Bioconversion of maize husk into value added ruminant feed by using white-rot fungus

Bioconversión de la tusa de maíz como valor agregado en la alimentación de ruminates mediante el uso de hongos de la pudrición blanca

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ABSTRACT

Treatment of crop wastes with some species of white-rot fungi can enhance the nutritive value. After solid state fermentation of maize (*Zea mays* L.) husk MH with four white-rot fungi (*Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus sajor caju* and *Lentinus subnudus*) for 40 days, the chemical composition and *in vitro* digestibility of the resulting substrates was evaluated. Biodegradation increased the crude protein from 7.44% for untreated MH, (UM, control) to 9.89% for *L. subnudus* (LSM); 9.67% for *P. tuber-regium* (PTM) and 9.90% for *P. pulmonarius* (PPM) and 9.55% for *P. sajor caju* (PSM). In contrast, growth of fungi reduced crude fiber (CF) from 30.45% (UM) to 22.27, 24.29, 19.07 and 14.14% for LSM, PTM, PPM and PSM, respectively. The preference for cellulose and hemicellulose utilization by the fungi was indicated by decrease in values obtained. LSM had the least value for cellulose (27.43 %) while PSM with a hemicellulose content of 18.46% recorded the highest reduction in hemicellulose. There were significant differences ($p \leq 0.05$) between the treated and untreated maize husk in terms of metabolisable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) as measured by the *in vitro* gas production method using ruminal microflora. OMD ranged from 38.28-48.97% with highest values for fungal treated husk and lowest for untreated substrate. LSM showed the highest ($p \leq 0.05$) values for SCFA and ME for all the substrates under study. All the fungal treated substrates had the highest *in vitro* fermentation characteristics and cumulative gas production. Result of this study show that fungal treatment of maize husk enhanced digestibility by increasing the crude protein and decreasing the crude fiber.

Key words: Maize husk, white-rot fungi, biodegradation, *in vitro* digestibility.

RESUMEN

El tratamiento de residuos de cosechas con algunas especies de hongos causantes de la pudrición blanca puede mejorar su valor nutritivo. Después de la fermentación en estado sólido de tusas de maíz (TM) (*Zea mayz* L.) con cuatro hongos causantes de la pudrición blanca (*Pleurotus tuber-regium*, *Pleurotus pulmonarius*, *Pleurotus sajor caju* y *Lentinus subnudus*) durante 40 días se evaluó la composición química y digestibilidad *in vitro* de los sustratos resultantes. La biodegradación incrementó la proteína cruda de 7,44% para las TM no tratadas (TMNT, control) a 9,89% para *L. subnudus* (TMTLS); 9,67% para *P. tubérculo-regium* (TMTPT); 9,90% para *P. pulmonarius* (TMTPP) y 9,55% para *P. sajor caju* (TMTPS). Por el contrario, el crecimiento de los hongos redujo la fibra cruda de 30,45% (TMNT) a 22,27; 24,29; 19,07 y 14,14% para TMTLS, TMTPT, TMTPP y TMTPS, respectively. La preferencia por la utilizacion de celulosa y hemicelulosa de los hongos fue indicada por la disminución en los valores obtenidos. TMTLS (27,43%) tuvo el menor valor para la celulosa, mientras que TMTPS (18,46%) registró la menor reducción de la hemicelulosa. Hubo diferencias significativas ($p \leq 0,05$) entre las TM tratadas y no tratadas en términos de energía metabolisable (EM), digestibilidad de la materia orgánica (DMO) y de ácidos grasos de cadena corta (AGCC). La DMO varió entre 38,28-48,97% y los mayores valores fueron aquellos de las TM tratadas con hongos y los menores para las no tratadas. TMTLS mostró el valor más alto ($p \leq 0,05$) para AGCC y EM para todos los sustratos en estudio. Todos los sustratos tratados con hongos tuvieron los mayores valores para las características de fermentación *in vitro* y producción acumulada de gas. Los resultados de este estudio mostraron que el tratamiento de TM con hongos mejoró la digestibilidad mediante el incremento de la proteína cruda y la disminución de la fibra cruda.

