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Effects of fungal (*Lachnocladium spp*.) pretreatment on nutrient and antinutrient composition of corn cobs

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The nutritive value of corn cob following pretreatment by fermentation with fungal species of *Lachnocladium spp*. was investigated. Corn cob was milled and subjected to incubation with fungal species for a period of one week. Significant increase ($p<0.05$) in protein, ash and some mineral elements were observed. Calcium, magnesium, zinc and sodium content were observed to be higher in fermented cobs while significant decrease ($p<0.05$) were observed in the fiber and antinutrient composition. Phytate, saponin, and oxalate levels were particularly lower in the fermented cobs. Biological pretreatment of corn cobs by fermentation with *Lachnocladium* species was found to significantly improve the nutritive value of corn cob, thus its potential usage in balanced feed for animal product was greatly improved.

Key words: Corn cob, nutrients, *Lachnocladium spp*., fermentation.

INTRODUCTION

Huge quantities of agro-industrial biomass are produced worldwide annually, although, these materials are potential feed resources for ruminant livestock, their use is limited due to high fiber components (Jarommi et al., 2011). Vast quantities of crop residues are generated as a result of agricultural practices. These residues pose both disposal and environmental pollution problem. These results in the loss of nutritionally valuable materials which when processed could yield various valuable products like biofuels, chemicals and cheap energy sources for fermentation, improved animal feeds and human nutrients (Soliman et al., 2013). There is tremendous potential of agro- industrial by-products and crop residues in upholding the aims of livestock production. However the high amount of lignin content of the residues underscores optimal utilization. Lignin interferes by acting as a physical barrier that prevents the contact of cellulase to cellulose and other nutrients (Umamaheswari et al., 2010). Currently, about eight million metric tonnes of corn are produced annually (Nwanma, 2009) and a production forecast for 2010-2015 envisaged a 23% growth. The maize plant comprises of the stalks, husks, shanks, silks, leaf blades, leaf sheaths, tassels and cobs. The corn cob carries the grain and together with associating husks, shanks and silks are harvested from the farm. The other parts are left on the farm to rot (Kludze et al., 2010). Corn cob has high percentage of lignin (45% cellulose, 35% hemicellulose and 15% lignin) and has low nutritive value and degradation rate (Sun and Cheng, 2002). This is because rumen micro flora lack enzymes for degradation of lignin and cellulose and hemicellulose are embedded within the lignin structures. Thus, the nutritive values of corn cob not only depend on the availability of nutrient but on such attributes as lignifications and crystallinity of cellulose.

The degree of utilization of crop residues or wastes by livestock is affected by pretreatment (Wong et al., 1991;

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Bolanle et al., 2012). Treatment of crop residues for improving their nutritional value has been undertaken since the beginning of the 20th century (Doyle et al., 1990). Since then, tremendous efforts has been directed towards treatment via physical, physiochemical, chemical and biological means. Physical treatment for example milling and pilleting, soaking, boiling, and steaming leads to increase in surface area and density and an increase in the metabolizable energy (Beadsley, 1993), but have high-energy cost and ineffectiveness in improving feeding value of crop residue. Chemical treatment methods involve steeping by use of chemicals basically acids and alkali like sodium hydroxide and calcium hydroxide. The chemical methods are also more effective compared to the physical methods but their limitations are numerous. Environmental concerns are associated with disposal of spent acids and alkali, and unspecific side reactions which occur to yield non-specific by products. Also, there is requirement of extreme corrosive conditions of high temperature and pH thus necessitating highly trained personnel and expensive equipment (Grethlein and Converse, 1991; Zhu et al., 2009). Biological treatments with microorganisms such as fungi for example white rot fungi have several advantages when compared with physical and chemical methods. In this case, hydrolysis of polysaccharides occurs via microbial enzymes though fermentation under much milder conditions, do not produce undesirable products and are environmentally friendly (Smith et al., 1997; Rubin, 2008; Palmqvist et al., 2000).

White rot fungi grow well and produce lignocellulosic enzymes under solid state fermentation (SSF) because the medium conditions are closer to their natural habitats. (Salvachúa, 2011; Davinia, 2013). Thus, considering the substantial amount of cobs available for free or sold at very low prices by agro-industries, the upgrading of residues from fermented cobs for use in balanced feed for animal production is of potential advantage (Stamford et al., 2004). This research work has thus been designed to investigate the ability of fungal *Lachnocladiun Spp.* pretreatment to upgrade the nutrient quality of corn cobs and enhance its potential usage.

MATERIALS AND METHODS

Collection and preparation of sample

Air dried corncob residues or agricultural waste were collected in clean bags from Samaru market opposite Ahmadu Bello University main Campus Samaru Zaria. The residues were milled in a mortar and subsequently sieved with a 40 mm mesh size.

