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Biodegradation of Cowpea Shells by Pleurotus Specie for it Use as Ruminant Feed

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Abstract: In this work we present the study of bioconversion of cowpea shells in solid state fermentation with edible mushrooms, *Pleurotus ostreatus* and *Pleurotus pulmonarius* so as to upgrade its nutritional values and digestibility for its use as ruminants feed. The results obtained showed an increase in the crude protein (CP) from 12.25% for the control to 17.04% for *P. ostreatus* degraded cowpea husk (POT) and 15.41% for the *P. pulmunarius* degraded cowpea husk (PPT). A significant ($P<0.05$) decrease in the values of neutral detergent fiber (hemicellulose. Cellulose and lignin) and acid detergent fiber (lignin and cellulose) were detected. The estimated organic matter digestibility (OMD) improved from 45.65% (control) 62.14% for POT and 59.52% for PPT. Similar trend was also observed in the estimated ME from 6.97 MJ/Kg DM to 9.41MJ/Kg DM for POT and 9.103 MJ/Kg DM for PPT, respectively. Wide variations also exist in the factional rate of gas production (c) and fermentation of the insoluble fraction (b).

Key words: Bioconversion % Cowpea shells % Solid state fermentation % Edible mushroom % Digestibility

INTRODUCTION

Huge tonnage of agricultural wastes and agro industrial products are generated annually all over the world. From the production, processing and even consumption, there are great varieties of left over, which creates problems of disposal. In Nigeria, appropriate method of recycling these wastes into form that will be useful to the animals has generated interest in recent times. The digestibility of lignocellulosics materials is very low because of the inherent lignin in the cellulose and hemicellulose matrix. Cell wall lignifications of crop residues have been reported as a major factor that limit the availability of cell wall structural carbohydrates for animal utilization [1]. The effect of lignin on cellulose degradation by cellulose and its role in cellulose recovery from organisms during hydrolysis of lignocellulosics reveal that lignin had an inhibitory effect on rumen micro organisms [2].

The natural ability of some fungi, particularly some species of Pleurotus to upgrade lignocellulosics materials into animal feeds is well documented in literature. Previous studies have shown the feasibility of using agricultural wastes to produce animal feed [3] and as substrates for mushroom production [4-6]. In the present work we study the biodegradation of cowpea shells by *P. ostreatus* and *P. pulmonarius* for its use as animal feed.

MATERIALS AND METHODS

Sample Collection: Dried samples of agricultural wastes (cowpea shells) 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.

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 [7].The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

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Degradation of Cowpea Shells by *P. ostreatus* and *P. pulmonarius*

Preparation of Substrate: The jam bottles (120mls) used for this study were thoroughly washed, dried for 10 min. at 100°C and 25.0 g of the dried milled substrate were weighed into each jam bottle and 70ml distilled water were added. The bottle was immediately covered with aluminum foil and sterilized in the autoclave at 121°C for 15 min. Each treatment was triplicates.

Inoculation: Each bottle was inoculated at the center of the substrate with 2, 10.0 mm mycelia disc and covered immediately. They were kept in the dark cupboard in the laboratory at 30°C and 100% Relative humidity (RH). 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: Nitrogen (N) content of the agricultural wastes was determined by the standard Kjeldhal method [8] 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 the methods proposed by Van Soest *et al*. [9]. 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 [10] 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 Menke and Steingass [11] 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 $MgSO_3$ 7H2O + 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 Fievez *et al.* [12]. 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 Menke and Steingass [11]. 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 [13]. 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 [11], organic matter digestibility (OMD) (%) was assessed as OMD = 14.88 + 889Gv + 0.45CP + 0.651XA [11], short chain fatty acids (SCFA) as 0.0239 V – 0.0601 [14] 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 [15] options.

