Libyan Agriculture Research Center Journal International 1 (3): 195-201, 2010
ISSN 2219-4304

Improving the Nutritive Value of Millet Stover Using Biological Treatment

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Abstract: This study was carried out to determine the impact of fungal treatment in a solid state fermentation on the nutritive value of millet stover. Two white-rot fungi: *Pleurotus ostreatus* and *Pleurotus pulmonarius* were cultured in triplicates on millet stover for 21 days after which the chemical composition, mineral composition and *in vitro* digestibility were determined. The result obtained showed an increase in the crude protein content (CP) from 3.02% for the control to 8.29% for *Pleurotus ostreatus* treated millet stover (POM) and 6.40% for *P. pulmonarius* treated millet stover (PPM).On the contrary, the percentage of crude fiber (CF), Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) were decreased consistently,while the content of ether extract (EE) increased. The major minerals (Mg, Na, K) and all the analyzed trace minerals were increased significantly ($p<0.05$) in all the treated millet stover. At 96h of incubation, gas volume was increased from 45.33mL for the control to 71.67mL for POT and 69.33mL for PPM. The estimated short chain fatty acid (SCFA), organic matter digestibility (OMD) and metabolisable energy (ME) also followed the same trend. The highest rate of gas production constant (c) was obtained in POM followed closely by PPM. The fermentation of the insoluble fraction (b) was increased significantly from 36.33mL (control) to 59.00mL for PPM and 62.67 ML for POM. These results showed that bioconversion of millet stover improved the chemical composition and the *in vitro* digestibility and therefore could be incorporated into the diet of ruminants.

Key words: Chemical composition % *In vitro* digestibility % Solid state fermentation % White-rot fungi

INTRODUCTION

Fibrous crop by-products farm wastes are characterized by extensive lignifications of the cellulose and hemicellulose and by low levels of protein, soluble carbohydrates and minerals [1]. [2] reported that about 82,000 tones of cowpea husks and straws are produced in Nigeria annually. The prohibitive cost of concentrate diet for ruminants in the tropics during the dry season necessitates continuous search for less expensive and high nutritive feedstuffs [3]. In view of this, it is therefore necessary, especially in the developing countries to develop an environmentally friendly recycling methodology that will convert the vast mass of millet stover produced annually, into value added ruminant feed.

Various methods of pre-treatment aimed at delignification have been studied; these include biological, chemical and physical interventions. Numerous researchers have conducted studies on chemical and physical treatments, but in the developing countries such as Nigeria there is paucity of information on biological treatment of agricultural wastes. The availability of edible mushrooms (white-rot) to degrade lignocellulosics abounds in literature. [4] noted that several white-rot fungi exhibit a capacity to increase the *in vitro* digestibility of wheat straw and offer possibilities for the production of ruminant feedstuff.

The present study was therefore aimed for evaluating the nutritive fungal treated stover and the effect of digestibility.

MATERIALS AND METHODS

Sample Collection: Dried samples of millet stover were collected from the Teaching and Research Farm,

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Nasarawa State University, Keffi. The materials were milled and oven-treated at 65°C until a constant weight was obtained for any 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 [5]. The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

Degradation of Maize Husk by *P. ostreatus* and *P. pulmonarius*

Preparation of Substrate: The jam bottles (200ml) used for this study were thoroughly washed, dried for 10 min. at 100°C. 25 g of the dried samples milled through 1mm screen 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 triplicated.

Inoculation: Each bottle was inoculated at the center of the substrate with 2, 10 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.

***In vitro* Gas Production:** Rumen fluid was obtained from three West African Dwarf female goats through a suction tube before the morning feed. The animals were fed with 40% concentrate feed (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 according to [6] in 120ml calibrated syringes in three batches at 39°C. To 200mg sample in the syringe was added 30ml inoculum contained cheese cloth strained rumen liquor and buffer (9.8g $NaHCO_3$ + 2.77g Na_2HPO_4 + 0.57g KCL + 0.47g NaCl + 0.12g $MgSO_4$. $7H_20$ + 0.16g $CaCI_2$. $2H_20$ 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 24h. After 24 hours of incubation, 4ml of NaOH (10M) was introduced to estimating the amount of methane produced, [7]. The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of gas production characteristics were estimated using the equation $Y = a + b (1 – e^{ct})$ described by [8], where Y = volume of gas produced at time't' a = intercept (gas produced from the soluble fraction, b = gas production from the insoluble fraction, a+b= final gas produced, c = gas production rate constant for the insoluble fraction (b), t = incubation time. The post incubation parameters such as metabolisable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) and short chain fatty acids (SCFA) were estimated at 24h post gas collection according to [6].

