MICROBIAL DEGRADATION OF MAIZE COB AND ITS ASSESSMENT FOR USE AS BROILER FEED SUPPLEMENT

BY

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ABSTRACT

Maize, *Zea mays* is extensively cultivated in Nigeria with estimated total grain yield of 1.37 million tonnes per year. The maize by-product which includes the cob is readily available. This project was designed to convert maize cob to value-added products in broiler feed through microbial degradation using batch fermentation.

One hundred and fifty kilogrammes of maize cob samples were collected from four dump sites in a farmers' market in Oyo town. Bacteria and fungi were isolated using nutrient agar and potato dextrose agar media respectively. Identification of isolates was done by conventional methods and cellulase enzyme assay carried out by dinitrosalicylic acid method. The isolates with cellulase activities were then used for solid state fermentation. The process was then sealed up in fabricated tanks for bulk fermentation using *Bacillus subtilis* and *Aspergillus niger* solely. The derivatives labelled Bacteria Enriched Cob Product (BECP) and Fungi Enriched Cob Product (FECP) were subjected to chemical and microbiological analyses. The BECP and FECP were each substituted at 18%, 50% and 82% inclusions in compounded feeds while commercial feed served as control. These were fed to four-week old broiler chicken for six weeks in a completely randomized experimental set up of seven groups with ten chicks per group. Mean Weight Gain (MWG), Feed Conversion Ratio (FCR), economic performance, haematological indices and organ histopathology were determined. Data was analysed using inferential statistics at $p = 0.05$.

Thirty-three (33) isolates made up of 19 bacteria and 14 fungi had cellulase activity. These were the genera *Bacillus (10), Pseudomonas (8), Proteus (1) Aspergillus (6), Penicillium (4), Rhizopus (1), Mucor (1), Botryotrichum (1)* and *Geotrichum (1).* The best cellulase producers were *Aspergillus niger*, 1.7 mg/ml and *Bacillus subtilis* 0.7 mg/ml with optimal activity at 40^{o}C, pH 4.0 and 28^{o}C, pH 7.0 respectively. Cellulose degradation ability of fungi 17.3% was higher than bacteria 6.6% while the crude protein levels were 7.5% and 9.5% respectively. The MWG (18.0g) of chicks fed BECP (82%), was much lower than the control (34.3g), FECP (18%), 38.9g; BECP (18%), 37.6g; FECP (50%) 31.0g and BECP (50%), 30.7g. The feed cost per kilogram gain for FECP (28%), ₦185.7; BECP (18%), ₦208.4 and FECP (50%) ₦227.4 were lower than the control ₦235.9. The FCR for BECP (82%), 8.4 was higher than the control value of 3.6. The red blood cell count for all treatments ($1.7 – 2.6 \times 10^{6}/mm^{3}$) did not differ significantly. However, white blood cell count was significantly higher in chicken fed with 82%, BECP ($27.2 \times 10^{3}/mm^{3}$) and 82% FECP, $20.4 \times 10^{3}/mm^{3}$ compared to the other diets ($11.2 – 16.2 \times 10^{3}/mm^{3}$). Only the kidneys and lungs of chickens fed with 82% BECP were severely congested.

Maize cob contained cellulolytic bacteria and fungi capable of degrading it at different extents. Substitution of either bacteria enriched product or fungi enriched product up to 50% were economically suiTable and safe for use as broiler chicken feed supplement.

Keywords: Maize cob, Microbial degraded cob, Broiler, Feed stuff.

Word Count: 482

CERTIFICATION

I certify that this work was carried out by **Olutoyin Omolara Bamigboye** under my supervision at the Department of Microbiology, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

To the glory of God, I dedicate this work to my God-given heritage Favour Oluwadara, Oluwafunbi Damilola and Mofiyinfoluwa Oluwafemi, to whom I am also dropping the academic baton to start where I have stopped.

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CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Corn also called Indian maize or maize (*Zea Mays*) is a cereal plant of the tribe Maydeae of the grass family gramineae originating in the Americas. Its edible grain is used as human food as well as livestock feed and as raw materials in industry (Encyclopedia Britannica, 2003). Maize (*Zea Mays L*) is the main cereal crop grown in southwestern Nigeria. It is used primarily as staple food for human consumption, animal feeds and a raw material for industrial purposes (Owolade *et al.*,2001). In Nigeria, the production of maize has been transformed from minor crop activities around homestead to a major commercial crop competing with sorghum and millet as a strategic crop in the grain economy of the nation. Maize is harvested twice in a year in the rain forest zone of southern Nigeria (Adebowale, 1988). The maize residue and by-products usually constitute a nuisance to the environment in the southwestern Nigeria especially during the harvesting seasons when the husk and cob form heaps in refuse dump and litters the environment.

Agriculture wastes are among the causes of environmental pollution. Their conversion into useful products may ameliorate the pollution menace. These wastes which include husk, straw, leaves and cobs are highly underutilized in Africa, particularly Nigeria. A large quantity is left on the farmlands to be decomposed by microorganisms such as bacteria and fungi (Okafor, 1988). In Nigeria, a large quantity of rice husks and maize cobs are produced annually and these by-products are left to rot away or burned like other agro-industrial wastes (Oladeji, 2010). These wastes are promising feedstuffs for the production of energy, food and chemicals (Andriani *et al.*, 2011).

Maize cobs may be relatively poor in nutritive value compared with some other locally available residues or roughages (Dzowela, 1987), but it has the advantage of wide availability in large quantities, easy and cheap procurement, large cellulose and hemicellulose reserve. Cellulose, which forms about 40-50% of plants' composition is the most abundant organic matter on earth (Milala *et al.*,2005). Cellulose is commonly degraded by enzyme called cellulase. This enzyme is produced by several microorganisms. commonly bacteria and fungi (Shin *et al.*,2000, Immanuel *et al.*,2006).

1.1 MAIZE COB AS AGRICULTURAL BY-PRODUCT

In Nigeria, maize residues and by-product were estimated to be about 4.11 million tonnes in 1989, consisting mainly of straw, husks, skins and trimmings, cobs and bran (Adebowale, 1992). For every kilogram of maize grain, 3 kilogrammes of fibrous by – products are produced (Kossila, 1984) indicating the production of about 2.25 million tonnes of fibrous by – products annually. Most of these by-products are allowed to waste. Maize cob is a waste product from shelling maize seeds and has been reported by Alokan (1988) to constitute about 60% of the maize "ear". It is one of the crop residues of interest for bioenergy production (Hay, 2010). It also has large cellulose and hemicellulose reserves.

1.2 CELLULOSE COMPONENT OF MAIZE COB

Cellulose is the most renewable biopolymer (Coral *et al.*, 2001) and the main polymeric component of the plant cell wall (Baldrian and Valaskova, 2008). Proper biotechnological utilization of these wastes in the environment will eliminate pollution and convert them to useful by-products. The chemical composition of maize cobs as reported by Tuah and Qrskov (1989) included cellulose, 38.01%; hemicellulose, 46.35%, lignins, 9.60%, nitrogen, 0.50%, and cell content, 6.04%. The general recalcitrance of cellulose, lignin and hemicellulose and the importance of their biodegradation in the environment had received much attention for several years (Erickson *et al.*, 1990). Cellulose exist in a number of crystalline and amorphous topologies. Its insolubility and heterogenicty makes native cellulose a recalcitrant substrate for enzymatic hydrolysis (Schwarz, 2001). Cellulose has been used by man for centuries, although, its enormous potential as a renewable source of energy was recognized only after cellulose-degrading enzyme; cellulases, had been identified (Bhat and Bhat, 1997).

1.3 CELLULASE ENZYME FOR CELLULOSE DEGRADATION

Cellulases (1, 4 – D – glucan glycanohydrolase, EC 3.2.1.4) are multienzyme complexes, comprising three main components made up of endo-glucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.9.1) and glucosidase (EC 3.2.1.2.1) which had been shown to act

synergistically in the hydrolysis of cellulose (Emert *et al.*,1974; Ryu and Mendels, 1980). Cellulase is the enzyme that hydrolyzes the β-1,4-glycosidic bonds in the polymer to release glucose units (Nishida *et al.*, 2007). Cellulase enzymes provide a key opportunity for achieving tremendous benefits of biomass utilization through the bioconversion of lignocellulosic biomass into the simplest carbohydrate monomer, glucose (Andriani *et al.*, 2011). Cellulases are being studied increasingly due to their application in the hydrolysis of cellulose, the most abundant biopolymer and potential source of utilizable sugars, which serve as raw materials in the microbial production for a wide variety of chemicals, food and fuel (Ekperigin, 2007). Cellulase acts collectively to hydrolyse cellulose of agricultural wastes to produce simple glucose units (Smith, 1996). By the action of cellulase produced during fermentation, glucose units within bundles of fibre will be released for the production of energy.

In recent years, the potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms. Cellulases are produced by large number of microorganisms. They are either cell-bound or extracellular. Although a large number of microorganisms can degrade cellulose, only a few of them produce significant quantitites of free enzyme capable of completely hydrolyzing crystalline cellulose (Koomnok, 2005). The biotechnology of cellulase and hemicellulases began in the early 1980s, first in animal feed and then in food application (Chesson, 1987; Coral, 2001; Camassola and Dillon, 2007; Zhang *et al.*, 2010). Today, these enzymes account for apporiximate 20 percent of the world enzyme market, mostly from *Trichoderma* and *Aspergillus*. Cellulase research has been concentrated mostly on fungi but there is increasing interest in cellulase production by bacteria (Crawford, 1986; Li and Gao; 1996; Krairitthichai and Thongwai, 2008; Lennox *et al.*, 2010, Andriani *et al.*, 2011).

1.4 CELLULASE PRODUCTION BY FUNGI

Cellulases are synthesized by cellulolytic fungi such as the *Chaetomium*, *Fusarium*, *Myrothecium* and *Trichoderma* species (Coblay and Steele, 1976). Other species include the *Penicillium* and *Aspergillus* species (Milala, *et al.*,2005). Although a large number of microorganisms are capable of degrading cellulose, only a few of these

produce significant quantities of cell free enzymes capable of completely hydrolysing crystalline cellulose *in vitro*. Fungi are the main cellulase producing microorganisms, though a few bacteria and actinomycetes have also been reported to yield cellulase activity (Immanuel, *et al.*, 2007). Filamentous fungi particularly *Aspergillus* and *Trichoderma* spp are well known efficient producers of plant cell wall-degrading enzymes. These organisms consist of three classes of enzymes endoglucanases, cellobiohydrolases, and B-glucosidases which are necessary to degrade cellulose (Gielkens *et al.*,1999, Bhat, 2000).

Studies have been carried out to produce cellulolytic enzymes from biowaste degradation process by many microorganisms including fungi such as *Trichoderma, Penicillium, Aspergillus* sp. (Mandels and Reese, 1988; Hoffman and Wood, 1985; Lak shmikant and Mathur 1990. Immanuel *et al,* (2007) also studied the production and partial purification of cellulase by *Aspergillus niger* and *Aspergillus fumigatus* fermented in coir waste and sawdust. Maximum enzyme production was obtained at 40^{o}C and 50^{o}C and pH 7 and pH 6 by *A. fumigatus* and *Aspergillus niger* respectively. However, fungal cellulolytic system differ from the complex cellulolytic systems of bacteria (Baldrian and Valaskova, 2008).

1.5 CELLULASE PRODUCTION BY *BACTERIA*

Cellulolytic property of bacterial species like *Pseudomonas, Cellulomonas, Bacillus, Micrococcus, Cellulovibrio* and *Sporospytophaga* spp have been reported (Nakamura and Kappamura, 1982; Immanuel *et al.*,2006). The specific cellulolytic activity shown by the bacterial species was found to depend on the source of occurrence of such bacteria (Saxena *et al.*,1993).

Cellulase activities have been reported in many nitrogen fixing bacteria such as *Sinorhizobuim fredii* (Chen *et al.,* 2004), *Bacillus sphenicus* (Singh *et al.,* 2004), *Bacillus circulans* (Baird *et al.*,1990), *PaeniBacillus azotofixatous* (Rosado *et al.*,1998), *Gluconacetobacter* (Emtiazi *et al.,* 2003) and *Azospirillum* (Steenhoudt and Vauderleyden, 2001). Although with the change of Bacillus classification, all nitrogen-fixing Bacillus strains such as *B. polymyxa, B. macerans, B. azotofixaus* are now assigned to *paeniBacillus* (Rosado, 1998, Kashen *et al.,* 2004) but there are other many spore

forming bacteria that might fix nitrogen. Emtiazi *et al.* (2007) studied the cellulase activities of *PaeniBacillus* strains. *PaeniBacillus* strain E produced 4µml^{-1} CMC ase when it was grown on CMC as the only carbon source. In his preliminary studies of cellulase production by two bacteria species *Acinetobacter anitratus* and *Branhamella species.* Ekperigin (2007) reported that enzyme production occurred at the late logarithm phase for each bacteria species. The kinetics of CMC and cellobiose hydrolysis were found to follow the Michaelis- Menten type with K_m values of 4.97 and 7.90mg/ml for *A anitratus* and *Branhamella* sp respectively.

1.6 FUNGAL DEGRADATION BY CELLULOSE

Cellulose degradation by fungi have been reported by many workers (Crawford, 1981; Bumpus and Aust, 1981, Aust, 1990; Aust and Benson 1993) but few works have been reported on the activity of fungi in degradation of maize cobs (Adejoye, 2007; Oduguwa *et al.,* 2007). Lennox *et al.* (2010) in their study of comparative degradation of sawdust by microorganisms isolated from it observed that, *Penicillium sp*. had the highest percentage degredation rate of 14.3% followed by *Mucor sp (*13.3%), *Trichoderma sp* (9.5%) and *Aspergillus sp* (4.3%)..

White rot fungi, which use cellulose as a carbon source, possess the unique ability to degrade lignin completely to carbondioxide in order to access the cellulose molecule (Aust and Benson, 1993). In their study on the effect of six species of white rot basidiomycetes on the chemical composition and rumen degradability of wheat straw; Jalc *et al.* (1997) studied changes in *in-vitro* dry matter digestability (IVDMD), volatile fatty acid (VFA) production and cell wall constituent degradation in wheat straw treated with six white rot fungi *Daedalea quercina, Hericium clathoideas, Phelinus leavigatus, Inonotus andersonii, Inonotus obliguos* and *Inonotus dryophilus*. The study suggested that the digestion enhancement of wheat straw colonized by white rot fungi was regulated by complex factors including the degradation of structural carbohydrates and lignin. Many species of white rot fungi had been screened on a variety of lignocellulosic substrate for their ability to improve the nutritional value of poor quality crop residues for use as a ruminant feedstuff (Fahey *et al.,* 1993; Reids, 1987; Arora *et al.*, 2002; Adejoye, 2007). Recently the advantage of the rumen simulating technique (RUSITEC) in studying

rumen fungi was demonstrated. Anaerobic fungi are an important part of the rumen and play a significant role in fiber degradation. The RUSTITEC provide a long term incubation with condition which are close to natural rumen ones (Koshyukousky *et al.*,1995). The efficiency and regulation of cellulose degradation differs among wood-rotting, litter decomposing, mycorrhizal or plant pathogenic fungi and yeasts due to the different roles of cellulose degradation in the physiology and ecology of the individual groups (Baldrian and Valaskova, 2008).

1.7 BACTERIA DEGRADATION OF CELLULOSE

Aerobic bacteria produce numerous individual, extracelular enzymes with binding modules for different cellulose conformations. Specific enzymes act in synergy to elicit effective hydrolysis. In contrast, anaerobic bacteria possess a unique multienzyme complex, called cellulosome. (Schwarz, 2001). Ruminants possess cellulolytic bacteria in their rumen, which degrade cellulose material abundant in their feeds. It has been shown that cellulolytic degrading bacteria increase when ruminants are fed with high fiber diet. Several experiment performed with cellulose degrading bacteria were done in the rumen of animals (Smith *et al.,* 1991, Tuah and Qrskov 1989). *Eubacterium cellulosoluvens*, a gram positive anaerobic and cellulolytic bacteria appears to be one of the predominant cellulolytic rumen bacteria in European cattle (Van glyswysk, 1990).

Varel and Pond (1985) reported that cellulolytic flora can be increased by prolonged feeding of gestating swine on high fiber diet. The diets each fed to five pigs were corn-soyabean, 20% corn cobs and 40 and 96% alfalfa meal while feacal samples showed increase in celllolytic flora with time. Two of the predominant cellulolytic species found in the rumen, *Bactericides succinogenes* and *Ruminococcus flavefaciens* were the predominant organisms in the large intestine of pigs. These may represent cultivable flora. In their study of comparative degradation of sawdust by microorganisms isolated from it, Lennox *et al.* (2010) found *cellulomonas sp* to be the most effective degrading agent among the bacteria with percentage degradation at 18.3%. Other bacteria isolate which include *Micrococcus sp*; *Pseudomonas sp* and *Bacillus sp* also degrade cellulose.

Reports on strains belonging to species such as *Bacillus sphaericus* and *B. subtilis* express high cellulose degradation activities (Singh *et al.*, 2004). Bacillus continue to be dominant bacterial cellulose degrader due to the capacity of some selected species to produce and secrete large quantities of extracellular enzymes (Rastogi *et al.*, 2010; Ariffin *et al.*, 2006).

1.8 FEATURES OF NATURAL CELLULOSIC MATERIAL

Some features of natural cellulosic materials are known to inhibit their degradation/bioconversion (Solomon *et al.*, 1988 and 1999). These are degree of crystalinity, liginification and the capillary structure of cellulose to cellulolytic enzymes and other hydrolytic agents (Fan *et al.*,1987). Crop residue and its by-products contain substantial quantities of plant nutrients most of which are not readily available because of their high lignocellulose contents (Dzowela, 1987). Lignocellulosic biomass, the most abundant organic raw material in the world, constitutes a major portion of agricultural and forest wastes (Andriani, 2011).

The hemicellulose content of maize cobs is very high (46.4%). As hemicellulose is more closely associated with lignin than any other polysaccharide fraction and is believed to be bonded by phenolic constitutents (Van Soest, 1982), the cellulose and hemicellulose of the maize cobs may therefore not be readily available for microbial degradation.

1.9 PRETREATMENT OF CELLULOSIC MATERIAL BEFORE BIODEGRADATION

Lignocellulosic biomass holds remarkable potential for conversion into commodity products presenting dual advantage of sustainable resource supply and environmental quality. Though their utilization does not compete with food and feed demand, its bio conversion and utilizability is facilitated by pretreatment (Damisa *et al.*, 2008).

However, many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulosic materials have been reported (Kamakura, 1997; Kanosh *et al.*,1999; Solomon *et al.*,1999). Oseni and Ekperigin (2007) in an attempt to pretreat, maize wastes of cobs or shafts were dried, pulverized, boiled for 45 minutes, before being sieved and dried in drying cabinet.

1.10.1 GENERAL METHODS OF PROCESSING THE MAIZE COB

Many workers have reported the use of alkaline hydrolysis to increase the digestability of maize cobs by ruminants by measuring the dry-matter disappearance (DMD) values of treated and untreated maize cob after being fed to ruminants (Tuah and Qrskov, 1989; Faniyi, 2006).

Nelson *et al.* (1984) using maize cob containing 40% moisture reported the 48h DMD values of 2, 3 and 4% ammonia treated samples to be 61.30, 61.69, and 65.94% respectively. Those values were higher than the 48% DMD values for ammonia treated maize cob obtained by Tuah and Qrskov (1989). Acid pretreatment of corn cob with 1% HCl was reported by Pan *et al.* (2009) during bihydrogen production. A maximum hydrogen yield of 107.9ml/g-TVS and hydrogen production rate 4.20 ml/g-TVSh-1 was obtained under the condition of 10g/l substrate concentration and initial pH 8.0.

The nutritive value of residues may be upgraded by treating them in some way. Treatment may be physical (soaking, grinding, pelleting, boiling, steaming under pressure or gamma irradiation), chemical (Sodium hydroxide, organic white ash, alkaline hydrogen, peroxide or urea/ammonia) physicochemical (particle size/chemicals, NaOH/pelleting or chemical/steaming) or biological (enzyme, white rot fungi or mushrooms).

1.10.2 ALKALINE HYDROLYSIS OF MAIZE COB

Alkali treatment disrupts the cell wall by solubilizing hemicellulose and lignin; hydrolyzing uronic acid and acetic acid esters and swelling cellulose (Jackson, 1977). The most utilized of the alkali is caustic soda. The use of this alkali is not feasible in Nigeria, however, since it has to be imported at high costs and is not easily available to local farmers. When available it is so caustic that farmers face the constant danger of chemical burns. A local substitute that continues to have wide acceptabilities is organic white ash (Adebowale, 1985[a]).

According to Chesson (1987), alkali treatment maximizes plant structure damage by promoting microbial colonization and prevents or slows down the rate of lignin accumulation on cell wall surfaces by the specific degradation of lignin or by promotion of its solubilization. This solubilization appears to be the result of the cleavage of the

specific alkali-liable linkage formed between the structural polysaccharides (hemicellulose) of the cell wall and lignin itself (Hartley, 1985).

The level of sodium hydroxide treatment improved the 48H DMD values of maize cobs and the highest level of treatment resulted in the highest DMD value. When the sodium treated samples were washed after treatment to reduce the sodium content the DMD values were not reduced compared to the unwashed sample (Tuah and Orskov 1989). Kategile and Frederikson (1979) report the *in vivo* organic matter digestibility of 10% sodium hydroxide treated maize cob to be 32.1%. Nagole *et al.* (1983) reported that treatment of maize cobs with 4.5% sodium hydroxide increased *in vivo* dry matter digestibility from 44.7 + 1.6 to 54.2 + 2.0%.

Other substrate which have been pretreated using alkaline solution include coir waste and sawdust (Immanuel *et al.*,2007; Solomon *et al.*, 1999).

