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# NUTRITIVE VALUE AND *IN VITRO* GAS PRODUCTION OF FUNGAL TREATED MAIZE COBS

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## ABSTRACT

Studies were conducted to evaluate maize cob cultured with white-rot fungi: *Pleurotus ostreatus* and *P. pulmonarius* for 21 days as a means of improving their nutritive value for ruminant animals. The substrates obtained after biodegradation were analyzed for the chemical composition, mineral content and *in vitro* fermentation. The results of the chemical composition showed an increase in the crude protein from 3.89% (control) to 10.11% for *Pleurotus ostreatus* treated maize cob (POC) and 7.46% for *P. pulmonarius* (PPC) treated maize cob. On the contrary, the crude fiber (CF) decreased significantly ($p<0.05$) from 28.69 % in the control to 19.53% in POC and 21.48% in PPC. Decrease in the values of neutral detergent fiber (hemicellulose, cellulose and lignin) and acid detergent fiber (lignin and cellulose) were detected. The value obtained for cellulose ranged from 28.70 to 34.70%; hemicellulose ranged from 19.05 to 23.18% and acid detergent lignin ranged from 12.44 to 16.88%. There were significant ($p<0.05$) increase in the Calcium, Magnesium, Iron and Manganese contents of the treated substrates compared with the untreated. The fractional fermentation rate (c $h^{-1}$) was highest for PPC followed by POC. The fermentation of the insoluble but degradable fraction (b) increased significantly ($p<0.05$) from 37.00% in the control to 52.33% in POC and 49.33% in PPC. Gas volume at different incubation period was highest in POC. Methane decreased from 15 ml to 11 ml in the control and PPC, respectively. There were significant ($p<0.05$) differences among the treated and untreated substrates in terms of estimated metabolisable energy (ME), short chain fatty acid (SCFA) and organic matter digestibility (OMD). The estimated metabolisable energy (ME) ranged from 6.63 to 8.59 MJ/Kg DM for the control and POC. The POC showed the highest values for SCFA 0.9517 (µm) and OMD (60.75%). The result showed that fungi treated maize cobs had potential of being converted to value added ruminant feed.

**Key words:** white-rot fungi, biodegradation, *in vitro* fermentation

## INTRODUCTION

The livestock sector plays a significant economic role in most developing countries, and is essential for food security of their rural population. Among the major constraints limiting the development of livestock production in many developing countries, inadequacy of animal feed resources is most often the crucial factor [1]. Cereal crops (maize, wheat or sorghum) and root crops (cassava and potato) are staple foods for the average Nigerian [2].The cost is increasing daily due to increase in ruminant population and increasing man's demand for these food items. In view of this, what many livestock farmers do is to alternatively feed the animals with agro industrial by-products (AIBs) [3]. The AIBs are of low nutritive values and some of them contain anti-nutritional factors [4]. However, agricultural waste such as maize cob may play an important role in animal nutrition if treated with white-rot fungi, especially in Nigeria where huge tonnage of this waste is generated annually. Recently, there has been significant interest in the efficient use of agro industrial residues [5] and agricultural wastes. Maize (*Zea mays*) is grown by an increasing number of small holder farmers, especially in the southern part of Nigeria; it is a prolific crop producing a high amount of maize stover and corn cobs. In the present day, after removal of the maize seeds, the cobs are burnt and sometimes left on the farm to rot. Recent interest in alternative use of agricultural waste has been developed and this includes fungal treatment of waste with the view of converting them into value added rumen ruminant feed. Bioconversion of maize cobs into ruminant feed will not only provide a basis for comparison with already existing chemical treatment but may help to solve environmental problems which are associated with burning and improper disposal. Thus fungal treatment is an environmental friendly recycling biotechnology.

*In vitro* gas production technique using syringes allow quick assessment of nutritional value of feedstuff [4] and where sophisticated equipment is lacking, it is a cheap means of evaluating the nutritive value of feedstuff for ruminants [6]. The method has been used for the estimation of the direct and the indirect gas production in syringes for the determination of short chain fatty acid [7]. The present study was undertaken to determine changes in chemical and mineral composition and *in vitro* digestibility of maize cobs.

## MATERIALS AND METHODS

### Sample Collection

Dried samples of agricultural wastes (maize cobs) were collected from the Teaching and Research Farm, Nasarawa State University, Shabu-Lafia, Nigeria. The samples were milled through a 1 mm screen and oven-treated at 65$^{o}$C until a constant weight was obtained for dry matter determination.