ME = 2.20 + 0.136* Gv + 0.057* CP + 0.0029*CF;
OMD = 14.88 + 0.88Gv + 0.45CP+ 0.651XA;
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.

Chemical Composition: DM was determined by oven drying the milled samples to a constant weight at 105°C for 8 hours. Crude protein was determined as Kjadhal nitrogen x 6.25. Ether extracts, crude fiber and ash were determined according to [9] method. Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) was determined using the method described by [10]. Hemicellulose was calculated as the difference between NDF and ADF while cellulose is the difference between ADF and ADL.

Statistical Analysis: Data obtained were subjected to analysis of variance (ANOVA).Where significant differences occurred, the means were separated using Duncan multiple range F-test of SAS [11]option.

RESULTS

The result of the chemical composition of millet stover before and after fungal treatment (Table 1) showed a significant ($P<0.05$) increase in the CP content of the fungal treated millet stover compared with the untreated.

The cumulative gas production profile corrected for blank fermentation of the treated and untreated millet stover is shown in Figure 1, while Table 3 showed the cumulative gas volumes at 24, 48, 72 and 96h after incubation. The gas volumes ranked from the highest to the lowest were POM, PPM and control.

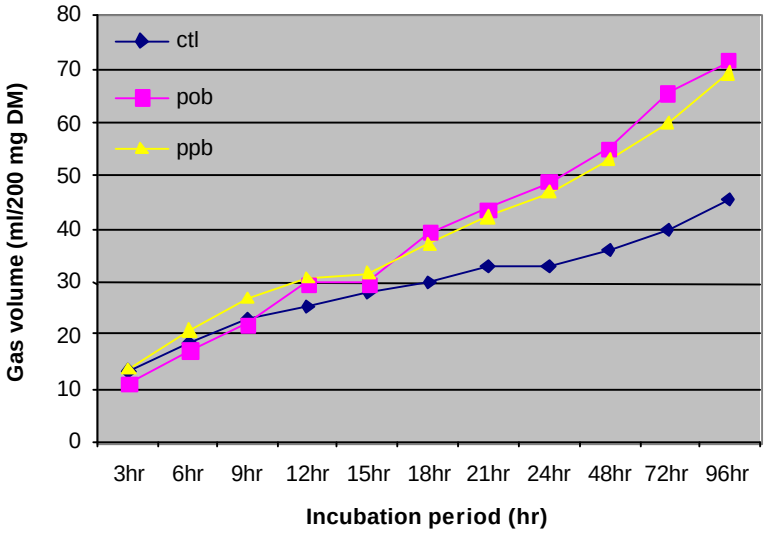

Fig. 1: *In vitro* gas production of fungal treated and untreated millet stover

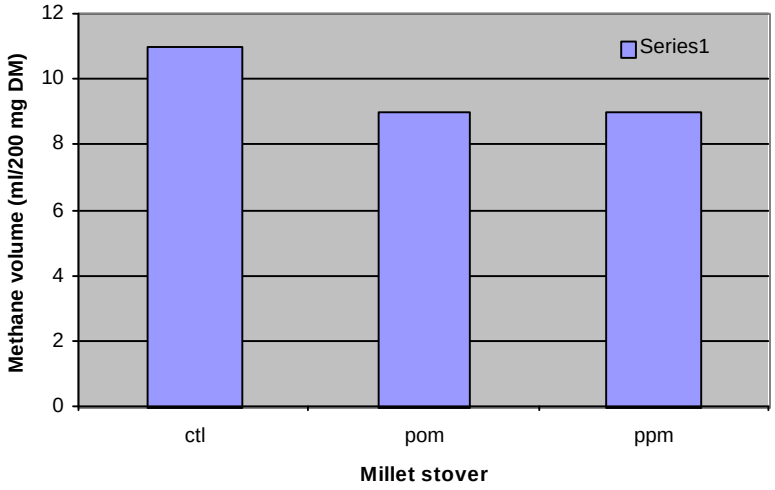

Fig. 2: Methane production of fungal treated and untreated millet stover

Table 1: Chemical composition (g/100gmDM) of treated and untreated millet stover

Parameters	Control	POM	PPM	SEM
Crude protein	3.85^{b}	5.99^{a}	4.64^{ab}	0.27
Crude fiber	31.53^{a}	24.49^{b}	24.55^{b}	0.78
Ether extract	5.78	5.44	4.95	0.33
Ash	6.78	6.90	6.20a	0.29
Neutral Detergent fiber	69.95^{a}	62.58^{b}	62.62^{b}	0.39
Acid Detergent fiber	46.94^{a}	42.29^{b}	40.45^{c}	0.29
Acid Detergent lignin	14.98^{a}	11.17^{c}	12.76^{b}	0.22
Cellulose	31.96^{a}	47.78^{b}	27.69^{a}	5.61
Hemicellulose	23.01	20.29	22.17	0.54
Nitrogen Free Extract	47.94^{a}	42.84^{ab}	40.34^{b}	1.16

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

Table2: Some major minerals and trace mineral composition of fungal treated millet stover

Major minerals (g/100gDM)	Control	POM	PPM	SEM
Calcium	9.34[b]	15.74[a]	5.03[c]	0.01
Phosphorus	0.54[ab]	0.104[b]	0.91[a]	0.09
Magnesium	0.76[c]	8.45[a]	8.08[b]	0.00
Potassium	0.03[b]	0.56[a]	0.56[a]	0.00
Sodium	0.43[b]	0.96[a]	097[a]	0.00
Trace minerals (ppm)				
Iron	1.09[b]	1.14[b]	2.14[a]	0.16
Copper	0.02[c]	0.04[b]	0.05[a]	0.00
Zinc	0.01[c]	0.06[a]	0.02[b]	1.36
Manganase	0.04[c]	0.08[b]	0.08[b]	0.00