1.10.3 USE OF ASH

Adebowale (1985[b]) used local alkali such as cocoa pod and palm bunch ashes to treat roughages for ruminants. Adeleye (1988) also used a similar approach to treat roughages for ruminants in his attempt to find alternative feeding stuffs for ruminants. Adopting Adeleye's (1988) method, Faniyi (1998) used "Iye" (wood ash alkali) and urea (fertilizer grade) to treat cowpea and sorghum seedhulls. Iye solution was obtained by thoroughly mixing one part wood ash with 9 parts of water (w/v based). The mixture was allowed to stand overnight and the solution (Iye) was then filtered (Adeleye, 1988) and used as required.

Pre-treatment (alkali treatments) and fermentation would have caused a breakdown of the structural components of the seedhulls into utilizable nutrients for broiler chickens. Hence better utilization of urea and Iye-treated seedhulls based diets than BDG based diet (Faniyi 1998; Faniyi and Ologhobo, 1999).

On a pragmatic basis, the use of organic waste ash (palm bunch or cocoa pod husk) (Adebowale, 1985[a] and 1985[b]) or urea (Sundstol *et al.*, 1978) is recommended for small holder livestock farmers.

1.11 NUTRIENT SUPPLEMENTATION

Supplementation is perhaps a cheaper and simpler way of feeding maize residue and by-products in situ, involving practical methods that are realistic for small farm situations (Adebowale, 1988). Since maize residues are low in nitrogen, one way of improving their nutritive value is to add cheap nitrogen sources to make up for the deficiency. It was found that treated maize residue must be supplemented or animals must be allowed to browse if they are to perform satisfactorily (Singh and Jackson, 1971; Adebowale, 1988). Various inorganic nitrogen soruces have been optimized by different workers to enhance degradation of waste and cellulase production and compliment production of single cell protein (Scherief *et al.*, 2010).

Tuah and Qrskov (2005) also fortified the maize residue before giving them to ruminants. Diets were fortified with cotton seed cake, molasses and mineral supplements. All animals were fed an additional 1kg of *Panicum maximum* per head/day. DMI increased from 0.75 kg/day for dry treated maize cobs to 1.23kg/day for wet treated maize. Both dry matter intake and live weight gain indicated better utilization when wet treated residue was fed rather than dry residues.

In an attempt to improve the nutritive value of peeled citrus pulp, Faniyi (1998) added urea fertilizer granules to broiler chicks diet. The intention was to take advantage of ammonium hydroxide which results from adding water to urea and which would play some roles in delignifying fibrous materials. The extent of utilization of such diets showed that the body weight at 5 weeks of the broiler chicks was lowest 263.51g when 0% urea was added and highest at 1% urea addition at 382.15g when the urea fertilization granules concentration was increased to 1.5% the broiler weight dropped to 370.24g (Faniyi, 2002). Feed consumption decreased as urea fertilizer increased in the diet. Urea can serve as supplemental source of non-proteinous nitrogen to the broiler chicks, hence, the improvement observed in the average body weight gain of the broiler chicks.

1.12. SOLID STATE FERMENTATION

Fermentation is a chemical change that occurs as a result of action of micro-organism such as yeast, fungi, bacteria and protozoa. The reaction occurs under reduced

oxygen but increased hydrogen (anaerobic) condition (Sarojini, 2000). The use of microorganism e.g. bacteria and fungi in the process of fermentation as it occurs in rumen, ceacum and other parts of the gastrointestinal tract can be employed to produce food for man. They can reproduce thousands of new proteins (SCP) within a short time, can be grown on substances like agricultural by-products e.g. corn cob, industrial waste such as methane, molasse's fruit pulp, paper and petroleum products, produce food and get rid of waste.

Solid state fermentation is the process whereby microorganisms are grown on moist solid materials in the absence of free flowing water. Substrate that had been traditionally fermented by solid state included a wide variety of agricultural products such as rice, wheat, maize, soybeans, barley etc. Fermentation technology has developed in which for example a considerable amount of unicellular biomass referred to as single cell protein, could be multiplied by using it to ferment a variety of industrial by-products containing cellulose which are not directly utilizable by humans but could serve as renewable raw materials (Maurice, 2001).

The ability of microorganisms to grow on a solid substrate is a function of their requirements for water activity, their capacity of adherence and penetration into the substrate and their ability to assimilate mixtures of different polysaccharides due to the often complex nature of the substrate used. Microbial cultures produce many useful enzymes which are needed in food production, citric acid dextrans and cellulose. In fact, microorganism and their enzymes are used to bring about chemical conversion because their enzyme process can occur at normal temperatures and pressures compared with non enzymic chemical conversions which require high temperature and pressure (Faniyi, 2006).

The filamentous fungi are the best adapted microorganisms for solid substrate fermentation owing to their physiological, enzymological and biochemical properties. The hyphal mode of fungal gives the filamentous fungi the power to penetrate into the solid substrate. This also gives them a major advantage over unicellular microorganisms for the colonization of the substrate and the utilization of the available nutrients. In addition, their ability to grow at low water activity and high osmotic pressure conditions

makes fungi efficient and competitive in natural microflora for bionconversion of solid substrate (Faniyi, 2006).

However, bacteria and yeasts have also been used in traditional cultivation in solid substrate fementation processes. Bacteria have been used for enzymes production composting, ensiling and some food processes. Bacteria fermentations involving proteolytic activity are expected to increase the biological availability of essential amino acids than yeast fermentation, which mainly degrade carbohydrates (Chaven and Kadam, 1989). In solid substrate fermentation, the solid serves both as a support and a nutrient source. The substrate are heterogenous, water insoluble materials from agriculture or by-products from food industry, which have an amylaceous or lignocellulosic nature.

In agro-based food industry, solid substrate fermentation has been applied in the process of transformation of food residues for the production of traditional fermented food, protein enrichment and single cell protein production (Updegraff, 1971). Agricultural waste which had been used in solid state fermentiton for feed production include sugar cane bargasse, corn cob, corn straw, wheat straw (Faniyi, 2006; Babayemi *et al.*, 2009).

The bioconversion of agricultural and industrial wastes to protein-rich food and feedstuffs has an additional benefit of making the final product cheaper. This would offset the negative cost value of waste used as substrate to yield single cell protein (Anupama, 2000).

1.13 POULTRY UTILIZATION OF CROP RESIDUE AND BY-PRODUCTS

Poultry utilization of crop residue and by-products had been confirmed by many workers (Ademosun, 1973; Faniyi, 1998; Faniyi and Ologhobo, 1999).

Brewers' dried grains (BDG) used to be an industrial brewery waste until Ademosun (1973) established its nutritional/feed value. Similarly, cowpea and sorghum seedhulls are crops by-products that are usually obtained after processing cowpea and sorghum. Faniyi (1998) collected the seedhulls, sundried for many days, threshed and winnow dropped with harmmer mill, ground at a plate mill and incorporated into farm ration for broiler chickens. When the seedhulls were used, it was observed that the finishing weight, body weight gain, feed intake, feed conversion ratio and feed utilization

of broiler chicken on BDG (conventional source of fibre), cowpea seedhull and sorghum seedhull-based diets were similar. It implies that cowpea and sorghum seedhulls too, though crop by-products have nutritional values.

In another experiment Faniyi (1998) used pretreated crop residue as poultry feeds. The importance of pretreatement of diet as feedstuff is clearly shown in the performance of broiler chickens and on the length and weight of parts of the tract. The fact that the enzymes can degrade lignin (Zeikus, 1981) and the fact that sorghum seedhull can respond positively to enzyme treatment is promising.

Poultry cannot fully utilize high fibre diets because they lack the digestive framework that can elaborately digest large amount of fibre. The problem of high fibre content can be solved by dietary inclusion of enzymes. (Ani *et al.,*1999). Treated and untreated cowpea and sorghum feedhulls can be used as feeding stuffs or ingredients in poultry and swine diets. Pretreatment may however make them more digestible. But the number of days allowed for fermentation, biodegradation, chemical treatment and a combination of any of them may have to be re examined so that over solubilization of fibrous materials will not lead to loss of sugar content of bundles of fibre in crop residues "Cracking" the fibre may be sufficient so that the nutrients are or will be delivered in the gastro intestinal tract of animals (Faniyi, 2006).

Other crop by-products that had been fed to broiler, pullet and cockerel other poultry birds included groundnut husk (Faniyi, 1996) peeled citrus pulp (Faniyi, 2002) rice husk, wheat offal, maize offal, palm kernel cake (Faniyi, 2002) and mango seed kernel (Faniyi, 2006).

1.14 POULTRY UTILIZATION OF MAIZE COB

Adequate provision of balanced diet for animal at a reasonable cost is one of the greatest constraints to the expansion of the poultry industry (Abu *et al.,* 2009). The unprecedented increase in the cost of conventional ingredients used in compounding livestock feed has necessitated intensive investigations into the use of agricultural and agro-based industrial by-products. The major problem with these agricultural by-products have always been availability in industrial quantity in an accessible location.

This prompted a quest for a more available agricultural by-product such as maize cob. (Adeyemi and Familade, 2003).

Maize cob is perhaps the most prominent cereal crop by-product in Nigeria. The use of maize cobs as a feedstuff for ruminants has been reported (Alokan, 1988). It is however indispuTable that poultry cannot properly handle fibrous material because their enzymatic digestion cannot breakdown the cellulose cell wall, thus making the nutrients locked up in materials like maize-cob unavailable. One of the ways of using feeds that are under normal circumstances indigestible is by the use of fermentation techniques (Dirar, 1992).

According to Hammer (1989), when insoluble fibres are present in the gastro-intestinal tract (GIT) of monogastrics, they pose certain physiological effects such as inhibition of digestive enzymes in the intestine as well as increase in the bacterial number excreted. However, locked-up nutrient in these fibres are usually poorly utilized due to the digestive limitation of these animals which include absence of hydrolytic enzyme to break them down (Onilude and Oso, 1999). This fermentation techniques is essential to increase the digestibility of the maize cob and for the poultry to be able to utilize the nutrient locked up in it.

The use of rumen bacteria *in vivo* to ferment maize cob before being fed to ruminant and poultry had been done by some workers (Krishna 1985; Odunlani 1988; Tuah and Qrskov, 1989). Pretreatment of cob with acid and alkali before being fed to livestock had also been experimented by other workers (Faniyi, 2006).

However, there are few reports on the use of natural microflora present on degrading maize cob for biodegradation process and these microorganism can be effective since they are found on the cob (Oduguwa *et al.*, 2007). Moreover, the resultant effect of such feed compounded from such fermentation on the chicken need to be determined. Nutritional studies should not be limited to performance, carcass quality and nitrogen balance alone. The effect of the diets on blood constituents is also very important since blood transports gases, nutrients, excretory products, homones etc. within the body (Aletor *et al.*,1982) and laboratory test on the blood are vital tools that help detect any deviation from normal in the animal or human body (Ogunbanjo *et al.*,2009).

Evaluation of blood parameters is very important to monitor and evaluate disease prognosis (Amakiri *et al.*,2009).

The aims of this research work therefore were:

- Isolation and characterization of bacteria and fungi found on deteriorating maize cobs
- Screening of microbial isolates for cellulase activity
- Solid state fermentation of ground maize cobs with best cellulase producing bacteria and fungi
- Chemical and mineral analysis of the degraded cob
- Feeding trials on broiler chicks
- Haematological and histopathological study of organs and blood of experimental birds

CHAPTER TWO

MATERIALS AND METHODS

2.1 Sample collection and treatment

Maize cobs were obtained from the dumping site of a local farmer market at Ajegunle in Oyo town, Oyo State in the southwestern Nigeria. The collection was during the dry season between October and December after the late maize harvest. The cobs were sundried to be more friable for grinding. They were then grounded through a laboratory hammermill with a screen of 2.5mm.

2.2 Media preparation

The media used were measured as indicated and according to manufacturers' specifications in g/l and autoclaved at 121^{o}C for 15 minutes. Solid media for plating were dispensed in 20ml quantities in 9cm diameter sterile glass petri dishes unless otherwise stated. Liquid media were dispensed in 10ml quantities in 2 by 7cm screw cap bottle.

2.2.1 Isolation of bacteria from degrading cob

The medium used for isolation of the bacteria was nutrient agar, both pour plate and spread plate techniques were used for the isolation. One gram of the ground maize cob was added into 10ml of sterile distilled water and serial dilutions were made up to 10^{-12}. One millilitre of dilutions were then plated using both the pour plate and spread plate technique. In addition the surface of ground degrading maize cobs were scraped into the sterile petri dishes and sterilized media were added. Inoculated plates were incubated in an incubator (P-selecta, Barcelona, Spain) at 32^{o}C for 24 hours.

2.2.2 Isolation of fungi

The media used for isolation of fungi from the degrading maize cobs were potato Dextrose agar and yeast extract agar. The same method used in section 2.2.1 was employed for isolation. Incubation was done at 32^{0}C for 5 days.

2.2.3 Maintenance of culture

2.2.4 Routine sub culturing

The medium used for short-term storage of the bacteria culture was nutrient agar while that for fungi culture was potato dextrose agar. Sub-cultures were carried out at two month intervals. The isolates were maintained on both plates and slant cultures.

2.3 Morphology of bacterial isolates

The bacterial cells were cultured overnight on nutrient agar plates. Each bacterial isolate was smeared with a drop of water on a cleaned, grease-free glass slide and air-dried. The prepared slides were Gram stained as suggested by Olutiola *et al.* (1991). The stained slides were examined under a light microscope with oil immersion objectives.

2.3.1 Motility test for the isolates

The hanging-drop method described by Olutiola *et al.* (1991) was used. Vaseline was placed round the edge of the hollow in a clean cavity slide and a loopful of the bacterial culture was dropped carefully on the coverslip placed on the bench. The cavity slide was inverted over the coverslip in such a way that the drop was at the center of the cavity and the cavity slide was pressed down gently to firmly keep the coverslip in the position with the vaseline. The cavity slide was inverted quickly and smoothly with the drop still hanging. This preparation was examined under the light microscope.

2.3.2 Endospore staining

The method of Olutiola *et al.* (1991) was used in determining the presence of endospore in the bacteria isolates. A heat-fixed smear of each bacterium was flooded with 5% malachite green stain and heated gently for 5 minutes. The stain was not allowed to dry during the heating by the addition of more stain. Thereafter, the stain was washed off with water and the smear counter-stained with 0.5% safranin for 30 seconds and then washed with water. The slide was then viewed under X 100 objective of light microscope using oil immersion. Bacterial spores were stained green while the vegetative cells were stained red.

2.4 Biochemical and physiological tests

2.4.1 Catalase test

A loopful of growth from a 24 hour culture on nutrient agar was transferred to a drop of a 3% solution of hydrogen peroxide. The evolution of gas bubbles indicated the presence of catalase.

2.4.2 Oxidase test

The modified method of Kovacs (1956) cited by Persley (1980) was used to detect the presence of oxidase. Filter paper strips were soaked in a 1% aqueous solution of N, N-dimethyl-p-phenylenediamine and dried in a fume cupboard. A loopful of bacteria growth from 24 hour culture on nutrient agar plate was smeared onto the paper with a platinum needle. The production of a purple colour within 10 seconds indicated a positive reaction.

2.4.3 Urease test

The method of Fahy and Hayward (1983) was used to detect the presence of urease. The basal medium contained in g/l $NH_4H_2PO_4$ 0.5; K_2HPO_4 0.5; $MgSO_4.7H_2O$ 0.2; NaCl 5; glucose 10; yeast extract (Difco) 10; cresol red 0.016; agar 15; autoclaved in a flask. A 20% solution of urea was filter-sterilised and added to the sterile basal medium to give a final concentration of 2% urea. The medium was dispensed in 10ml quantities to 25ml screw-capped bottles and the bottles were slanted so as to give slope with about apChemically 2.5cm depth and a slant of 4.0cm length. The slant agar was inoculated with each isolate and incubated at 30^{o}C. Production of a red-violet colour on the slant within 3 days indicated a positive reaction.

2.4.4 Indole test

The bacteria isolates were grown in tryptone (1.0%) yeast extract (0.5%) broth for 5 days on a shaker incubator according to the method of Olutiola *et al.* (1991). To 1 ml of the broth culture was added Kovac's reagent. The development of a cherry red colour that faded away after 15min indicated a positive reaction.

2.4.5 Starch hydrolysis test

The method of Fahy and Hayward (1983) was used to detect starch hydrolysis. Soluble starch (1%) was added to nutrient agar and sterilized. The starch plates were streaked and incubated for 7 days; thereafter they were flooded with iodine solution. Starch stained blue-black, a clear zone around the line of growth showed amylase activity.

2.4.6 Methyl red test

The method cited by Skerman (1967) was used for the methyl red test. Cultures were grown in glucose-phosphate-peptone water, which contained in g/l peptone, 5; K_2HPO_4, 5; and glucose, 5. The first two ingredients were dissolved by steaming and the solution cooled and adjusted to pH 7.4. The glucose was then added and 5ml of the media was dispensed into a screw-capped bottle and autoclaved at 108^{o}C for 25 minutes. After inoculation and 4 days incubation, three drops of methyl red solution were added to each tube. The formation of a red colour indicated a positive test.

2.4.7 Voges-proskauer test

Glucose-phosphate-peptone broth prepared as described above was inoculated with bacteria isolates and incubated at 37^{o}C for 4 days. To 1ml of the culture medium was added 1ml of 40% potassium hydroxide and 3ml of 5% ethanolic solution of naphtol (solution of naphtol in absolute ethanol) and the bottle was shaken thoroughly. The formation of a pink colour in 2-3 minutes indicated a positive reaction (Skerman, 1967).

2.4.8 Citrate utilisation test

Simmons' citrate medium was prepared by reconstituting oxoid dehydrated medium and dispensing it in 5ml quantities to test tubes. The slopes were inoculated and incubated for 2 days. A change in colour of the medium from green to blue indicated a positive reaction (Skerman, 1967).

2.4.9 Carbohydrate metabolism test

Acid production from carbohydrate was determined using a modified method of Fahy and Hayward (1983). A basal medium containing NaCl, 0.2g; peptone 5.0g; phenol red (2ml of 1% solution per litre). Filtered carbon sources were added aseptically to give 1% final concentration after autoclaving. A change of colour of the media after incubation for 4 days from pink to yellow confirmed acid production.

2.4.10 Hydrogen Sulphide Production Test

The modified method of Hayward and Hodgkins (1961) cited by Olutiola *et al.* (1991) was employed to detect the production of hydrogen sulphide. The medium contained peptone, 1.0g; L-cysteine, 0.01g; Na_2SO4, 0.05g. The pH was adjusted to 7.0 with a 40% sodium hydroxide solution. The medium was dispensed in 5ml quantities to 10ml screw-capped bottles. The production of hydrogen sulphide was detected by the blackening of lead acetate paper, which was suspended over the medium. Observations were recorded 7 days after inoculation.

2.4.11 Gelatin hydrolysis test

Nutrient broth supplemented with 10-15% gelatin was prepared and dispensed into test tubes and sterilized in the autoclave. Cultures were inoculated into the nutrient-gelatin broth and incubated for 7 days at 30^{o}C. The tubes were thereafter refrigerated to solidify any unhydrolysed or undenatured gelatin. Liquefaction of the gelatin confirmed a positive test (Hayward and Hodgkins, 1961).

2.4.12 Case in utilisation

Skim milk agar was prepared by adding 1% (v/w) skim milk to nutrient agar (Harrigan and McCance, 1966). The agar was sterilized by autoclaving at 110^{0}C for 10 minutes. On cooling, the medium was dispensed into sterile Petri dishes and left to solidify. The plates were then streaked across once with the isolate and then incubated for 3 days at 35^{0}C. Uninoculated plates served as control. At the end of incubation, a clear zone around the line of streaking indicated casein hydrolysis while the absence of a clear zone indicated negative result.

2.4.13 Coagulase test

The method of Fahy and Hayward (1983) was used to detect casein hydrolysis. The basal medium contained peptone, 0.5g; yeast extract, 0.3g and agar, 2.0g in 100 ml of distilled water. The pH was adjusted to 7.2 and a solution of 4% skim milk was filtered, sterilized and added to the sterile basal medium to give final concentration of 0.4% milk. ApChemically 15-20ml was dispensed into sterile plates and inoculated after solidification. The plates were examined for hydrolysis after 3 days of incubation over a light pack.

2.4.14 Nitrate reduction

The medium used to detect the reduction of nitrate to nitrite by the isolates contained peptone, 0.1g; NaCl, 0.5g; KNO_3, 0.2g; agar, 0.3g all in 100ml of distilled water and was dispensed in 5ml amounts to 10 ml screw-capped bottles. The cultures were tested for the presence of nitrite after seven days of incubation using the starch-iodine test. The test reagents were a starch-iodine solution containing starch, 0.4g Znl_2, 2.0g in 100ml of distilled water and a solution containing 16ml concentrated hydrochloric acid and 84ml distilled water. A drop of each reagent was added to the culture. The formation of a blue colour indicated the presence of nitrite (Skerman, 1967).

2.4.15 Production of ammonia from arginine

The modified method of Thonley (1960) was used to detect the anaerobic breakdown of arginine. The medium contained peptone, 0.1g; NaCl, 0.5; KH_2P04, 0.03; L arginine, 1.0g; phenol red, 0.001. The pH was adjusted to 7.2 with 40% sodium hydroxide solution. The media were dispensed 3ml into test tubes. Each test tube was stab inoculated with growth from 48 hours culture on nutrient agar and sealed with a 2gm layer of sterile 4% agar. The tubes were examined for a change in the colour of the indicator after four days.

2.4.16 Growth in 4% sodium chloride broth

The test organisms were inoculated into screw-cap tubes containing 10mls of MRS broth containing 4% sodium chloride. Incubation was carried out at 30^{o}C for 72 hours. Increased turbidity of the medium was recorded as positive for growth. The uninoculated tubes served as control.

2.4.17 Growth at different pH values and temperatures

Nutrient broth (from Sigma – Aldrich chemical G-mbit Steinhein, India) was weighed and prepared as specified by the manufacturer in already prepared potassium phosphate buffer and the pH of the broth was adjusted to desired range with 1M sodium hydroxide solution and concentrated hydrochloric acid. The broth was homogenized and dispensed in screw-capped bottles in 5ml quantities before sterilization in the autoclave at 121^{0}C for 15 minutes. The bacteria were inoculated from 24 hour-old cultures and incubated at 30^{o}C for 48 hour. The pH range 3.9 and 9.2 were maintained. Inoculated nutrient broth incubated at different temperatures of 3, 15, 45 and 60 for 48 hours were used for growth temperature study. The optical density of broth cultures were determined from a UV/visible spectrophotometer.

