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Research Article

CORN COB AS A FEED COMPONENT THROUGH FUNGAL FERMENTATION USING *ASPERGILLUS NIGER*

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ABSTRACT
Corncob is commonly regarded as a waste product in agricultural processing because of its high cellulosic nature. In Africa, it is usually abandoned on farm lands after the maize processing, or used as a source of cooking fuel in rural areas where it generates a lot of smoke, thus constituting a source of environmental pollution. However, if properly processed and harnessed, it could be utilized as an energy source in feed formulation through fungal degradation. This is because corn cob is made up of glucose molecules which could be obtained on microbial hydrolysis of cellulose. In this study, corncob cellulose was pre-treated and subjected to solid state fermentation by *Aspergilus niger*. Corn cob was milled using a Pertern Laboratory Mill 3100 of 0.8mm sieve size and oven dried at 60^0C. *Aspergilus niger* was obtained from the Bacterial Research Division of the National Veterinary Research Institute. Fermentation was carried out in a corn cob based broth medium. 250ml of the broth medium was inoculated with 3.02 X 10^7 spores of *A. niger*. Twenty five grams of the milled corn cob was aseptically inoculated with *Aspergilus niger* of 50ml inoculum per sample. Treatment was done in quadruplets (n=4). Moisture level was adjusted every six hours to keep the sample moist. Fermentation lasted 10 days. The results showed an increase in protein (from 3.01 % in the raw corn cob to 4.06 % in *Aspergillus niger* fermented corn cob) and lipid content (from 0.70 % to 1.30 % on fermentation). Fermentation reduced the crude fiber (from 42.70 % in the raw to 30.70 % in the fermented corn cob) and enhanced the mineral profile of the treated corn cob. We can thus suggest that fungal fermentation of corn cob using *Aspergillus niger* is an effective and cheaper means of enhancing the nutritional quality of corn cob for use as an energy source in livestock feed formulation.

Keywords: *Corn Cob, Aspergillus Niger, Fermentation, Feed, Nutritional Quality*

INTRODUCTION
Cereals form the major energy source in compounded poultry and livestock feed, Mathlouthi *et al*., (2002); Lazaro, (2003); Safaa, (2009). Cereals also serve as staple foods, providing a large sustenance support for the ever growing human population especially in Africa and some parts of Asia, Liu, (1999). This poses a challenge as the quantity of grains available for the expanding human population in addition to animals is limited. Other factors complicating this include poor storage facilities in most African countries and environmental factors like flooding which reduces the end quantity of cereals available for use as food and feed, leading to competition between humans and animals for grains. The overt result is a continuous rise in the market price of grains as reported by Teguia and Beynen, (2005). The high cost of feed is reported to be a major factor militating against increased commercial poultry farming, and a consequently high cost of poultry protein. In fact, feed costs amount to about 70% of the total production costs of broiler meat in Cameroon, Teguia and Beynen, (2005). All of this explains why poultry meat is expensive in most African markets.
Locally, corncob poses an environmental challenge, as it is usually disposed of improperly, littering the environment and blocking drainages in urban areas after consumption. In the rural areas, it is used as a source of firewood fuel for cooking thereby producing smoke which results in poor air quality and respiratory illness caused by carcinogenic haze. This is most prominent in women and children who are usually around the cooking fire as reported by Ezzati and Kammen (2002); Desai, (2004); Shrestha, (2005). Unfortunately, the production and end use of such biomass as fuel is done under suboptimal

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conditions, contributing enormously to the greenhouse gas burden, which is of consequential effects to us all, and the future of the earth as our habitat.

The major factors militating against the utilization of corncob in poultry nutrition are its cellulosic nature, high fibre content, low protein as well as lipid and mineral value (Chen, 2010). Corn cob consists of cellulose, hemicellulose, and lignin (Chen, 2010). Cellulose is a polymer of glucose molecules linked by beta 1,4 bonds. Cellulose is difficult to hydrolyze due to two main reasons. First of all, cellulose is insoluble in water and forms crystals. Secondly, cellulose of practical interest is rarely pure, but coexists with lignin and hemicellulose in well-defined anatomical structures. Also, lignin forms a physical seal around cellulose, which makes it very resistant to efficient degradation by acid hydrolysis (Chen, 2010). Lignin also reduces the accessibility of cellulose to cellulase enzymes. Poultry animals cannot use cellulose as an energy source because they lack cellulase: the enzyme that hydrolyses the beta 1,4 linkages.