### The fungus

The sporophores of *Pleurotus ostreatus* and *Pleurotus pulmonarius* growing in the wild were collected from Ibadan University botanical garden. These were tissue

cultured to obtain fungal mycelia [8].The pure culture obtained was maintained on plates of potato dextrose agar (PDA).

**Degradation of maize cob by *P. ostreatus* and *P. pulmonarius***

**Preparation of substrate**

The jam bottles (120 ml) used for this study were thoroughly washed, oven dried for 10 min. at 100$^{o}$C. About 25 g of the dried milled substrate were weighed into each jam bottle and 70 ml distilled water were added. The bottle was immediately covered with aluminium foil and sterilized in the autoclave at 121$^{o}$C for 15 min .Each treatment was in triplicates.

**Inoculation**

Each bottle was inoculated at the center of the substrate with two 10.00 mm mycelia disc and covered immediately. They were kept in the dark cupboard in the laboratory at 30°C and 100% relative humidity (RH) by placing water soaked foam on the floor of the experimental room. After 21 days of inoculation, the experimental bottles were harvested by autoclaving again to terminate the mycelia growth. Samples of the biodegraded samples were oven dried to constant weight for chemical analysis and *in vitro* digestibility.

**Chemical analysis**

Crude protein, crude fibre, ether extract and ash contents of the samples were carried out in triplicates as described [9] and amount of crude protein were calculated (N x 6.25). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were assessed using the methods proposed by Van Soest [10]. 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 multipurpose West African Dwarf female goats through suction tube before the morning feed. The goats were 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* (guinea grass).The incubation procedure was as described [11]. The 120ml calibrated transparent plastic syringes with fitted silicon tube at the mouth were used while incubation was in three batch incubation. The incubation temperature was maintained at 39±1$^{o}$C. The buffer (pH 7) containing $NaHCO_3$ + $Na_2HPO_4$ + KCl + NaCl + $MgSO_4.7H_20$ + $CaCl_2$. $2H_2O$ was used and kept in the incubator for warming. About 200mg (n=3) of the feed samples (substrate) was measured and carefully dropped into syringes after removing the plunger. The plunger was returned by pushing the substrate upward the syringe. The rumen liquor was strained through a four layer cheese cloth. Rumen liquor and buffer were mixed together (1:4 v/v) as inoculums, all under continuous flushing with streams of $CO_2$. Using 50 ml plastic calibrated syringe, 30ml inoculums containing cheese cloth strained rumen liquor and buffer were dispensed into the substrate through the silicon tube. The silicon tube in the syringe was then tightened by a metal

clip so as to prevent escape of gas. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, 24, 48, 72, and 96 h. At post incubation period, 4 ml of NaOH (10 M) was introduced using 5 ml capacity syringe to estimate the methane production. The content was inserted into the silicon tube, which was fastened to the 120 ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was agitated while the plunger began to shift position to occupy the vacuum created by the absorption of $CO_2$. The volume of methane was read on the calibration. To determine the actual gas produced, the average of volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

## STATISTICAL ANALYSIS

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 GV – 0.0601 [12] 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 SAS [13] options.

## RESULTS

### Chemical composition

The chemical composition of the fungal treated and untreated maize cob is presented in Table 1. Crude protein, (CP) crude fibre, (CF), neutral detergent fibre (NDF), Acid detergent fibre (ADF), Acid detergent lignin (ADL), cellulose and hemicellulose were affected by fungal treatment. CP increased from 3.89% in the control to 10.11% for the *Pleurotus ostreatus* treated maize cob (POC) and 7.46% for the *P Pulmonarius* treated maize cob (PPC). There was consistent decrease in the CF, NDF, NDL, ADL and cellulose in the fungal treated maize cob.

### Mineral composition

Table 3 shows the major and trace mineral composition of the treated and untreated maize cob. Wide variations exist in the results obtained for the mineral composition. The calcium levels ranged from 0.422% in the control to 0.987 % POC. Phosphorus content ranged from 0.026% in the control to 0.168% (POC). Sodium content was generally low and ranged from 0.038% in POC to 0.351% (control). Potassium content ranged from 0.157% in POC to 0.841 % in control. The content of iron ranged from 0.3560 ppm in control to 3.36 ppm in PPC. Copper was lowest in POC (0.034 ppm) and highest in the control (0.078 ppm). There was significant decrease in the manganese content ranging from 0.113 ppm in POC to 0.155 ppm in PPC and 0.065 ppm in control. Zinc content also differed significantly ($p<0.05$) between the treated and untreated maize cobs.