2.5 Preliminary screening for enzyme synthesis

2.5.1 Media for enzyme production

The different fungi and bacteria isolates were grown in chemically defined media composed of KH_2PO_4 (1.0g1^{-1}), $MgSO_4.7H_2O$ (0.5 g 1.0^{-1}), yeast extract (1.0g 1^{-1}). $CaCl_2$, $2H_2O$ (0.14g1), carboxymethyl cellulose 10g and thiamine (0.0025g 1^{-1}), (Vahidi *et al.*, 2004).

2.5.2 Optimization of cultural conditions for enzyme production

This was done by varying the different parameters that affect the production of cellulolytic enzymes to determine the best conditions for the production.

2.5.3 Influence of temperature

Bacteria and fungi isolates were cultivated in the chemically defined medium of Vahidi *et al.* (2004) as explained in 2.5.1. Ten millilitres of the chemically defined medium was dispensed into 30ml MacCatney bottles and sterilized, then inoculated with one 8mm alga disc plug of the fungi and incubated at 28, 32 and 40^{o}C for 0.4, 7, 14, 21 and 28 days. Thereafter, the culture was harvested and the mycelium free filtrate was used to determine the cellulase enzymes activity. For the bacteria isolates a loopful of the isolate was taken and inoculated into the chemically defined medium and incubated at 36^{0}C for 0, 4, 7, 14, 21 and 28 days filteration was done using sintered glass crucible and filterate was used to determine the cellulase activity.

2.5.4 Influence of pH

The bacteria and fungi isolates were cultivated in the chemically defined medium (Vahidi *et al.*,2004). 10ml of the chemically defined medium was dispensed into 30 Maccartney bottles and pH adjusted by using 0.1M HCl and).1M NaOH to 4.0, 5.5 and 7.0 and sterilized, then inoculated with one 8mm alga disc plug of the fungi and incubated at 28^{o}C, 32^{o}C and 40^{o}C for 0, 7, 14, 21 and 28 days. Thereafter, the culture was harvested and the mycelium free filtrate was used to determine the cellulase enzymes activity. The bacteria isolate was inoculated as described in 2.5.3

2.5.5 Influence of nitrogen sources

The isolates were cultivated in chemically defined media containing 1% carboxyl methyl cellulase and supplemented with different nitrogen sources of 1% (w/v) (Vahidi *et al.*, 2004). The nitrogen sources included yeast extract, peptone, urea, $(NH_4)_2$ SO_4 and $NaNO_2$ Ten millilitres of each were dispensed into 30 ml MacCartney bottles and sterilized, then inoculated with one 8mm disc plug of the fungi and incubated at 30^{o}C for 0, 4, 7, 14, 21, and 28 days. Thereafter, the culture was harvested and the mycelium free filtrate was used to determine the cellulase enzyme activity. The bacteria isolates were also inoculated as described in section 2.5.3

2.6 Enzyme activity in fungi and bacteria isolates

2.6.1 Cellulase activity in fungi

After growth had been allowed to proceed for the required length of time (at intervals between 0 – 28 days) at room temperature, the cultures were filtered through sintered glass crucible and the cellulolytic activity of the filtrates was determined using the method of Reese and Mandels (1963). The assay medium was 0.55% carboxymethyl cellulose (CMC) in 0.55M acetate buffer (pH 5.5), and nine millilitre of the assay medium were incubated with 1ml of the fungus filtrate for 1 hour at 32^{o}C. The filtrate of the uninoculated control was also obtained and similarly assayed. To estimate the amount of reducing sugar released, 1ml of dinitrosalicylic acid (DNSA) reagent was added to 1ml of the filtrate-CMC reaction mixture and the absorbance was set at 540nm using an SP 600 Spectrophotometer. Absorbance was set at 100% with the filtrate-CMC reaction mixture of the uninoculated control. Dinitrosalicylic acid reagent was prepared by combining 1.0g DNSA with 20ml 2M NaOH 20g potassium sodium tartrate in 100ml distilled water. The absorbance of standard aqueous solution of D-glucose of various concentrations (0-10 mg/ml) was determined and used to construct a graph of % transmittance as related to mg of glucose per ml, the amount of reducing sugar produced by 1ml of fungus filtrate from the CMC assay medium was calculated from the graph. Cellulolytic activity of the filtrate was then expressed in terms of the amount of total reducing sugars (RS) per ml. three replicates were determined and the mean of the three was taken.

2.6.2 Cellulase activity in bacteria

The same method in 2.6.1 was used and 1ml of Bacteria filterate was used to determine the cellulase activity using DNSA method.

2.7 Solid state fermentation

After the result of the cellulase assay two Bacterial isolate *Bacillus subtilis* and *Bacillus licheniformis I* and two fungal isolate *Aspergillus niger* and *Aspergillus oryzae* were chosen for solid state fermentation.

2.7.1 Growth of isolate

Bacteria isolate were sub cultured from slant by using a sterile loop to streak on Nutrient agar plates and incubated at 32^{o}C for 48 hours.

2.7.2 Growth of the fungal isolate

Fungi isolates were subcultured from slant by point inoculation in the centre of potato dextrose agar plate and incubated at 32^{o}C for 5 to 7 days to obtain more isolates. Spores were then harvested for fermentation.

PREPARATION OF INOCULA

2.7.3 Preparation of bacteria inocula

Preparation of broth culture of bacteria isolates 1.65g of peptone water (oxoid) was dissolved in series of 100ml of distilled water.

These were homogenized and sterilized at 121^{o}C for 15 minutes. The broth were allowed to cool. A loopful of each of the Bacteria isolate was inoculated into each of the four conical flask in replicate and a fifth serve as control. These were incubated at 32^{o}C for 48 hours. Centrifugation of broth culutre was done at 3000rpm for 5 minute and the Bacteria cell were then suspended in N saline and concentrated to 0.5 optical density.

2.7.4 Preparation of fungal isolates for substrate fermentation

Fungi isolate growing on PDA for 7 days were scraped into conical flask containing 100ml of sterile distilled water and shaken gently to release the spores. Spore counts were carried out using haemocytometer to determine the number of spores per ml of sterile distilled water. 10ml of sterile distilled water containing fungal spores will be used as inoculum.

2.7.5 Substrate preparation and fermentation

The substrate was treated according to the modified method of Braun *et al.* (2000). 25g each of the gritisized dry substrate particle size (1mm) were weighed into 22 wide mouth screw cap bottles of 5.5cm diameter and 75ml of distilled water were added 7 of the bottles contained substrates which were not pretreated, 7 contained NaoH

pretreated substrate while 7 contained ssh pretreated substrate. The last bottle contained maize cob only and this served as the control. Out of the 7 bottles in each category peptone water was added as nutrient supplement to 2 of the bottles and inoculated with each of the bacteria isolate. The substrate in2 bottles was supplemented with chicken extract and inoculated with Bacteria isolate. 2 bottles were inoculated only without supplementation while one bottle served as control without inoculation. The set up was repeated based on the days of sampling. The same method was used for substrate preparation and fermentation by fungi. All the bottles were sterilized prior to inoculation with inoculum.

2.7.6 Pretreatment of substrate

The maize cobs were sun dried for several hours and crushed through the laboratory hammer mill. The crushed substrate was then sieved in 1mm sieve to get the powdered form or gritisized form.

2.7.7 Pretreatment with NaOH

The gritisized cobs were soaked in 1% Na0H solution in ratio 1:10 substrate; solution for 2 hours at room temperature. It was then washed free of chemicals and filtered using distilled water.

2.7.8 Pretreatment with wood ash

The substrate was soaked in 1% ash solution in ratio 1:10, substrate, solution for 2 hours. It was then washed free of the alkali and filtered using distilled water.

2.7.9 Nutrient supplementation of substrate

One percent of peptone solution was prepared and 10ml dispensed into each bottle. Similarly, 1% chicken extract solution was prepared and 10ml of it was dispensed into the specified bottles.

2.8 Physico-chemical and nutritional analysis mineral composition determination

2.8.1 Potassium

Flame photometric method of Associaiton of official analytical chemist (A.O.A.C) (1980) was used to determine the potassium content of the fermented maize cob sample. Five grams of sample were ashed in a furnance at 500^{o}C for 4 hours. The residue was cooled in desiccator and weighed. Ten milliliters of concentrated HNO_3, 5ml of $HClO_4$ and 10ml of 6N HCl were added and mixed until dissolved. The solution was placed in a boiling water bath and evaporated to almost dryness. De-ionized water was added and transferred to 250ml volumetric flask quantitatively and made up to the mark with deionized water. The absorbance of the resulting mixture was read at 766.5mm. The concentration of the element was read from the standard curve.

2.8.2 Phosphorus

Determination of the phosphorus was carried out by adding 45ml of de-ionized water to 5ml of each sample in a 100ml flask. Twenty milliliters of vanodomolybdate solution was added to each flask and diluted to volume within 5 minutes. These were mixed thoroughly and allowed to stand for 10 minutes. The phosphorus content was determined colorimetrically with Technicon Analyser (EEL, 201).

2.8.3 Calcium

Each of the samples was placed in separate volumetric flasks. Calcium standards of varying concentrations were prepared in 200ml volumentric flask containing 80 ml of H_2SO_4 and 20ml of lanthanum stock solution (A.O.A.C., 1980). The samples and their standards were analysed for their calcium content by using Atomic Absorption Spectrophotometer (Perkin – Elmeyer, Corporation, 1980). The concentrations read out of the samples were calculated from the standard curve.

2.8.4 Magnesium

The same method as in calcium determination was carried out for magnesium except that ammonia buffer of pH 10 was used in place of NaOH and Eriochrome black indicator was used. The mixture was titrated from purple to blue end point.

2.8.5 Iron

The sample was mixed thoroughly and 50.0ml was measured into a 125ml Erlenmeyer flask. Two millilitre concentrated HCl, 1 millilitre NH_20H. HCl solution and glass beads were added and heated to boiling. It was allowed to boil until volume was reduced to 15 to 20 millilitre to ensure dissolution of iron. It was allowed to cool to room temperature and transferred to a 100ml volumetric flask. Ten milliliter of NH_4C_2 H_30_2 buffer solution and 4 millilitre pheonnanthroline solution was added and diluted to mark with water. It was mixed thoroughly and left for 10 to 15 minutes for maximum colour development.

2.8.6 Dry Matter (DM) determination

Two grams of the sample were weighed into a previously weighed crucible. The crucible plus sample was then transferred into the oven (set at 100^oC) to dry sample to a constant weight. At the end, the crucible plus sample was removed from the oven and transfered to a desiccator, cooled for ten minutes and weighed.

$$\%DM\ (\%\ dry\ matter) = \frac{W_3 - W_0}{W_1 - W_0} \times \frac{100}{1}$$

Where

Weight of empty crucible is W_0

Weight of crucible plus sample is W_1

Weight of crucible plus oven dried sample W_3

2.8.7 Determination of ash content

One gram of the sample was quantitatively transferred into pre-weighted porcelain crucibles. The weight of the samples with the crucibles was recorded. The crucibles containing the samples were placed in a pre-heated furnace at 600^{o}C for 6 hours after which they were removed and cooled to room temperature in desiccators and weighted. The difference between the final weight and the porcelain dish gave the ash content, which was expressed as a percentage of the initial weight (A.O.A.C., 1980).

2.8.8 Crude Fber Determination

The crude fiber content of the maize cob sample was determined using the method of A.O.A.C. (1980).

The weighed samples were first defatted using a solvent system composed of 49% ethanol, 50% acetone and 10% HCl. The samples were air dried and put in a liter conical flask. Two hundred milliliters of 0.255 M H_2SO_4 were added and brought to boil prior to which antifoaming agent was added. The mixture was boiled gently for 30 minutes using the reflux condenser after which it was left for 1 minute and poured at once into a shallow layer of hot water under gentle suction so that the two hundred milliliters could be filtered within 10 minutes. The insoluble matter was washed with boiling water until the washing was free from acid and then washed back into the original flasks by means of wash bottle with 200ml of 0.313 M NaOH. All the insoluble materials were transferred to the filter paper and washed using boiling water until they were acid free. The insoluble matter was also washed twice with ethanol and thrice with ether and transferred to a dried, weight ash less filter paper No. 54 and dried at 100^{o}C to a constant weight. The paper was incinerated with contents at 600^{o}C for 6 hours in Gallenkamp furnace.

The amount of crude fiber was obtained by subtracting the weight of the ash from the increased weight of the paper due to the insoluble material (Person. 1975).

2.8.9 Estimation of crude protein

Kjedahl method of nitrogen/protein determination was used (A.O.A.C. 1980). Two grams of sample were weighed in duplicates into digestion tubes. Twelve milliliters (12ml) of 95-97% sulphuric acid were added into each of the tubes (A.O.A.C.,1980). The

samples were digested at 420^{o}C using the digestion system for 30-45 minutes until they become clear. After cooling, 250ml of distilled water was added into each of the tubes followed by dispensing 50ml of 40% sodium hydroxide solution. The tube content was distilled for 3-5 minutes into receiving flasks containing 250ml each of mixed indicator (14% boric acid with bromocresol green/methyl red indicator solution) using the 10002 distilling system. Then, each of the samples in the flasks was titrated against 0.1N NaOH solution until a neutral green colour was obtained. Twenty five milliliters of mixed indicator were transferred into another receiver flask and a blank (no sample, only chemicals) was distilled into it. The distillate from the bank was also titrated with 0.1N NaOH solution until a neutral grey colour was obtained. The crude protein was calculated as N x 6.25.

2.8.10 Lignin determination

This acid detergent lignin of the sample was determined according to the method of A.O.A.C. (1980) as modified by the method of Nahm (1982). Two grams of dry agricultural waste samples were weighed into 250ml beaker suiTable for refluxing. One-hundred milliliter of cold acid detergent solution and 2ml dehydronapthalene were added and the contents were heated to boiling in 5-10 minutes. Refluxing of the sample was done for 60 minutes from onset of boiling and the sample filtered after refluxing using a vacuum pump. The sample was washed repeatedly with acetone until it removes no more colour and then dried at 105^{o}C for eight hours or overnight. This was then allowed to cool in a desiccator to room temperature and the weight determined.

$$\text{ADF} = \frac{\text{Weight of crucible with samples} - \text{Weight of crucible}}{\text{Sample weight}} \times 100/1$$

The ADF sample obtained was covered with 72% H_2SO_4 and stirred with a glass rod to a smooth paste, by breaking all the sample lumps. This was allowed to stand for three hours, after which the acid was filtered off as much as possible with a vacuum pump. The sample residue was washed with hot water (85 to 95^{o}C) until it was free from acid. This was dried in crucible overnight at 100^{o}C and re-weighed. The crucible with the content was ignited in a muffle furnace at 500^{o}C for 3 hours. The samples were allowed

to cool to 250^{o}C and then transferred to desiccator to cool to room temperature and the weight determined. The lignin content was thus calculated as:

$$\text{ADL} = \frac{\text{dry weight of crucible and content} - \text{dry weight of crucible after ashing} \times 100}{\text{Original weight of ADF sample}} \quad 1$$

2.8.11 Cellulose

This was determined according to the method of Van Soest and Wine (1968). The cellulose content was calculated thus:

ADF value – ADL value = Cellulose

2.9 Microbially Degraded Cob (MDC) feed preparation

2.9.1 Preparation of inoculum for MDC production

Large quantities of corn cobs were sundried to be more friable for crushing by using laboratory hammermill. The crushed substrates were then sieved to get the required particle size (1mm). Distilled water was added in ratio 1:2 substrate; water and chicken extract solution was added in ratio 2:1 substrate; chicken extract solution. Sterilization was done at 121^{o}C for 15 minutes. On cooling, it was inoculated and left to ferment at 30^{o}C for 28 days.

2.9.2 Scale up of feed production

Large scale production of poultry feed was done by using a fabricated silo prepared from used cylindrical steel drum (60cm diameter 110 cm height). The inner portion of the silo was lined with 0.25mm polyethylene for each experiment bags in which Bacteria and fungal inoculate were used solely, 24kg of corn cob containing other additive laws sterilized in a giant autoclave and loaded into polythene bag, inoculated with the inocula and then sealed up after which it was covered with metallic lid. Fermentation was allowed to take place for 28 days at room temperature.

The microbial degraded cob produced included the derivative labeled Bacteria Enriched Cob Product (BECP) and Fungi Enriched Cob Product (FECP) which was used in compounding dietary feeds. Seven dietary feeds containing basal diets were prepared in which BECP and FECP were each substituted for maize at 18, 50 and 82% while the

commercial feed served as the control. The experimental diets and clean drinking water were provided *ad libitum* throughout the experimental period. Conventional management practical were observed in all the seven treatments.

2.9.3 Experimental birds

Four weeks old seventy (70) broiler chicken (abro acha breed) obtained from Gbeemu farm, Osogbo were used in the study. They were individually weighed and randomly assigned to seven treatments (diets) in groups of ten (10) birds per treatment labeled as G_1D_1 G_2D_2 to G_7D_7. The treatments were replicated two times with 5 birds per replicate using A and B to distinguish between replicate of the same group. The birds were maintained on the various diets for six weeks.

2.9.4 Performance characteristics

The feed intake, feed conversion ratio, feed efficiency, body weight and daily weight gain of the experimental birds were determined on weekly basis for the period of six weeks. The daily feed intake was obtained by subtracting the left-over from the total amount of feed supplied. Birds in each group tagged G_1D_1A, G_1D_1B to G_7D_7A, G_7D_7B were weighed at the inception of the experiment and weekly thereafter to obtain the weekly and daily weight gain. The economic implication of including BECP and FECP into diets of broiler chicken was assessed by calculating the cost per kilogram of each diet, total feed cost and feed cost per kilogram of weight gain by the broiler.

2.9.5 Analysis of experimental diets

The Chemical analysis of the experimental diets, BECP and FECP were carried out according to AOAC (1980) method. The parameters determined included the crude protein, crude fiber, moisture content, Ether extract, Ash and cellulose content. The minerals which included calcium, iron, magnesium phosphate and potassium were also analyzed.

2.9.6 Haematological and biochemical indices

The blood constituent of the broiler chicken fed with the experimental diets were analysed after six weeks of the experiment. Seven birds, one bird per diet batch were selected and blood samples taken through cardiac puncture. Blood parameters which included packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), lymphocytes, neutrophils, monocytes and eosinophils were assessed using standard principles according to Barker and Silverton (1985).

Biochemical indices which included total protein, albumium globulin, getinine aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also determined.

2.9.7 Blood samples collection

Blood samples were collected after cardiac puncture into anticoagulant bottle. They were analyzed to determine the packed cell volume (PCV), red blood cell count, white blood cell count, differential lymphocyte, neutrophil count etc, and using conventional methods as described by Barker and Silverton (1985).

2.9.8(i) Determination of Packed Cell Volume (PCV) of blood samples

The blood samples collected with anticoagulant bottles were used; plain capillary tubes were filled up to about two third of the length of the tube with the blood samples. The vacant ends were sealed with plasticin. The capillary tubes were arranged in the haematocrit centrifuge and centrifuged for 5 minutes 3,000 revolutions per minute and the percentage of the packed cell volume were determined using the graphic reader (Barker and Silverton, 1985). Haemoglobin was determined using automated electronic machine.

2.9.8(ii) Erythrocyte count (RBC)

This was determined using a haemocytometer. Erythrocyte diluting pipette was used to draw the blood samples to 0.5 marks on the pipette. The tip of the pipette was wiped using sterile cotton wool. The pipette was filled to 1.01 point with blood cell diluting fluid and then gently mixed. The cotton wool was used to discard some of the diluted blood at the tip of the haemoacytometer and the counting chamber was filled.

The counting chamber was mounted on light electroscope and the cells were viewed at high magnification. The erythrocyte in 5 of the 25 squares in the central area of each chamber of the haemocytometer was counted taking the four Maizeer squares and the central one. The total number of the erythrocytes obtained was multiplied by 10,000.

2.9.8(iii) Leucocytes count

Leucocytes count was determined by drawing up the blood samples to 0.5 mark of the haematocytometer and filled to the 1.01 mark with leucocytes diluting fluid. The white blood cell (WBC) in the four large Maizeer squares of the haematocytometer chamber was counted and the total multiplied by 50.

2.9.8(iv) Differential white cell count

Thin smear of the blood samples were made, the slides were allowed to dry in air and fixed with absolute methanol for 5 minutes. The smears were stained with Giemsa stain and were examined for the various leucocyte count. Other haemogram parameters determined were by simple calculation.

2.9.8(v) Histo-pathological examination

Seven broiler chicks (one from each diet) were selected and slaughered by cervical dislocation. The internal organs which included the liver, kidney, lungs and pancreas were dissected and preserved in 10% formalin solution for histo-pathological examination.

2.10 Histological procedures

Histopathological processing (preparation of organs and tissues for microscopic examination) of the excised tissues of the lungs, liver, kidney and pancreas from the sacrificed broiler of all the diets were performed using Medical Laboratory Technology and Clinical Pathology (MLTCP) and MPAMA'S Procedure as follows:

2.10.1 Slide Preparation

Fixation:- The constitutents of the cells and tissues were fixed in a physical and partly chemical state to withstand subsequent treatment with various reagents e.g. ethanol, xylene,without loss or decomposition by endogenous or exogenous factors. The fixatives denatured the proteins and formed a mesh work that held the cell constituents. The fixatives were 10 to 20 times the volume of the specimen, and were fixed for twenty four (24) hours before dehydration.

Dehydration:- In place of automated, manual process was used for removing the inherent water content of the tissue specimen in a gradual way, considering osmotic dynamics (Shandon Elliot). First at 70% ethanol for 2 hours, 95% ethanol for 2 hours and 100% ethanol, times three for 2 hours (x3).

