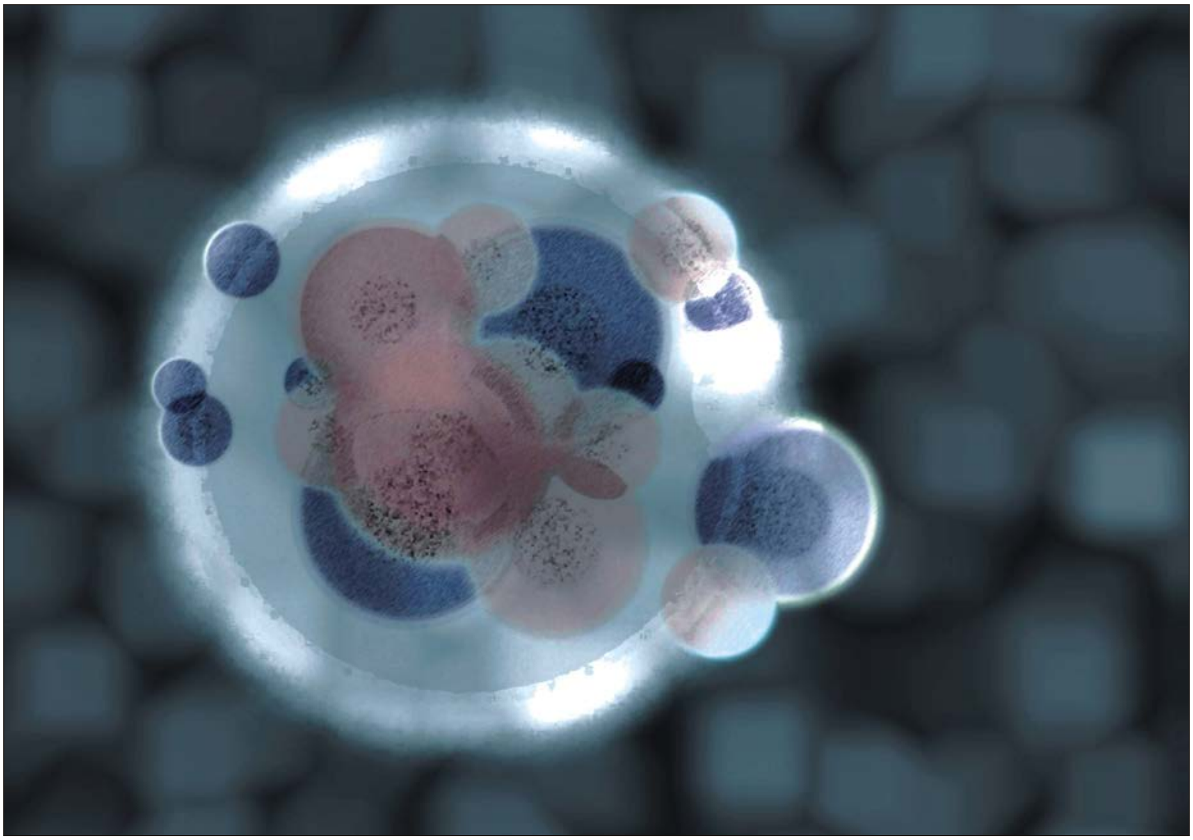

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An *in vitro* Evaluation of *Pleurotus ostreatus* EM-1-modified Maize (*Zea mays*) Cob as a Non-conventional Energy Source for Livestock in Ghana

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Abstract: Treatment with *Pleurotus ostreatus* strain EM-1 recently has been proposed as an effective means of transforming maize cob into nutritive animal feed for livestock production in the West African sub-region. This study compares *P. ostreatus* strain EM-1-treated maize cob with peels of cassava and plantain, widely-accepted complementary feedstuffs in West Africa, in terms of *in vitro* biodegradability and composition. Subjection of milled maize cob samples to solid state fermentation by *P. ostreatus* strain EM-1, until complete mycelial colonization, resulted in an increase of 107.3% in cell extractives and a 41.2% reduction in lignin content. The cellulose content of the treated maize cob exceeded that of plantain peel and cassava peel by 44.9 and 71.2%, respectively, while protein and lipid content did not differ significantly from mean values obtained for cassava peel. Cellulosic sugar production from treated maize cob, measured at 37°C for up to 3 h in the presence or absence of 0.05 U mL^{-1} cellulase, surpassed that of cassava peel by 52.3% ($p<0.05$) but was significantly lower than that of plantain peel. The data indicate that the potential metabolizable energy of *P. ostreatus* strain EM-1-modified maize cob far exceeds that of cassava peel. Based on the present findings, maize cob treated with *P. ostreatus* strain EM-1 should serve as an excellent complementary energy source for small ruminants in the West African sub-region.

