

Full Length Research Paper

Conversion of sorghum stover into animal feed with white-rot fungi: *Pleurotus ostreatus* and *Pleurotus pulmonarius*

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Treatment of crop residues with some species of white-rot fungi can enhance the nutritive value. After the fungal treatment of sorghum (*Sorghum bicolor*) stover with two white-rot fungi in a solid state fermentation, the chemical composition and *in vitro* digestibility of the resultant substrate was determined. The results show a significant ($p < 0.05$) increase in crude protein contents from 2.54% for the control to 4.51% for *Pleurotus ostreatus* (POS) and 4.59% for *Pleurotus sajor pulmonarius* (PPT). The nitrogen free extract (NFE) content also increased significantly ($p < 0.05$). The crude fibre decreased significantly from 31.65% for the control to 27.49% for POS and 23.54% for PPS. There were also consistent significant decreases ($p < 0.05$) in the values obtained for NDF, ADF ADL. Significant differences were also observed in the hemicellulose and cellulose contents. Fermentation of the insoluble fraction (b) was enhanced by the fungal treatment. Wide variations were also observed in the mineral contents of the different substrates. The estimated organic matter digestibility (OMD) ranged from 42.99 to 57.75%, short chain fatty acid ranged from 0.56 to 0.94 μM and metabolisable energy (ME) ranged from 5.97 to 8.21 MJ/Kg DM. This result suggests that fungal treatment of sorghum stover resulted in improved CP and digestibility, hence its potential in ruminant nutrition.

Key words: Chemical composition, crop residues, *in vitro* digestibility, solid state fermentation, sorghum stover, white-rot fungi.

INTRODUCTION

The Nigerian livestock industry competes with other sectors for the consumption of conventional ingredients.

This competition often pushes the prices of finished feed upwards (Iyayi and Aderolu, 2004). A search for the alternative use of lignocellulosic materials is thus neces-

sary as a way of finding lasting solution to shortage of feedstuffs.

Sorghum stover is available in plenty from the middle belt to the northern areas of Nigeria (Belewu, 1999). Klopfenstein (1978) reported that there is an estimate of about 1 kg of residue from 1 kg of grain produced on the field. After the removal of grains, the stovers are left in the field where natural breakdown occurs while a larger fraction is burnt to generate steam for the stripping. The feeding value of these stovers is very low because of its high crude fiber and lignin, low crude protein vitamins and minerals. physical or chemical methods, but chemical method of improvement has been greatly limited, especially in the developing country such as Nigeria, by high cost of chemicals, safety concerns and the possible environmental consequences. Recent advances in fungal

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Abbreviations: CP, Crude protein; CF, crude fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; POS, *Pleurotus ostreatus*; PPT, *Pleurotus sajor pulmonarius*; NFE, nitrogen free extract; OMD, organic matter digestibility; ME, metabolisable energy; DM, dry matter; PDA, potato dextrose agar; SCFA, short chain fatty acid.

treatment of lignocellulosics have shown that certain strains of white-rot fungi have the natural ability in upgrading lignocellulosics.

This study was therefore conducted to investigate the improvement in the nutritive value and *in vitro* digestibility of sorghum stover after biodegradation with white-rot fungi.

MATERIALS AND METHODS

Sample collection

Dried samples of agricultural wastes (sorghum stover) 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°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 University of Ibadan botanical garden, Nigeria. These were tissue cultured to obtain fungal mycelia (Jonathan and Fasidi, 2001). The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

Degradation of cowpea shells by *P. ostreatus* and *P. pulmonarius*

Preparation of substrate

The jam bottles (120 ml) used for this study were thoroughly washed and dried for 10 min at 100°C. 25.00 g of the milled dried samples, were weighed into each jam bottle and 70 ml 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, 10.00 mm mycelia disc and covered immediately. They were kept in the dark cupboard in the laboratory at 30°C and 100% relative humidity (RH). 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 (AOAC, 1991) 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. (1991). 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% *Panicum 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 (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/liter) of 9.8 NaHCO₃ + 2.77 Na₂HPO₄ + 0.57 KCl + 0.47 NaCl + 2.16 MgSO₃ 7H₂O + 16 CaCl₂ 2H₂O (1:4 v/v) under continuous flushing with CO₂ 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°C and the volume of 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 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 produced 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), while organic matter digestibility (OMD) (%) was assessed as OMD = 14.88 + 889Gv + 0.45CP + 0.651XA (Menke and Steingass). Short chain fatty acids (SCFA) were obtained as 0.0239 V – 0.0601 (Getachew et al., 1999) where Gv, CP CF and XA are total gas volume, Crude protein, crude fiber and ash, respectively.

Statistical Analysis

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 (1988) option.