Clearing:- For removing the ethanol that the tissues had been bathed in and to imitate a complete process that will make cells transparent at microscopic level, three changes of the clearing agent (in xylene for 2 hours at three consecutive time) was used.,

Infiltration:- Leaving xylene, the tissues were transferred to molten paraffin wax which serves as support for subsequent state of sectioning. Paraffin wax permeates into the tissues to fill up vacuoles that have been left by dehydration. The first, second and third wax at two hours each were completed in a wax high temperature oven.

Embedding:- The processed, infiltrated tissues were positioned in molten paraffin wax within a mould enclosure. They were left to solidify.

Blocking:- The tissues were cut into little chunks or blocks, held firmly by paraffin wax. These blocks are placed with spatula and attached to pieces of wood that served for clamping and positioning for sectioning. The tissue blocks are then trimmed, ready for sectioning.

Sectioning:- Microtone machine was used to cut thin slice of the original tissue at a preset thickness of 4um (a sedition). The sections are floated in water bath, and the satisfactory ones are picked up with microscope glass slides that had been coated (only one side) with glycerine-egg albumin. The slides were air dried for

30 minutes, labeled with their respective specimen number with a diamond pencil and arranged in a slide carrier, ready for staining.

Staining:- Haematoxyline and eosin staining procedure was used as follows:

Dewaxed in xylene (3x) for 3 minutes each

Hydrated in 100% ethanol for 3 minutes

Hydrated in 95% ethanol for 3 minutes

Hydrated in 75% ethanol for 3 minutes

Watered for 3 minutes

Stained in haematoxylin for 15 minutes

Excess stain was washed off

Differentiated in 1% acid alcohol for 3-5 seconds

Acid was washed off

Blue in water for 3-5 minutes

Counterstained in 1% Eosin for 3 seconds

Dehydrated through in 50%, 70%, 95%, 100% and another 100% Ethanol respectively.

Cleared in first, seconds and third xylene

The slides were retained in the last xylene before they were mounted in a good transparent mutants that had a refractive index similar to that of the glass slide.

Analysis of data

Data from this study were analysed with SAS and SPSS a computer software package to determine analysis of variance and the test of significance were carried out using Duncan's multiple range tests.

CHAPTER THREE

RESULTS

3.1 Microbiological characterization of the bacteria isolates associated with degrading maize cob

Thirty three microbial isolates (19 bacteria and 14 fungi)were obtained from the degrading maize cob collected from the local dump at the farmers' market in Ajegunle Oyo, Oyo State. The bacteria isolate included *Bacillus subtilis* (2), *Bacillus licheniformis* (4), *Bacillus cereus* (4), *Bacillus megaterium* (2), *Pseudomonas putida* (2), *Pseudomonas cepacia* (1), *Pseudomonas aerugnosa* (3) and *Proteus mirabilis* (1).

Morphological and biochemical characterization of bacteria isolates shown in Table 1 revealed that all the *Bacillus* species were gram-positive rod while the *Pseudomonas* species and *Proteus* species were gram-negative rods. They were all motile, catalase positive, and were able to hydrolyse gelatine. They were all capable of growth at 45^{o}C and 4% sodium chloride concentration. Moreover, all the *Bacillus species* were positive for oxidase test and able to hydrolyse casein and arginine. However they were negative for indole, coagulase and urease test and could not grow at 60^{o}C. Only *Bacillus subtilis* could not grow at 15^{o}C, negative for Voges Proskauer test but positive for methyl red, citrate utilization and nitrate reduction tests. All the *Bacillus licheniformis* species were negative for citrate utilization but reduced nitrate.. *Bacilus cereus* and *B megaterium* however showed varied reaction to citrate utilization. Three out of the four species of *B cereus* strains and one of the two species of *B megaterium* utilized citrate coud not.

All the *Pseudomonas* species were negative for indole test, did not grow at pH 3.9 and 60^{o}C and were not able to hydrolyse starch or casein. They were however positive for urease, coagulase, methyl red, oxidase and catalase test. They utilized nitrate and grew at 15^{o}C. *P putida* could grow at 3^{o}C and negative for Voges-Proskauer test while *P. aerugniosa* and *P. capacia* were negative at 3^{o}C and positive for Voges-Proskauer test.

All the Bacteria isolates oxidatively metabolized glucose but had varied reaction in other sugars as shown in Table 2. All the *Bacillus* species metabolised lactose, galactose, rhaminose, xylose except *B licheniformis*. Both *B subtilis* and *B licheniformis* metabolised sucrose mannitol and raffinose while *B. cereus* and *B. megaterium*

metabolised fructose. Only *B. licheniformis* metabolised arabimose but *B subtilis* could not metabolise maltose.

All the pseudomonas species could metabolize glucose, sucrose, mannitol, lactose but could not metabolise raffinose and ramminose. All *Pseudomonas* could metabolize maltose and galactose except *P. cepacia*, only *P. putida* could utilize arabinose while *P. cepia* alone metabolised fructose.

Table 1: Biochemical test for bacteria isolates obtained from deteriorating corn cob

											Growth at different temp °C													
Code	**Grain rx**	**Cell morph**	**Catalase**	**Oxidease**	**Casein hyd**	**Gelatin hyd**	**Methyl red**	**Nitrate utilization**	**H_2S**	**Vhoges Prockauer**	**15°**	**45°**	**6o°**	**3°**	**Coagulase**	**Urrease**	**Growth at pH 3.9**	**Growth at pH 9.2**	**NH_3**	**Starch**	**Growth in 4% Nacl**	**Citrate utilization**	**Motility**	**Indole Test**	**Probable Identity**
BA	-	R	+	+	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	-	*Pseudomonas putida*
BB	-	R	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+	-	*Pseudomonas aeruginosa*
BC	+	R	+	+	+	+	+	+	+	+	+	+	-	-	-	-	(+)	+	+	+	+	+	+	-	*Bacillus cereus*
BD	+	R	+	+	+	+	+	+	+	+	+	+	-	-	-	-	(+)	+	+	+	+	+	+	-	*Bacillus cereus*
BE	+	R	+	+	+	+	+	+	+	+	+	+	-	-	-	-	(+)	+	+	+	+	-	+	-	*Bacillus cereus*
BF	-	R	+	+	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	-	*Bacillus cereus*
BG	+	R	+	+	+	+	-	+	-	-	+	+	-	-	(-)	-	+	+	+	+	+	-	+	-	*Bacillus licheniformis*
BH	-	R	+	+	-	+	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	-	*Pseudomonas putida*
BI	-	R	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+	-	*Pseudomonas aeruginosa*
BJ	-	R	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+	-	*Pseudomonas aeruginosa*
BK	-	R	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	(+)	-	-	+	+	+	-	*Proteus mirabilis*
BL	+	R	+	+	+	+	-	-	(+)	+	+	+	-	-	-	-	-	+	+	+	+	+	+	-	*Bacillus megaterium*
BM	-	R	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+	-	*Pseudomonas cepacia*
BN	+	R	+	+	+	+	-	-	(+)	-	+	+	-	-	-	-	+	-	+	+	+	-	+	-	*Bacillus megaterium*
BO	+	R	+	+	+	+	+	+	-	-	-	+	-	-	-	-	(+)	(+)	+	+	+	+	+	-	*Bacillus subtilis*
BP	+	R	+	+	+	+	+	+	-	-	-	+	-	-	-	-	(+)	(+)	+	+	+	+	+	-	*Bacillus subtilis*
BQ	+	R	+	+	+	+	-	+	-	-	+	+	-	-	-	-	+	+	+	+	+	-	+	-	*Bacillus licheniformis*
BR	+	R	+	+	+	+	-	+	-	-	+	+	-	-	-	-	+	+	+	+	+	-	+	-	*Bacillus licheniformis*
BS	+	R	+	+	+	+	-	+	-	-	+	+	-	-	-	-	+	+	+	+	+	-	+	-	*Bacillus licheniformis*

Table 2: Sugar fermentation test for bacteria isolates obtained from deteriorating corn cob

S/N	Code	Glucose	Fructose	Maltose	Lactose	Sucrose	Galactose	Xylose	Ara*Bacillis*inose	Raffinose	Ramminose	Manitol	Dulc	Pro*Bacillis*a*Bacillis*le Identity
1	BA	+	-	+	+	+G	+	-	-	-	-	+G	+	*Pseudomonas putida*
2	BB	+	-	(+)	=	+	+G	-	+G	-	-	(+)	(+)	*Pseudomonas aeruginosa*
3	BC	+	+	+G	=	-	+G	-	-	-	+	-	(+)G	*Bacillus cereus*
4	BD	+	+	+G	+	-	+G	-	-	-	+	-	(+)G	*Bacillus cereus*
5	BE	+	+	+G	+	-	+G	-	-	-	+	-	(+)G	*Bacillus cereus*
6	BF	+	-	-	+	+G	+	-	-	-	-	+G	+	*Bacillus cereus*
7	BG	+G	-	+	+G	+G	+	+	+G	+	+	+	-	*Bacillus licheniformis*
8	BH	+	-	+	+	+G	+	-	-	-	-	+G	+	*Pseudomonas putida*
9	BI	+	-	(+)	+	+	+G	-	+G	-	-	(+)	(+)	*Pseudomonas aeruginosa*
1o	BJ	+	-	-	+	+	+G	-	+G	-	-	(+)	(+)	*Pseudomonas aeruginosa*
11	BK	+G	+	-	=	(+)G	+G	+G	-	-	-	-	-	*Proteus mirabilis*
12	BL	+G	+	+	+G	+	(+)G	+	+	-	-	+	+	*Bacillus megaterium*
13	BM	+	+G	-	+G	+	-	+	+G	-	-	(+)G	(+)	*Pseudomonas cepacia*
14	BN	+G	-	+G	+	+	(+)	-	-	(+)	-	-	-	*Bacillus megaterium*
15	BO	+G	-	-	+	+G	+	-	-	+	+	+G	-	*Bacillus subtilis*
16	BP	+G	-	-	+	+G	+	-	-	+	+	+G	-	*Bacillus subtilis*
17	BQ	+G	-	+	+G	+G	+	+	+G	+	+	+	-	*Bacillus licheniformis*
18	BR	+G	-	+	+G	+G	+	+	+G	+	+	+	-	*Bacillus licheniformis*
19	BS	+G	-	+	+G	+G	+	+	+G	-	+	+	-	*Bacillus licheniformis*

3.2 Morphological, growth characteristics and identification of fungal isolates

The fungal isolates were identified using their surface growth characteristics on laboratory media and morphological characteristics as shown in Table 3. The microscopic examination revealed the characteristics features of the fungi. There were six *Aspergillus* species; 4 *Penicillium* species, 1 each species of *Rhizopus*, *Mucor*, *Botryotrichum* and *Geotrichum*.

All the *Aspergillus* species showed roughened or warty conidia at maturity, the plates reverse were cream coloured except in *Aspergillus niger* which was brown. The *Penicillium* species had roughened globose to subglobose conidia and there were presence of ellipsoidal penicilli which exhibited slow growth rate except *Penicillium expansum*

Rhizopus sp possessed hyaline stolon and sporangiophore arising from the stolon with black sporangia. *Mucor racemosus* had intact conidia head with non septate hyphae *Botryotrichum piluliferum* showed a fast growing rate with light brown powdery mycelicim with white hyphae. Geotrichum candidum were fast growing, with white butyrous or membranous colonies with advancing hyphae dichotomously branched.

Table 3: Morphological, growth characteristics and identification of fungal isolates obtained from degrading corn cob

Isolate code	Surface growth characteristics on la*Bacillis*oratory media	Description of the characteristic features of fungus	Growth rate	Reverse coloured or uncoloured	Identification
FA	White to grayish brown colonies about 1cm high with tendency to collapse black spores	Colonies were raised, about 1-2cm high. Stolons hyaline. Sporangiophore arising directly form stolon or aerial hyphae sporangia black	Fast	Cream	*Rhizopus sp*
FB	Dirty green colonies	Colonies were raised, conidiophore with large globose vesicles. The metulae are inflated and club-shaped and were borne on the coidiophore	Fast	Cream	*Aspergillus flavus*
FC	White fluffy cotton-wool like colonies	Colonies were raised, intact conidia heads, non-septate hyphae	Fast	Cream	*Mucor racemosus*
FD	Dark brown or chocolate brown colonies	Intact conidia heads, conidiophore arising from thick walled conidia conidiophore hyaline to brown mostly smooth walled conidia at maturity globose irregularly roughened with conspicous ridges	Fast	Brown	*Aspergillus niger*
FE	Dirty/army green mycelium	Conidiophore arising from long broad, thick walled, sometimes branched foot cell.	Fast	Cream	*Aspergilus flavus*
FF	Grey colonies	Brownish grey with much aerial mycellium and rather few conidiophore, reverse yellow conidiophore arising from creeping aerial hyphae, short, mostly monoverticillate, bearing few small phialides into the conidia bearing necks. Conidia in short chains, globoses conspiously roughened	Slow	Yellow	*Penicililum restrictum*

Morphological, growth characteristics and identification of fungal isolates obtained from degrading corn cob

FG	Grey colonies with white surrounding raised colonies	Densely branched conidiophores straight elevated to ellipsoida Branches arsing fairly low on the conidiophore, conidia globose, conspicuously roghened.	Slow	Cream	*Penicilium atrovenetum*
FH	Dirty green mycellum	As in FB and FE Conidiophore warty, rarely smooth conida slightly roughened or finely echinulate	Fast	Cream	*Aspergillus flavus*
FI	Yellow green mycelium old colonies brown	Colonies reaching 5-6cm diameter in 1o days. Conidiophore warty conidia slightly roughened or finely echcnulate. Uniseriate conidia head	Fast	Cream pink	*Aspergillus oryzae*
FJ	Grayish-green mycelium	Colonies fast growing, reaching 4-5 conidia after 1o days. Conidia subglobose or ellipsoida penicilii-two-three stage branched with numerous usually some what-appressed metulae	Fast		*Penicilium expansum*
FK	Light brown powdery mycelium white hyphae	Colonies spreading broadly with abundant aerial mycelium late become bluff from production of rough walled sterile sepae	Fast	Light brown	*Botryotrichum piluliferum*
FL	Brownish green mycelium	Large globose conidiophore arising from long broad thickened foot cell	Fast		*Aspergillus oryzae*
FM	White fluffy mycelium	Fast growing white colonies, white butyrous or membraneus advancing hyphae dichotomously branched. Conidia chain mostly aerial, and erect.	Fast		*Geotrichum candidum*
FN	Bluish green mycelium	Coniodia globose to elipsoid smooth walled. Colonies blue green or grey green, vellvety reverse intensely yellow.	Slow	Yello	*Penicillum chrysogenum*

3.3 Preliminary screening of bacteria and fungal isolates for cellulase production in liquid medium

The Bacteria and fungi isolates were screened for production of cellulase. The Bacteria isolates had their highest reducing sugar values on day 7 (Fig. 1). The highest amount of reducing sugar produced was by *Bacillus subtilis* 1 (BO) with a value of 0.7 mg/ml on day 7 followed by *Pseudomonas putida I* with the value of 0.2 while the lowest was obtained in *Pseudomonas aeruginosa* with a value of 0.02 mg / ml on day 7.

Figure 2 shows the result of the cellulase assay of the fungal isolates with days of incubation. The highest cellulase activity for the fungal isolate was obtained on day 14. The highest amount of reducing sugar produced was from *Aspergillus niger* with a value of 1.7mg/ml while the lowest value was found in *Mucor racemosus* with a value of 0.7mg/ml., *Penicillium expansum*, 1.6mg/ml; *Botryotrichum* sp, 1.4 mg/ml; *P. atrovenetum*, 1.4 and *A. flavus* 1.4mg/ml.

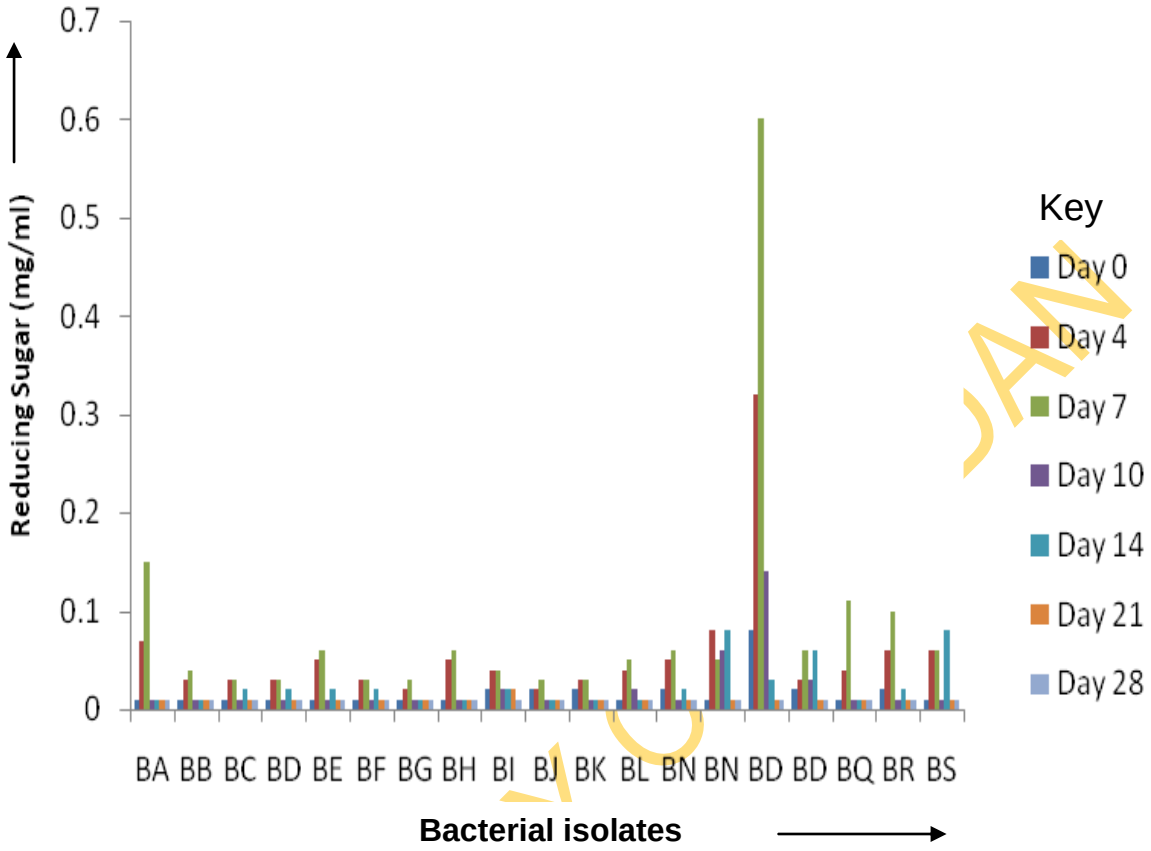


Fig. 1: Reducing sugar (mg/ml) produced by bacterial isolates

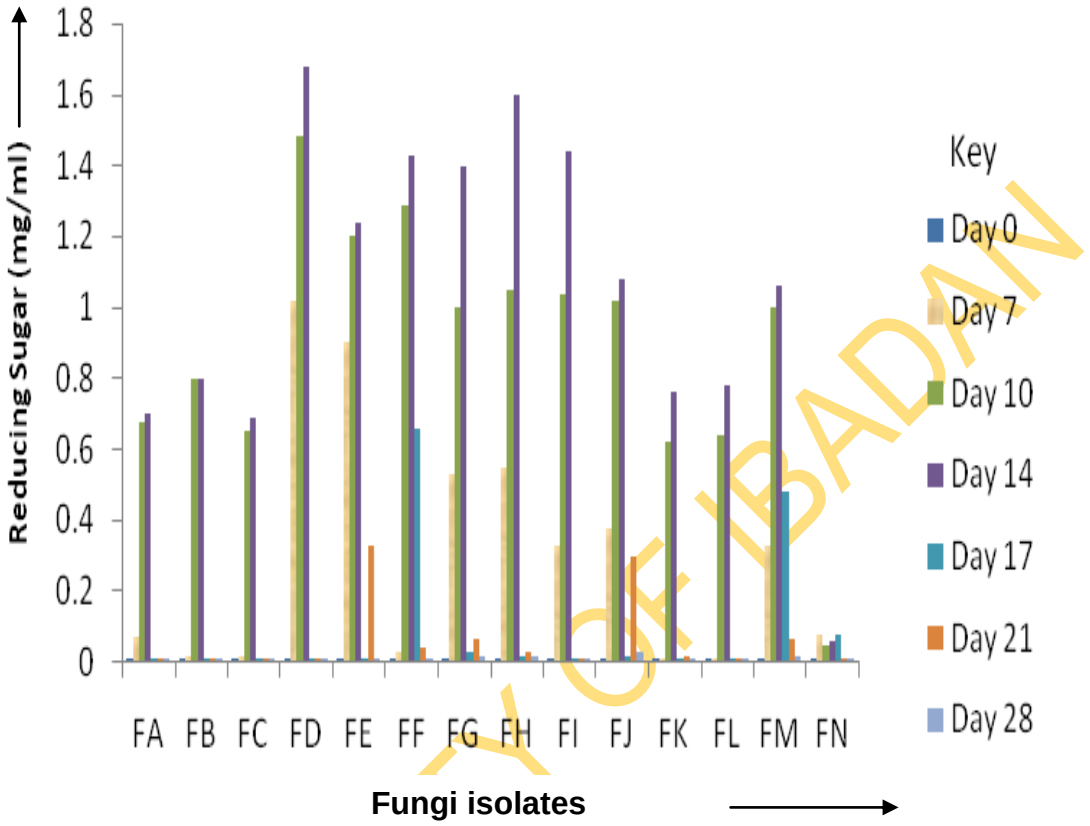


Fig. 2: Reducing sugar (mg/ml) produced fungal isolates (mg/ml)

3.4 Effect of physiological conditions on the cellulase activity of the bacterial isolates

The production of cellulase under different physiological conditions by *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus licheniformis* I and *Bacillus licheniformis* II in liquid medium was studied. Effect of temperature on cellulase production by the bacteria isolates is shown in Fig 3. Cellulase activity was observed highest for *Bacillus subtilis* at 28^{o}C with a value of 0.5 mg/ml while *Bacillus licheniformis* I and B. *licheniformis* II had the cellulase value of 0.5 mg/ml and 0.4mg/ml. *Bacillus subtilis* also has the highest cellulase production of 0.4mg/ml on day 4 at 40^{o}C.