The oldest methods known to convert cellulose to glucose are based on acid hydrolysis. Though acids can breakdown the lignins and cellulose to release sugars through hydrolysis reaction, they have low specificity (Grethlein, 1978) and the by-production of caramelization products and radicals, as well as the specialized materials of construction required, are serious disadvantages of this process. During chemical pre-treatment using dilute acid hydrolysis, inhibitors such as furfural are derived from the dehydration of pentoses and hexoses, (Lewkowski, 2001). Aliphatic acids, such as acetic, formic, and levulinic acid are also formed by de-acetylation of hemicellulose. Furfurals reduce yeast enzymatic and biological activities, break down DNA, and inhibit protein and RNA synthesis which are all detrimental to nutritional value optimization (Liu *et al*., 2008**)**.

Inspite of the odds, utilization of agricultural wastes should be encouraged since recycling of wastes can minimize environmental pollution and enhance the income of farmers by creating new products from wastes, such as animal feed. Biotechnological research must emphasize research on the bio-efficient management of wastes, its processing technology, and evaluate their potential for use as feed for poultry and livestock.

While the potential for agro-byproducts conversion and utilization are very obvious, feed from such wastes in diet formulation has been given minimal consideration due to the constraints imposed by several nutritional and technical considerations. Solid fermentation of agro-industrial residues is thus proposed as a suitable pre-treatment that can lead to the use of corncob as an animal feed. Solid state fermentation can upgrade the nutritive value of agricultural byproducts and presents potential solution to feeding animals in developing countries.

Some studies have been carried out on the potential of corn cob for use as an energy source using fungal fermentation. The impact of boiling on the chemical composition of maize cobs using *Geotrichum albidum*, *Neurospora crassa* and *Rhizopus* stolonifer has been investigated by Akpomedaye and Gabriel-Ajobiewe, (2011**)** while *Pleurotus pulmonarius* has also been used to ferment pretreated cellulose for in vitro gas production (Akinfemi *et al*., 2009).

In this study, *Aspergilus niger* was used to hydrolyse corn cob cellulose through solid state fermentation because *Aspergilus niger* is known to secrete cellulose which breaks down cellulose in corn cob to glucose. During the growth on such substrates, hydrolytic exo-enzymes are expected to be synthesized by the micro-organisms and excreted outside the cells, which results in metabolic reactions that hydrolyze cellulose to glucose.

MATERIALS AND METHODS

Solid State Fermentation

Corn cob was obtained from a Farm settlement in Plateau State, North Central Nigeria. They were cleaned, sundried and ground using a Pertern Laboratory Mill 3100 of 0.8mm sieve size. The milled corn cob was oven dried at 60^0C. .*Aspergillus niger* was obtained from the Bacterial Research Division of the National Veterinary Research Institute (NVRI) Vom, Nigeria. The *Aspergillus niger* was sub cultured on PDA and refrigerated at 4^0C. Fermentation was carried out in a corn cob based broth medium (CBB).

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CBB is a modified Mandel broth medium and consists of the following per liter: 1.4g of $(NH_4)_2SO_4$, 2.04g of KH_2PO_4, 0.3g of $CaCl_2$, 0.3g of $MgSO_4.7H_2O$, 0.25g of citric acid, 2ml of tween 80, 10g of Avicel cellulose, 1g of yeast (Zakpaa *et al.*, 2009). Two hundred and fifty milliliters of the corn cob based broth medium was inoculated with 3.02 X 10^7 spores of *A. niger* and incubated on a shaker (G24 Environmental incubator shaker, New Brumswick Co. USA) at 120rpm at 25^0C.

Four (4) 250mL volumetric flasks containing 25g each of the milled corn cob were autoclaved at 121^0C for 15 minutes. After sterilization, the flasks containing the samples were aseptically inoculated with *Aspergilus niger* of 50 milliliter of inoculum per sample. The seeding density was 4.01 x 10^6 spores per twenty five (25) grams of the sample. Treatment was done in quadruplets (n=4) to ensure repeatability and accuracy of results.

Group I represents the control group which was not subjected to any fermentation treatment, Group II represents the *Aspergilus niger* treated corn cob sample. Moisture level was adjusted every six hours to keep the sample moist by adding 50 milliliter of distilled water and the fermentation mash stirred thereafter. Fermentation was carried out for 10 days. Fungal degradation was terminated by oven drying at 50^0C for 36 hours, to a constant weight. The control group was similarly treated.

Proximate composition analysis was carried out on both the raw corn cob and fermented corn cob according to the Standard Methods of the Association of Official Analytical Chemists (AOAC, 1990). Their available carbohydrate content was calculated by difference.

Statistical Analysis

The results obtained are presented as Mean ± SEM and difference between treatments was analyzed by unpaired t- test using Graph pad version 4.03. P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

The proximate composition and mineral content of raw (untreated) corn cob is shown in Table 1 and Table 2 respectively. Proximate composition of corn cob after the fermentation treatment with *Aspergillus niger* is shown in Table 3 and the mineral content for the treated corn cob is shown in Table 4.

