EVALUATION OF THE NUTRITIVE VALUE OF SORGHUM THRESHED TOP TREATED WITH WHITE-ROT FUNGI

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SUMMARY

Sorghum threshed top can be converted into value added ruminant feed. In this study edible mushrooms: *Pleurotus ostreatus* and *Pleurotus pulmonarius* was cultivated on sorghum threshed top in a solid state fermentation for a period of 21 days. The resultant substrates were subjected to chemical and minerals analysis and the *in vitro* digestibility was determined. The results obtained showed a significant ($p<0.05$) increase in the crude protein contents from 7.24% in the control to 12.97% for *Pleurotus ostreatus* treated sorghum threshed top (POT) and 14.89% for *Pleurotus pulmonarius* treated sorghum threshed top (PPT). On the other hand there were decreases in the crude fiber (CF) and CF fractions. The contents of calcium, potassium and iron also increased significantly. Gas production rate constant (c) increased from 0.010 (h^{-1}) in control to 0.019(h^{-1}) for POT and 0.030(h^{-1}) for PPT. The estimated organic matter digestibility (OMD), short chain fatty acid (SCFA) and metabolisable energy (ME) were observed to highest in POT followed by PPT. The same trend was observed for the gas volume at different incubation period.

***Keywords:** edible mushroom, solid state fermentation, in vitro digestibility*

INTRODUCTION

By the year 2020, the global population is expected to increase by more than 40%, possibly exceeding the billion mark (Belewu *et al* 2005). Feeding these additional population with a limited supply of suitable farmland and livestock poses a problem of immense proportions in the developing countries where 800 million people suffer from malnutrition today (Jarven, 2003).The application of biotechnology in bridging the gap between animal nutritional requirements and available feedstuffs is thus necessary. In Nigeria, large quantities of sorghum threshed top is generated annually especially in the northern part of the country where the cultivation of sorghum is common. Bioconversion of these wastes into ruminant feed will improve livestock feeding in Nigeria. El-Ashry *et al*, (2003) and El-Kady *et al*, (2006) applied *Trichoderma viride* to improve the nutritive value and digestibility of poor quality roughages. So, exogenous enzymes obtained from fungal treatment will lead to beneficial effects on animal performance. Cultivation of edible mushrooms *Pleurotus ostreatus* and *P. pulmonarius* on agro wastes are likely to improve the nutritive value of the wastes. The objective of this work was to study the effect of cultivation of two edible mushrooms on the chemical and mineral compositions, and *in vitro* digestibility of sorghum threshed top.

MATERIALS AND METHODS

Sample Collection:

Dried samples of agricultural wastes (sorghum threshed top) 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 65^0C 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 (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 (120mls) used for this study were thoroughly washed, dried for 10 min. at 100°C and 25.00g of the dried milled substrate 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 30^0C 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, 1995) 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% *Pannicum maximum* at

5% body weight. The rumen liquor was collected into the thermo flask that had been pre warmed to a temperature of 39^0C from the goats before they were offered the morning feed. Incubation procedure was as reported by Menke and Steingass (1998) 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 $MgSO_3$ $7H_2O$ + 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 ± 1^0C 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.*, (2005). 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 (1988). 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 (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), organic matter digestibility (OMD) (%) was assessed as OMD = 14.88 + 889Gv + 0.45CP + 0.651XA (Menke and Steingass), short chain fatty acids (SCFA) as 0.0239 V – 0.0601 (Getachew *et al.*., 1999) 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 (1989) options.

RESULTS AND DISCUSSION

Alteration in the chemical composition:

After 21 days fungal incubation of sorghum husk, the chemical composition was altered (Table 1). The two fungi used increased crude protein (CP) content and may have been a result of increased fungal biomass (Chen *et al.*, 1995). The increase in CP content may 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 (Kadiri, 1999). These results agreed with those obtained by Akinyele and Akinyosoye, 2005; Belewu and Belewu, 2005 and El Shafie *et al*, 2007; Isikhuemhen and Okhuoya 1999). The fungi used decreased NDF concentration mainly due to the extensive utilization of hemicellulose (Chen, *et al*, 1995). Similar decrease was also observed for the cellulose contents of the fungal treated sorghum threshed tops. This may probably be the result of the utilization of the cellulose by the fungi as an energy source during growth. The variations observed in the cellulose and hemicellulose content of the substrates could be the result of physiological behaviour of these fungi with respect to lignocellulose degradation (Isihkuemhen *et al.*, 1996). Consistent decrease in ADL was also obtained in the fungal treated wastes compared with the untreated. The extent of lignin degradation of these fungi may be related to their ability to produce lignin-degrading enzymes, such as lignin peroxidase and manganese peroxidase (Nerude *et al.*, 1991). A consistent significant decrease was also observed in the CF contents of the treated wastes compared with the untreated, and may have been the result of extracellular enzymes produced by the fungi used capable of reducing the fiber contents (Belewu and Belewu, 2005).