The effect of pH on cellulase production by the Bacteria isolates was observed to be significantly different. Cellulase production was observed to be highest at pH 7.0 on day 7 in *Bacillus subtilis* with a value of 0.5 mg/ml. Other Bacteria species (Bacillus lichenformis II, 0.4 mg/ml; *Pseudomonas putida* 0.5 mg/ml) also showed highest cellulose production at pH 7.0 except *Bacillus licheniformis* I whose highest production of 0.4mg/ml was found in pH 5.5. The lowest production level of cellulase was found in *Bacillus subtilis* at pH 4.0 on day 0 with a value of 0.02 mg/ml. (Fig. 4).

Peptone was observed to induce the highest cellulase activity with values of reducing sugar of 0.8 mg/ml on day 4 in *Bacillus licheniformis* II. *Bacillus licehniformis* I had its highest value 0.7 mg/ml on day 4 in NH_4Cl while *Bacillus subtilis* had its highest production with value of 0.7 mg/ml in Urea on day 4. The lowest value of 0.02 mg/ml was found in day 0 for *pseudomonas putida* in Ammonium chloride (NH_4Cl) and sodium nitrite ($NaNO_2$) (F`ig. 5a and 5b). *Bacillus subtilis* and *Bacillus licheniformis* I had the highest reducing sugar production in yeast extract, peptone and urea.

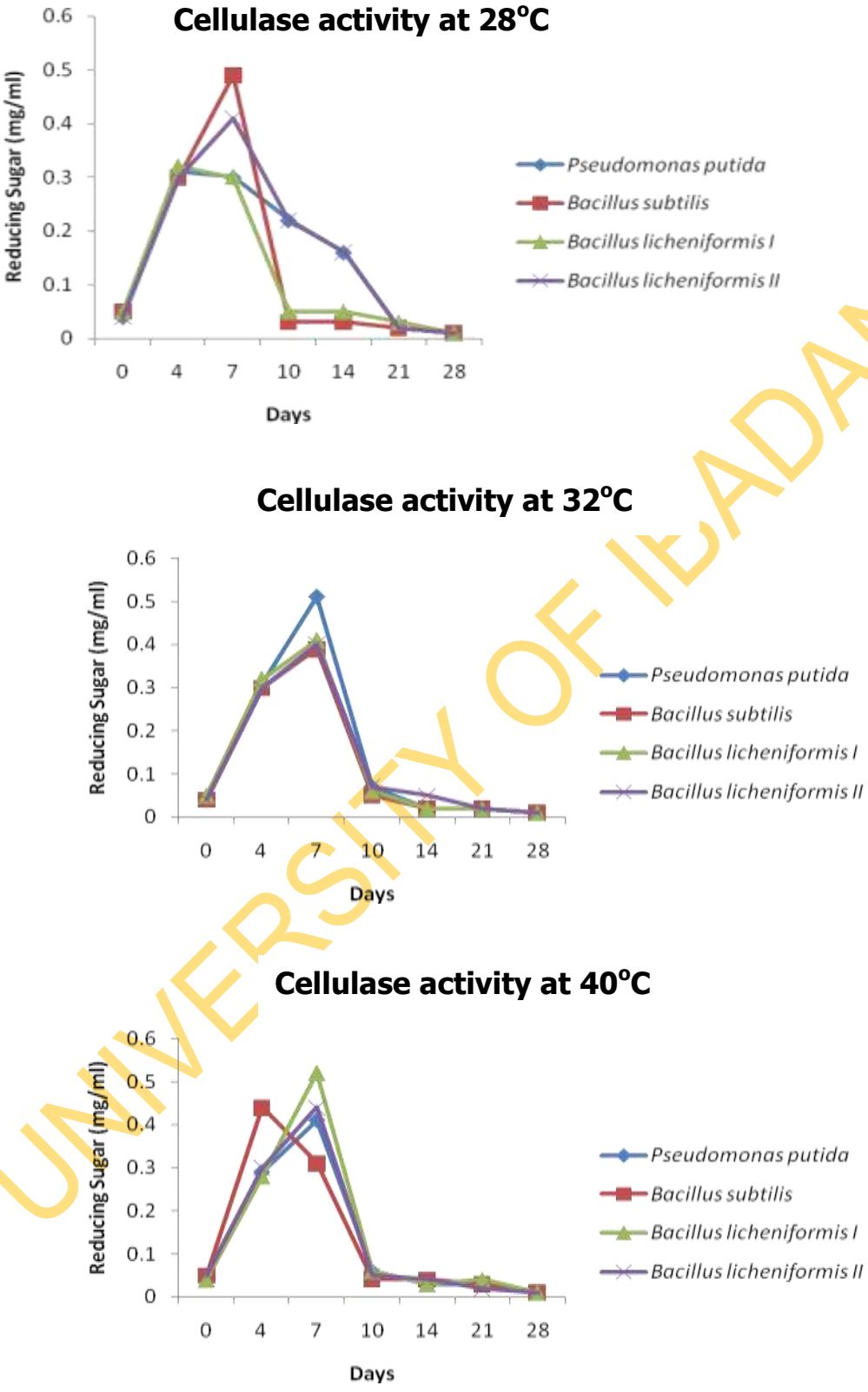


Fig.3: Effect of temperature on cellulase production by bacterial isolates

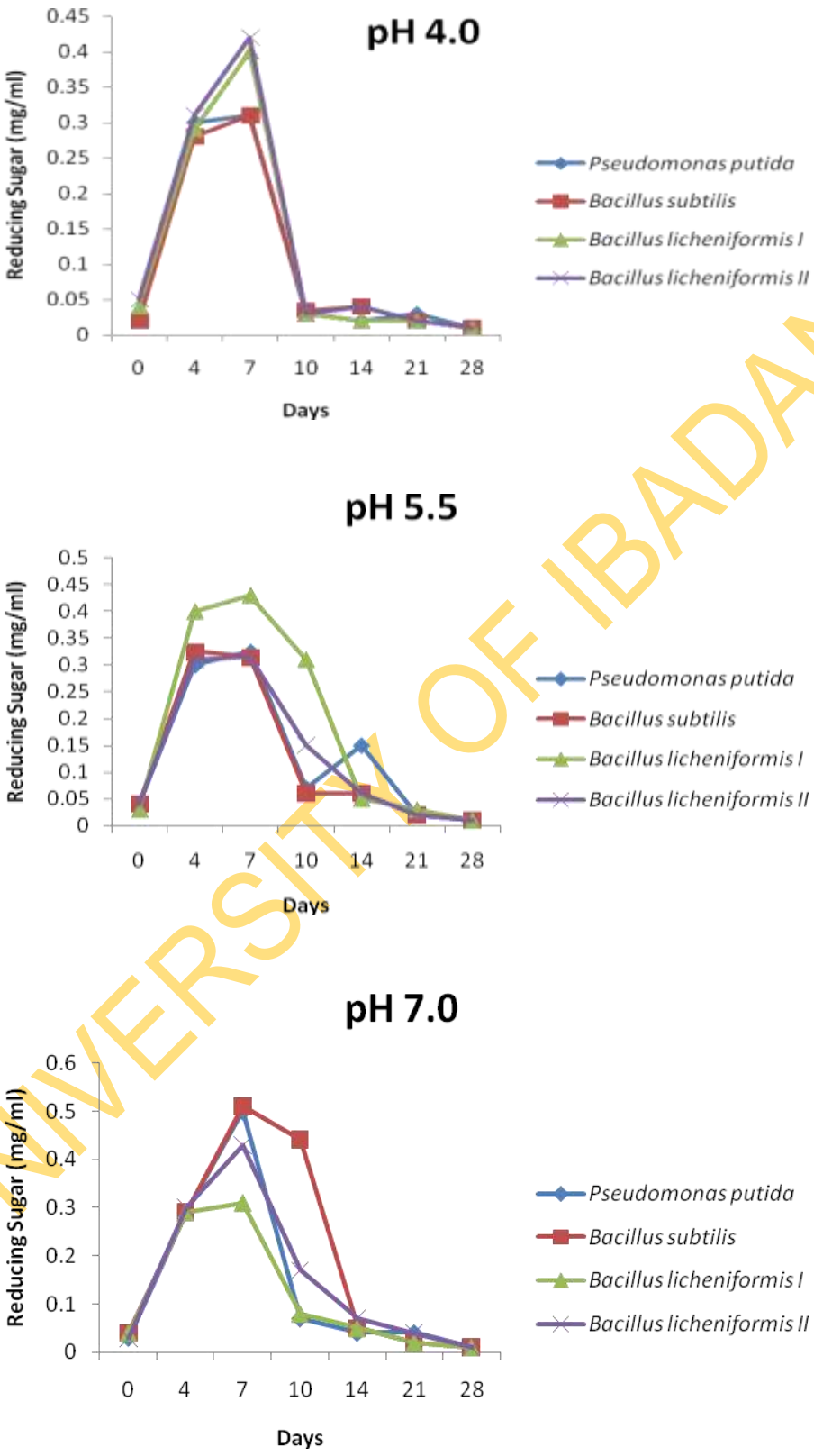


Fig.4: Effect of pH on cellulase production by bacteria isolates

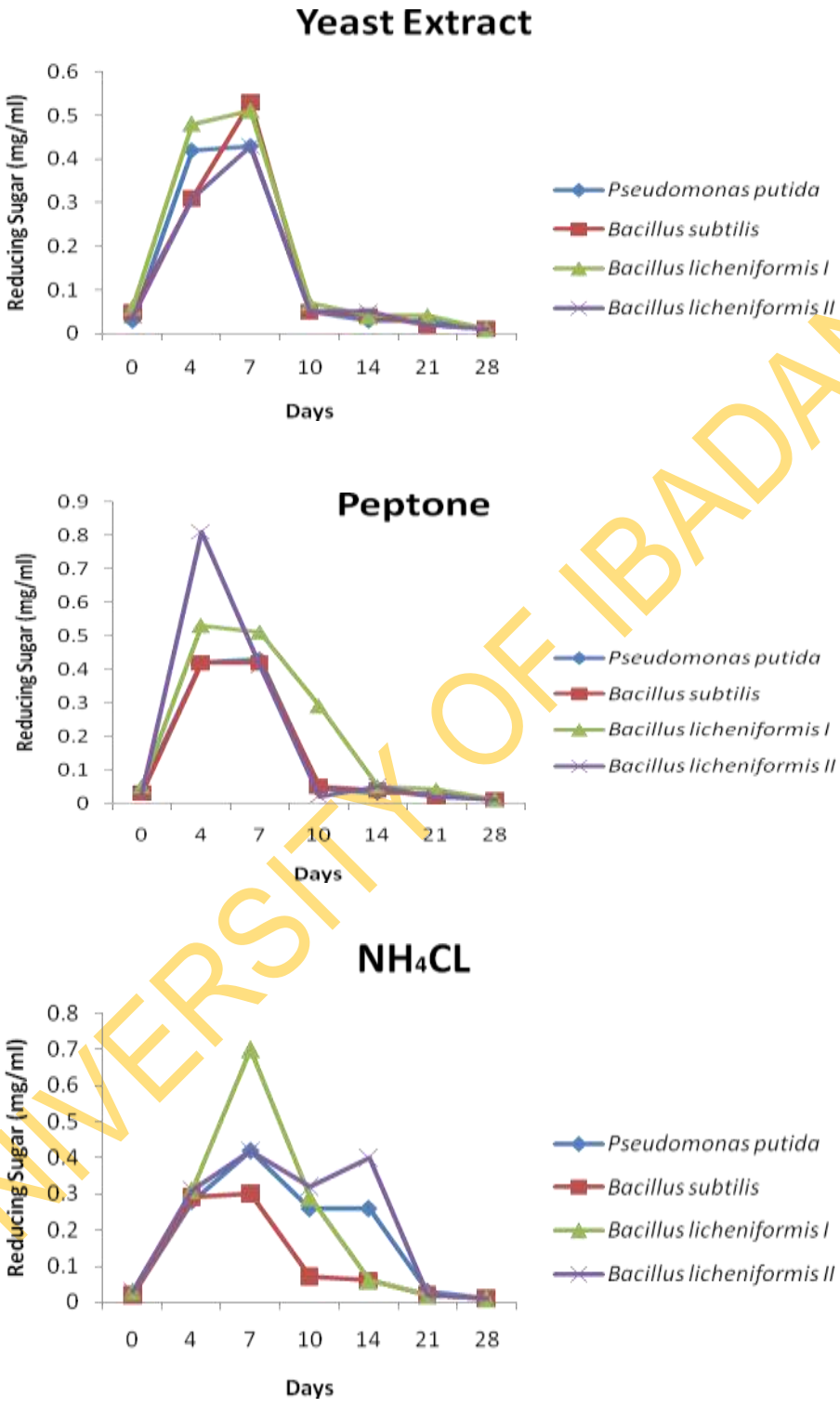


Fig.5a: Effect of different nitrogen sources on cellulase production by bacteria isolates

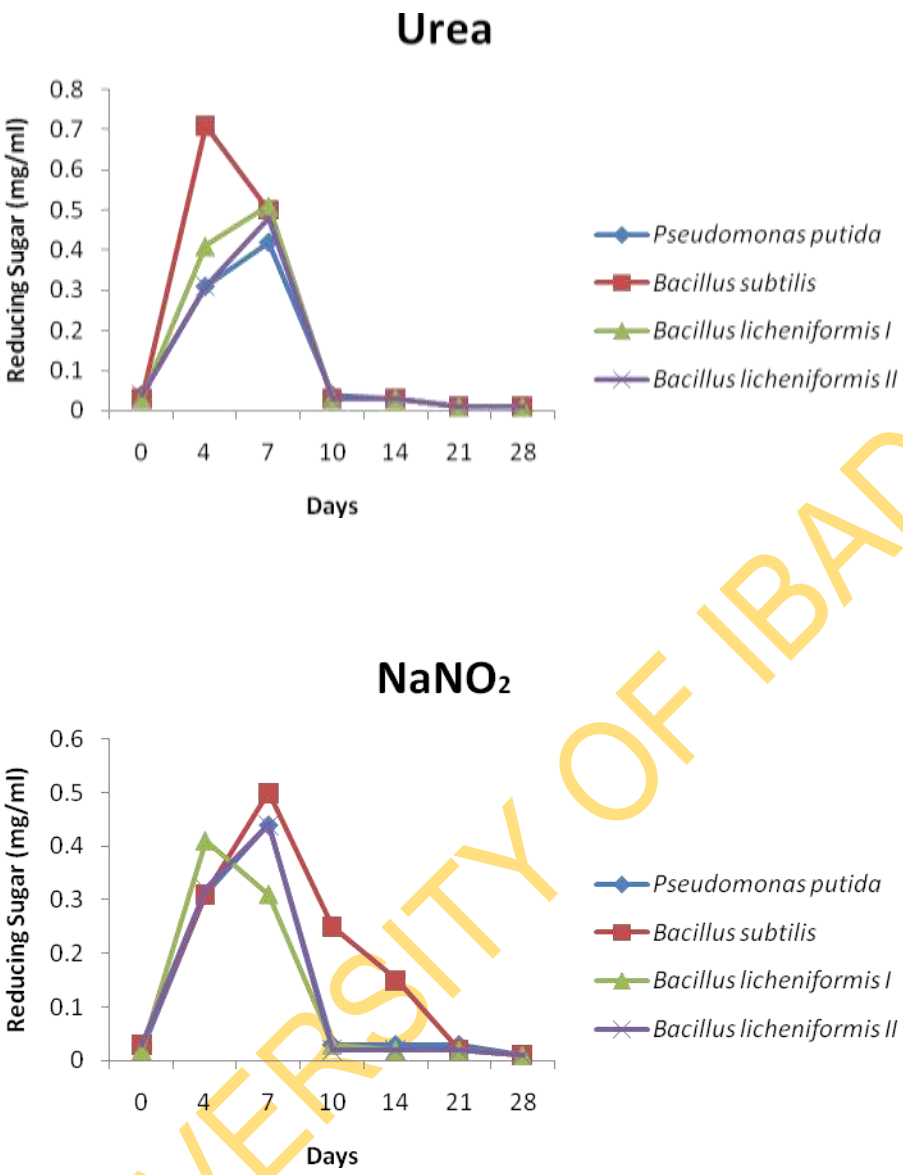


Fig.5b: Effect of different nitrogen sources on cellulase production by bacteria isolates

3.5 Effect of physiological conditions on the cellulase activity of the fungal isolate

Cellulase activity under different physiological conditions by *Rhizopus oryzae, Aspergillus niger, Aspergillus flavus, Penicillium expansum* and *Aspergillus oryzae* in liquid medium was studied. The result of the cellulase activity at different temperature by the fungal isolate is shown in Fig 6. Cellulase activity was observed to be highest at 40^{o}C, with a value of 1.3 mg/ml of reducing sugar in *Aspergillus niger* on day 14 while the lowest value of 0.5mg/ml was observed in *Rhizopus oryzae* at 32^{o}C on day 14. All the fungal isolate had their highest reducing sugar production at 40^{o}C except *Penicillium expansum* that had its highest value of reducing sugar of 1.3mg/ml at 32^{o}C on day 14.

The effect of pH on cellulase production by the isolates was observed to be significantly different. Cellulase production was observed to be highest at pH 4.0 in *Aspergillus niger* with the value of 1.5 mg/ml on day 14 while the lowest was found in *Rhizopus oryzae* with pH 5.5 with a value of 0.5mg/ml on day 14. All the isolate had their optimum cellulase activity at pH 4.0 on day 14 except *Penicillium expansum* that had its highest value of 1.2 mg/ml at pH 5.5 (Fig. 7). Effect of different Nitrogen sources on the cellulase activity of the fungal isolate were also examined. The highest cellulase activity was observed in *Aspergillus flavus* with value of 1.5mg/ml in sodium nitrite ($NaNO_2$) on day 14 while the lowest was found in *Rhizopus oryzae* with a value of 0.7mg/ml in both Urea and peptone on day 14. *Aspergillus niger* has its highest value of 1.3 mg/ml in Ammonium chloride (NH_4Cl) on day 14 while *Aspergillus oryzae* had its highest value of 1.3 mg/ml in Peptone on day 14 while the highest value of 1.2mg/ml was recorded for *Penicillium expansum* in NH_4Cl on day 14. (Fig 8a and 8b).