RESULTS AND DISCUSSION

Chemical composition and mineral contents of treated and untreated sorghum stover

Table 1 presents the chemical composition of both the treated and untreated sorghum stover. The Crude Protein (CP) contents of the fungal treated substrates increased from 2.54% for the control to 4.51% for *Pleurotus ostreatus* (POS) and 4.59% for *Pleurotus Pulmonarius* (PPS) treated substrates, respectively. The increase in the CP contents may be due to secretion of certain extra cellular enzymes which are proteineous in nature into the waste during their breakdown and its subsequent metabolism (Kadiri, 1999). CP increase could also be due to the capture of excess nitrogen by aerobic fermentation

Table 1. Chemical composition (g/100gDM) of treated and untreated sorghum stover.

Parameters	Control	POS	PPS	SEM
Dry matter	91.23 ^a	81.42 ^b	80.56 ^c	0.00
Crude protein	2.54 ^b	4.51 ^a	4.59 ^a	0.07
Crude fiber	31.65 ^a	27.49 ^b	23.54 ^c	0.78
Ether extract	6.19 ^a	4.99 ^b	6.26 ^a	0.11
Ash	6.28	6.35	6.39	0.17
NFE	53.34 ^c	56.57 ^b	59.30 ^a	0.26
NDF	70.23 ^a	66.43 ^b	66.58 ^b	0.15
ADF	46.69 ^a	43.08 ^b	38.87 ^c	0.77
ADL	15.21 ^a	13.39 ^b	12.56 ^c	0.10
Cellulose	31.48 ^c	29.69 ^b	26.31 ^c	0.22
Hemicellulose	23.54 ^b	23.35 ^b	27.71 ^a	0.22

^{a,b,c}Means on the same row with different superscripts are significantly varied ($P < 0.05$).
 POS = *P. ostreatus* degraded sorghum stover, PPS = *P. pulmonarius* degraded sorghum stover, and SEM = standard error of mean.

(Sallam et al., 2008) suggesting that the treated substrates are good source of protein for livestock. This agrees with the findings of Zadrazil (1993), Belewu and Okhawere (1998), and Iyayi and Aderolu (2004).

On the other hand, the decreasing of CF and CF fractions (NDF, ADF and ADL) in the treated sorghum stover may be the result of cellulase enzymes secreted by cellulolytic fungi. White-rot fungi produce extracellular lignin modifying enzymes, the best characterized of which are laccase, lignin peroxidase and manganese peroxidases (Isikhuemhen and Nerude, 1999). Belewu and Balewu (2005) reported that decrease in the fiber fractions could be due to the production of various enzymes during the vegetative and reproductive phases with lignocellulose degrading properties. This agrees with the result obtained in this study. The degradation of lignin, a complex polymer (Kuforiji and Fasidi, 2004) is important because using lignin degrading fungi is to make as much as possible the digestibility of the substrates degraded (Adenipekun and Fasidi, 2005). Zadrazil (2000) observed that increase in lignin content in plants materials used as feed correlates with the decrease of digestibility for rumen micro organisms. The over utilization of the cellulose as energy source for the fungi used may be responsible for the depletion of the cellulose contents. Whereas, the hemicellulose was much reduced in the POS, it however increased in PPS, this suggest energy availability for the animals. The variations observed in the results obtained for the two fungi used could be due to specie differences. More work is therefore required in this area to determine the optimal fer-

mentation period. There were variations in the mineral contents of the substrates under study. However, no significant difference ($P > 0.05$) was observed in the calcium, iron and magnesium contents. Mushrooms contain appreciable amount of potassium, phosphorous copper and iron but low level of calcium (Anderson and Feller, 1942). The variations in the mineral content

observed in this study, may not be unconnected with the type of substrates used, duration of fermentation and the specie of fungi used (Isikhuemhen et al., 1996).