Table (1): The chemical composition (g/100g DM) of sorghum threshed tops treated with white-rot fungi

Parameters	Control	POT	PPT	SEM
Dry matter	91.10	92.41	91.10	0.00
Crude protein	7.24^{c}	12.97^{b}	14.89^{a}	0.10
Crude fiber	31.77^{a}	28.43^{b}	26.44^{c}	0.09
Ether extract	6.28^{a}	6.03^{b}	5.70^{c}	0.09
Ash	7.17^{a}	7.17^{a}	6.43^{b}	0.11
NFE	47.52^{a}	47.30^{a}	44.22^{b}	0.22
NDF	70.28^{a}	60.04^{b}	60.03^{b}	0.02
ADF	48.62^{a}	42.79^{b}	41.68^{b}	0.05
ADI	14.94^{a}	12.65^{b}	10.63^{c}	0.09
Cellulose	33.68^{a}	31.05^{b}	30.14^{c}	0.08
Hemicellulose	21.66^{a}	18.36^{b}	17.25^{c}	0.07

a,b,c, 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

Table (2): Some major (g/100g DM) and trace minerals (ppm) composition of treated and untreated sorghum threshed tops.

Major minerals	Control	POT	PPT	SEM
Calcium	0.522^{c}	2.3275^{a}	0.6245^{b}	0.00
Phosphorus	0.078^{b}	0.093^{a}	0.097^{a}	0.00
Magnesium	0.366^{b}	0.653^{a}	0.344^{c}	0.00
Sodium	0.00556^{b}	0.0456^{a}	0.048^{a}	0.00
Potassium	0.0658b	5.45a	0.05b	0.00
Trace minerals				
Iron	0.753^{c}	1.38^{b}	3.61^{a}	0.04
Copper	0.0022^{b}	0.043^{a}	0.050^{a}	0.00
Zinc	0.011^{b}	0.041^{a}	$0.011b^{a}$	0.00
Manganese	0.012^{b}	0.012^{b}	$0.077a^{a}$	0.02

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 shel

Table (1) showed that there are no significant differences among treated wastes in DM content. El Marakby (2003) pointed that there are slightly decreased in DM content after treated wheat straw with *Agaricus bisporus*. The fungi used decreased EE content due to consuming some fatty acids by microorganisms as a suitable energy source for growth.

These results agreed with those obtained by Sabry (2007) who found that fungal treated led to decrease EE content.

Changes in the Mineral compositions:

Wide variations exist in the results of the minerals contents of the treated substrates compared with the untreated (Table 2). The major minerals and micro minerals with the exception of manganese were significantly ($P<0.05$) higher in POT especially the critical minerals: calcium and phosphorus. Mushrooms are good source of protein, vitamins, and minerals (Khan *et al.*, 1981). Mushrooms contain appreciable amount of potassium, phosphorous, copper and iron but low level of calcium (Anderson and Feller, 1942).

Gas volume and gas production characteristics:

The results of gas volume and gas production characteristics are shown in Table (3). A comparison of gas volume and gas production characteristics showed significant differences ($P<0.05$) in the result obtained. The gas volume at asymprote (b) describes the fermentation of the insoluble fraction. The fermentation of the insoluble fraction of the control differed significantly ($P<0.05$) from POT but not significantly different ($P>0.05$) from PPT, although the value obtained for (b) in POT was slightly higher than control. The higher values observed in the fungal treated substrates could possibly due to reflection of lignin removal (Table 1) (Chumpawadee *et al.*, 2007). Digestibility of lignocellulosics has been known to be correlated with lignin content (Zadrazil, 1982, 1985). This observation agrees with the findings of Melaku *et al.*, (2003) who found that fibrous constituents especially lignin influence *in vitro* gas production.