Organism and fermenting conditions

The test organism *Lachnocladium* spp. (white rot fungi), was cultivated on potato dextrose agar (PDA) slants until sporulation. The spores were harvested using 0.1% between 80 and spore number was estimated by direct microscopic counting using a haemocytometer. The white rot fungi was cultivated in mineral salt agroresidue media as described by Ali et al. (1991), in a 250 ml conical flask containing 30 ml in g/l of 10.0, KH_4PO_4; 10.5, $(NH_4)_2SO_4$; 0.33, $MgSO_4.7H_2O$; 0.5, $CaCl_2$; 0.013, $FeSO_4.7H_2O$; 0.004, $MnSO_4.H_2O$; 0.5% yeast extract and 10 g of corn cobs. The agroresidue media was inoculated with spore suspension of 7.5 ± 10^5 spores/ml and incubated at 28 ± 3°C. Another set of fermenting conditions was set up as above but without inoculation with the fungi to serve as the unfermented control, while the dried milled residues served as pure control for both media.

Chemical analysis

The proximate composition of the fermented, unfermented and control samples was carried out. Samples were analyzed for moisture, dry matter, crude protein, lipid, total carbohydrate, fiber, organic matter and mineral matter (ash) using AOAC (1990) methods. Antinutrients components determined include tannin, using Trease and Evans (1978), Saponin (Hudson and El-Difrawi, 1979), Phytate and oxalate (AOAC, 1990) and cyanide (Ikediobi et al., 1980). Sodium and potassium analysis were carried out using flame photometer. Atomic absorption spectroscopy (AAS) was used to determine Ca, Fe, Mn, Zn, and Mg.

Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) and least of significant difference (LSD) at 0.05 probability level. All statistical analyses of data were performed using SPSS 17.0 software and the data were reported as mean values± standard deviation (SD).

RESULTS

The effects of fungal fermentation on proximate composition of corn cobs are shown in Table 1. Proximate analysis of all the three group of samples showed significant difference ($p<0.05$) in the amount of crude protein, ash content and crude fiber in the fermented cobs compared to the unfermented and control corn cobs. Significant difference at $p<0.05$ was not observed in the fat, moisture, total carbohydrate, dry matter and organic matter in all the three group of samples. Table 2 shows the results of mineral analysis on the three groups of samples. Significant difference ($p<0.05$) was observed in sodium, potassium, and Zinc between the fermented and control samples.

The mineral values for sodium, potassium and zinc in the unfermented samples did not show significant difference when compared to the control and fermented samples except sodium which did not vary significantly with either the fermented or the control samples. Other minerals; calcium, iron, manganese, and magnesium determined in the three samples did not show significant variability. The results of antinutrients analysis on the fermented, unfermented and control samples are shown in Table 3. Significant difference was observed in levels of phytate, saponin, and oxalate as the levels decreased in fermented samples as compared to the unfermented and control samples. Other antinutrients; cyanide and

Table 1. Effects of *Lachnocladium spp*. fermentation on proximate composition of corn cobs.

Parameter	Fermented cobs	Unfermented cobs	Control
Crude protein	4.79 ± 0.017[a]	4.10±0.0057[a]	3.42± 00.010[b]
Fat	9.69 ± 0.005[a]	9.96±0.0059[a]	9.55± 0.005[a]
Moisture	3.98±0.010[a]	5.42±0.021[a]	5.43±0.017[a]
Ash	5.69±0.010[a]	4.46±0.340[b]	4.41±0.004[b]
Carbohydrate	74.51±0.006[a]	74.51±0.0055[a]	74.51± 0.0061[a]
Fibre	6.83±0.023[a]	7.72±0.011[b]	7.3-± 0.001[b]
Dry matter	94.55± 0.0058[a]	94.56±0.0056[a]	94.52±0.035[a]
Organic matter	95.59± 0.0058[a]	94.31± 0.011[a]	95.34± 0.011[a]

Values are means of triplicate determination ± standard deviations (n= 3). Values not having the same superscript on the row are significantly different (p<0.05).

Table 2. Effect of *Lachnocladium spp*. fermentation on mineral composition of corn cobs.

Parameter	Fermented cobs	Unfermented cobs	Control
Calcium	0.024 ± 0.0057[a]	0.021 ± 0.0001[b]	0.025 ± 0.010[a]
Sodium	0.056±0.0057[a]	0.049 ± 0.005[ab]	0.041± 0.005[b]
Potassium	0.311±0.50[a]	0.241 ± 0.0057[b]	0.241 ± 0.047[b]
Magnesium	0.044±1.00[ab]	0.047 ± 0.010[a]	0.031± 0.013[b]
Iron	0.0025±0.001[a]	0.0024 ± 0.010[a]	0.0024± 0.057[a]
Manganese	0.0033±0.0011[a]	0.033±0.011[a]	0.0031 ± 0.019[a]
Zinc	0.0063 ± 0.0058[a]	0.0018±0.015[b]	0.0018± 0.120[b]

Values are means of triplicate determination ± standard deviations (n= 3). Values not having the same superscript on the row are significantly different (p<0.05).

Table 3. Effect of *Lachnocladium* spp. fermentation on antinutrients composition in corn cobs.

