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Treatment of Maize Stover with White Rot Fungi (*Pleurotus tuber-regium* and *Lentinus subnudus)* and Their Effect on *in vitro* Digestibility

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Abstract: Investigations were conducted for 40 days on the biodegradation of maize stover using two white rot fungi :*Pleurotus tuber–regium(*PT) and *Lentinus subnudus* (LSM) collected from the Botanical Gardens, University of Ibadan, Ibadan, Nigeria. The crude protein (CP), crude fat (CF) and ash contents (AC) of the treated stover were observed to increase at the end of solid state fermentation.There was a significant consistent reduction in the proportion of crude fibre content (CFC) in the fungal treated stover from 14.42% (LSM) to 19.06% (PT) ($P < 0.05$). Cellulose was observed to be more depleted in PT than LSM (29.79 and 30.89 g/100g DM respectively) in degraded stover while; hemicellulose was more depleted in LSM than PT with values of 18.51 and 20.23 in g/100g DM of degraded stover.Final gas production were observed to be higher in the fungal treated substrate increasing from 13.00ml to 15.00ml and 22.00ml in PT and LSM respectively ($P < 0.05$),. Gas production from the insoluble fractions,b(ml) increased significantly from 10.67 to 17.50ml and 18.00ml in PT and LSM respectively.The CF fractions decreased significantly in all the treated subsrate ($p<0.05$).However, the two fungi has greater affinity to degrade acid detergent fibre(ADF), acid detergent ligrain(ADL) and neutral detergent fibre (NDF). The methane (CH_4) production was nevertheless, higher in the untreated substrate with a value of 13 mmol.

Key words: Maize stover · Edible mushroom · Fermentation · *in vitro* gas production

INTRODUCTION

Fleshy edible fungi could be found growing on varieties of habitats in different climatic regions of the world. These habitats include, richly loamy soil, agricultural soils, partially buried decaying roots, palm wastes,rice straw, cotton wastes,cassava wastes,animal dung, damp rotten log of wood, trunk of trees, decaying organic matter in damp soil rich in organic substrate [1-4]. Edible mushrooms are highly nutritious and food values could be compared with some rich vegetables [3,5]. Apart from the benefits derived from the consumption of mushrooms,the substrates on which they grow could be upgraded into livestock feeds.Lignolytic microorganisms are wood inhabiting fungi which are able to colonise different plant residues and increase the digestibility of these substrates [6]. The ability of edible mushrooms in reducing the lignin content of lignocellulose is well reported in literatures [7-10]. Very huge ammount of maize stover is produced annually in Nigeria. A large percentage of this agricultural wastes are burnt especially in developing counteries, while small amount is utilized as animal feeds [6]. Digestibility of maize stover is known to be correlated with the lignin and crude fibre contents. The objectives of this study was to use *Pleurotus tuber-regium* and *Lentinus subndus* which are two white rot fungifrom Nigeria to upgrade maize stover to provide suitable diet for animal feeds by means *in vitro* digestibility.

MATERIALS AND METHODS

Samples: Maize stovers were collected from the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The materials were milled and oven-treated at 65°C until a constant weight was obtained. Cultures of *Pleurotus tuber regium* and *Lentinus subnudus* were obtained from stock culture at the Mycology section, Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria and were regularly subcultured on plates of potato dextrose agar (PDA).

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Degradation of Maize Stover by *P. Tuber Regium* and *L. Subnudus*: The experimental bottles used for this study were washed and oven dried for 30 min. at 100°C and 25.00g of the moistened sterilized milled maize stovers were weighed into each bottles in triplicate. The bottle was immediately covered with aluminum foil and sterilized in the autoclave at 121°C for 15 min.After cooling, the substrate were inoculated with 5% (w/w) active fungal culture. These inoculated bottles were incubated in a well ventilated dark room at 30°C and 100% relative humidity.After 40 days of incubation, the biodegraded samples were subbjected to chemical analysis and *in vitro* digestibility.

Gas Production: Rumen fluid was obtained from three - non lactating West African dwarf female goats through a suction tube before the morning feed. The animals received 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. Rumen fluid from the three goats were combined, stored in a warm insulated flask filled with CO_2, filtered through cheesecloth and mixed (1:4) with anaerobic strained rumen liquor/buffer solution as described by Menke and Steingass, [11] in 120ml calibrated syringes in three batches at 39°C. To 200mg sample in the syringe was added 30ml inoculum under continous 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 estimate the amount of methane produced. Gas productions were corrected for blank gas production (i.e.,gas production in buffered rumen fluid without sample). The volume of gas production characteristics were estimated using the equation $Y = a + b (1 - e^{ct})$ described by Qrskov and McDonald [12] 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. Metabolizable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) were estimated on established Menke and Steingass [11] and short chain fatty acids (SCFA) was calculated as reported by Getachew *et al,* [13].

$$ME = 2.20 + 0.136* GV + 0.057* CP + 0.0029*CF;$$
$$OMD = 14.88 + 0.88GV + 0.45CP\ 0.651XA;$$
$$SCFA = 0.0239*GV - 0.0601;$$

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: Crude protein (Kjedhal nitrogen x 6.25), ether extracts and ash were determined according to AOAC, [14] method. Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent liquin (ADL) was determined using the method described by Van Soest *et al* [15]. 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) and mean were separated by Duncan multiple range test using Statistical Analysis System (SAS) package [16].