Key words: Mushroom, maize cob, delignification, animal feed, cellulose, biodegradability

INTRODUCTION

Ghana generates vast quantities of maize (*Zea mays*) cob annually from the cultivation of maize (Agyare *et al*., 2006; Abunyewa *et al*., 2007) but derives little benefit from this crop residue, much of which is disposed of by burning. Maize cob contains a considerable amount of cellulose, a linear biodegradable polymer comprising β-(1, 4)-D-glucopyranose units (Kumar *et al*., 2010). Cellulolytic microorganisms in the rumen have the capacity to convert the cellulose fraction of lignocellulosic materials such as maize cob into metabolisable sugars (Kuan and Liong, 2008; Israel *et al*., 2008). However, the use of maize cob in particular as a complementary feedstock is severely constrained by the fact that its rate of enzymatic degradation is probably one of the lowest recorded for lignocellulosic residues. This is largely due to the presence of lignin, a complex branched aromatic heteropolymer of phenylpropane units highly resistant to biodegradation. It provides plant cell walls with rigidity and protection by forming a matrix around structural polysaccharides (Van Parijs *et al*., 2010). The degree of association between cellulose and this recalcitrant biopolymer is believed to be the single most important factor influencing the susceptibility of the cellulose component of lignocellulose to enzymatic degradation (Ahmed *et al*., 2001; Besombes and Mazeau, 2005; Ndubuisi *et al*., 2008).

In a previous study, we demonstrated that treatment of maize cob meal with the EM-1 strain of *Pleurotus ostreatus* caused a marked improvement in cellulose biodegradability (Adamafio *et al*., 2009a). *Pleurotus ostreatus* strain EM-1 is an edible mushroom that is cultivated in commercial quantities all year round in Ghana because of its, high yield and environmental adaptability (Obodai and Vowotor, 2002). Like other white-rot fungi, it produces various extracellular lignin-degrading enzymes during cultivation (Isikhuemhen and Mikiashvilli, 2009; Dashtban *et al*., 2009).

A comparative assessment of the biodegradability and biochemical composition of *Pleurotus ostreatus* strain EM-1-treated maize cob is critical to its acceptance

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by the livestock sector in Ghana. The present study was therefore carried out to compare the enhanced biodegradability and composition of *P. ostreatus*-treated maize cob with those of plantain (*Musa paradisiaca*) peels and cassava (*Manihot esculenta*) peels, lignocellulosic complementary feedstuffs which are widely-used in the West African sub-region (Danso *et al*., 2006; Onyimonyi and Ugwu, 2007; Duku *et al*., 2010).

MATERIALS AND METHODS

Air-dried maize cobs, unripe plantain peel and cassava peel were obtained locally and from the Forest and Horticultural Crops Research Centre of the University of Ghana at Kade. The cobs and peels were shredded in a hammer mill and then ground in a disc attrition mill, oven-dried at 60°C to constant weight and stored at 4°C. *P. ostreatus* (Jacq. ex. fr) Kummer strain EM-1was obtained from the Council for Scientific and Industrial Research-Food Research Institute (CSIR-FRI), Ghana.

Mushroom cultivation: *P. ostreatus* (Jacq. ex. fr) Kummer strain EM-1, was maintained on potato dextrose agar slants and spawn was prepared on sorghum grains (Zadrazil, 1978). Both cultures and spawn were incubated in the laboratory at 26-28°C and 60-65% relative humidity. Substrate preparation and inoculation were carried out till the end of spawn run as previously described (Adamafio *et al*., 2009b). The colonised substrate was then were oven-dried at 60°C to constant weight and stored at 4°C.

Cellulosic sugar release and Soluble sugar content: The *in vitro* rate of cellulosic sugar release was determined using exogenous cellulase as previously described (Adamafio *et al*., 2009a). Results are expressed as Mean±SEM of four determinations. Soluble sugar content of each by-product was estimated by refluxing samples weighing between 7 to 10 g with 100 mL of deionised water for 16 h. The resulting aqueous extracts were stored at -20°C until analyzed for reducing sugars.