Table 1: Proximate composition of Unfermented Corn cob in g/100g dry matter

Nutrient	Value
Moisture	3.20
Crude Protein	3.10
Crude Fibre	42.70
Lipids	0.70
Total Ash	2.35
Nitrogen Free Extract	48.04

Values are means of three determinations ± SD

Table 2: Mineral Element Composition of Unfermented Corn cob (mg/100g dry matter)

Mineral	Value
Calcium	0.10
Phosphorus	0.013

Table 3: Proximate composition of fermented Corn cob in g/100g dry matter

Nutrient	Value
Moisture	4.20
Crude Protein	4.06
Crude Fibre	30.70
Lipids	1.30
Total Ash	2.45
Nitrogen Free Extract	57.29

Values are means of three determinations ± SD

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Table 4: Mineral Element Composition of fermented Corn cob (mg/100g dry matter)

Mineral	Value
Calcium	0.13
Phosphorus	0.015

The fermentation of corn cob using *Aspergillus niger* altered the texture and color of corn cob causing it to be more coarse and darker in colour compared to the unfermented corn cob. The process of fermentation increased the protein content from 3.01 % in the untreated corn cob to 4.06 %, reduced the crude fiber content from 42.70 % in the untreated corn cob to 30.70 % and the lipid profile of the corn cob was enhanced from 0.7 % in the untreated corn cob to 1.30 % in the treated corn cob samples.

Moisture levels increased from 3.20 % in the untreated sample to 4.20 % after treatment with *Aspergillus niger*. Ash levels increased from 2.35 % before fermentation to 2.45 % after fermentation while the energy content (nitrogen free extract) increased from 48.04 % in the unfermented corn cob to 57.29 % after fermentation. Fermentation also enhanced the mineral profile of fermented corn cob: Calcium and Phosphorus levels increased from 0.10mg/100g to 0.13mg/100g and from 0.013mg/100g to 0.015mg/100g after fermentation respectively.

The increase in the protein content after fermentation can be attributed to the contribution of fungal protein from the introduction of *Aspergillus niger* to carry out the fermentation, Belewu and Belewu, (2005) and from cell growth by *Aspergillus niger* which causes the breakdown of polysaccharides and release of bound proteins thereby increasing the protein content and making the substrate nutritionally better, Belewu *et al*.,(2003). Increase in protein could also be as a result of the increase in the microbial biomass due to growth and proliferation of *Aspergillus niger* resulting in increased fungal content as well as the enzymes secreted by *Aspergillus niger* during the fermentation process. All enzymes are proteins, and fungi are known to secrete enzymes like amylase and cellulase.

Reduction in the crude fibre content could be indicative of the degradation of the cellulosic cell wall component of the corn cob substrate by extra cellular enzymes of *Aspergillus niger*. The lower crude fibre content reported here agrees with the results of other studies by Belewu *et al*., (2006); Belewu and Sam, (2010). Increase in Lipid content is evidently due to the ability of *Aspergillus niger* to synthesize long chain fatty acids from acetyl coenzymes A and other complex unsaturated lipids, Iyayi and Aderolu, **(**2004).

An increase in the energy content (nitrogen free extract) observed could be attributed to the hydrolysis of insoluble crude fiber to energy source by *Aspergillus niger* on fermentation. Fermentation enhanced the mineral profile of fermented corn cob by increasing the calcium and phosphorus values and this could have come from the fungal biomass as well as the minerals contained in the fermentation broth medium.

In conclusion, the result of this study shows that corn cob can serve as an energy source for feed formulation using fungal fermentation by *Aspergillus niger*. The advantages of this technology could include a reduction in the cost of feed production, cost of animal production and a potential reduction in the market price of livestock and poultry meat. A combination of the above factors will enhance food availability and consequently food security, which is a target of the millennium development goals. The environmental challenges as a result of indiscriminate disposal of corncob will also be addressed as Farmers will be discouraged from using corncob as firewood which produces smoke and chlorofluorocarbons which damage the ozone layer, contributing to global warming.

Further studies using different concentrations of *Aspergillus niger* is recommended to evaluate which concentration of the microorganism yields optimal hydrolysis of corn cob cellulose to glucose. Studies using animal trials with *Aspergillus niger* fermented corn cob incorporated into their feed will provide adequate information about in *vivo* digestibility levels of such formulated diet. Such research will bring us closer to the desired goal of using corn cob as an unconventional feed resource for feed formulation, to reduce the cost of animal production.

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