RESULTS

Table 1 shows the result of the proximate compositions of maize stover degraded by *Lentinus subnudus* (LSM) and *Pleurotus tuber-regium* (PT) after 40days. The crude protein (CP) increased significantly in the fungal treated maize stover compared with the untrated stover (control). *Lentinus subnudus* (LSM) had the highest crude protein 14.34% while PT had 8.45%.These values showed significant increse when compared with that of the control (3.72%) ($P<0.05$). There was a consistent decline in the proportion of Crude fibre content (CFC) in the fungal treated stover from 14.42% (LSM) to 19.06% (PT) while that of control is 31.84%. (Table1). Dry matter content (DM) reduced significantly from control (88.74%) to 86.80 and 86.55 % in LSM and PT respectively. In regard to crude fat, (CF) and nitrogen free extract (NFE), the values, increased significantly from control (CF :0.82 and NFE :54.14) to 1.61, CF and 59.47% NFE in LSM and,1.22, CF and 61.36% NFE in PT respectively ($P<0.05$) (Table 1).

Table 2 reveales the results of fiber analysis. There were consistent decrease in the values obtained in the treated substrates compared with the control..There were no significant difference in value of acid detergent fibre obtained for LSM and PT 42.73 and 41.81% but differed significantly from the control 46.53%. ($p<0.05$) The highest value (67.85%) of Neutral detergent fibre was obtained in the control while the least value (6124%) was observed in LSM. Acid detergent ligrain decreased from 13.63% (control) to 12.63% in PT and 11.83% in LSM

Table 1: Proximate composition (g/100g DM) of *Lentinus subnudus* and *Pleurotus tuber regium* degraded maize stover

Parameters	UM	LSM	PT	SE
Dry Matter	88.74^{a}	86.80^{c}	86.55^{b}	±0.01
Crude Protein	3.72^{c}	14.34^{a}	8.45^{a}	±0.07
Crude fat	0.82^{b}	1.61^{a}	1.22^{b}	±0.07
Ash	9.45^{c}	10.15^{a}	9.90^{b}	±0.04
Crude fiber content	31.84^{a}	14.42^{c}	19.06^{b}	±0.05
Nitrogen free extract	54.14^{a}	59.47^{a}	61.36^{b}	±0.12

a,b,c, means on the same column with different superscripts are significantly varied (P < 0.05),UM= control(untreated stover) LSM = *Lentinus subnudus* degraded maize stover, PT = *Pleurotus tuber- regium* degraded maize stover,SE = Standard error

Table 2: Fiber analysis (g/100g DM) of *Lentinus subnudus* and *Pleurotus tuber regium* degraded maize stover

Parameters	UM	LSM	PT	SE
Neutral detergent fiber	67.85^{a}	61.24^{c}	62.04^{b}	±0.01
Acid detergent fiber	46.53^{a}	42.73^{b}	41.81^{b}	±0.01
Acid detergent ligrain	13.63^{a}	11.83^{c}	12.63^{b}	±0.01
Cellulose	32.90^{a}	30.89^{b}	29.79^{c}	±0.06
Hemicellulose	21.32^{a}	18.51^{c}	20.23^{b}	±0.02

a,b,c, means on the same column with different superscripts are significantly varied (P < 0.05), UM= control(untreated stover) LSM = *Lentinus subnudus* degraded maize stover, PT = *Pleurotus tuber- regium* degraded maize stover, SE = Standard error

respectively. Cellulose was observed to be more depleted in PT (29.79%) than LSM (30.89%) while hemicellulose was more depleted in LSM (18.51%) than PT (20.23%).

Presented in Table 3 is the *in vitro* gas production characteristics. Final gas produced, a+b (ml) were observed to be significantly higher in the fungal treated substrate increasing from 13.00ml to 15.00ml and 22.00ml in PT and LSM respectively (P<0.05). The highest value was recorded in LSM.Gas production from the insoluble fractions, b(ml) increased significantly p<0.05) from 10.67 UM (control) to 17.50ml and 18.00ml in PT and LSM respectively.There were significant difference (p>0.05) in the volume of gas produced,y (ml) in the control (11.33ml) and LSM (16.33ml). However, the value obtained for LSM was not significantly different from tthat of PT, (P<0.05) . The gas production rate c(h^{-1})was highest in PT (0.57ml) followed by the control (0.31ml) and LSM (0.18ml).