Gas Production Characteristics

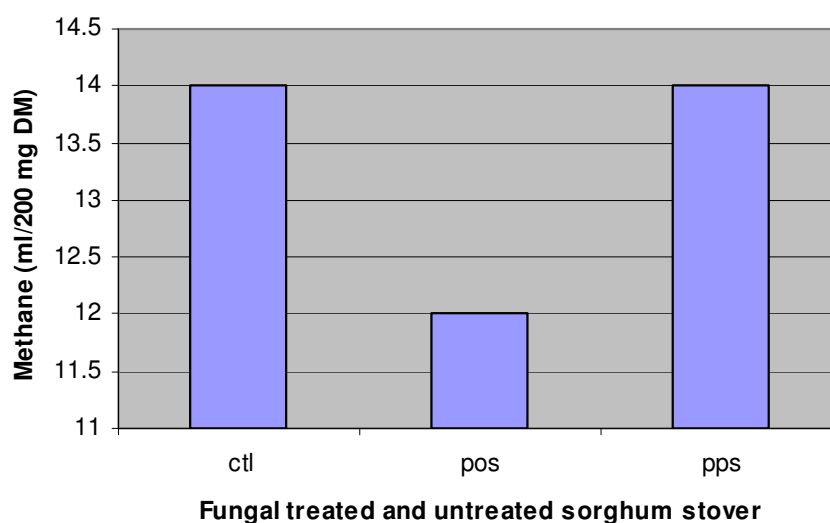
Gas production from the fermentation of the treated and untreated sorghum stover were measured at 3, 6, 12, 15, 18, 21, 24, 48, 72 and 72 h using *in vitro* gas production of (Menke and Steingass, 1988). Table 2 shows the results of gas production characteristics. The production from the insoluble but degradable fractions (b) was significantly higher in the treated substrates. This suggests that the carbohydrates fractions were readily available to the microbial population (Chumpawadee et al., 2007). This could also be traceable to the depletion of the lignin content (Table 1) of the treated substrates. The gas volume at the different incubation hours 24, 48, 72 and 96 h were also higher in all the treated substrates. This is due to high NFE contents of the fungal treated substrates. Tavendale et al. (2005) stated that rapidly fermented feeds (as observed in the fungal treated stover) are likely to produce a lower proportion of acetate and higher proportion of propionate and butyrate and cell wall content (NDF and ADF) are negatively correlated with gas production, its reduction therefore enhances gas production. Also the high level of lignin in the control explains in part the low gas production thus confirming the low feeding value of this feed. Low degradation has been associated with the lignin contents. From the present study, the lignin contents of the fungal treated sorghum stover had been depleted hence opening up the carbohydrate contents to the attacks of the rumen microbes and this in turn increase gas production. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Wolin, 1960; Steingass and Menke, 1996) and substantial

Table 2. Some major (g/100gDM) and trace (ppm) mineral composition of fungal treated sorghum stover.

Parameters	Control	POS	PPS	SEM
Major minerals				
Calcium	2.88	2.88	0.20	0.00
Phosphorus	0.97 ^a	0.09 ^b	0.096 ^b	0.00
Magnesium	7.70 ^a	0.77 ^b	0.147 ^c	0.00
Sodium	0.076 ^a	0.07 ^a	0.031 ^b	0.00
Potassium	0.914 ^a	0.914 ^a	0.01 ^c	0.00
Trace minerals				
Iron	3.59 ^a	3.59 ^a	1.24 ^b	0.04
Copper	0.040 ^b	0.04 ^b	0.054 ^a	0.00
Zinc	0.026 ^a	0.026 ^a	0.012 ^b	0.00
Manganese	0.027	0.027	0.029	0.00

^{a,b,c}Means on the same row with different superscripts are significantly varied ($P < 0.05$).

POS = *P. ostreatus* degraded sorghum stover, PPS = *P. pulmonarius* degraded sorghum stover, and SEM = standard error of mean.

**Figure 1.** Methane (ml/200 mg DM) production of the fungal treated and untreated sorghum stover.

changes in carbohydrates fractions were reflected by total gas produced (Deville and Givens, 2001). Gas production rate constant (c) also differed significantly with faster rates obtained in the treated substrate compared with the untreated. The high rates obtained may be related with its high CP content and low content of NDF, ADF and ADL (Osuga et al., 2006). Nsahlai et al. (1994) and Larbi et al. (1998) reported that there were a positive correlation between NDF and ADF and the rate the rate and extent of gas production. Shown in Figure 1 is the graph of methane production. From the graph, methane production was at equal levels in the control and PPS. As in the present study, reduction of methane production in POS could be due to the conversion of CO₂ and H₂ to

acetate instead of CH₄ (Miller, 1995). Figure 2 shows *in vitro* gas production pattern for a period of 96 h.

Estimated organic matter digestibility (OMD) (%), short chain fatty acid (SCFA) (mm) and metabolisable energy (ME) (MJ/ Kg DM)

The results of the OMD, SCFA and ME are presented in Table 3. The results showed higher values estimated for OMD in all the fungi treated substrates. OMD has been shown to have high correlation with gas volume (Sommart et al., 2000; Nitipot and Sommart, 2003). The high volume obtained for OMD in this study implies that the microbes