Palabras clave: Tusa de maíz, hongos causantes de la pudrición blanca, biodegradación, digestibilidad *in vitro*.

INTRODUCTION

In Nigeria, ruminant animal suffer from under feeding especially during the dry seasons due to shortage of forages and more so because of low nutritive value of available crop wastes. The available wastes such as maize stover, corn cob, and maize husk cereal straws are not able to meet the nutritional requirements of ruminants. To dispose these wastes, they are usually burnt in heaps thereby releasing offensive odor and gases into the atmosphere. Some are even thrown into the rivers and streams thereby endangering aquatic life (Jonathan *et al*, 2008). Maize husks are fibrous leafy materials covering the maize ear. They are usually heaped in refuse dump, farm lands and sometimes near homes. They usually constitute nuisance to the environment and are rarely relished by ruminant animals because of their tough nature and low nutritive value.

Maize husk in spite of its limitations could be recycled and used as a source of valuable lignocellulosics biomass for animals if treated with fungi. Belewu and Okhawere (1998), reported on the delignification and nutritive values of rice husk and sorghum stover treated with *Trichoderma harzanium*. They observed increase in crude protein content and decrease in crude fiber content of the fungal treated substrates. Cultivation of edible mushrooms like *Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus sajor caju* and *Lentinus subnudus* on lignocellulosic wastes may thus be valuable for converting these materials, which are considered to be waste into protein rich ruminant feeds. The cultivation and harvest of these fungi on maize husk, apart from enriching the substrate, may also offer economic incentive for agribusiness. In view of the paucity of information on this subject, it is therefore necessary to examine the influence of cultivating edible mushrooms on the chemical composition and *in vitro* fermentation of maize husk. This study was therefore conducted to provide information on the possibility of converting maize husk into a value added feedstuff for ruminant feed via solid state fermentation.

MATERIALS AND METHODS

Sample Collection

Dried samples of maize residues (maize husk) and were collected from the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The materials were milled and oven-treated at 65^0C until a constant weight was obtained for any dry matter determination.

The fungus

The sporophores of *Pleurotus tuber-regium, Pleurotus pulmonarius, Pleurotus sajor caju* and *Lentinus subnudus* growing in the wild were collected from Ibadan University botanical garden. These were tissue cultured to obtain fungal mycelia (Jonathan and Fasidi, 2001). The pure culture obtained was maintained on plate of potato dextrose agar.

Degradation of maize husk by *P. tuber-regium, P. pulmonarius, P. sajor caju* and *L. subnudus*

Preparation of substrate

Jam bottles used for this study were thoroughly washed and dried for 10 min at 100^oC. Twenty five grams of the dried milled substrate was weighed into each jam bottle and 70ml distilled water was added. The bottles were immediately covered with aluminum foil and sterilized in the autoclave at 121^oC for 15 min .Each treatment was conducted in triplicate.

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 a dark cupboard in the laboratory at 30^0C and 100% relative humidity. After 40 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 Menke and Steingass (1998) in 120ml calibrated syringes in three batches at 39^0C. To 200mg sample in the syringe was added 30ml inoculum containing

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 $CaCI_2$. $2H_20$ at a ratio of 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 estimate the amount of methane produced (Fievez *et al*., 2005). The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The gas production characteristics were estimated using the equation $Y = a + b (1-e^{ct})$ described by Ørskov and McDonald (1979), 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 dry matter (DM)) and organic matter digestibility (OMD %) and short chain fatty acids (SCFA) were estimated at 24h post gas collection according to Menke and Steingass, (1988).

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 105^0C for 8 hours. Crude protein was determined as Kjeldhal nitrogen x 6.25. Ether extracts, crude fiber and ash was determined according to (AOAC, 1995) method. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was determined using the method described by Van Soest *et al* (1991). 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 (Statistical Analysis System Institute Inc., 1998) option.

RESULTS AND DISCUSSION

Chemical composition

Shown in Table 1 are the results of chemical composition (g/100g DM) of maize husk treated with four different fungi. A wide variation exists in the different results obtained. The results show a significant ($p \leq 0.05$) increase in the crude protein (CP), ether extract (EE) and ash contents after the fungal treatment. The CP increased from 7.44% (UM)

Table 1. Chemical composition (g/100g DM) of degraded maize husk by four strains of fungi.