RESULTS AND DISCUSSION

Changes in the Chemical Composition: The results obtained for the chemical composition is presented in Table 1. The two fungi used increased significantly ($P<0.05$) the crude protein (CP) contents of the cowpea shells compared with the untreated. The value obtained increased from 12.25% for the untreated (control) to 17.04% for the POT and 15.41% for PPT. The CP increase may have been a result of increased fungal biomass [16]. CP increase could also be due to secretion of certain extra cellular enzymes which are proteineous in nature into the waste during their breakdown and its subsequent metabolism [17]. This result is in agreement with the findings of Akinyele and Akinyosoye [18] who obtained

Table 1: The chemical composition (g/100g DM) of cowpea shell treated with white-rot fungi

Parameters	Control	POT	PPT	SEM
Dry matter	90.83^{a}	89.16^{c}	89.69^{a}	0.06
Crude protein	12.25^{b}	17.04^{a}	15.41^{a}	0.28
Crude fiber	30.79^{a}	24.18^{b}	23.52^{b}	0.11
Ether extract	6.13^{a}	5.18^{b}	5.39^{ab}	0.14
Ash	7.64^{a}	6.13^{c}	7.09^{b}	0.09
NFE	43.19^{b}	47.47^{a}	48.59^{a}	0.40
NDF	71.44^{a}	65.54^{b}	61.76^{c}	0.21
ADF	52.35^{a}	45.23^{b}	42.11^{c}	0.41
ADL	17.15^{a}	14.18^{b}	11.41^{c}	0.84
Cellulose	35.20^{a}	31.05^{b}	30.70^{b}	0.50
Hemicellulose	19.09^{a}	21.31^{b}	19.65^{a}	0.44

a,b, 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, POT= *Pleurotus ostreatus* degraded cowpea shells, PPT = *Pleurotus pulmonarius* degraded cowpea shells, SEM=Standard error of mean

Table 2: Some major (g/100g DM) and trace minerals (ppm) composition of treated and untreated cowpea shells

Major minerals	Control	POT	PPT	SEM
Calcium	7.36^{a}	0.847^{b}	0.417^{c}	0.01
Phosphorus	0.91^{a}	0.074^{b}	0.082^{b}	0.02
Magnesium	5.34^{a}	0.426^{b}	0.395^{b}	0.02
Sodium	0.514^{a}	0.041^{b}	0.029^{b}	0.01
Potassium	0.345^{b}	1.120^{a}	0.321^{b}	0.01
Trace minerals				
Iron 4.98^{b}	0.112^{c}	6.84^{a}	0.02	
Copper	0.02^{b}	0.043^{a}	0.0462^{a}	0.00
Zinc 0.0586^{a}	0.0574^{a}	0.0315^{b}	0.00	
Manganese	0.1613^{a}	0.018^{c}	0.077^{b}	0.01

a,b,c, means on the same row with different superscripts are significantly varied ($P < 0.05$),SEM= standard error of mean, POT= *Pleurotus ostreatus* degraded cowpea shells, PPT = *Pleurotus pulmonarius* degraded cowpea shells

an increase in CP contents *Volvariella volvacea* treated agro- wastes. On the other hand, the ether extracts (EE) decreased in the treated samples and may have been due to the consumption of some fatty acids by micro organisms as a suitable energy source for growth [19]. Likewise, the crude fiber (CF) consistently decreased in the treated samples. The overall means were 30.97%, 24.18% and 23.52% for the control, POT and PPT respectively. This might be due to the ability of the fungi used to produce certain enzymes during the vegetative and reproductive phases with lignocelluloses degrading properties [20]. There was a significant depletion in the cellulose contents of the treated samples, while there were no significant difference ($P>0.05$) in the hemicellulose content of the control and PPT, however, it differed significantly from POT. Decrease in cellulose may be due to the ability of the fungi used to utilize cellulose and hemicellulose through enzymatic system which could degrade polymer in cellulose and hemicellulose to its monomer which can serve as a carbon source by the fungus to produce biomass [21-23]. In this case, the cellulose was over utilized causing it to be depleted. Whereas, the variations in the hemicellulose contents of the fungal treated samples may be due to specie differences.