Parameter	Fermented cobs	Unfermented cobs	Control
Phytate	0.803 ± 0.0057[a]	1.32± 0.0061[b]	1.32 ± 0.010[b]
Cyanide	0.75±0.005[a]	0.75 ± 0.01[a]	0.75 ± 0.005[a]
Saponin	2.21± 0.0043[a]	3.84 ± 0.030[b]	3.86 ± 0.032[b]
Tannin	0.022 ± 0.002[a]	0.024± 0.0033[a]	0.024 ± 0.0083[a]
Oxalate	0.024±0.001[a]	0.087 ± 0.005[b]	0.088 ± 0.0055[b]

Values are means of triplicate determination ± standard deviations (n= 3). Values not having the same superscript on the row are significantly different (p<0.05).

tannin were not found to be significantly different in the three samples analyzed.

DISCUSSION

The proximate analysis of the fermented cobs showed a significant increase in crude protein. The increase in the protein content of the corn cobs fermented with *Lachnocladium* spp. could be attributed to the possible secretion of laccase and manganese peroxidase and some extracellular enzymes (proteins) such as amylases, and cellulases (Oboh and Akinwumi, 2003) into the fermenting media by the fermenting organism (Sidharth et al., 2013) as well as increase in the growth and proliferation of the fungi in the form of single cell proteins (Omer et al., 2012). Most agricultural wastes are known to support the growth of microorganism as single cell protein and thus enhance feed quality (Sharma and Arora, 2013). For example, fermented cassava has been reported for similar potentials by Obuekwe (1993) using Rhizopus as fermenting organism. Fermentation was also seen to generally improve the protein value of the same seed flour (Aderonke and Beatrice, 2013). The reduction of fiber content in fermented corn cobs is of significant importance, as fiber is often used as a negative index of nutritive value in the prediction of total digestible nutrients (TDN) and net energy. It is assume that higher fiber means lower digestibility.

The physical characteristics of fiber (particularly particle size) are also important in regulating rate of passage, rumination, insalivations and the pH of the rumen (Mahesh and Madhu, 2013). The total fiber or cell wall fraction of plants comprises cellulose, hemicellulose, lignin, cutin, silca and a variety of minor substances. The proportions of these components vary among parts of the same plant and also change as plants mature. The proximate analysis of the fermented, unfermented and control samples did not show any significant difference at $p< 0.05$ in the percent-tage dry matter, organic matter, moisture, moisture content and crude fat. The dry matter and organic matter are common denominators for comparing nutrient contents of feeds; other determinations in proximate analysis are expressed on dry matter basis. The results obtained have shown that fermentation does not have effect on these compositions.

Another factor limiting the wider feed use of many crop residues is the ubiquitous occurrence of a diverse range of natural compounds which act to reduce nutrient utiliza-tion and low food intake which are referred to as antinutritional factors (Osagie, 1998; Sarwar, 2012). Fermentation brings about numerous biochemical and nutritional changes in the raw materials, including the breakdown of certain constituents, the reduction of antinutritional factors and the synthesis of B vitamins (Egounlety and Aworh, 2000). Analysis of fermented corn cobs showed a significant reduction ($p<0.05$) in the saponin, oxalate, and phytate, while cyanide and tannin did not show significant changes. Saponins are steroid or triterpinoid glycosides which are characterized by their bitter or stringent foaming properties and their hydrolytic effects on red blood cells. But the effectiveness of saponins and other antinutrients depend on the amount present in the feed and effect of pretreatment process on these feeds.

Thus, a significant reduction in the level of the saponin following fermentation is quite advantageous. (Adeniran, 2013). Phytates are hexaphoshate derivative of inositol and storage form of phosphorus in plants. Phytate are insoluble and form salt with metals such as calcium, iron, zinc and magnesium, rendering these metals unavailable for absorption (Osagie, 1998). A reduction in phytate observed in fermented corn cobs may be due to secretion of phytates bond or due to change in the pH of the medium which affects the attachment of water and thus configuration of phytic acid by altering the strong water molecules attached (Onigbinde, 2005; Adeniran, 2013). The ability of fermentation process to reduce phytate levels have been reported by Mulimani et al. (2003) and Marfo et al. (1990). Oxalate is a dicarboxilic acid anion present as insoluble salts of potassium, sodium and ammonium or as calcium oxalate. It can be toxic when consumed in large quantities. Thus, a reduction in its level through fermentation is quite beneficial to the feeding value of corn cobs. The ash content which is defined as the total mineral was seen to have increased in value. Specific analysis of the mineral elements showed changes in calcium, potassium and zinc. This could be attributed to the effect of fermentation on reducing the levels of antinutrients; phytate and oxalate which increase the bioavailability of mineral elements. (Onyango, 2013)

Conclusion

Fermentation with white rot fungi could serve as a good means of pre-treating corn cobs to improve its nutritional value, as it has been demonstrated to improve the protein content, fiber level, ash and some mineral elements like calcium, sodium, potassium and zinc. It has also been seen to reduce the level of some antinutrients like saponin, phytate and oxalate. With the combined effects of fungal fermentation on the nutritive value of corn cobs, its usage as an animal feed has been greatly improved. With this alternative use, environmental pollution concerns by maize cobs available for free or sold at very low prices by agro industries and the upgrading of residues from fermented cobs for use in balanced feed for animal production is of potential advantage.

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