Parameters	UM	LSM	PTM	PSM	PPM	SEM
Dry Matter	88.85^a	86.78^b	86.89^b	86.44^b	87.70^a	0.00
Crude Protein	7.44^b	9.89^a	9.67^a	9.55^a	9.90^a	0.30
Ether extract	1.27^b	2.52^a	1.75^a	2.78^a	2.82^a	0.21
Ash	3.32^c	3.53^b	3.90^a	3.86^a	10.37^a	0.26
Crude fiber	30.45^a	22.27^c	24.29^b	14.15^e	19.07^d	0.00
NFE	42.48^a	36.43^b	39.49^{ab}	39.49^{ab}	42.18^a	0.63
ADF	49.15^a	39.27^d	41.05^c	44.09^b	43.94^b	0.30
NDF	71.14^a	58.96^c	60.58^c	62.55^b	63.13^b	0.33
ADL	14.87^a	11.54^b	11.89^b	11.27^b	12.14^b	0.28
Cellulose	34.25^a	27.73^d	29.43^c	32.82^b	31.80^b	0.24
Hemicellulose	21.99^a	19.69^b	19.53^b	18.46^b	19.90^b	0.33

Small case letters imply means in the same row with different superscripts are significantly varied ($p \leq 0.05$).
UM = untreated maize husk (control), LSM = *Lentinus subnudus* degraded maize husk, PTM = *Pleurotus tuber-regium* degraded maize husk, PSM = *Pleurotus sajor caju* degraded maize husk, PPM = *Pleurotus pulmonarius* degraded maize husk, SEM = Standard error of the mean, NFE = Nitrogen Free Extract, ADF = acid detergent fibre, NDF = neutral detergent fiber and ADL = acid detergent lignin.

to 9.90% (PPM). The improved CP value obtained in the fungal treated maize husk could be due to the release of polysaccharide bound protein and this makes the substrate nutritionally better (Belewu *et al*, 2003). This agrees with the report of Broerse and Visser (1996) who stated that the extra cellular enzymes secreted by the fungus contains amorphous homo and heteropolysaccharides which often in association with protein. Rice husk treated with *Trichoderma harzanium* recorded a similar increase in nutrient composition and this has been found to compensate for the low and poor protein content of concentrate diets of raw straw and hay consumed by animals in the tropical environment (Belewu 2001; Belewu and Banjo, 1999). Crude fiber contents (NDF, ADF, ADL, cellulose and hemicellulose) which were observed to consistently reduce in all the four fungi treated substrates agrees with report of Belewu (2001). The decrease in NDF concentration may be attributed mainly to the extensive utilization of hemicellulose by fungi (Chen *et al*, 1995).The preferential degradation of cellulose and hemicellulose could be the result of type of substrate, duration of degradation and physiological behaviors of the fungi used.

Gas volume

The *in vitro* gas production over the period of 24h is shown in Table 2. As could be seen from the trend displayed by the treated substrates more gas production is still possible beyond 24h. There are many factors that may determine the amount of gas produced during fermentation, depending on the nature and level of fiber (Babayemi, *et al*., 2004) and potency of the rumen liquor used for incubation (Babayemi, 2007). Generally, as cited by Babayemi (2007) gas production is a function and mirror of degradable carbohydrate and therefore, the amount of gas produced depends on nature of the carbohydrates (Demeyer and Van Nevel,1975; Bummel and Becker, 1997). All the four fungi used improved gas production, an indication of better digestibility of the treated substrate. Sommart *et al*., (2000) suggested that 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. Methane productions (Figure 1) were highest in the substrate treated by LSM and PTM with the lowest methane production in PSM. Methane production is an energy loss to the animal; this implies that there would be the need for energy supplementation in the diet of the ruminant to replenish this loss.