The major mineral contents with the exception of potassium were more utilized by the fungi used resulting in it depletion. The cowpea shells were high in Ca, P and Mg. It is thus expected that on decomposition that these would be released to influence the growth of fungi used. This however, depends on the physiological behaviour of the fungi used, type of substrate and the duration of fermentation. A depletion of this nature will require fortification of minerals by the introduction of minerals lick or mineral addictives.

Gas Volume: Table 3 showed that the results of the gas volume produced at 24, 48, 72 and 96 h of incubation. A significant difference in volume of gas produced at different incubation period was observed in

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

Parameters	Control	POT	PPT	SEM
C	0.002^{C}	0.009^{b}	0.013^{a}	0.00
b	34.33^{C}	38.00^{b}	43.67^{a}	0.19
Gv 24	33.00^{b}	49.00^{a}	47.00^{a}	0.58
Gv 48	36.00^{b}	55.33^{a}	53.00^{a}	0.97
Gv 72	40.00^{C}	65.07^{a}	60.00^{b}	0.87
Gv 96	45.00^{b}	71.67^{a}	69.33^{a}	0.51
CH_4	10.00^{a}	6.00^{b}	4.00^{c}	0.67

a,b,c, means on the same row with different superscripts are significantly varied ($P < 0.05$), SEM= standard error of mean, POT= *Pleurotus ostreatus* degraded cowpea shells, PPT= *Pleurotus pulmonarius* degraded cowpea shells, b= fermentation of the insoluble fraction, c= gas production rate constant, CH_4 = methane

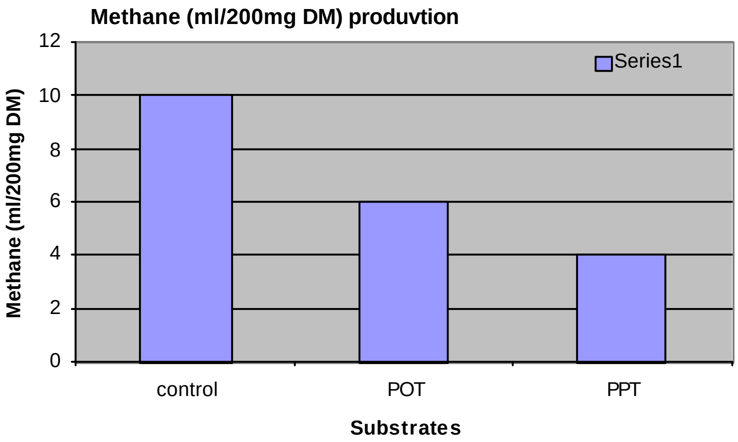

Fig. 1: Methane (ml/200mg DM) production of treated and untreated cowpea shells

all the treatment. Higher gas volumes were obtained in the treated shells compared with the untreated. High gas production at incubation may be due to high soluble carbohydrate fraction [24]. Since gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation and carbohydrates fractions, so the higher gas volume observed in the treated cowpea shells could be related to depletion in the fiber fractions [25]. This is consistent with De Biover *et al*. [26] who reported that gas production was negatively related with NDF content and ADL provided a favorable environment for microbial attachment to the treated substrates as the incubation time progresses hence, the variations in gas volume at different incubation time. Figure 2 shows that *in vitro* gas production pattern over a period of 96 hours of incubation.

Gas production rate constant (c), also differed significantly with the fastest rate obtained in PPT followed by POT. The faster rates obtained in the fungal treated substrates could be due to the depletion in the lignin contents. Nsahlai [27] and Larbi *et al.* [28] reported that there were positive correlation between CP and the rate of gas production and negative correlation between NDF and ADF and the rate and extent of gas production. Kinetics of gas production is dependent on the relative proportions of soluble and insoluble particles of the feed [29].

Figure 1 shows that bar chart of methane (CH_4) production. The reduction in the volume of methane (CH_4) produced in the treated substrates could be due to the conversion of CO_2 and H_2 to acetate instead of CH_4 [30]. This process mainly occurs when low roughages diets containing high proportions of sugars and protein are fed [31]. Tavendale *et al*. [32] stated that rapidly fermented feeds as observed in the treated cowpea shells are likely to produce a lower proportion of acetate and a higher proportion of propionate and butyrate. Methane production is an energy loss to the animals, so a lower production as obtained in this study implies energy conservation for the ruminants. Figure 2 shows the *in vitro* gas production of treated and untreated cowpea shells.