Short chain fatty acid (SCFA) differed significantly(p<0.05) between the substrates but were not significantly different between the LSM and PT. (Table 4). Organic matter digestibility (OMD) were different significantly between the control (28.68 MJ Kg/ DM) and PT and LSM with 32.66 and 41.55 MJ Kg/ DM respectively. Likewise, there were signifficant differences

Table 3: *in vitro* gas production characteristics of treated maize stover

Parameters	UM	LSM	PT	SE
a+b(ml)	13.00c	22.00^{a}	15.00^{b}	±1.00
b(ml)	10.67^{b}	18.00^{a}	17.50^{a}	±1.07
y(ml)	11.33^{b}	16.33^{a}	12.67^{ab}	±1.10
c(h^{-1})	0.31^{a}	0.18^{b}	0.57^{a}	±0.01

a,b,c, means on the same column with different superscripts are significantly varied (P < 0.05), Y = vol. of gas produced at time t. b = gas production from the insoluble fraction, c = gas production rate constant for the insoluble fraction., UM= control(untreated stover) LSM = *Lentinus subnudus* degraded maize stover, PT = *Pleurotus tuber- regium* degraded maize stover, SE = Standard error

Table4: Short chain fatty acid (mol) organic matter digestibility (%) and metabolisable energy (MJ/KgDM) of treated maize stover

Parameters	UM	LSM	PT	SE
SCFA	0.37c	0.55a	0.42b	±0.10
OMD	28.68^{b}	41.55^{a}	32.66^{a}	±1.10
ME	4.24^{c}	6.10^{a}	4.82^{b}	±1.07

a,b,c, means on the same column with different superscripts are significantly varied (P < 0.05),SCFA = Short chain fatty acid,OMD = organic matter digestibility,ME = metabolisable energy, UM= control(untreated stover) LSM = *Lentinus subnudus* degraded maize stover, PT = *Pleurotus tuber-regium* degraded maize stover, SE = Standard error

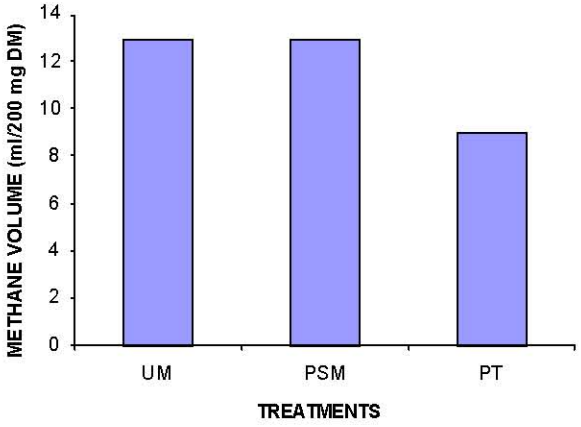

Fig. 1: Methane production from *in vitro* gas production of maize stover treated with two strains of mushroom.
UM= control, PSM= *Lentinus subnudus*, PT=*Plurotus tuber-regium*

(p>0.05) in the Metabolising energy (ME) in the UM, PT and LSM. Fig.1 shows the *in vitro* gas production pattern of UM (control), PT and LSM over a period of 24 hours while Fig.2 shows the bar chart of *in vitro* methane production.Methane production was observed to be similar in the control and LSM while the least volume was obtained in PT.

DISCUSSION

The crude protein which was observed to be higher in LSM and PT (Table 1), may be due to the addition of fungal biomas [17] to the substrate during colonization. The CP values obtained, is comparable to most cereal crops. This agrees with the report of Jacqueline *et al* [18] who suggested that the extracellular enzymes secreted by the fungi contained amorphous homo and heteropolysaccharides which are often associated with fungal proteins. The decrease in crude fibre fraction could be due to the production of various enzymes during the vegetative and reproductive phases with lignocellulose degrading properties. The solubulisation of the lignin occurs during the vegetative phase and enzymes like laccase,manganese peroxidasee and lignin peroxidase are secreted while cellulose degrading enzymes is secreted during the reproductive phases [19]. The actions of these enzymes may also be responsible for the depletion of the cellulose and hemicellulose contents of LSM and PT treated substrates. The two fungi decreased NDF concentrations mainly due to the extensive utilization of hemicellulose during growth as a source of energy for the fungi [20] . The extent of lignin degradation by these fungi may be related to their ability to produce lignin degrading enzymes such as lignin peroxidase and manganese peroxidase [10]. Final gas producded, a+b (ml), potential gas production from the insoluble, b(ml) and volume of gas produced,y (ml) were higher in LSM and PT suggesting better fermentation. The enhanced CP and softened CF and CF fractions most likely contributed to the best values observed for OMD and ME in LSM and PT respectively.Since SCFA was better in LSM followed by PT, this indicates more energy potential for the ruminant (21).CH_4 production is an indication of energy loss to the animal.This was obseved to be lowest in the treated substrate suggesting better energy conservation. The differences in the SCFA,OMD and ME of LSM and PT may be as a result of specie differences in the fungi used. Although CH_4 produced in LSM was at equal level with that produced by the control,this could however be compensated for in the higher SCFA and ME obtained in LSM. This study revealed that treatment of maize stover with edible fungi resulted in enhanced crude protein and reduced cude fibre fraction indicating that it is beneficial to ruminants.

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