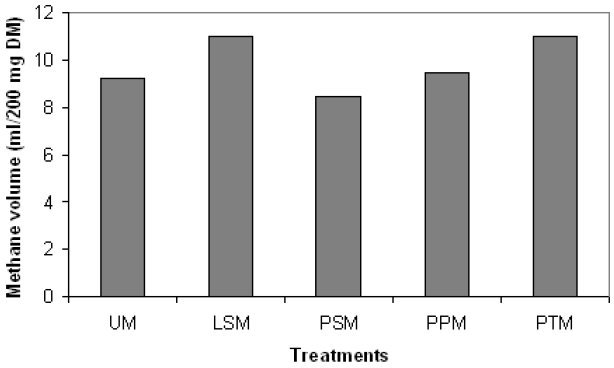

Figure 1. Methane productions from in vitro gas production of maize husk treated with four strains of mushroom. UM = untreated maize husk (control), LSM= *Lentinus subnudus* degraded maize husk ,PTM = *Pleurotus tuber-regium* degraded maize husk, PSM= *Pleurotus sajor caju* degraded maize husk, PPM= *Pleurotus pulmonarius* degraded maize husk.

Table 2. *In vitro* gas production (ml/200 mg DM incubated) from maize husk degraded by four strains of mushroom for a period of 24 hours.

	Incubation period (h)							
Treatments	3	6	9	12	15	18	21	24
UM	8.67^{b}	12.50^{b}	14.00^{b}	14.67^{b}	16.00^{b}	17.67^{b}	19.00^{b}	20.33^{d}
LSM	13.00^{a}	16.33^{a}	17.67^{a}	19.00^{a}	20.30^{a}	21.67^{a}	22.67^{a}	29.00^{a}
PTM	12.00^{a}	15.00^{a}	17.67^{a}	19.33^{b}	19.67^{a}	22.00^{a}	22.67^{a}	23.67^{c}
PSM	12.50^{a}	16.00^{a}	18.33^{a}	19.33^{b}	20.33^{a}	21.33^{a}	22.00^{a}	26.33^{b}
PPM	12.50^{a}	14.67^{a}	18.00^{a}	19.33^{b}	20.33^{a}	20.67^{ab}	22.67^{a}	26.00^{b}
SEM	0.32	0.56	0.28	0.40	0.40	0.64	0.90	1.20

Small case letters imply means in the same column with different superscripts are significantly varied ($p \leq 0.05$), UM = untreated maize husk (control), LSM= *Lentinus subnudus* degraded maize husk ,PTM = *Pleurotus tuber-regium* degraded maize husk, PSM= *Pleurotus sajor caju* degraded maize husk ,PPM= *Pleurotus pulmonarius* degraded maize husk and SEM = Standard error of the mean

Gas production characteristics

Gas production from the fermentation of treated and untreated maize husk was measured at 3, 6, 9, 12, 15, 18, 21 and 24 hours using *in vitro* gas production technique. The results are presented in Table 3. The gas volumes at asymptote (b) describe the fermentation of the insoluble but degradable fraction. It can be seen from the result obtained from this study that value obtained for (b) in the treated maize husk was higher than the untreated, possibly a reflection of the decreased CF and ADL. Furthermore, the fungi used enhanced the CP of the treated substrates. Getachew *et al*., (1999) reported that gas production is basically the result of fermentation of carbohydrate into acetate, propionate and butyrate. The high fermentation obtained for all the fungi treated maize husk may possibly be influenced by the carbohydrate fractions readily available to the microbial population (Chumpawadee *et al*.2007). The fast rate of gas production (c) obtained in the PTM treated maize husk and the untreated substrate (UM) could mean that the carbohydrate were readily available to rumen microbial population. Slower rates were however obtained in LSM, PSM and PPM may be due to specie differences of the fungi used.

Potential extent of gas production (a + b) ml

The potential extent of gas production, (a + b) ml as observed in the results obtained in this study, shows that the fungal treated maize husk were more fermented (Table 3). This implies that the treated substrates were highly available in the rumen. It could be seen from this result also that the entire treated maize husk had a lower NDF compared with the untreated. Therefore, fungal treatment of maize husk resulted in a more easily degradable substrate. This agrees with the findings of Sommart *et al*.,(2000) and Nitipot and Sommart (2003) who stated that energy feeds with lower NDF showed a higher potential extent of gas production (Table1 and Table 2). It could also be stated that the lowest value of potential extent of gas production (a+b) ml, obtained in the untreated maize husk could be the result of the carbohydrate fraction having a high proportion of lignified cell walls, with the resulting low fermentation, and thus low gas production. This agrees with the findings of Melaku *et al*., (2003) who found that fibrous constituents, especially lignin negatively influence *in vitro* gas production.