Estimated Short Chain Acid (SCFA) Organic Matter Digestibility OMD and Metabolisable Energy (ME): The results of SCFA, OMD and ME at 24h after

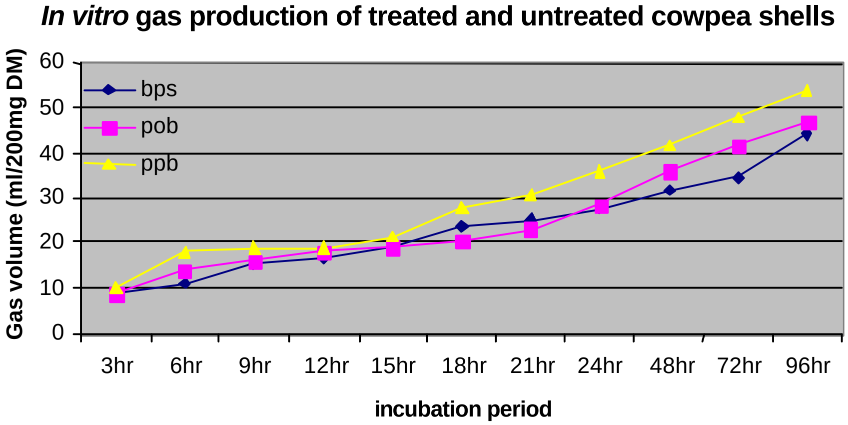

Fig. 2: *In vitro* gas production of treated and untreated cowpea shells

Table 4: Short chain fatty acid (SCFA), organic matter digestibility (%) and metabolisable energy (MJ/Kg DM)

Parameters	Control	POT	PPT	SEM
SCFA (μM)	0.849^{b}	1.23^{a}	1.18^{a}	0.01
OMD (%)	45.68^{b}	62.14^{a}	59.52^{a}	0.54
ME(MJ/Kg DM)	6.97^{a}	9.41^{a}	9.03^{a}	0.08

a,b,c, means on the same row with different superscripts are significantly varied ($P < 0.05$), SEM = standard error of mean, POT= *Pleurotus ostreatus* degraded cowpea shells, PPT = *Pleurotus pulmonarius* degraded cowpea shells, SCFA= short chain fatty acid, OMD= organic matter digestibility, ME= metabolisable energy, MJ/Kg DM= mega joule per kilogram dry matter

incubation are shown in Table 4. Increase in SCFA was observed in the treated substrates. Blummel and Orskov [33] stated that gas production from different class of feeds incubated *in vitro* in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation. A high gas production is thus expected to yield higher values of SCFA. SCFA is an indication of energy availability to the animal, a higher values as indicated by the results obtained in this study suggest that more energy are available to the ruminant in the fungal treated substrates compared with the untreated.

The OMD was highest for POT and this is not significantly different from PPT but differed significantly from the control which had the least OMD. Increase in OMD may be due to the breakdown of the CF and ADF contents of the treated substrates and increase in its CP contents. It could also be due to breakdown of cell wall bonds during the fermentation of the substrates by the fungi [34, 35]. There is a correlation between CP content and OMD and it is negatively correlated with CF, ADF and NDF [36, 37].

The predicated ME profile were widely varied between the treated and untreated samples with the highest value obtained in the treated substrates. The data showed that there was no significant difference among the treated substrates. There was a positive correlation between ME calculated from *in vitro* gas production together with CP and EE with ME value of conventional feeds measured *in vitro* [12].

CONCLUSION

Biological treatment of cowpea husk is a promising way of converting wastes into value added ruminant feeds. Apart from the increase in CP contents of the biodegraded samples, increased digestibility was also observed. This suggests that biological treatment of wastes is suitable for conversion of wastes into ruminant feeds. However, more work in these areas is necessary to verify the effectiveness of these degraded substrates on the performance of live animals.

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