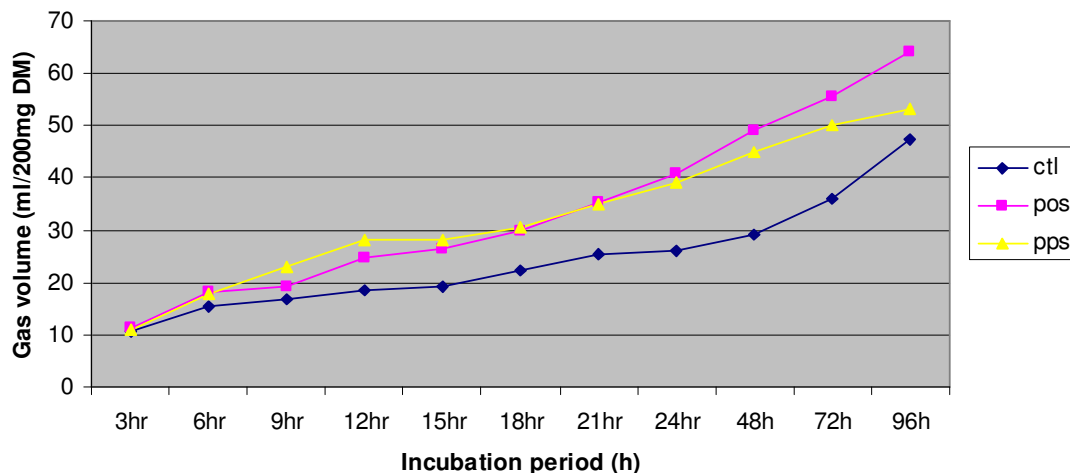


Figure 2. *In vitro* gas production of fungal treated and untreated sorghum stover.

Table 3. Gas volume and *in vitro* digestibility characteristics.

Parameters	Control	POS	PPS	SEM
Gas production characteristics				
b (mL)	36.67 ^c	53.00 ^a	42.33 ^b	0.63
c (h ⁻¹)	0.0105 ^c	0.2618 ^a	0.0149 ^b	0.00
Gas volume				
Gv 24	26.00 ^b	41.67 ^a	39.00 ^a	0.78
Gv 48	29.00 ^b	49.00 ^a	45.00 ^a	0.93
Gv 72	36.00 ^c	55.67 ^a	50.00 ^b	0.40
Gv 96	47.33 ^c	64.33 ^a	53.33 ^b	0.43

^{a,b,c}Means on the same row with different superscripts are significantly varied ($P < 0.05$).

POS = *P. ostreatus* degraded sorghum stover, PPS = *P. pulmonarius* degraded sorghum stover, SEM = standard error of mean, b = fermentation of the insoluble fraction, c = gas production rate constant, and CH₄ = methane.

in the rumen and animal have high nutrient uptake (Chumpawudee, 2006). The reduced CF contents (Table 1) of the fungal treated substrates probably influenced improvement in OMD, since high NDF and ADL contents in feedstuffs result in lower fiber degradation (Van Soest, 1988). Additionally, tropical forages and concentrates feedstuffs have a large proportion of lignified cell walls with low fermentation rates and digestibility rates and limited intake (Ibrahim et al., 1995; Hindrichsen et al., 2001), this is also true of tropical agricultural wastes.

The estimated ME were varied and particularly higher in the treated substrates. There was a positive correlation between metabolisable energy (ME) calculated from *in vitro* gas production together with CF and EE content with metabolisable energy value of conventional feeds measured *in vivo* (Menke and Steingass, 1988).

The SCFA estimated (Table 4) from gas production were 0.56, 0.94 and 0.87 μM for the control, POS and PPS, respectively. The higher values obtained in the

fungal treated substrates is due to the high gas production which is evident in the first 24 h of incubation (Figure 1). The gas production of different classes of feeds (Blummel and Orskov 1993) incubated *in vitro* in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrates fermentation (Sallam et al., 2007). Getachew et al. (2002) reported the close association between SCFA and gas production *in vitro*, and the use of this relationship between SCFA and gas production to estimate the SCFA from gas values, which is an indicator of energy availability to the animal. In this study, the higher values obtained for the treated substrates implies more energy content in the substrates.

Conclusion

In conclusion, the biodegradation of sorghum stover using two different fungi resulted in increased crude

Table 4. Estimated short chain fatty acid (SCFA, μM), Organic matter digestibility (OMD, %) and metabolisable energy (ME, MJ/Kg DM).

Parameters	Control	POS	PPS	SEM
ME	5.97b	8.21a	7.83a	0.10
SCFA	0.56b	0.94a	0.87a	0.02
OMD	42.99b	57.75a	55.39a	0.61

^{a,b}Means on the same row with different superscripts are significantly varied ($P < 0.05$).

SEM = Standard error of mean, POS = *P. ostreatus* degraded sorghum stover, PPS = *P. pulmonarius* degraded sorghum stover, SCFA = short chain fatty acid, OMD = organic matter digestibility, ME = metabolisable energy (MJ/Kg DM)

protein (CP) and decrease in the CF fraction with the consequent result on increase in the digestibility of the resultant substrates. This implies that the substrates can be included with other feedstuffs in the diet of ruminant animals.

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