Chemical composition: Protein, lipid and lignocellulose were determined using the macro-Kjedahl method (AOAC, 1970), soxhlet extraction using petroleum ether and a gravimetric method (Van Soest and Robertson, 1980), respectively. Extractives and moisture content were also estimated (Sluiter *et al*., 2008; AOAC, 1970). Tannin content was determined using the vanillin-HCl assay (Price *et al*., 1978). Potential metabolizable energy was taken to be the sum total of the products of metabolizable bio-and macromolecule content and the appropriate literature value for energy content (carbohydrate: 15.8; lipid: 37.8 KJ g^{-1}). Protein was excluded since most protein-derived amino acids do not contribute to energy metabolism in the fed state. The enzyme inhibition index was calculated as the sum of condensed tannin and lignin content. Mean values were expressed on dry weight basis.

Statistical analysis: Analysis of variance (ANOVA) tests along with Least Significant Difference (LSD) post-hoc comparisons were conducted using Excel Data Analysis Statistical Software and Statgraphics-plus Software Programme (Version 3.0). The level of significance was set to $p<0.05$. Differences among means with $p<0.05$ were accepted as representing statistically significant differences.

RESULTS

Pleurotus ostreatus EM-1 was successfully cultivated on non-supplemented milled maize cob. The colonization of the maize cob substrate by mycelia was completed 21 days after inoculation. Susceptibility to the action of exogenous cellulase was taken to be a measure of *in vitro* biodegradability and was measured as the rate of release of reducing sugars from incubated samples. The degradability of maize cob substrate, obtained at the end of spawn run, was compared with that of milled peels of plantain and cassava.

As shown in Fig. 1, the amount of cellulosic sugar released from *P. ostreatus* strain EM-1-treated maize cob after a 3 h incubation was 52.3% higher ($p<0.05$) than the mean value for cassava peel samplesbut significantly lower ($p<0.05$) than the mean value for plantain peel

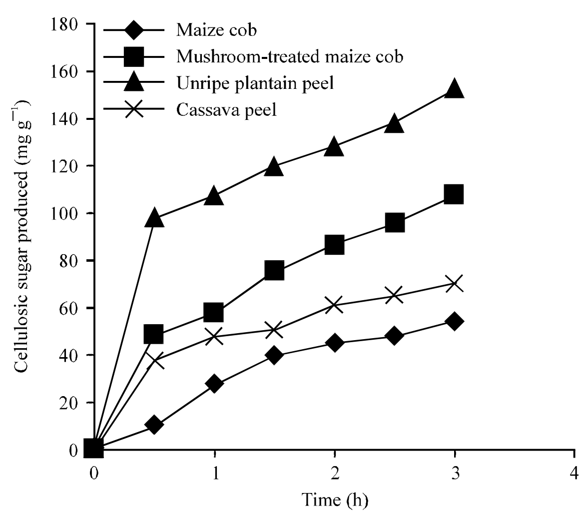

Fig. 1: Rate of cellulase-induced sugar production *in vitro*

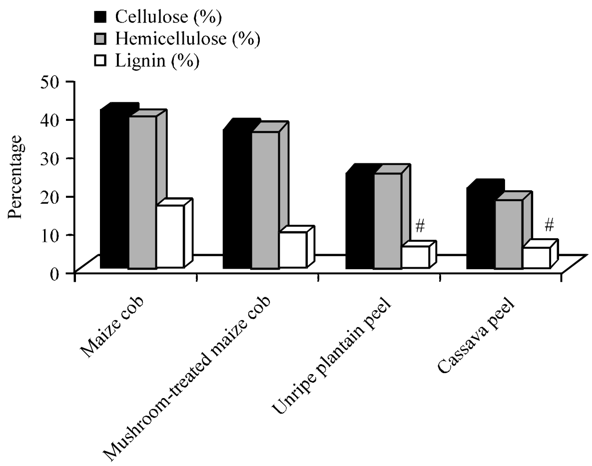

Fig. 2: Lignocellulose profile of by-products. Similar symbols denote a lack of significant difference between values

samples (Fig. 1). Also, the amount of cellulosic sugar released from the mushroom-treated maize cob was approximately twice that of untreated maize cob (Fig. 1).