**Gas volume and *in vitro* gas production characteristics**

Gas volume and *in vitro* gas production characteristics are presented in Table 3. The result indicated that cumulative gas volumes at 24, 48, 72 and 96 h after incubation were significantly different ($p<0.05$). The gas volumes ranked from highest to the lowest were POC, PPC and control. Figure 1 shows the *in vitro* gas production. The gas volume at asymptote (b) described the fermentation of the insoluble fraction. The fermentations of the insoluble fraction of the fungal treated and untreated maize cob were significantly different ($p<0.05$).However, the values obtained for POC and PPC were not significantly different ($p>0.05$). The fermentation of insoluble fractions of the control, POC, and PPC were 37.00, 52.33 and 49.33 ml, respectively. The fermentation rate ranged from 0.02929 to 0.1246 ($h^{-1}$). There were significant differences in the values obtained for the fermentation rates. The fastest rates were obtained for PPC followed by POC and the slowest rate observed in the control. Methane production was 11 ml in PPC, 12 ml in POC and 15 ml for the control.

**Estimated metabolisable energy (ME), short chain fatty acid (SCFA), and organic matter digestibility (OMD)**

The ME value for the treated and untreated maize cob was calculated from the amount of gas produced at 24hr of incubation with the supplementary CP and EE analysis. The estimated metabolisable energy (ME, MJ/kg DM), short chain fatty acid (SCFA, µm) and organic matter digestibility (OMD %) from gas production for the control, POC and PPC are presented in Table 4. The estimated ME for the different substrates differed significantly ($p<0.05$). The values obtained increased from 6.63 MJ/kg DM in the control to 8.13 MJ/kg DM for PPC and 8.59 MJ/kg DM for POC.

The SCFA estimated for the control, POC, PPC were 0.6649, 0.9517 and 0.8959 µm. SCFA estimate for control differed significantly ($p<0.05$) from the others. The OMD differed significantly ($p<0.05$) between the control and the fungal treated maize cobs although higher value was obtained in POC.