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

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

Parameters	Control	POM	PPM	SEM
b mL	36.33[c]	62.67[a]	59[b]	0.19
c (hG^{1})	0.0122[c]	0.0159[a]	0.0147[b]	0.00
Gv24	32.00[b]	37.00[a]	35.00[ab]	0.67
Gv48	36.67[b]	41.67[a]	39.67[a]	0.38
Gv72	38.67[b]	43.67[a]	41.67[ab]	0.77
Gv96	41.00[b]	46.00[a]	44.00[ab]	0.67
CH_4	11.00 [a]	9.00[b]	9.00[b]	0.00

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

Table 4: Estimated short chain fatty acid (SCFA), Organic matter digestibility (OMD) and metabolisable energy (ME)

Parameters	Control	POM	PPM	SEM
SCFA (µM)	0.7047[c]	0.8242[a]	0.7764[b]	0.00
OMD (%)	49.47[c]	54.97[a]	52.33[c]	0.00
ME (MJ/Kg DM)	6.86	7.64	7.26	0.19

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

The results of the methane and gas production characteristic is shown in Table 3, while Figure 2 shows the bar chart of methane production. The methane production was the highest in POM and this does not differ significantly (P>0.05) from the control.

The mineral composition of the treated and untreated millet stover is shown in Table 3. The calcium constituent ranged between 0.27% in POM and 2.23 in the control. Phosphorus content ranged from 0.07 in control to 0.34 in PPM. Magnesium content ranged from 0.22% in PPM to 0.45% in POM. Sodium content was generally low in all the samples and it ranged from 0.0031% in the control to 0.0345% in PPM, while potassium content ranged from 0.0925% in the control to 0.098% in POM.

The values obtained for SCFA was significantly higher in the treated millet stover compared with the untreated. However, there was no significant difference (p>0.05) in the fungal treated stovers. The valued ranged from 0.85µM in the control to 1.23 µM in POM.

DISCUSSION

The ability of the fungi used to increase the protein content of lignocellulose is well documented in literature [12, 13]. The increase in the CP content of the treated millet stover may be due to the secretion of certain extra cellular enzymes which are proteineous in nature into the waste during their breakdown and its subsequent

metabolism [14]. CP increase could also be due to the addition of the fungal mycelia into the substrates. This agrees with the findings of [15] and [16]. On the other hand, the CF and CF fractions (NDF, ADF and ADL) decreased in all the treated substrates. The decreasing CF and CF fractions could be as a result of the activities of cellulolytic bacteria [17]. During fungal growth, part of the cell wall is converted into soluble sugars to provide energy [18], a phenomena that could be responsible for decrease in major fiber (cellulose and hemicellulose) components [19].

