43^{b}	41.75^{bc}	0.14
ADL	14.71^{a}	11.93^{b}	11.45^{b}	11.54^{b}	0.11
Cell	31.27^{a}	29.34^{c}	30.98^{a}	30.21^{b}	0.14
Hcell	24.96^{d}	34.10^{a}	26.82^{c}	28.68^{b}	0.06

abc mean on the same row with different superscripts are significantly different ($p<0.05$)
DM= dry matter, CP= crude protein, CF= crude fiber, EE= ether extract NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL =acid detergent lignin, Cell= cellulose, Hcell= hemicellulose, SEM= Standard error of mean.

Table 2: In vitro gas production (ml/200 mg DM) of fungal treated maize husk over a period of 24 hours

Treatment	3h	6h	9h	12h	15h	18h	21h	24h
UNMH	2.0^{b}	4.0^{b}	7.0^{b}	7.0^{a}	8.0^{c}	11.0^{c}	13.0^{b}	15.0^{c}
PTMH	5.0^{a}	7.0^{a}	10.5^{a}	12.0^{a}	15.0^{b}	17.5^{b}	17.5^{b}	20.0^{b}
PPMH	2.67^{ab}	4.0^{a}	10.0^{a}	12.0^{a}	17.0^{ab}	20.0^{b}	24.0^{a}	30.0^{a}
POMH	5.0^{a}	7.5^{a}	8.0^{a}	12.0^{a}	22.0^{a}	24.0^{a}	24.0^{a}	24.0^{b}
SEM	0.42	0.19	0.44	0.17	1.01	0.69	0.96	0.78

abc mean on the same row with different superscripts are significantly different ($p<0.05$). UNMH = untreated maize husk, *Pleurotus tuber-regium* treated maize husk, *Pleurotus pulmonarius* treated maize husk, *Pleurotus ostreatus* treated maize husk, SEM= Standard error of mean.

improvement in the digestibility of the fungal treated substrate, which can be ascribed to the increase in CP and decrease in the fiber components. This result is consistent with reports elsewhere (16, 17 and 18). Furthermore, The high volume of gas obtained in the treated substrates may be the results of treatment effects on the cell wall content (NDF and ADF). This findings is in agreement with the assertion elsewhere (17) which stated that cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters.

In Table 3, the results of *in vitro* gas production characteristics and estimated gas parameters are shown. Wide variations were observed in the fermentation of the insoluble but degradable fractions (b, ml) with values ranging from 13 (UNMH) to 27.33 (PPMH); High fermentation of the insoluble fraction (b) observed in the treated maize husk is possibly influenced by the carbohydrate fractions readily available to rumen microbial population; this same reason is responsible for the fast rate of gas production in the fungal treated samples.Organic matter digestibility (OMD %) ranged from 33.22 (UNMH) to 46.99 (PPMH), metabilizable energy (MJ/KG DM) ranged from 5.39 (UNMH) to 8.19 (PPMH) and short chain fatty acid (mol) ranged from 0.298 (UNMH) to 0.657 (PPMH).

Table 3: Gas production characteristics and estimated gas production parameters

Parameters	UNMH	PTMH	PPMH	POMH	SEM
gas prod. Characteristics					
b (ml)	13.00^{c}	15.0^{bc}	27.33^{a}	19.0^{b}	0.89
c (h-1)	0.043^{a}	0.071^{a}	0.038^{a}	0.081^{a}	0.01
estimated parameters					
OMD%	33.22^{c}	38.55^{b}	46.99^{a}	41.70^{b}	0.70
SCFA (mol)	0.298^{c}	0.418^{b}	0.657^{a}	0.514^{b}	0.02
ME (MJ/Kg DM)	5.39^{0}	6.20^{bc}	8.19^{a}	7.05^{b}	0.15

abc mean on the same row with different superscripts are significant ly different ($p<0.05$) b=fermentation of the insoluble but degradable fraction c=gas production rate constant, OMD= organic matter digestibility, SCFA=short chain fatty acid. ME= metabolisable energy SEM= Standard error of mean.

The increase contents of OMD observed in the fungal treated maize husk may be because the limiting lignin and crude fiber contents have be reduced (3) coupled with the increase in CP contents. Thus the release of the substrates carbohydrate for fermentation by amylolytic bacteria and protozoa was enhanced (19). This result implies that the rumen microorganisms and the animal have high nutrient uptake. The SCFA which was generally higher in the entire fungal treated husk implies energy availability to the animals. A number of other factors could be responsible for this, such as high gas production in the treated substrate and this is more evident throughout the period of fermentation (20). The estimated ME differed significantly ($P<0.05$) and are comparable to those obtained for corn meal (21) and *Tephrosia candida*/guinea grass mixtures (22).Previous (8) study showed a strong correlation between ME values measured *in vivo* and predicted from 24h *in vitro* gas production and chemical composition of feed. In addition estimated ME in the present study was found to be lower than that reported (22).

The *in vitro* gas production method has been successfully used to evaluate the energy value of several classes of feed (11,22 and 23).Others, (23 and 21) suggested that the *in vitro* gas production technique helps to better quantity nutrient utilization and its accuracy in describing digestibility in animals has been validated in numerous experiments

Conclusion and Application

The results obtained in this study suggest that the treatment of maize husk with the use of fungi will help in conversion of agricultural wastes to value added ruminant feeds. It is therefore recommended that much research should be directed to this area to properly harness the potential benefits.

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