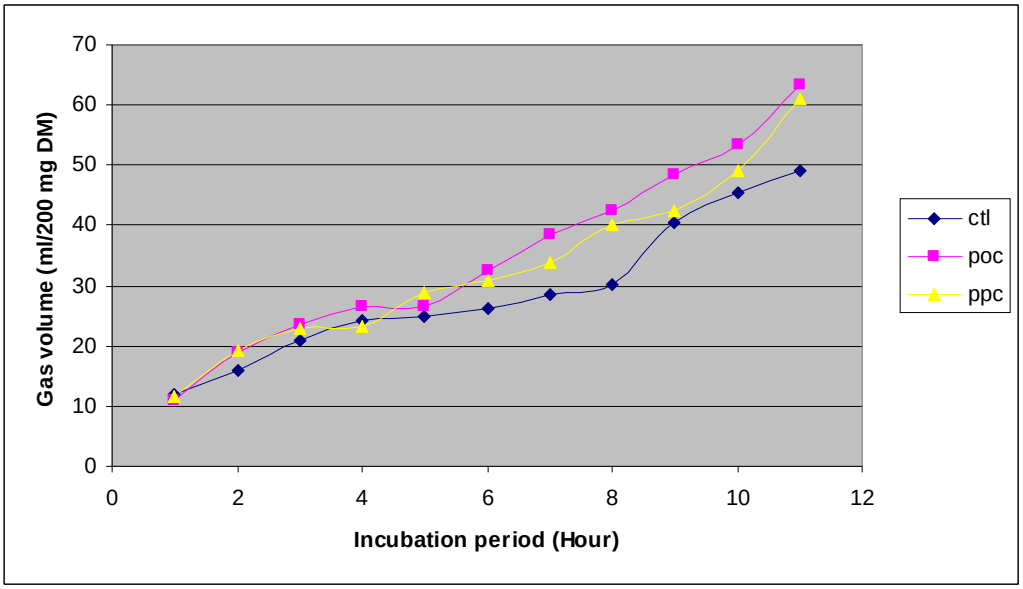


**Figure 1: *In vitro* gas production pattern of fungal treated and untreated maize cob**

## DISCUSSION

The content of crude protein (CP) in the treated substrate was higher compared with the untreated due probably to the addition of fungal protein during solubilization and degradation [14]. The CP increase could also be due to the increased fungal biomass [15]. This result agrees with earlier findings [16, 17]. On the other hand, the decreasing of crude fibre (CF) and CF fractions in the treated substrate could be due to the ability of the fungi to secrete hydrolyzing and oxidizing enzymes, which could aid the decomposition of recalcitrant compounds in the waste into utilizable compounds [18, 19]. This observation confirms that maize cobs, which are nuisance in the environment, could be successfully recycled into value added substrate for ruminant feeds.

Generally, the major minerals except calcium and magnesium after fungal treatment were within the range values previously reported [20]. The values are adequate to meet the requirement for growth, reproduction and milk production in West African sheep and goat [21]. The calcium and phosphorus ratio observed in both treated and untreated maize cob were not within approved 1:1 to 1:2 range recommended [20]. All the trace minerals observed in both the treated and untreated substrates were extremely deficient. This, therefore, implies that provision of feeds fortified with minerals in form of either salt lick or diet inclusion would be necessary [21].

It can be seen from the results obtained in the study that fermentation of the insoluble fraction (b) of the fungal treated substrate was higher compared with the control, possibly a reflection of improved lignin content. It has been reported that the high

fermentation of the insoluble fraction obtained in the treated substrates may also be influenced by the carbohydrate fraction readily available to the microbial population [8, 14, 17, 22].

The high rates of gas production in the treated substrates could be related to its high CP content and low content of CF, NDF, ADF and ADL [23]. The relatively low content of fibre can facilitate the colonization of the feed by the rumen microbial population, which in turn might induce higher fermentation rate, therefore improving digestibility [24]. As the fermentation process is partially regulated by the fibrous content of the feeds [4], the treated maize cob fermented faster than the untreated.

The cumulative gas volume at different hours of incubation was higher in the treated compared to the untreated. Other authors have suggested that gas volume at 24 h after incubation is in direct relationship with ME [25] and the 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 [26]. Methane production has negative effect on the animal on one hand as it is an energy loss to the animal while on the other hand it accumulates in the rumen resulting in bloating [20].

The lower SCFA recorded in the control is expected because of the lower production and this is evident in the first 24 h of incubation (Fig. 1).The gas production from cereal straws and different classes of feeds [27] incubated *in vitro* in buffered rumen fluid was closely related to the production of SCFA, which was based on carbohydrate fermentation [1]. Other researchers reported a close association between SCFA and gas production in which the higher values of SCFA obtained in the treated substrate implies energy availability to the animal. High digestibility of organic matter estimated for the fungal treated maize cob suggested that the microbes in the rumen and animals had high nutrient uptake [8]. The higher fibre content of the control probably resulted in lower OMD since high NDF and ADF content in feedstuff resulted in lower fibre degradation [24]. The estimated ME was comparatively higher in the treated substrates suggesting an improvement in the energy station of the substrate, and thus the potential of been incorporated in conventional feed mixtures.
A mutual relationship exists between total gas production and ME, OMD, and SCFA [28].

## CONCLUSION

The outcome of this study showed that fungal treatment of bean pods might be useful protein and energy supplement in animal feeding. The digestibility of the fungal treated maize cobs was significantly higher, within the treated substrates. This could be traced to the fact that digestibility is influenced by fungal species, type of substrate and the fermentation period. More work is, therefore, advocated where other strains of fungi would be tested on different substrates.

**Table 1: Chemical composition (g/100gDM) of fungal treated and untreated maize cobs**

| PARAMETERS | CONTROL | POC | PPC | SEM |
|---|---|---|---|---|
| Crude protein | 3.89[c] | 10.11[a] | 7.46[b] | 0.13 |
| Crude fiber | 28.69[a] | 19.53[c] | 21.48[b] | 0.11 |
| Ether extract | 5.68[a] | 5.31[b] | 5.99[a] | 0.11 |
| Ash | 7.67[a] | 6.25[b] | 7.24[a] | 0.08 |
| Dry matter | 90.83[a] | 89.16[a] | 89.69[a] | 0.06 |
| Neutral detergent fiber | 70.63[a] | 64.59[c] | 66.74[b] | 0.22 |
| Acid detergent fiber | 51.58[a] | 41.67[c] | 43.56[b] | 0.12 |
| Acid detergent lignin | 16.88[a] | 12.44[c] | 14.86[b] | 0.03 |
| Cellulose | 34.70[a] | 29.23[b] | 28.70[b] | 0.10 |
| Hemicellulose | 19.05[b] | 22.92[a] | 23.18[a] | 0.17 |