***In vitro* organic matter digestibility (OMD) and short chain fatty acid (SCFA)**

The result of *in vitro* OMD is shown in Table 3. High digestibility of organic matter was observed in all the fungal treated maize husk, probably because the limiting lignin and crude fiber contents has been reduced, coupled with an increase in crude protein contents. Thus, the release of the substrate's carbohydrate for fermentation by amylolytic bacteria and protozoa was enhanced (Kotarski *et al*., 1992).This result implies that the rumen and animal have high nutrient uptake. The result obtained for short chain fatty acid (SCFA) (Table 3), showed significant difference ($p>0.05$) in the values obtained from treated and untreated substrate. SCFA which was generally higher in all the treated substrate

Table 3. Estimated gas production characteristics, estimated organic matter digestibility (OMD), short chain fatty acid (SCFA) and metabolisable energy (ME).

Parameters	UM	LSM	PTM	PSM	PPM	SEM
Gas production characteristics						
(a+b) (ml)	20.33^{d}	29.00^{a}	23.67^{c}	26.33^{b}	26.00^{b}	0.33
b (ml)	16.66^{e}	16.00^{a}	11.67^{d}	13.83^{b}	13.50^{c}	0.02
c (h^{-1})	0.029^{b}	0.016^{e}	0.032^{a}	0.022^{d}	0.025^{c}	0.01
In vitro digestibility						
OMD (%)	38.28^{d}	45.99^{b}	42.60^{c}	44.89^{bc}	48.97^{a}	0.77
SCFA (mol)	0.55^{d}	0.75^{a}	0.69^{b}	0.69^{b}	0.63^{c}	0.07
ME (MJ/kg DM)	5.45^{d}	6.75^{a}	6.37^{b}	6.37^{b}	6.34^{b}	0.70

Small case letters imply means in the same row with different superscripts are significantly varied ($p \leq 0.05$)
a+b= final gas produced, b = gas production from the insoluble fraction, c = gas production rate constant for the insoluble fraction, UM = untreated maize husk (control) LSM = *Lentinus subnudus* degraded maize husk ,PTM = *Pleurotus tuber-regium* degraded maize husk, PSM = *Pleurotus sajor caju* degraded maize husk, PPM= *Pleurotus pulmonarius* degraded maize husk and SEM = Standard error of the mean.

implies energy availability to the animals. A number of factors could be responsible for this, such as high gas production in the treated substrate and this is more evident throughout the period of fermentation. Gas production from different classes of feeds incubated *in vitro* in buffered rumen fluid is closely related to the production of SCFA which is based on carbohydrate fermentation (Sallam *et al*., 2007)

Estimated metabolisable energy (ME)

Metabolisable energy was predicted using the equation of Menke and Steingass, (1988). The estimated ME values for the different substrates are shown in Table 3.The values obtained differed significantly. The ME obtained for the fungal treated maize husk are similar to those obtained for corn meal (Chumpawadee *et al*., 2007) and *Tephrosia candida*/Guinea grass mixtures (Babayemi, 2007). Menke and Steingass, (1988) reported a strong correlation between ME values measured *in vivo* and predicted from 24h *in vitro* gas production and chemical composition of feed. The *in vitro* gas production method has been successfully used to evaluate the energy value of several classes of feed (Getachew *et al*, 1998; Getachew *et al*, 2002; Babayemi, 2007; Sallam *et al*., 2007). Sallam *et al*., 2007 suggested that the *in vitro* gas production system helps to better quantify nutrient utilization and its accuracy in describing digestibility in animals has been validated in numerous experiment.

CONCLUSIONS

The results obtained in this study suggest that the treatment of maize husk by the application of fungi will help in conversion of agricultural wastes to higher quality ruminant feed thereby enhancing their digestibility by ruminants. It is therefore recommended that more work should geared towards this direction to harness the hidden potentials of agricultural wastes for the benefit of the developing countries.

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