[20] suggested that gas volume at 24h after incubation is indirect relationship with metabolisable energy in feedstuffs. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate [21, 22]. [23] suggested that gas volume is a good parameter from which you predict digestibility, fermentation end product and microbial protein synthesis of the substrates by rumen microbes in the *in vitro* system. Furthermore, *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume [23, 24]. The highest gas production obtained in the treated millet stover could be the result of the reduced CF and CF content. [17] reported that cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. This reduction in the CF fraction could facilitate microbial activity through improvement of the microbial environment as incubation advances. Also the reduced ADL in the fungal treated substrate may explain in part the high amount of gas produced. The result of the present study agreed with the findings of [17, 25]. Additionally, since gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation and carbohydrates fractions, the lowest gas production from the untreated stover, could be related to the feeding value of this agricultural wastes [17].

Methane production has negative effects on the animals in one hand as it is energy loss to the animal and on the other hand, when accumulates in the rumen, it results in bloat [3]. Therefore, a reduction in the methane production is saving of energy for the animal. The highest rate (c) and fermentation of the insoluble fraction (b) observed in the treated millet stover could be related with its high CP content and low content of NDF, ADF and ADL. [26] and [27] reported that gas production and estimated parameters are negatively correlated with NDF and ADF. In this study, POM and PPM which had lower fiber contents had high gas production and fermentation characteristics.

The major mineral composition differed significantly with higher increase obtained in the fungal treated millet stover, this agrees with the findings of [28] who reported that mushroom contain appreciable amount of potassium, phosphorous, copper and iron but low level of calcium. The increase in the mineral content of the treated substrates could have been the results of the release of minerals contained in the fungi used into the substrates.

The content of iron ranged from copper, zinc and manganese were generally very low in the control compared with the fungal treated stover. The calcium and phosphorus ratio observed in the PPB treated stover were within the approved 1:1 ranged recommended by [29], whereas that of the control and POM were not within the specified range. This suggests that the feed may need fortification of minerals in form of mineral lick or diet inclusions. Generally, the major minerals Mg, Na and K in the present study were extremely deficient in the fungal treated substrates.

The result obtained in the present study for all the trace elements showed extreme deficiency in both the treated and the untreated, although the deficiency were more with the control. Therefore, supplementation with minerals will be required to jack up the deficiency observed.

The values obtained for SCFA was significantly higher in the treated millet stover compared with the untreated. However, there was no significant difference in the fungal treated stovers. The valued ranged from 0.85µM in the control to 1.23µM in POM. This is comparable with values obtained for the different parts of *Enterolobium cyclocarpon* [3] but lower than that reported for some ruminant feedstuffs. [17]. The gas production from different classes of feeds [21] incubated *in vitro* in buffered rumen was closely related the production of SCFA which was based on carbohydrate fermentation [17]. [30] reported close association between SCFA and gas production *in vitro* and used the relationship between SCFA and gas production to estimate the SCFA, which is an indicator of energy availability to the animals.

The organic matter digestibility (OMD) was the highest in POM followed by PPM with the least from the control. The high organic matter digestibility observed in the treated substrate could be linked to the improvement in the CP. This result implies that the microbes in the rumen and animals have high nutrient uptake [25]. Additional, lower fiber content (Table 1) of the treated substrates resulted in higher OMD, since low NDF and ADL content in feedstuffs is expected to result in fiber

degradation. Besides, the removal of the lignin contents also has a contribution in the digestibility. Digestibility of lignonellulosics has been known to the correlated with lignin content [31, 32].

The values estimated for ME differed significantly between the treated and untreated substrates. [33, 6] and [34] concluded that the prediction of ME is more accurate when based on gas and chemical constituents measurements as compared to calculations based on chemical constituents only. There was a positive correlation between ME calculated from in vitro gas production together with CP and fat content with ME value of conventional feeds measured *in vitro* [6].

The result obtained showed an improvement in the CP, CF and CF fractions which resulted in increase digestibility of the fungal treated substrates. The results also showed a considerable improvement in the digestibility of organic matter, short chain fatty acids and estimated metabolisable energy. This implies that fungal treated millet stover can be incorporated with other feedstuffs in the diet of ruminants. However, more research is needed to feed the resultants substrates to live animals.

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