Means on the same row with different superscripts are significantly different ($p < 0.05$) POC = *Pleurotus ostreatus* degraded maize cob, PPC = *Pleurotus pulmonarius* degraded maize cob, SEM= standard error of the mean.
NB: 1. Ether extract means do not have the superscripts
2. NDF and Dry matter superscripts have 'a'and 'c' and no ' b'. Why?
3. The arrangement of values down the columns is not straight

**Table 2: Major mineral (mg/kg) and trace minerals (ppm) composition of fungal treated maize cob**

| MINERALS | CONTROL | POC | PPC | SEM |
|---|---|---|---|---|
| Major minerals | | | | |
| Calcium | 0.422[c] | 0.987[a] | 0.490[c] | 0.01 |
| Phosphorus | 0.026 | 0.168 | 0.144 | 0.03 |
| Magnesium | 0.084[c] | 0.347[a] | 0.237[b] | 0.00 |
| Potassium | 0.841[a] | 0.157[c] | 0.693[b] | 0.00 |
| Sodium | 0.35[a] | 0.038[b] | 0.062[b] | 0.00 |
| Trace minerals | | | | |
| Iron | 0.356[c] | 1.830[b] | 3.36[a] | 0.06 |
| Copper | 0.078[a] | 0.034[b] | 0.041[b] | 0.00 |
| Zinc | 0.057[b] | 0.038[c] | 0.145[a] | 0.00 |
| Manganese | 0.065[c] | 0.113[b] | 0.155[a] | 0.00 |

Means on the same row with different superscripts are significantly differen ($p < 0.05$) POC = *Pleurotus ostreatus* degraded maize cob, PPC = *Pleurotus pulmonarius* degraded maize cob, SEM= standard error of the mean.

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

| | CONTROL | POC | PPC | SEM |
|---|---|---|---|---|
| Gas production characteristics | | | | |
| b (ml | $37.00^{b}$ | $52.33^{a}$ | $49.33^{a}$ | 0.57 |
| c ($h^{-1}$) | $0.0292^{c}$ | $0.1225^{b}$ | $0.124^{a}$ | 0.03 |
| Gas volume (ml) | | | | |
| GV 24 | $30.33^{b}$ | $42.33^{a}$ | $40.00^{a}$ | 0.53 |
| GV48 | $40.33^{b}$ | $48.33^{a}$ | $42.33^{b}$ | 0.51 |
| GV 72 | $45.33^{c}$ | $53.33^{a}$ | $49.00^{b}$ | 0.53 |
| GV 96 | $49.00^{a}$ | $63.33^{a}$ | $61.00^{a}$ | 0.48 |
| $CH_4$ | $15.00^{a}$ | $12.00^{b}$ | $11.00^{b}$ | 0.33 |

Means on the same row with different superscripts are significantly different ($p < 0.05$) POC = *Pleurotus ostreatus* degraded maize cob, PPC = *Pleurotus pulmonarius* degraded maize cob, SEM= standard error of the mean. b= fermentation of the insoluble but degradable fraction, c= gas production rate constant, GV = gas volume

**Table 4: Metabolisable energy (ME) (MJ/kg DM), Short chain fatty acid (SCFA) (µm) and organic matter digestibility (OMD) (%)**

| PARAMETERS | CONTROL | POC | PPC | SEM |
|---|---|---|---|---|
| ME | $6.63^{c}$ | $8.59^{a}$ | $8.13^{b}$ | 0.07 |
| SCFA | $0.665^{b}$ | $0.9517^{a}$ | $0.8959^{a}$ | 0.01 |
| OMD | $48.32^{b}$ | $60.75^{a}$ | $58.15^{a}$ | 0.48 |

Means on the same row with different superscripts are significantly different ($p < 0.05$) POC = *Pleurotus ostreatus* degraded maize cob, PPC = *Pleurotus pulmonarius* degraded maize cob, SEM= standard error of the mean. ME = metabolisable energy, SCFA= short chain fatty acid, OMD= organic matter digestibility.

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