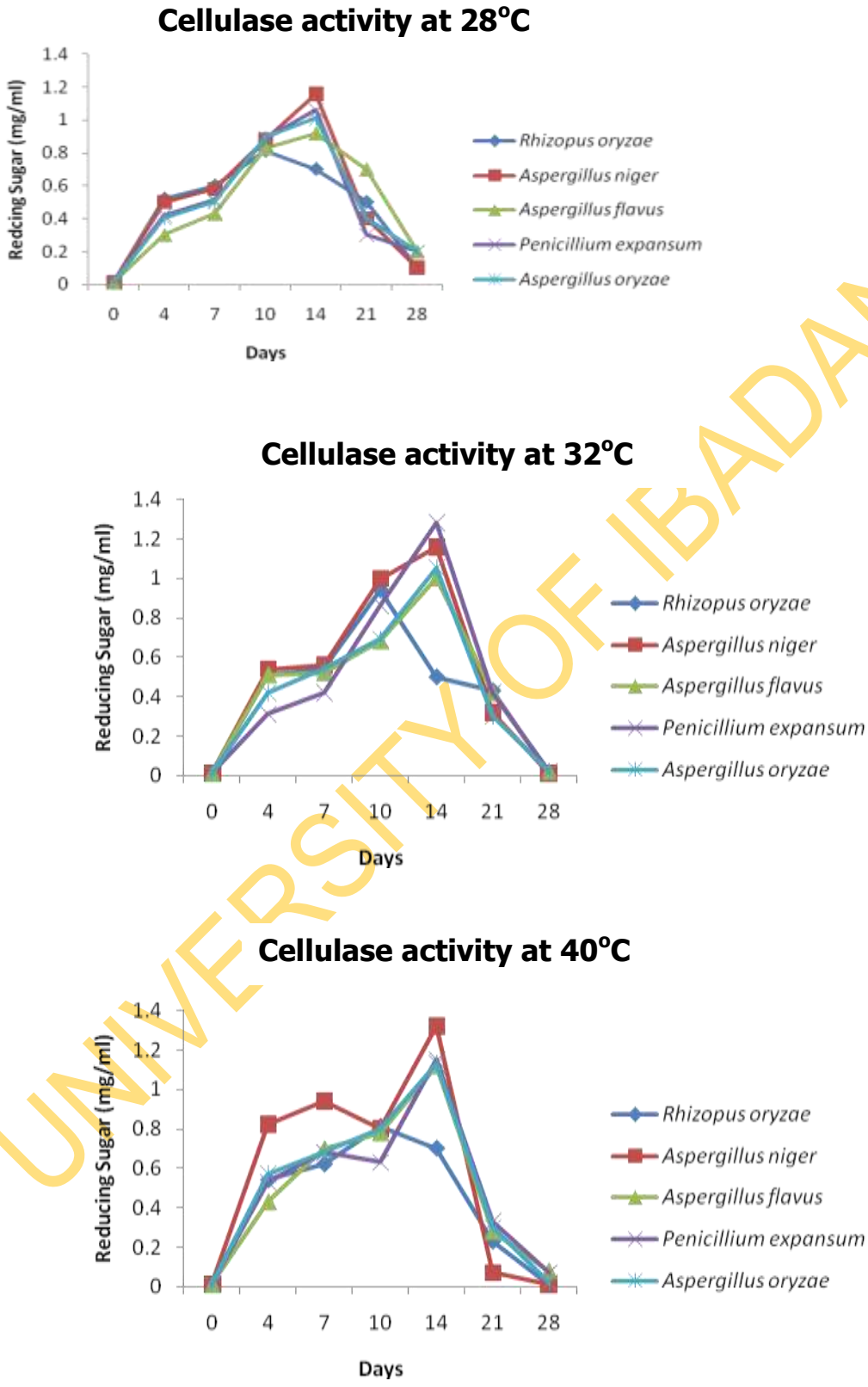


Fig.6: Effect of temperature on the cellulase production by fungal isolates

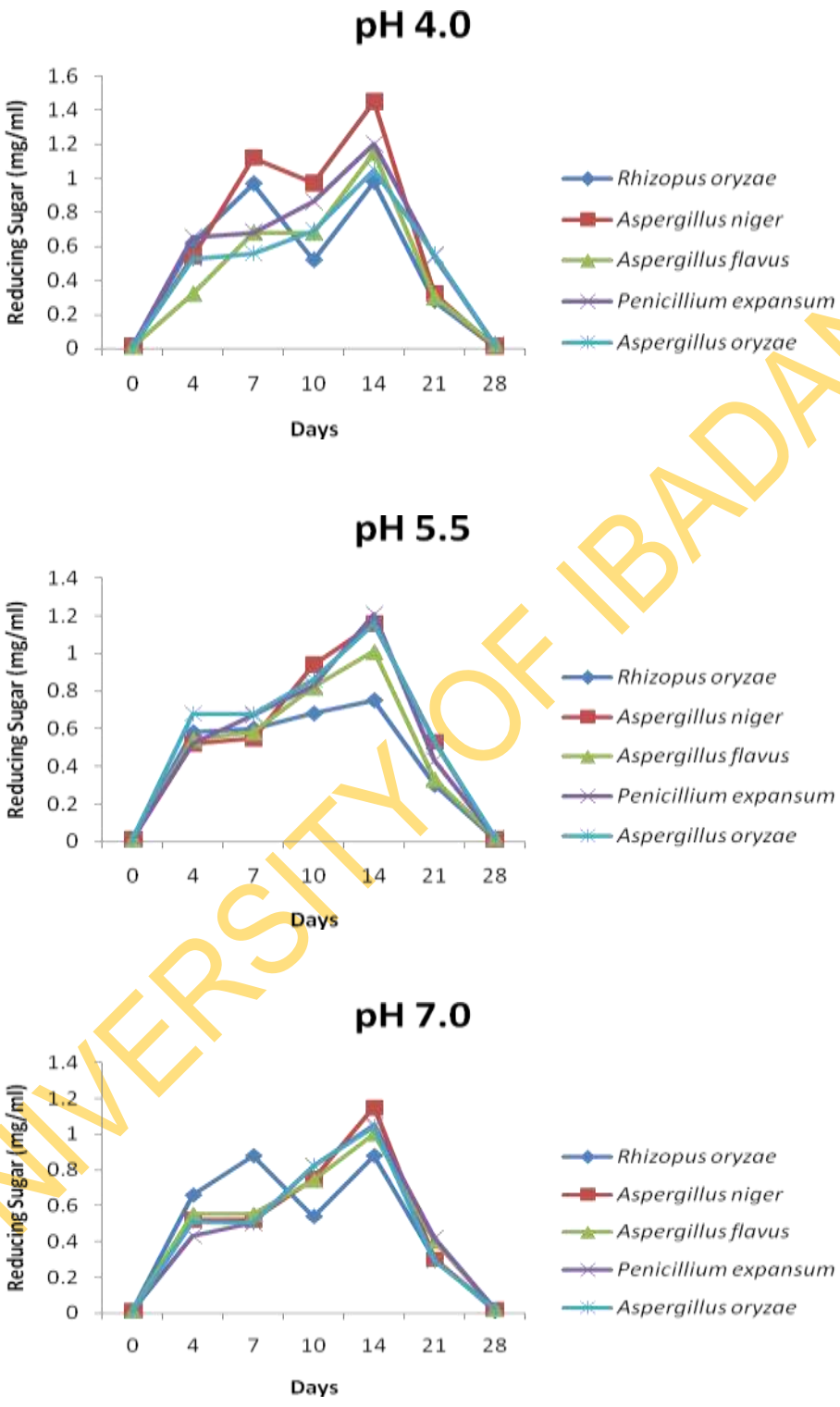


Fig.7: Effect of pH on the cellulase production by fungal isolates

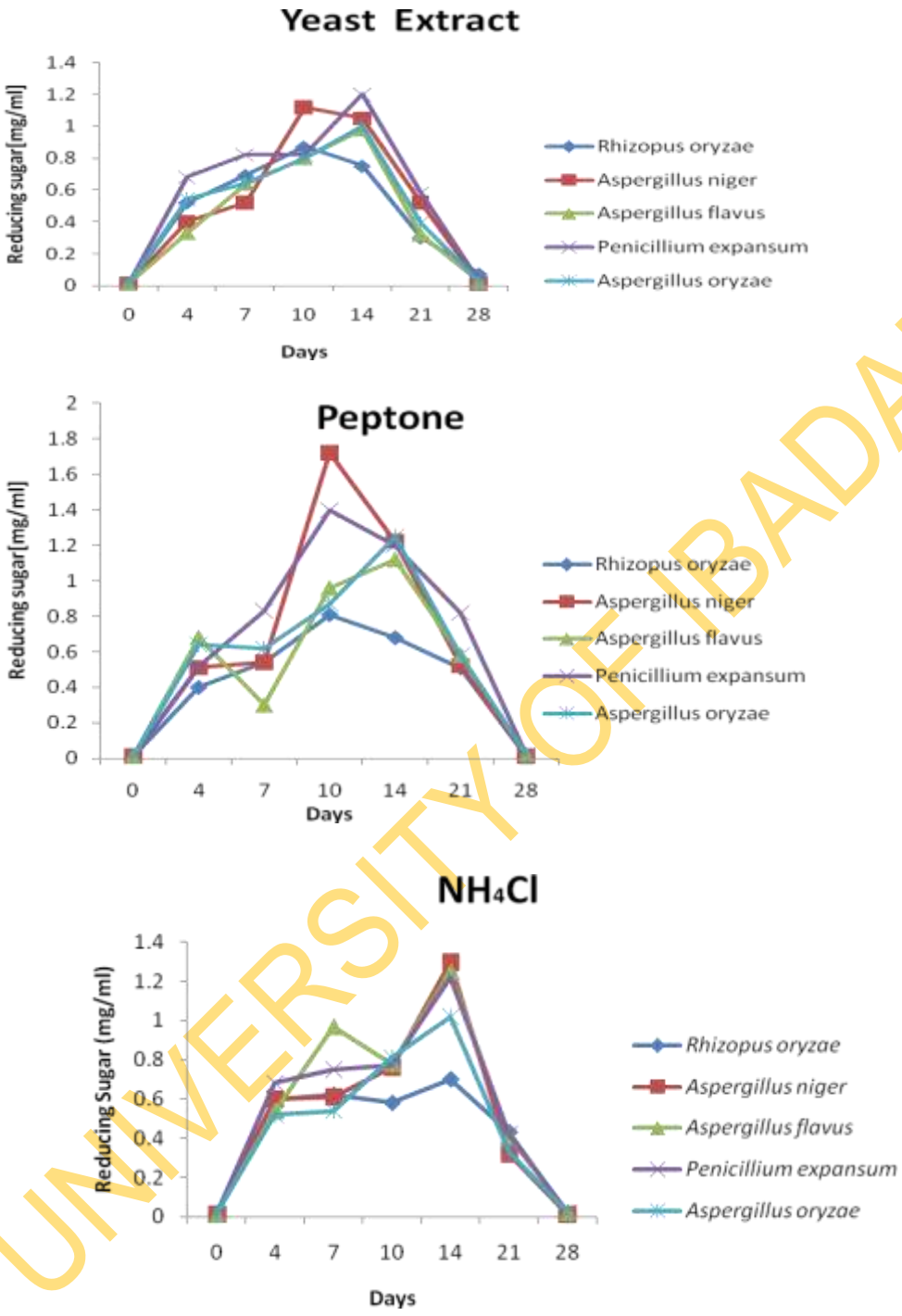
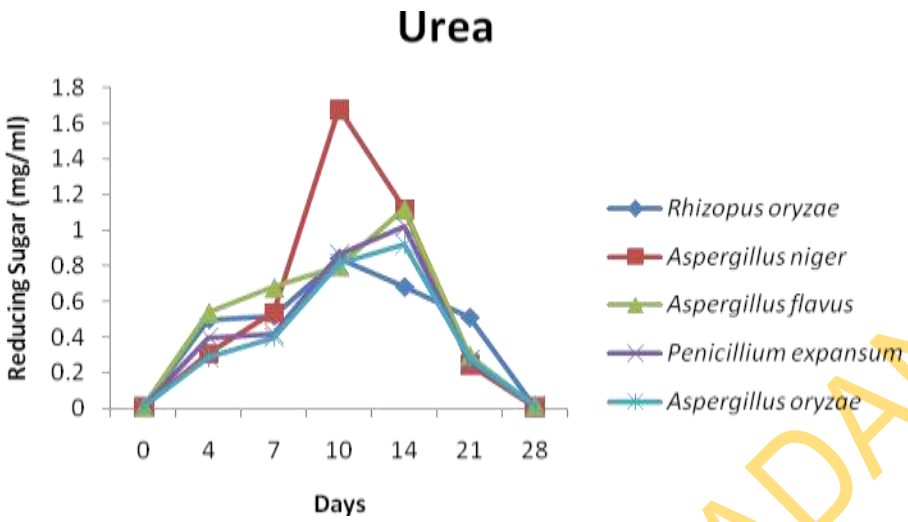


Fig.8a: **Effect of different nitrogen sources on cellulase production by fungal isolates**



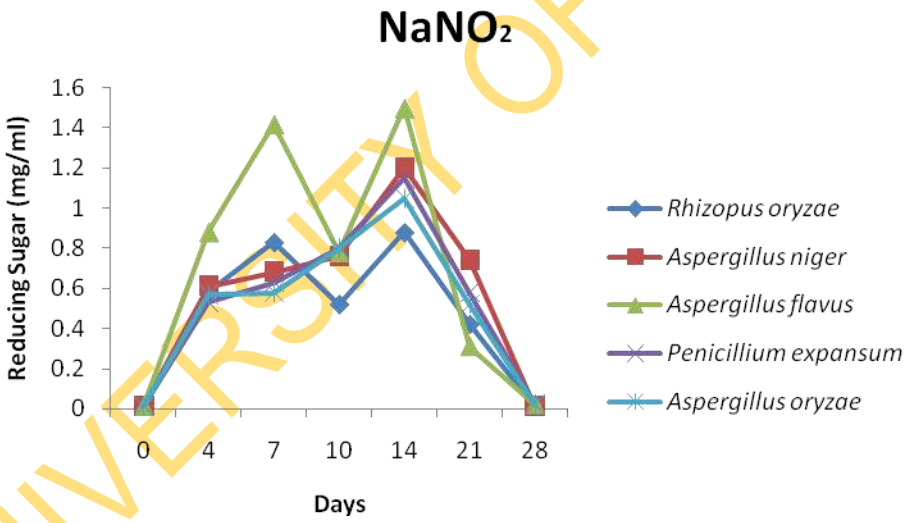


Fig.8b: Effect of different nitrogen sources on cellulase production by fungi isolates

3.6 Effect of bacteria fermentation, nutrient supplementation and alkali pretreatment on substrate's chemical composition

The effect of nutrient supplementation and alkali pretreatment on the Chemical composition of maize cob fermented with *Bacillus subtilis* and *Bacillus licheniformis* was studied as shown in Tables 4 - 10.

There were significant increase in the crude protein throughout the period of fermentation. The crude protein values were higher at day 35 in untreated maize cob in all the treatments except in Table 8 where it was higher in ash pretreated maize cob inoculated with *Bacilus subtilis* with a value of 7.9% compared with 7.7% in untreated and 6.9% in ash pretreated maize cob. For example in Table 4, crude protein on day 35 for the untreated maizecob was 8.7% compared to 7.6% in NaOH pretreated and 7.7% in ash pretreated maize cob. The lowest percentages of crude protein were found in NaOH pretreated maize cob throughout the Tables. The highest crude protein value of 9.5% was found in Table 7 where untreated maize cob was supplemented with chicken extract and inoculated with *Bacillus licheniformis* followed by Table 5 with a value of 9.1% where untreated maize cob was supplemented with chicken extract and inoculated with *Bacillus subtilis*.

There were lower initial crude protein levels in NaOH pretreated (2.6%) and ash pretreated (3.2%) compared to 4.3% in untreated corn cob (Table 5). However, there was increase in the protein content though the period of incubation with the highest value on day 35 found in untreated corn cob (9.1%), while the NaOH pretreated and Ash pretreated value are 7.6% and 7.5% respectively.

The highest percentage of cellulose degraded was found in Table 5 i.e. 6.6% where untreated maize cob was supplemented with chicken extract and inoculated with *Bacillus subtilis.* The initial cellulose content in the NaOH pretreated (34.0%) and ash pretreated (34.6%) was lower than the untreated corn cob (35.1%) in all the treatment as seen in Table 6. However, the percentage cellulose degraded was higher in untreated than in NaOH (0.8) and ash pretreated maize cob (2.5%).

The percentage protein and cellulose degraded was higher in chicken extract supplemented maize cob than in peptone supplemented maize cob. For example the highest crude protein percentage of 8.7 in untreated maize cob supplemented with

peptone and inoculated with *B subtilis* (Table 4) was lower than 9.1 in chicken extract supplemented untreated cob in Table 5 while the cellulose degraded in untreated corn cob supplemented with chicken extract and inoculated with *B licheniformis* (4.8%) (Table 7) was greater than peptone supplement version of 2.8% in Table 6.

Percentage crude protein was lower in untreated maize cob inoculated with *B subtilis* 7.7% (Table 8) and *B lichenformis* 8.0% (Table 9) with no supplementation compared to the supplemented one (9.5%) in Table 6. The lowest crude protein percentage was found in NaOH pretreated, unsuplemented, uninoculated maize cob with a value of 6.2% (Table 10).

There was significant decrease in the dry matter, ether extract, and crude fiber while the ash content increased throughout the period of fermentation.

Ash content of untreated maize cob increased from 2.8 to 3.5% for NaOH pretreated 2.7 to 3.6% and ash pretreated 2.6 to 3.5% while other extract decreases from 3.1 and 2.4% untreated, 4.0 to 2.6% in NaOH pretreated and 3.4 to 2.4% in ash pretreated.