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

Parameters	Control	POT	PPT	SEM
c (h^{-1})	0.010^{b}	0.019^{ab}	0.030^{a}	0.00
b (mL)	51.67^{b}	61.67^{a}	54.00^{b}	0.19
Gv after 24	35.67^{c}	60.00^{a}	47.00^{b}	0.48
Gv after 48	42.33^{c}	63.00^{a}	49.67^{b}	1.12
Gv after72	54.33^{C}	69.67^{a}	61.00^{b}	0.46
Gv after 96	61.33^{c}	76.33^{a}	69.00^{b}	0.50
CH_4 ml				

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

The results of gas volume at 24, 48, 72, and 96h (Table 3) showed a significant high gas volume produced by the fungi treated substrates. Menke and Steingass (1988) reported that gas volume at 24h incubation is indirect relationship with metabolisable energy in feedstuffs. Fievez *et al* (2005) suggested that although the gas production from *in vitro* fermentation is a nutritional waste, it remains one of the reliable means to measure quality of feeds. 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*, 2004b) and potency of the rumen liquor for incubation (Babayemi, 2007). Gas production has also been shown to have a close relationship with feed intake (Blummel and Becker, 1997). The fast rates of gas production obtained in the treated substrates could be the influence of carbohydrates fractions readily available to the microbial population (Chumpawadee *et al.*,2007). Nsahlai *et al.*, (1994) and Larbi *et al* (1998) reported that there were positive

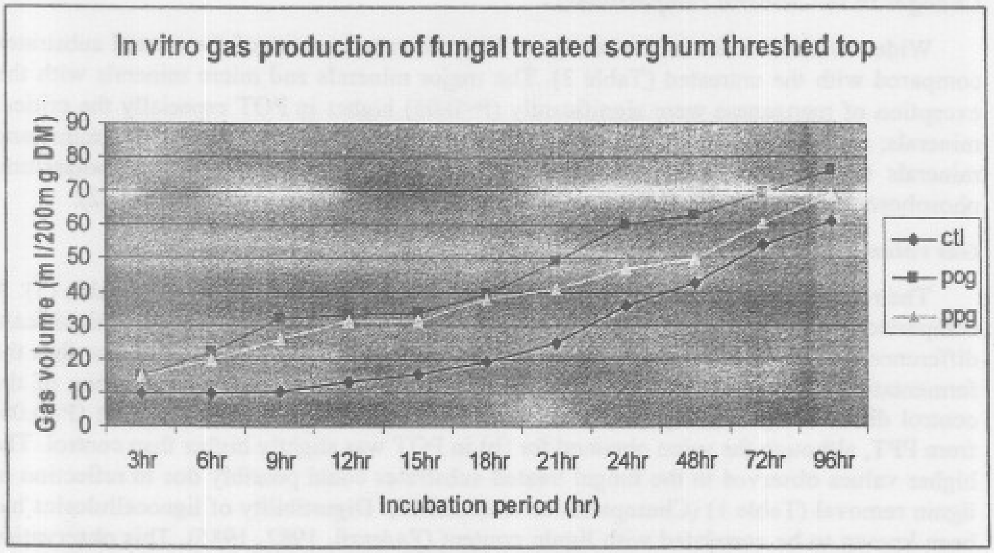

Figure (1): *In vitro* gas production of fungal treated and untreated sorghum threshed top

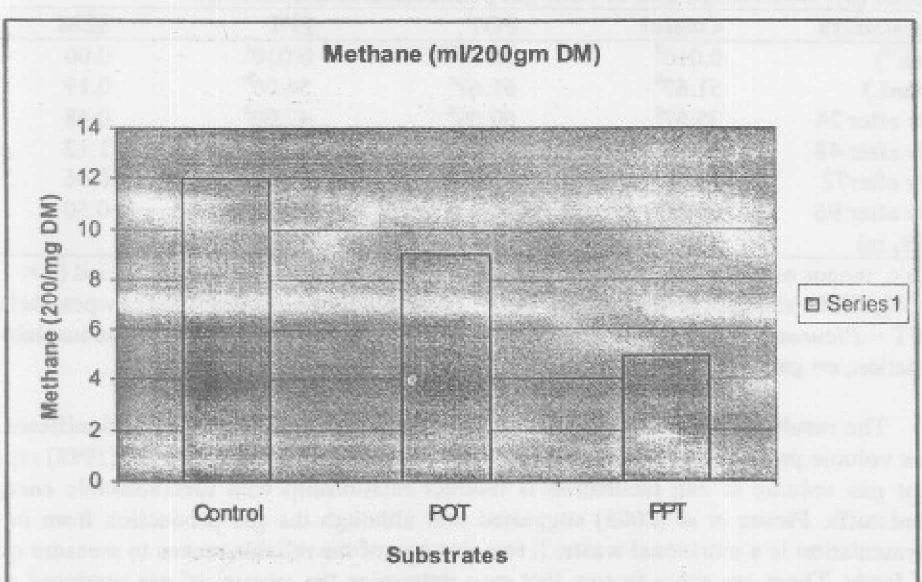

Figure 2: Methane production of fungal treated and untreated sorghum threshed tops

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 (Cone *et al.*, 1997). The fermentation of insoluble fraction were significantly higher in all the treated substrates compared with the untreated, possibly a reflection of reduced lignin contents and enhanced CP. The result of *in vitro* gas production pattern (Fig. 1) over an incubation period of 96 hr while figure 2 show the methane production of the different substrates under study. Methane production has negative effects on ruminants in one hand as it is an energy loss to animal and on the other hand, when accumulates in rumen, it results in bloat (Babayemi, 2006).