The lignocellulose profiles of the by-products are presented in Fig. 2. Although treatment with *P. ostreatus* strain EM-1 resulted in a considerable reduction in the acid detergent lignin content of maize cob (from 13.1-8.4%), the level of the recalcitrant biopolymer remained significantly higher ($p<0.05$) than values for plantain peel (5.6%) and cassava peel (5.1%). Nonetheless, the ratio of structural carbohydrate to lignin increased considerably from 4.90 to 7.55 which was comparable to 8.61 and 6.98 for plantain peel and cassava peel, respectively (Fig. 3). The amount of cellulose present in treated maize cob was approximately 45 and 71% higher than mean values obtained for plantain peel and cassava peel, respectively. Similarly, as shown in Fig. 3, the hemicellulose content of treated maize cob was considerably higher ($p<0.05$) than that of plantain peel (42%) and cassava peel (98%). All of the differences recorded in cellulose content of the by-products were statistically significant ($p<0.05$). This was also true of hemicellulose content (Fig. 2).

As expected, treatment with the mushroom caused significant increases in both the protein content (80.9%) and cell extractives content (107.3%) of maize cob. The soluble sugar content of the maize cob increased significantly after treatment with *Pleurotus ostreatus* EM-1 but was low compared with that observed for plantain peel (Table 1). A slight reduction in the potential metabolizable energy of maize cob occurred following treatment with *P. ostreatus*; nonetheless it exceeded the mean value for cassava peel by 81.8% (Fig. 3). The enzyme inhibitor index of the treated maize cob was less than 50% of the value for plantain peel (Fig. 3).

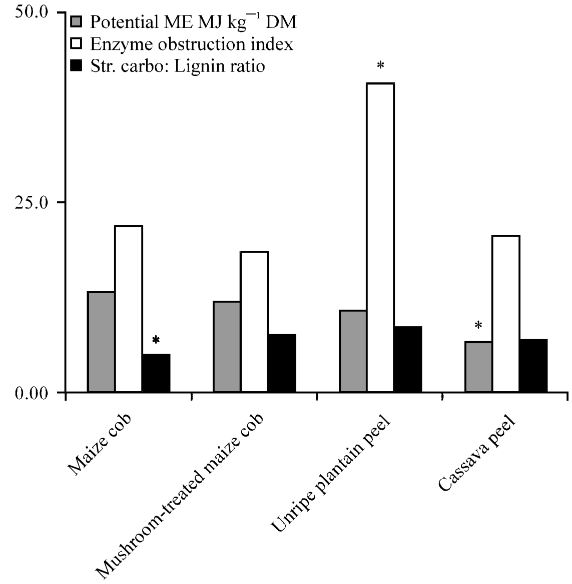

Fig. 3: Potential metabolizable energy, enzyme inhibition index and structural carbohydrate to lignin ratio. Asterisks denote significant difference ($p<0.05$) from value for *P. ostreatus*-treated maize cob

Table 1: Selected constituents of crop residues/by-products

	DW (%)				
Crop by product	Dry matter	Lipid	Soluble sugars	Protein	Cell extractives
Maize cob	91.6	0.52	0.92	2.41*	6.8*
Treated maize cob	94.2	0.73	1.52	4.36	14.1
Unripe plantain peel	87.1	4.64*	5.71*	6.92*	6.4*
Cassava peel	83.5	0.62	0.55*	4.62	7.5*

*Mean value is significantly different from value for *P. ostreatus*-treated maize cob ($p<0.05$)

DISCUSSION

The most important considerations in the evaluation of any lignocellulosic material as an energy source for ruminants are the rapidity with which cellulose is depolymerized and the size of the cellulose fraction. Not surprisingly, treatment with *P. ostreatus* strain EM-1 led to an enhanced production of cellulosic sugar *in vitro*, reflecting greater cellulose biodegradability. This is attributable to the significant reduction in lignin content caused by *P. ostreatus* strain EM-1 and is consistent with the well-documented ability of mycelia of *Pleurotus* species to synthesize extracellular ligninolytic enzymes including laccase and manganese peroxidase