Table 4: Chemical composition (%) of maize cob supplemented with peptone and inoculated with *Bacillus subtilis*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	39.56^{d}	2.73^{f}	3.79^{e}	3.26^{a}	24.44^{c}	36.21^{a}	0.00^{e}	20.47^{b}
	4	39.82^{c}	3.06^{e}	5.17^{d}	3.06^{b}	24.88^{b}	36.17^{b}	0.11^{e}	20.37^{c}
	7	39.95^{b}	3.15^{d}	5.24^{e}	2.90^{c}	24.93^{b}	35.21^{c}	2.76^{d}	20.77^{a}
	14	39.12^{e}	3.34^{c}	5.28^{e}	2.83^{c}	23.82^{d}	34.57^{d}	4.53^{c}	19.65^{d}
	21	39.07^{f}	3.45^{b}	5.22^{ed}	2.64^{d}	23.68^{f}	34.47^{e}	4.81^{b}	19.63^{e}
	28	39.04^{g}	3.61^{a}	5.42^{b}	2.58^{d}	23.51^{g}	34.45^{f}	4.86^{b}	19.55^{f}
	35	39.99^{a}	3.60^{a}	8.70^{a}	2.41^{e}	23.72^{e}	34.35^{g}	5.14^{a}	19.42^{g}
NaOH pretreated maizecob									
	0	39.53^{c}	2.68^{f}	2.63^{g}	3.99^{a}	24.47^{c}	34.50^{a}	0.00^{f}	20.11^{a}
	4	39.72^{b}	2.84^{e}	4.17^{f}	3.84^{b}	24.78^{b}	34.36^{b}	0.41^{e}	20.10^{a}
	7	39.99^{a}	3.18^{d}	4.38^{e}	3.68^{c}	24.86^{a}	34.34^{b}	0.46^{d}	20.08^{a}
	14	39.25^{d}	3.34^{e}	4.49^{d}	3.45^{d}	23.48^{d}	34.30^{b}	0.58^{c}	19.50^{b}
	21	39.08^{e}	3.38^{c}	4.52^{c}	3.23^{e}	23.33^{e}	34.08^{c}	1.22^{b}	19.20^{c}
	28	39.02^{f}	3.54^{b}	4.62^{b}	2.95^{f}	23.15^{g}	32.94^{d}	4.52^{a}	19.18^{c}
	35	38.97^{g}	3.60^{a}	7.55^{a}	2.55^{g}	23.22^{f}	32.94^{d}	4.52^{a}	19.07^{d}
Ash pretreated maizecob									
	0	38.53^{b}	2.63^{f}	2.94^{g}	3.03^{a}	25.05^{c}	34.69^{a}	0.00^{g}	20.45^{b}
	4	38.02^{c}	2.74^{e}	4.02^{f}	2.96^{b}	25.42^{b}	34.66^{a}	0.09^{f}	20.10^{c}
	7	47.97^{a}	2.90^{d}	4.16^{e}	2.85^{c}	25.52^{a}	34.65^{a}	0.12^{e}	21.02^{a}
	14	37.53^{e}	3.17^{c}	4.27^{d}	2.84^{c}	24.18^{d}	34.00^{a}	1.99^{d}	20.06^{d}
	21	37.41^{f}	3.18^{d}	4.34^{e}	2.62^{e}	23.88^{e}	33.82^{ab}	2.51^{c}	19.88^{e}
	28	37.37^{g}	3.51^{a}	4.50^{b}	2.73^{d}	23.68^{g}	33.76^{ab}	2.68^{b}	19.52^{f}
	35	37.71^{d}	3.33^{b}	7.66^{a}	2.42^{f}	23.73^{f}	33.00^{b}	4.87^{a}	19.35^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 5: Chemical composition (%) of maize cob supplemented with chicken extract and inoculated with *Bacillus subtilis*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	39.62^{c}	2.78^{f}	4.29^{f}	3.13^{a}	25.62^{a}	35.10^{a}	0.00^{c}	20.78^{b}
	4	39.72^{b}	2.96^{e}	5.47^{e}	3.04^{b}	25.02^{c}	34.81^{b}	0.83^{b}	20.96^{a}
	7	39.88^{a}	3.05^{d}	5.50^{d}	2.90^{e}	25.10^{b}	34.41^{c}	1.97^{ab}	20.91^{a}
	14	39.22^{e}	3.25^{c}	5.61^{cd}	2.89^{c}	24.05^{d}	32.85^{d}	6.41^{a}	20.40^{c}
	21	39.18^{f}	3.35^{b}	5.67^{c}	2.74^{d}	23.89^{e}	32.85^{d}	6.41^{a}	20.24^{d}
	28	39.03^{g}	3.51^{a}	5.81^{b}	2.61^{e}	23.71^{g}	32.82^{d}	6.49^{a}	20.16^{e}
	35	39.58^{d}	3.50^{a}	9.05^{a}	2.41^{f}	23.77^{f}	32.80^{d}	6.55^{a}	20.08^{f}
NaOH pretreated maizecob									
	0	39.53^{c}	2.68^{f}	2.63^{g}	3.99^{a}	24.47^{c}	34.50^{a}	0.00^{f}	20.11^{a}
	4	39.72^{b}	2.84^{e}	4.17^{f}	3.84^{b}	24.78^{b}	34.36^{b}	0.41^{e}	20.10^{a}
	7	39.99^{a}	3.18^{d}	4.38^{e}	3.68^{c}	24.86^{a}	34.34^{b}	0.46^{d}	20.08^{a}
	14	39.25^{d}	3.34^{e}	4.49^{d}	3.45^{d}	23.48^{d}	34.30^{b}	0.58^{c}	19.50^{b}
	21	39.08^{e}	3.38^{c}	4.52^{c}	3.23^{e}	23.33^{e}	34.08^{c}	1.22^{b}	19.20^{c}
	28	39.02^{f}	3.54^{b}	4.62^{b}	2.95^{f}	23.15^{g}	32.94^{d}	4.52^{a}	19.18^{c}
	35	38.97^{g}	3.60^{a}	7.55^{a}	2.55^{g}	23.22^{f}	32.94^{d}	4.52^{a}	19.07^{d}
Ash pretreated maizecob									
	0	39.72^{c}	2.55^{f}	3.02^{g}	3.41^{a}	24.40^{c}	34.40^{a}	0.00^{e}	20.28^{c}
	4	39.97^{b}	2.71^{e}	4.47^{f}	3.24^{b}	24.68^{b}	34.30^{a}	0.29^{d}	20.38^{b}
	7	40.52^{a}	2.95^{d}	4.57^{e}	3.15^{c}	24.93^{a}	34.24^{a}	0.47^{c}	20.58^{a}
	14	39.56^{d}	3.17^{c}	4.62^{d}	3.17^{d}	23.59^{d}	34.00^{a}	1.16^{b}	19.36^{d}
	21	39.51^{e}	3.20^{b}	4.83^{c}	3.00^{d}	23.42^{e}	33.99^{a}	1.19^{a}	19.16^{e}
	28	39.23^{f}	3.44^{a}	5.17^{b}	2.95^{e}	23.20^{g}	33.99^{a}	1.19^{a}	19.08^{f}
	35	39.18^{g}	3.45^{a}	7.45^{a}	2.41^{f}	23.39^{f}	33.99^{a}	1.19^{a}	18.97^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 6: Chemical composition (%) of maize cob supplemented with peptone and inoculated with *Bacillus licheniformis*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	39.58^{b}	2.68^{f}	3.79^{g}	3.29^{a}	24.28^{c}	35.10^{a}	0.00^{e}	20.66^{b}
	4	39.58^{b}	2.79^{e}	5.69^{f}	3.19^{b}	24.78^{b}	35.00^{a}	0.28^{d}	20.56^{c}
	7	39.67^{a}	3.10^{d}	5.72^{e}	3.05^{c}	24.96^{a}	34.85^{a}	0.71^{c}	21.16^{a}
	14	38.95^{d}	3.29^{e}	5.76^{d}	2.97^{d}	23.44^{d}	34.12^{b}	2.79^{b}	20.33^{d}
	21	38.88^{d}	3.37^{b}	5.79^{c}	2.82^{e}	23.24^{e}	34.10^{b}	2.84^{a}	20.16^{e}
	28	38.78^{e}	3.63^{a}	5.97^{b}	2.79^{e}	23.05^{f}	34.12^{b}	2.79^{b}	20.00^{f}
	35	38.71^{f}	3.58^{a}	7.95^{a}	2.40^{f}	23.15^{g}	34.12^{b}	2.79^{b}	19.88^{g}
NaOH pretreated maizecob									
	0	39.82^{a}	2.65^{e}	2.26^{e}	3.56^{a}	24.25^{c}	34.02^{a}	0.00^{f}	20.42^{b}
	4	39.23^{c}	2.74^{d}	4.07^{d}	3.47^{b}	24.58^{b}	33.94^{b}	0.24^{e}	20.42^{b}
	7	39.01^{f}	2.90^{c}	4.27^{cd}	3.33^{c}	24.78^{a}	33.87^{c}	0.44^{d}	20.93^{a}
	14	39.12^{d}	3.22^{b}	4.39^{cd}	3.22^{d}	23.38^{d}	33.85^{c}	0.50^{c}	19.41^{c}
	21	39.07^{e}	3.23^{b}	4.92^{bc}	3.05^{f}	23.18^{f}	33.85^{c}	0.50^{c}	19.25^{d}
	28	39.38^{b}	3.37^{a}	5.00^{b}	3.11^{e}	23.11^{g}	33.80^{c}	0.65^{b}	19.21^{e}
	35	38.84^{g}	3.35^{a}	7.13^{a}	2.55^{g}	23.28^{e}	33.75^{d}	0.79^{a}	19.18^{f}
Ash pretreated maizecob									
	0	38.78^{a}	2.65^{g}	2.77^{f}	3.64^{g}	25.06^{c}	34.60^{a}	0.00^{e}	20.17^{b}
	4	38.07^{b}	2.84^{f}	3.39^{c}	3.57^{f}	25.17^{b}	34.50^{b}	0.29^{d}	20.07^{c}
	7	37.80^{c}	3.18^{e}	3.01^{e}	3.43^{e}	25.26^{a}	34.31^{c}	0.84^{c}	20.68^{a}
	14	37.32^{d}	3.35^{d}	3.26^{d}	3.37^{d}	24.48^{d}	33.82^{d}	2.25^{b}	19.37^{d}
	21	37.15^{e}	3.40^{c}	3.29^{d}	3.17^{c}	24.15^{e}	33.77^{d}	2.40^{a}	19.26^{e}
	28	37.07^{f}	3.73^{a}	3.84^{b}	3.14^{b}	24.08^{f}	33.76^{d}	2.43^{a}	19.14^{f}
	35	36.98^{g}	3.55^{f}	7.10^{a}	2.55^{a}	23.16^{g}	33.75^{d}	2.46^{a}	19.07^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 7: Chemical composition (%) of maize cob supplemented with chicken extract and inoculated with *Bacillus licheniformis*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	39.78^{c}	2.58^{f}	4.52^{f}	3.36^{a}	24.18^{c}	35.81^{a}	0.00^{g}	20.15^{b}
	4	39.95^{b}	2.71^{e}	5.82^{e}	3.16^{b}	24.56^{b}	35.60^{b}	0.59^{f}	20.05^{c}
	7	39.55^{e}	2.90^{d}	5.96^{d}	3.10^{b}	24.77^{a}	35.30^{c}	1.42^{e}	20.80^{a}
	14	39.05^{f}	3.12^{b}	6.04^{c}	2.99^{c}	23.15^{d}	34.90^{d}	2.54^{d}	19.45^{d}
	21	39.02^{g}	3.15^{b}	6.19^{b}	2.62^{d}	23.02^{e}	34.78^{d}	2.88^{c}	19.26^{e}
	28	39.72^{d}	3.31^{a}	6.19^{b}	2.59^{d}	22.96^{f}	34.13^{e}	4.69^{b}	19.22^{e}
	35	42.34^{a}	3.05^{c}	9.45^{a}	2.44^{e}	23.03^{e}	34.09^{e}	4.80^{a}	19.13^{f}
NaOH pretreated maizecob									
0	39.61^{a}	2.83^{f}	3.28^{f}	3.92^{a}	24.55^{c}	34.83^{a}	0.00^{f}	20.35^{b}	4
7	38.98^{b}	3.10^{d}	4.12^{d}	3.43^{c}	25.08^{a}	34.70^{a}	0.37^{e}	20.94^{a}	
	21	38.05^{e}	3.33^{c}	4.29^{c}	3.25^{d}	24.47^{d}	33.81^{c}	2.93^{c}	19.66^{e}
	28	38.52^{c}	3.55^{a}	4.50^{b}	3.16^{d}	24.27^{f}	33.77^{c}	3.04^{b}	19.57^{f}
	35	38.45^{c}	3.48^{b}	7.40^{a}	2.70^{e}	23.39^{g}	33.57^{d}	3.62^{a}	19.56^{f}
Ash pretreated maizecob									
	0	38.23^{a}	2.60^{f}	3.13^{g}	3.24^{a}	22.44^{c}	34.62^{a}	0.00^{g}	20.22^{b}
	4	37.98^{b}	2.74^{e}	3.67^{e}	3.14^{b}	22.58^{b}	34.43^{b}	0.55^{f}	20.12^{c}
	7	37.82^{c}	3.20^{d}	3.29^{f}	3.10^{b}	22.77^{a}	34.20^{c}	1.21^{e}	20.76^{a}
	14	37.25^{d}	3.43^{c}	3.94^{c}	3.07^{b}	21.59^{d}	33.71^{d}	2.63^{d}	19.56^{d}
	21	37.07^{e}	3.47^{c}	3.90^{d}	2.90^{c}	21.26^{e}	33.70^{d}	2.65^{c}	19.45^{e}
	28	36.88^{f}	3.92^{a}	4.22^{b}	2.97^{c}	21.10^{f}	33.69^{d}	2.69^{b}	19.37^{f}
	35	36.81^{g}	3.63^{e}	6.65^{a}	2.42^{d}	20.16^{g}	33.44^{e}	3.41^{a}	19.22^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 8: Chemical composition (%) of maize cob inoculated with *Bacillus subtilis*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	39.67^{e}	2.76^{e}	3.28^{g}	3.67^{a}	24.22^{c}	35.62^{a}	0.00^{g}	20.15^{b}
	4	39.89^{c}	2.81^{d}	4.72^{f}	3.47^{b}	24.86^{b}	35.46^{b}	0.45^{f}	20.05^{c}
	7	40.12^{b}	3.00^{bc}	4.89^{e}	2.15^{g}	24.98^{a}	35.36^{c}	0.73^{e}	20.86^{a}
	14	39.75^{d}	3.02^{bc}	5.29^{d}	3.06^{c}	23.77^{d}	34.99^{d}	1.77^{d}	19.17^{d}
	21	39.80^{c}	3.05^{b}	5.42^{b}	2.92^{d}	23.55^{e}	34.96^{e}	1.85^{c}	19.11^{e}
	28	39.23^{f}	3.13^{a}	5.22^{d}	2.64^{e}	22.82^{f}	34.66^{f}	2.70^{b}	19.05^{f}
	35	41.98^{a}	2.98^{c}	7.70^{b}	2.46^{f}	22.25^{g}	34.56^{g}	2.98^{a}	18.89^{g}
NaOH pretreated maizecob									
	0	39.58^{a}	2.73^{f}	2.70^{f}	3.72^{a}	24.36^{c}	34.69^{a}	0.00^{e}	20.19^{b}
	4	39.23^{b}	2.81^{e}	3.01^{e}	3.59^{b}	24.65^{b}	34.43^{b}	0.75^{d}	20.10^{b}
	7	38.89^{c}	3.00^{d}	4.12^{d}	3.38^{c}	24.94^{a}	34.39^{b}	0.86^{c}	20.67^{a}
	14	38.43^{d}	3.22^{c}	4.19^{ed}	3.27^{d}	23.66^{d}	34.30^{b}	1.12^{b}	19.42^{d}
	21	38.31^{e}	3.23^{c}	4.23^{b}	3.15^{f}	23.36^{e}	34.30^{b}	1.12^{b}	19.49^{e}
	28	38.12^{f}	3.45^{a}	4.32^{a}	3.18^{e}	23.06^{g}	34.10^{v}	1.70^{a}	19.37^{d}
	35	38.07^{g}	3.33^{b}	6.90^{a}	2.83^{g}	23.11^{f}	34.10^{c}	1.70^{a}	18.88^{e}
Ash pretreated maizecob									
	0	38.72^{a}	2.70^{e}	2.86^{f}	3.44^{a}	25.18^{c}	35.07^{a}	0.00^{e}	20.28^{a}
	4	38.22^{b}	2.79^{e}	4.68^{e}	3.34^{b}	25.36^{b}	34.87^{b}	0.57^{d}	20.15^{b}
	7	38.03^{c}	3.10^{d}	4.88^{d}	3.15^{c}	25.86^{a}	34.60^{c}	0.77^{e}	20.11^{c}
	14	37.86^{d}	3.30^{c}	4.88^{d}	3.14^{c}	24.55^{d}	34.44^{d}	1.89^{b}	20.01^{d}
	21	37.53^{e}	3.38^{c}	4.96^{c}	2.95^{d}	24.37^{e}	33.35^{e}	4.90^{a}	19.95^{e}
	28	37.23^{f}	3.73^{a}	5.28^{b}	2.86^{e}	24.24^{f}	33.34^{e}	4.93^{a}	19.82^{f}
	35	37.18^{g}	3.55^{b}	7.95^{a}	2.43^{f}	23.35^{g}	33.34^{e}	4.93^{a}	19.55^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 9: Chemical composition (%) of maize cob inoculated with *Bacillus licheniformis*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	39.52^{e}	2.81^{f}	3.04^{f}	3.16^{a}	24.35^{b}	35.11^{a}	0.00^{g}	20.55^{b}
	4	39.95^{c}	2.94^{e}	4.32^{e}	3.06^{b}	24.96^{a}	35.01^{b}	0.28^{f}	20.67^{a}
	7	40.05^{b}	3.10^{d}	4.51^{d}	3.05^{bc}	25.02^{a}	34.92^{c}	0.54^{e}	20.17^{d}
	14	39.56^{d}	3.25^{c}	4.52^{d}	3.04^{c}	24.00^{c}	34.23^{d}	2.51^{d}	20.21^{c}
	21	39.52^{e}	3.33^{b}	4.59^{c}	2.69^{d}	23.93^{d}	34.12^{e}	2.82^{b}	20.14^{e}
	28	39.34^{f}	3.49^{a}	4.65^{b}	2.64^{e}	23.66^{e}	34.13^{e}	2.79^{c}	20.05^{f}
	35	40.67^{a}	3.43^{a}	7.98^{a}	2.44^{f}	23.92^{d}	33.12^{f}	5.67^{a}	19.94^{g}
NaOH pretreated maizecob									
	0	38.72^{a}	2.58^{f}	2.63^{g}	3.49^{a}	24.88^{a}	35.60^{a}	0.00^{d}	20.18^{c}
	4	38.65^{b}	2.66^{e}	3.42^{f}	3.34^{b}	24.14^{c}	35.10^{b}	1.40^{c}	20.35^{b}
	7	38.23^{c}	2.90^{d}	3.63^{e}	3.18^{c}	24.42^{b}	35.10^{b}	1.40^{c}	20.75^{a}
	14	37.92^{d}	3.12^{c}	3.72^{d}	3.09^{d}	23.25^{d}	35.08^{b}	1.46^{c}	19.28^{d}
	21	37.72^{e}	3.13^{c}	4.83^{e}	2.95^{e}	23.18^{e}	34.40^{c}	3.37^{b}	19.18^{e}
	28	37.58^{f}	3.46^{a}	5.09^{b}	2.92^{f}	23.06^{g}	34.04^{d}	4.38^{a}	19.09^{f}
	35	37.52^{g}	3.35^{b}	7.20^{a}	2.55^{g}	23.13^{f}	34.04^{d}	4.38^{a}	18.83^{g}
Ash pretreated maizecob									
	0	37.98^{a}	2.66^{f}	2.44^{g}	3.99^{a}	25.05^{c}	35.49^{a}	0.00^{g}	20.17^{g}
	4	37.65^{b}	2.61^{f}	4.69^{f}	3.82^{b}	25.18^{b}	35.40^{a}	0.25^{f}	20.15^{f}
	7	37.42^{c}	2.78^{e}	4.82^{e}	3.65^{c}	25.55^{a}	35.26^{b}	0.65^{e}	20.09^{e}
	14	37.05^{d}	3.17^{d}	4.88^{d}	3.45^{d}	24.62^{d}	35.02^{c}	1.32^{d}	19.75^{d}
	21	36.92^{e}	3.20^{c}	4.92^{c}	3.18^{f}	24.42^{e}	34.96^{c}	1.49^{c}	19.48^{c}
	28	36.75^{f}	3.69^{a}	5.13^{b}	3.25^{e}	24.23^{g}	34.53^{d}	2.70^{b}	19.34^{b}
	35	36.63^{g}	3.45^{b}	6.98^{a}	2.70^{g}	23.33^{f}	34.00^{e}	4.20^{a}	19.33^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 10: Chemical composition (%) of maize cob soaked in water

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	39.67^{f}	2.65^{f}	3.03^{g}	3.84^{a}	24.39^{c}	35.41^{a}	0.00^{e}	20.18^{d}
	4	39.85^{c}	2.79^{e}	4.66^{f}	3.72^{b}	24.75^{b}	35.36^{a}	0.14^{d}	20.51^{b}
	7	40.32^{b}	3.08^{d}	4.77^{e}	3.43^{c}	24.95^{a}	35.30^{a}	0.31^{c}	20.77^{a}
	14	39.75^{d}	3.27^{c}	4.81^{d}	3.20^{d}	23.66^{d}	34.63^{b}	2.20^{b}	20.45^{c}
	21	39.71^{d}	3.28^{c}	4.86^{c}	3.02^{e}	23.56^{e}	34.61^{b}	2.26^{b}	19.32^{e}
	28	39.58^{g}	3.51^{a}	4.93^{b}	2.87^{f}	23.27^{g}	34.60^{b}	2.29^{b}	19.27^{f}
	35	40.78^{a}	3.33^{b}	8.05^{a}	2.44^{g}	23.36^{f}	34.46^{c}	2.68^{a}	19.22^{g}
NaOH pretreated maizecob									
	0	37.67^{e}	2.68^{e}	2.36^{g}	3.24^{a}	24.95^{c}	34.80^{a}	0.00^{f}	20.11^{b}
	4	37.56^{f}	2.89^{d}	3.56^{f}	3.19^{a}	25.03^{b}	34.52^{b}	0.80^{e}	20.09^{c}
	7	41.12^{a}	3.10^{c}	3.74^{e}	3.05^{b}	25.18^{a}	34.33^{c}	1.35^{d}	20.22^{a}
	14	39.85^{b}	3.27^{c}	3.81^{d}	3.02^{b}	24.25^{d}	34.22^{d}	1.67^{c}	19.54^{d}
	21	39.73^{c}	3.15^{b}	3.91^{e}	2.82^{c}	23.17^{f}	33.93^{e}	2.50^{b}	19.41^{e}
	28	39.60^{d}	3.31^{b}	4.24^{b}	2.67^{d}	24.08^{e}	33.86^{f}	2.70^{a}	19.33^{f}
	35	39.85^{b}	3.38^{a}	6.20^{a}	2.46^{e}	23.17^{f}	33.83^{f}	2.79^{a}	19.16^{g}
Ash pretreated maizecob									
	0	37.85^{b}	2.76^{e}	2.85^{g}	3.24^{a}	25.11^{c}	34.79^{a}	0.00^{f}	20.77^{a}
	4	37.56^{c}	2.91^{d}	4.12^{f}	3.16^{b}	25.22^{b}	34.30^{b}	1.41^{e}	20.51^{b}
	7	37.12^{e}	3.10^{c}	4.23^{e}	3.03^{c}	25.63^{a}	34.30^{b}	1.41^{e}	20.09^{c}
	14	37.03^{f}	3.30^{b}	4.27^{d}	2.99^{c}	24.18^{e}	33.63^{c}	3.33^{d}	20.04^{d}
	21	36.89^{g}	3.43^{b}	4.38^{c}	2.80^{d}	24.06^{f}	33.62^{c}	3.36^{c}	19.66^{e}
	28	37.23^{d}	3.73^{a}	5.28^{b}	2.86^{d}	24.24^{d}	33.35^{d}	4.14^{b}	19.65^{e}
	35	38.58^{a}	3.38^{b}	7.68^{a}	2.55^{e}	23.44^{g}	33.35^{d}	4.41^{a}	19.65^{e}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

3.7 Effect of fungal fermentation, nutrient supplementation and alkali pretreatment on substrate chemical composition

The effect of nutrient supplementation and alkali pretreatment on the Chemical Composition of maize cob fermented with *Aspergillus niger* and *Aspergilus oryzae* was studied as shown in Table 11 – 17.

There was significant increase in the crude protein in all Tables. The highest crude protein value of 7.6 was found in Table 12 where Ash pretreated maize cob was supplemented with chicken extract and inoculated with *Aspergillus niger*. The highest percentage of cellulose degraded (17.3%) was also found in Table 12. This represent the highest value in all the treatment.

A higher crude protein value was observed in NaOH pretreated and ash pretreated maize cob in all Tables except in the Table 16 and control, Table 17 where the untreated is a bit higher for example in Table 11 (maize cob supplemented with peptone and inoculated with *A niger*) the percentage crude protein on day 35 for the untreated was 6.9, NaOH pretreated 7.2 and Ash pretreated 7.5, whereas in Table 16 in maize cob inoculated with *Aspergillus oryzae* the percentage crude protein for untreated was 7.0 compared with 6.6 in NaOH pretreated and 6.2 in ash pretreatred. While in water soaked maize cob, (Table 17) the crude protein values (6.8%) was higher than NaOH pretreated (6.1) ash pretreated (6.3). The initial crude protein value was lower in NaOH pretreated cob than in untreated as seen in Table 11 where untreated was 3.3% and NaOH pretreated was 2.9%.

The initial cellulose content was lower in NaOH pretreated (34.0) and Ash pretreated (35.8) than in untreated maize cob (36.7) (Table 11). However, the percentage cellulose degraded was higher in untreated maize cob (15.6%) on day 35 than NaOH pretreated 11.2% and ash pretreated 12.5% (Table 11). The same trend was observed in Table 13 with maize cob supplemented with peptone and inoculated with *A oryzae* where the percentage cellulose degraded in untreated was 14.6%, NaOH pretreated, 10.4%, ash pretreated, 7.8%. However, percentage cellulose content (Table 12) in untreated corn was lower (14.7) than in ash pretreated (17.3%) while NaOH pretreated was lowest (12.5). Table 14, 15, 16 and 17 also follow the same trend in cellulose percentage as seen in Table 12. The percentage cellulose degraded was also higher when chicken extract

was the supplement than peptone was seen in Table 13 (7.8%) (peptone) Table 14 (12.3 %in Chicken Extract).

The crude fiber value for the untreated was higher in most of the Tables than the NaOH pretreated and ash pretreated. For example in Table 13 where Maize cob was supplemented with peptone and inoculated with *A oryzae* The crude fiber value at day O for the untreated was 25.1, NaOH pretreated, 22.9 and ash pretreated, 24.0.

There was significant decrease in the dry-matter, ash, crude fiber and liguin content of the maize cob as the time course of the fermentation increased, while crude protein and other extract content increased. The ash content decreased throughout the Tables for example in Table 15 content of the untreated decreased from 3.1 to 2.7%, NaOH pretreated 2.9 to 2.8% and ash pretreated 3.0 to 2.9% while ether extract increased from 2.9 to 6.0% in the untreated 3.1 to 6.1% in NaOH pretreated and 3.4 to 3.8% in ash pretreated. The dry matter also decreased 43.5 to 38.5%, 42.5 to 37.4%, 38.9 to 36.6%. The crude fiber decreased 24.3 to 21.2%, 23.5 to 21.4% and 24.2 to 21.6%. These decreases were significantly different at $p = 0.05$.