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

In vitro OMD at 24h after incubation is shown in Table (4). The value obtained for OMD differed significantly ($P<0.05$) among the different substrates with the highest value obtained in POT followed by PPT. High digestibility of organic matter was observed in the fungal treated substrates. These results implies that the microbe in the rumen and animal have high nutrient uptake. The lower fiber content (Table 1) of the fungal treated substrates probably resulted in higher *in vitro* organic matter digestibility since low NDF and ADL content of feedstuff is expected to favour higher degradation (Van Soest, 1988). Generally large proportion of lignin in the cell wall constituents does not favour fermentation and digestibility. The results obtained in this study showed that fungi used did not only increase the protein contents of the substrates but also reduced significantly the recalcitrant lignin present in the untreated sorghum threshed top.

Table (4): Short chain fatty acid (SCFA), organic matter digestibility (%) as affective by treatments after 24 h of incubation

Parameters	Control	POT	PPT	SEM
SCFA (µM)	0.792^{c}	1.373^{a}	1.063^{b}	0.01
OMD (%)	54.20^{c}	78.48^{a}	67.13^{b}	0.43
ME(MJ/Kg DM)	7.56^{c}	11.18^{a}	9.52^{b}	0.68

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.

Metabolisable energy was predicted by the equation of Menke and Steingass (1988). The ME of the different substrates is shown in Table (4). Menke and Steingass (1988) reported a strong correlation between ME values measured *in vivo* and predicted from 24h *in vitro* production and chemical composition of feed. The *in vitro* gas production method has also been widely used to evaluate the energy value of several classes of feed (Getachew *et al.*, 1999; Aiple *et al.*, 1996). The use of the *in vitro* gas method to estimate the ME of feedstuff should be encouraged because it reduces cost of labor saves time and is less expensive.

The SCFA was also estimated using the methods of Menke and Steingass (1988). The results obtained in this study showed a significant (P<0.05) variations in the results obtained. Higher values were obtained in the fungal treated substrates compared with the untreated. Higher production of gas and the eventual preponderance of SCFA in the fungal treated substrate probably showed an increase proportion of acetate and butyrate but may mean a decrease in propionate production (Babayemi et al 2004b) This high value of SCFA in the treated substrates suggest a potential to make energy available to the ruminants (Babayemi and Bamikal, 2006a).

CONCLUSION

Nutrient composition of the fungal treated sorghum husk showed an increase in the CP and this means it might be useful in combination with other feedstuffs as energy and nitrogen easily to digest. The ADL and CF contents were reduced in the treated samples with the consequent result of increased digestibility of the feedstuffs; therefore we suggest to use these biological treatment for the diet of the ruminants

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تقييم القيمة الغذائية لقيم الذرة السورجم المعاملة بفطر عيش الغراب

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في هذا البحث تم استخدام قمم السورجم المقطعة بعد معاملتها بالفطر الصالح للاستهلاك مثل *Pleurotus Ostreatus, Pleurotus Pulmonarius* حيث تم زراعتها على مخلف السورجم لمدة ٢١يوم.

وقد تم دراسة تأثير هذه المعاملة البيولوجية على التركيب الكيماوى والمعدني وكذلك *In vitro* على المخلف المعامل.

وأظهرت النتائج زيادة المحتوى من البروتين الخام ٧,٢٤ % إلى ١٢,٩% بالنسبة للفطر الأول وكذلك ارتفاعه الى ١٤,٨٩ % بالمعاملة بالفطر الآخر وكذلك أدت المعاملة بالفطر إلى انخفاض المحتوى من الألياف الخام وكذلك مكونات جدر الخلية بالمعاملة وكذلك زاد المحتوى من الكالسيوم والبوتاسيوم والحديد.

أما بالنسبة لحجم الغاز المنتج فقد زاد من ٠,٠١ (h^{-1}) في المخلف الغير معامل الى ٠,٠١٩ (h^{-1}) عند المعاملة بالفطر الآخر.

كذلك وجد زيادة في محتوى المادة العضوية المهضومة وكذلك الأحماض الدهنية قصيرة السلسلة والطاقة الممثلة خاصة في المخلف المعامل بالفطر الأول مقارنة بالفطر الثاني.

مما تقدم ينصح باستخدام المعاملات البيولوجية في تحسين القيمة الغذائية للمخلفات الزراعية وإدخال هذه المخلفات المعاملة في تغذية الحيوانات المجترة.