(Ahmed *et al*., 2001; Olfati and Peyvast, 2008). The extent of lignin depolymerisation (35.6%) in the present study exceeded the values (23-30%) reported by Rani *et al*. (2008) for lignocellulosic wastes treated with other strains of *P. ostreatus*. Contrary to expectations, the inverse correlation between the rate of cellulosic sugar release and lignin content was extremely weak (-0.61). However, there was a strong positive correlation between the rate of sugar release and the ratio of structural carbohydrate to lignin (r = 0.93), suggesting that this ratio might be the single most important determinant of lignocellulose biodegradability. The loss of cellulose (11.6%) recorded by the end of mycelia colonization in the present study is approximately three-fold greater than losses recorded for other strains of *P. ostreatus* in previous studies (Suguimoto *et al*., 2001), raising the possibility that genetic differences in the biological efficiency of strains might influence the amount of cellulose metabolized (Mirzaei *et al*., 2007). It is not clear whether the loss of cellulose can be minimized through manipulation of the solid-state culture conditions.

A number of researchers have reported satisfactory performance characteristics of small ruminants on cassava peel based diets (Ahamefule *et al*., 2006; Baiden *et al*., 2007; Lounglawan *et al*., 2011). Our *in vitro* assessment suggests that small ruminants might perform better on *P. ostreatus*-modified maize cob-based diets since the modified cob was superior to cassava peel with respect to biodegradability, structural carbohydrate content and potential metabolizable energy. Thus, a greater amount of energy should be generated from treated maize cob. An added advantage to the use of *P. ostreatus*-treated maize cob as a complementary feedstuff is the absence of the potentially toxic cyanogenic glycosides found in cassava peel (Adamafio *et al*., 2009b; Jorgensen *et al*., 2011).

In all likelihood, *P. ostreatus*-treated maize cob might also prove to be useful as a non-conventional energy source for non-ruminants such as pigs and poultry. Although monogastric animals do not degrade structural carbohydrates to the same extent as ruminants, a significant degree of cellulose degradation does occur in the small intestine and caecum of non-ruminants. Digestibility coefficients for cellulose ranging from 39.7 to 43.8 in pigs have been reported (Keys *et al*., 1969; Adeyemi and Familade, 2003; Shakouri *et al*., 2006). The ability of pigs and poultry to utilize cassava peel-based diets has been demonstrated unequivocally (Adesehinwa *et al*., 2011; Augustine *et al*., 2011). Since the treated maize cob displayed greater biodegradability than cassava peel, it is reasonable to expect that non-ruminants would degrade the former more readily.

The comparison between treated maize cob and plantain peel presented a mixed picture. *P. ostreatus* EM-1-treated maize cob was less degradable than plantain peel but contained a greater amount of structural carbohydrates and appeared to have slightly higher potential metabolizable energy. The positive influence of plantain peel on the performance characteristics of rabbits, pigs and cattle is well-documented (Omole *et al*., 2008; Ogunsipe and Agbede, 2010; Emaga *et al*., 2011). Ultimately, feeding trials would have to be conducted to determine whether or not the response to treated maize cob *in vivo* would compare favourably with that of plantain peel. It is interesting to note that plantain peel displayed superior biodegradability *in vitro* despite its high enzyme obstruction index. The index predicts the magnitude of the combined negative influence of tannins and lignin on the digestion process *in vivo*. Tannins form stable complexes with digestive enzymes thereby rendering them ineffective, while the association between lignin and structural carbohydrates restricts enzyme access (Lamy *et al*., 2011). Of course, many other factors affect the rate of lignocellulose digestion. For instance, the susceptibility of cellulose to enzymatic attack is not solely dependent on the degree of association with ligninbut also on the physical form of the cellulose. Crystalline cellulose which is more tightly packed, is not easily penetrated by water and enzyme, making it more resistant to enzymatic degradation than amorphous cellulose.

CONCLUSION

In conclusion, the present findings provide unequivocal evidence, for the first time, that treatment of maize cob with *P. ostreatus* strain EM-1 upgrades its biodegradability to a level comparable with that of cassava peel, a by-product that is commonly used in the West African sub-region as a feedstuff for ruminants. The use of *P. ostreatus* strain EM-1-treated maize cob as a non-conventional energy source would provide a partial solution to the critical deficit of dry season feed for livestock in Ghana. It would also minimize the environmental consequences of the inappropriate disposal of vast quantities of cob through burning.

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