Table 11: Chemical composition (%) of maize cob supplemented with peptone and inoculated with *Aspergillus niger*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	42.58^{a}	3.43^{a}	3.29^{c}	3.01^{f}	24.42^{a}	36.70^{a}	0.00^{g}	20.24^{a}
	4	41.12^{b}	3.23^{b}	3.45^{c}	3.23^{c}	24.37^{b}	34.76^{b}	5.29^{f}	20.15^{b}
	7	39.88^{c}	2.91^{c}	3.41^{c}	3.18^{d}	24.32^{c}	33.76^{e}	8.01^{e}	20.05^{c}
	14	39.25^{f}	2.75^{de}	3.39^{c}	3.11^{e}	23.66^{d}	33.13^{d}	9.73^{d}	19.66^{d}
	21	39.65^{d}	2.60^{e}	5.62^{b}	3.46^{b}	23.44^{e}	32.08^{e}	12.95^{c}	19.66^{d}
	28	39.55^{e}	2.80^{cd}	6.19^{b}	3.69^{a}	22.66^{f}	31.17^{f}	15.07^{b}	19.65^{d}
	35	38.58^{g}	2.60^{e}	6.86^{a}	3.68^{a}	21.44^{g}	30.98^{g}	15.59^{a}	19.36^{e}
NaOH pretreated maizecob									
	0	42.58^{a}	3.33^{a}	2.87^{g}	2.94^{e}	23.88^{a}	34.01^{a}	0.00^{f}	20.74^{b}
	4	40.38^{b}	3.30^{a}	3.17^{f}	3.30^{b}	23.57^{c}	32.36^{c}	4.85^{d}	20.92^{a}
	7	39.52^{c}	2.91^{cd}	3.47^{e}	3.19^{c}	23.66^{b}	32.96^{b}	3.19^{e}	20.26^{c}
	14	38.58^{e}	3.01^{b}	3.65^{d}	3.01^{d}	22.68^{d}	32.93^{b}	3.18^{e}	20.02^{d}
	21	38.78^{d}	2.70^{e}	6.15^{c}	3.15^{c}	22.47^{e}	30.70^{d}	9.73^{b}	19.44^{c}
	28	38.23^{f}	2.95^{bc}	6.46^{b}	3.39^{b}	22.17^{g}	30.83^{d}	9.35^{c}	19.25^{g}
	35	37.58^{g}	2.82^{de}	7.18^{a}	6.19^{a}	21.46^{f}	30.20^{e}	11.20^{a}	19.28^{f}
Ash pretreated maizecob									
	0	38.23^{a}	3.53^{a}	3.32^{g}	3.03^{c}	23.65^{c}	35.80^{a}	0.00^{g}	21.42^{b}
	4	37.23^{b}	3.44^{b}	3.57^{f}	3.33^{c}	23.66^{c}	35.63^{b}	0.47^{f}	21.55^{a}
	7	36.56^{d}	2.93^{d}	3.75^{e}	3.45^{b}	23.88^{a}	35.50^{bc}	0.84^{e}	21.05^{c}
	14	35.58^{f}	3.20^{c}	4.00^{d}	3.54^{a}	23.78^{b}	35.40^{c}	1.12^{d}	20.42^{d}
	21	35.83^{e}	2.67^{f}	5.70^{c}	3.13^{d}	23.62^{d}	33.02^{d}	7.77^{c}	20.16^{e}
	28	35.23^{g}	2.95^{d}	6.88^{b}	3.30^{c}	22.98^{e}	32.32^{e}	9.72^{b}	20.15^{e}
	35	36.64^{c}	2.85^{e}	7.47^{a}	3.29^{c}	21.42^{f}	31.33^{f}	12.48^{a}	19.77^{f}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 12: Chemical composition (%) of maize cob supplemented with chicken extract and inoculated with *Aspergillus niger*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	42.34^{a}	3.35^{a}	3.02^{g}	3.12^{f}	24.18^{c}	36.00^{a}	0.00^{f}	21.42^{a}
	4	41.04^{b}	2.97^{b}	3.27^{f}	3.34^{c}	24.25^{a}	36.00^{a}	0.00^{f}	20.46^{b}
	7	39.77^{c}	2.91^{bc}	3.19^{e}	3.17^{e}	24.22^{b}	34.43^{b}	4.36^{e}	20.45^{b}
	14	39.05^{e}	2.74^{d}	3.51^{d}	3.18^{e}	23.78^{d}	34.19^{c}	5.03^{d}	20.33^{c}
	21	39.55^{d}	2.57^{e}	4.77^{c}	3.23^{d}	23.35^{e}	32.55^{d}	9.58^{c}	20.24^{d}
	28	39.02^{f}	2.80^{c}	5.40^{b}	3.69^{a}	22.77^{f}	31.95^{e}	12.36^{b}	19.47^{e}
	35	38.48^{g}	2.74^{d}	6.71^{a}	3.47^{b}	21.52^{g}	30.77^{f}	14.69^{a}	19.45^{f}
NaOH pretreated maizecob									
	0	42.78^{a}	3.22^{a}	2.99^{g}	3.04^{g}	24.33^{a}	35.46^{a}	0.00^{g}	21.04^{b}
	4	40.28^{b}	3.08^{b}	3.25^{f}	3.53^{c}	23.65^{b}	35.43^{b}	0.08^{f}	21.25^{a}
	7	39.55^{c}	2.83^{c}	3.49^{e}	3.19^{f}	23.58^{c}	35.35^{c}	0.31^{e}	20.44^{c}
	14	38.75^{e}	2.81^{d}	3.66^{d}	3.46^{d}	22.95^{d}	34.38^{d}	3.05^{d}	20.23^{d}
	21	38.95^{s}	2.65^{f}	5.78^{c}	3.23^{e}	22.25^{e}	33.54^{e}	5.41^{c}	20.12^{e}
	28	38.53^{f}	2.84^{c}	6.13^{b}	3.64^{b}	22.06^{f}	33.05^{f}	6.80^{b}	19.74^{f}
	35	37.67^{g}	2.72^{e}	7.00^{a}	3.81^{a}	21.45^{g}	31.04^{g}	12.46^{a}	19.68^{g}
Ash pretreated maizecob									
	0	38.95^{b}	3.65^{a}	3.03^{g}	3.03^{g}	23.48^{c}	36.83^{a}	0.00^{g}	20.42^{a}
	4	36.95^{d}	3.60^{a}	3.36^{f}	3.41^{e}	23.59^{b}	35.91^{b}	2.50^{f}	20.28^{b}
	7	36.04^{e}	3.16^{b}	3.55^{e}	3.47^{c}	23.60^{b}	33.68^{c}	8.55^{e}	19.85^{c}
	14	35.82^{g}	2.99^{c}	3.77^{g}	3.43^{d}	23.96^{a}	32.51^{d}	11.73^{d}	19.44^{d}
	21	35.97^{f}	2.57^{f}	6.00^{c}	3.18^{f}	23.41^{d}	32.00^{e}	13.11^{c}	19.42^{e}
	28	38.01^{c}	2.90^{d}	6.36^{b}	3.62^{b}	23.11^{e}	31.87^{f}	13.47^{b}	19.27^{f}
	35	39.75^{a}	2.75^{e}	7.57^{a}	3.86^{a}	22.36^{f}	30.57^{g}	17.31^{a}	19.09^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 13: Chemical composition (%) of maize cob supplemented by peptone and inoculated with *Aspergillus oryzae*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	42.63^{a}	3.71^{a}	3.12^{f}	2.81^{e}	25.05^{a}	37.81^{a}	0.00^{g}	20.35^{b}
	4	40.98^{b}	3.01^{b}	3.47^{e}	3.07^{d}	24.77^{b}	36.37^{b}	1.94^{f}	20.77^{a}
	7	39.22^{c}	2.60^{e}	3.49^{e}	3.39^{b}	24.60^{c}	36.33^{c}	2.05^{e}	20.33^{c}
	14	38.98^{d}	2.87^{c}	3.64^{d}	3.18^{c}	23.95^{d}	35.42^{d}	4.50^{d}	20.05^{d}
	21	38.89^{e}	2.67^{e}	4.39^{c}	3.20^{c}	23.58^{e}	33.35^{e}	10.03^{c}	20.01^{e}
	28	38.12^{f}	2.89^{c}	4.57^{b}	3.47^{a}	23.05^{f}	32.48^{f}	12.43^{b}	19.74^{f}
	35	37.80^{g}	2.77^{d}	6.35^{a}	3.52^{a}	22.52^{g}	31.69^{g}	14.56^{a}	19.73^{f}
NaOH pretreated maizecob									
	0	45.55^{a}	2.98^{a}	2.12^{d}	2.78^{b}	22.88^{c}	35.77^{a}	0.00^{g}	21.16^{b}
	4	41.56^{b}	2.84^{b}	3.44^{c}	3.27^{ab}	23.05^{b}	34.89^{d}	2.46^{f}	21.33^{a}
	7	40.64^{c}	2.78^{b}	3.62^{c}	3.10^{ab}	23.17^{a}	35.24^{b}	1.48^{e}	20.78^{c}
	14	39.02^{c}	2.82^{b}	3.74^{c}	3.25^{b}	22.88^{c}	35.11^{c}	1.85^{d}	20.33^{d}
	21	39.45^{d}	2.67^{c}	4.67^{c}	3.30^{ab}	22.56^{d}	33.44^{e}	6.51^{c}	20.28^{e}
	28	38.89^{f}	2.90^{b}	5.33^{b}	3.50^{a}	22.16^{e}	32.63^{f}	8.78^{b}	20.18^{f}
	35	37.52^{g}	2.80^{b}	6.35^{a}	3.60^{a}	21.28^{f}	32.06^{d}	10.37^{a}	20.03^{g}
Ash pretreated maizecob									
	0	39.12^{a}	3.37^{a}	3.30^{g}	3.12^{f}	24.02^{a}	34.67^{a}	0.00^{g}	20.36^{e}
	4	37.23^{b}	3.30^{a}	3.65^{f}	3.46^{c}	23.84^{b}	34.00^{b}	1.93^{f}	20.18^{e}
	7	36.05^{e}	3.02^{c}	3.94^{e}	3.38^{d}	23.44^{c}	33.94^{c}	2.11^{e}	19.78^{d}
	14	35.98^{f}	3.22^{d}	4.14^{d}	3.53^{b}	23.18^{d}	33.92^{d}	2.16^{e}	19.55^{d}
	21	36.23^{d}	2.62^{f}	5.98^{c}	3.28^{e}	23.02^{e}	33.02^{e}	4.76^{c}	19.55^{c}
	28	35.98^{f}	2.84^{d}	6.77^{b}	3.53^{b}	22.88^{f}	32.71^{f}	5.65^{b}	19.36^{b}
	35	36.72^{c}	2.72^{e}	7.21^{a}	3.55^{a}	21.62^{g}	31.90^{g}	7.76^{a}	19.35^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 14: Chemical composition (%) of maize cob supplemented with chicken extract and inoculated with *Aspergillus oryzae*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	43.58^{a}	3.81^{a}	3.01^{d}	2.71^{f}	24.66^{a}	36.75^{a}	0.00^{g}	21.18^{b}
	4	41.56^{b}	2.84^{b}	3.42^{c}	2.99^{e}	23.95^{c}	36.44^{b}	0.84^{f}	21.45^{a}
	7	40.34^{c}	2.63^{f}	3.37^{c}	3.15^{c}	23.97^{b}	36.14^{c}	1.65^{e}	20.47^{c}
	14	39.58^{e}	2.75^{c}	3.54^{c}	2.98^{e}	22.87^{d}	35.99^{d}	2.07^{d}	20.15^{d}
	21	39.78^{d}	2.65^{e}	5.25^{b}	3.08^{d}	22.48^{e}	34.93^{e}	4.95^{c}	20.05^{e}
	28	39.08^{f}	2.83^{b}	5.33^{b}	3.50^{b}	22.15^{f}	34.00^{f}	7.48^{b}	19.25^{f}
	35	38.77^{g}	2.72^{d}	6.09^{a}	3.68^{a}	21.25^{g}	32.73^{g}	10.93^{a}	19.22^{g}
NaOH pretreated maizecob									
	0	42.58^{a}	2.74^{c}	2.79^{e}	2.94^{e}	23.58^{c}	36.95^{a}	0.00^{g}	21.41^{a}
	4	40.32^{b}	2.83^{b}	3.10^{de}	3.37^{c}	23.77^{a}	35.73^{b}	3.30^{f}	20.47^{c}
	7	38.88^{c}	2.78^{bc}	3.50^{cd}	3.22^{d}	23.62^{b}	35.12^{c}	4.95^{e}	20.66^{b}
	14	38.12^{e}	2.73^{c}	3.86^{c}	3.33^{c}	22.95^{d}	34.95^{d}	5.41^{d}	20.36^{d}
	21	38.56^{d}	2.72^{c}	3.91^{c}	3.40^{b}	22.36^{e}	34.47^{e}	6.71^{c}	20.17^{e}
	28	38.12^{e}	3.00^{a}	4.94^{b}	3.47^{b}	22.02^{f}	34.10^{f}	7.71^{b}	20.05^{f}
	35	37.41^{f}	2.72^{c}	6.19^{a}	6.27^{a}	21.66^{g}	32.79^{g}	11.26^{a}	19.17^{g}
Ash pretreated maizecob									
	0	39.32^{a}	3.20^{b}	3.33^{g}	3.23^{e}	23.62^{c}	35.73^{a}	0.00^{g}	21.42^{a}
	4	37.14^{b}	3.34^{a}	3.72^{f}	3.58^{bc}	23.99^{a}	34.97^{b}	2.13^{f}	20.68^{b}
	7	36.22^{e}	3.15^{c}	3.75^{e}	3.42^{d}	23.88^{b}	34.94^{b}	2.21^{e}	20.39^{c}
	14	35.23^{g}	3.38^{a}	3.92^{d}	3.66^{ab}	23.47^{d}	34.03^{c}	4.76^{d}	20.12^{d}
	21	36.31^{d}	2.72^{f}	5.65^{c}	3.40^{d}	23.27^{e}	33.94^{d}	5.01^{c}	19.77^{e}
	28	35.34^{f}	2.95^{d}	6.46^{b}	3.50^{c}	22.69^{f}	33.50^{e}	6.24^{b}	19.55^{f}
	35	36.35^{c}	2.83^{e}	6.64^{a}	3.73^{a}	21.45^{g}	31.34^{f}	12.29^{a}	19.42^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 15: Chemical composition (%) of maize cob inoculated with *Aspergillus niger*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	43.52^{a}	3.08^{a}	3.31^{f}	2.90^{e}	24.28^{b}	35.85^{a}	0.00^{g}	20.42^{d}
	4	41.55^{b}	3.03^{ab}	3.54^{e}	3.35^{b}	23.48^{e}	35.73^{b}	0.33^{f}	20.44^{c}
	7	40.22^{c}	2.66^{c}	3.53^{e}	3.13^{c}	24.58^{a}	35.11^{c}	2.06^{e}	21.16^{a}
	14	39.23^{e}	2.65^{c}	3.67^{d}	3.06^{d}	23.62^{c}	34.89^{d}	2.68^{d}	20.77^{b}
	21	39.56^{d}	2.60^{c}	5.32^{c}	3.15^{c}	23.52^{d}	33.42^{e}	6.78^{d}	20.24^{e}
	28	39.12^{f}	2.97^{b}	5.48^{b}	3.39^{b}	22.41^{f}	32.82^{f}	8.14^{b}	20.06^{f}
	35	38.53^{g}	2.67^{c}	6.24^{a}	6.03^{a}	21.15^{g}	32.10^{g}	10.46^{a}	20.24^{e}
NaOH pretreated maizecob									
	0	42.45^{a}	2.92^{ab}	3.04^{c}	3.06^{e}	23.47^{b}	33.97^{a}	0.00^{f}	23.15^{a}
	4	42.45^{a}	2.80^{b}	3.14^{bc}	3.26^{e}	23.51^{a}	31.77^{b}	6.47^{e}	22.56^{c}
	7	39.92^{b}	2.66^{d}	3.23^{bc}	3.16^{d}	23.47^{b}	31.76^{b}	6.50^{d}	22.77^{b}
	14	38.41^{d}	2.97^{a}	3.51^{b}	3.54^{b}	22.75^{c}	31.48^{c}	7.33^{c}	21.47^{d}
	21	38.78^{c}	2.70^{cd}	5.70^{a}	3.25^{c}	22.29^{d}	31.31^{d}	7.83^{b}	21.44^{e}
	28	38.04^{e}	2.95^{a}	6.00^{a}	3.53^{b}	22.16^{e}	31.30^{d}	7.86^{b}	21.33^{f}
	35	37.42^{f}	2.83^{bc}	6.38^{a}	6.11^{a}	21.42^{f}	30.97^{e}	8.83^{a}	21.18^{g}
Ash pretreated maizecob									
	0	38.89^{a}	3.03^{b}	3.29^{g}	3.39^{g}	24.18^{d}	36.94^{a}	0.00^{f}	21.14^{a}
	4	37.23^{b}	2.98^{bc}	3.55^{f}	3.71^{b}	24.28^{a}	35.70^{b}	3.36^{e}	20.66^{b}
	7	36.27^{e}	2.81^{e}	3.92^{e}	3.47^{f}	24.18^{b}	35.69^{b}	3.38^{e}	20.47^{c}
	14	35.72^{f}	3.14^{a}	4.03^{d}	3.64^{c}	23.87^{c}	35.64^{b}	3.52^{d}	19.88^{d}
	21	36.53^{d}	2.70^{e}	4.19^{c}	3.51^{e}	23.65^{d}	35.45^{bc}	4.03^{c}	19.05^{e}
	28	35.17^{g}	2.92^{cd}	5.03^{b}	3.53^{d}	22.88^{e}	34.91^{c}	5.50^{b}	18.74^{f}
	35	36.55^{c}	2.88^{d}	6.43^{a}	3.78^{a}	21.62^{f}	32.67^{d}	11.56^{a}	18.17^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 16: Chemical composition (%) of maize cob inoculated with *Aspergillus oryzae*

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	43.37^{a}	3.35^{a}	3.02^{d}	2.93^{c}	24.18^{c}	36.31^{a}	0.00^{g}	21.15^{a}
	4	40.37^{b}	3.24^{b}	3.34^{f}	3.34^{c}	24.25^{b}	35.90^{b}	1.13^{f}	20.46^{b}
	7	39.88^{c}	2.63^{g}	3.46^{e}	3.16^{d}	24.33^{a}	35.40^{c}	2.51^{e}	20.39^{c}
	14	39.42^{e}	2.84^{d}	3.50^{d}	3.15^{d}	23.77^{d}	35.29^{d}	2.81^{d}	20.06^{d}
	21	39.65^{d}	2.67^{f}	5.95^{c}	3.28c	23.41^{e}	32.98^{e}	9.17^{c}	19.78^{e}
	28	38.82^{f}	2.89^{c}	6.31^{b}	3.67^{b}	22.66^{f}	32.80^{f}	9.67^{b}	19.47^{f}
	35	37.85^{g}	2.75^{e}	6.95^{a}	6.40^{a}	21.44^{g}	31.90^{g}	12.14^{a}	19.25^{g}
NaOH pretreated maizecob									
	0	38.88^{a}	3.42^{a}	3.45^{d}	3.40^{e}	24.11^{c}	34.29^{a}	0.00^{g}	22.15^{b}
	4	35.75^{b}	3.41^{a}	3.89^{c}	3.58^{d}	24.28^{a}	34.03^{b}	0.76^{f}	22.35^{a}
	7	35.12^{d}	3.08^{c}	3.84^{c}	3.79^{c}	24.18^{b}	33.22^{c}	3.12^{e}	20.15^{c}
	14	34.58^{f}	3.24^{b}	4.11^{c}	3.99^{b}	23.18^{d}	33.13^{d}	3.38^{d}	19.88^{d}
	21	34.75^{e}	2.65^{f}	5.52^{b}	3.35^{f}	23.18^{d}	32.29^{e}	5.83^{c}	19.66^{e}
	28	34.23^{g}	2.83^{d}	5.76^{b}	3.45^{e}	23.01^{e}	31.94^{f}	6.85^{b}	19.44^{f}
	35	35.55^{c}	2.70^{e}	6.61^{a}	6.14^{a}	22.55^{f}	31.27^{g}	8.81^{a}	19.18^{g}
Ash pretreated maizecob									
	0	39.06^{a}	3.12^{b}	3.12^{g}	3.25^{f}	23.77^{a}	36.47^{a}	0.00^{g}	21.15^{a}
	4	36.98^{b}	3.14^{b}	3.49^{f}	3.60^{b}	23.71^{c}	36.09^{b}	1.04^{f}	20.88^{b}
	7	35.82^{d}	3.04^{c}	3.74^{e}	3.55^{d}	23.74^{b}	35.78^{c}	1.87^{e}	20.77^{c}
	14	35.11^{f}	3.33^{a}	3.39^{d}	3.62^{b}	23.66^{d}	35.62^{d}	2.33^{d}	20.47^{d}
	21	35.89^{c}	2.65^{e}	4.74^{c}	3.38^{e}	23.28^{e}	34.74^{e}	4.74^{c}	19.66^{e}
	28	34.98^{g}	2.84^{d}	5.74^{b}	3.56^{cd}	22.49^{f}	33.91^{f}	7.02^{b}	19.53^{f}
	35	35.53^{e}	2.80^{d}	6.17^{a}	3.93^{a}	21.48^{g}	31.88^{g}	12.59^{a}	19.36^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 17: Chemical composition (%) of maize cob soaked in water

Treatments	Days	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Cellulose content	% cellulose degraded	Lignin
Untreated maizecob									
	0	43.15^{a}	3.20^{a}	3.13^{f}	2.69^{b}	23.66^{b}	34.54^{a}	0.00^{e}	21.74^{a}
	4	41.55^{b}	3.06^{b}	3.38^{e}	3.09^{ab}	23.55^{c}	34.06^{b}	1.39^{d}	21.36^{b}
	7	40.23^{c}	2.63^{e}	3.53^{d}	3.03^{ab}	23.88^{a}	33.71^{c}	2.40^{c}	20.42^{c}
	14	39.22^{e}	2.60^{e}	3.54^{d}	3.01^{ab}	22.48^{d}	33.69^{c}	2.46^{c}	19.88^{d}
	21	39.58^{d}	2.65^{e}	4.24^{c}	3.05^{ab}	22.33^{e}	33.69^{c}	2.46^{c}	19.33^{f}
	28	38.89^{f}	2.86^{c}	6.13^{b}	3.48^{a}	22.01^{f}	32.99^{d}	4.49^{b}	19.25^{g}
	35	37.65^{g}	2.78^{d}	6.77^{a}	3.52^{a}	21.36^{g}	31.69^{e}	8.25^{a}	19.78^{e}
NaOH pretreated maizecob									
	0	37.65^{a}	3.29^{a}	3.40^{f}	3.27^{e}	24.05^{d}	35.13^{a}	0.00^{g}	21.05^{c}
	4	36.12^{b}	3.32^{a}	3.71^{e}	3.68^{a}	24.26^{c}	34.04^{b}	3.10^{f}	21.74^{a}
	7	35.88^{c}	2.95^{b}	3.60^{e}	3.54^{b}	24.62^{b}	33.96^{c}	3.33^{e}	21.15^{b}
	14	34.58^{f}	3.27^{a}	3.90^{d}	3.59^{b}	24.74^{a}	33.51^{d}	4.61^{d}	20.74^{d}
	21	34.97^{e}	2.62^{d}	4.46^{c}	3.20^{e}	24.05^{d}	32.94^{e}	6.23^{c}	20.28^{e}
	28	34.56^{g}	2.89^{c}	5.29^{b}	3.42^{c}	23.55^{e}	32.81^{f}	6.60^{b}	20.24^{f}
	35	35.64^{d}	2.80^{c}	6.14^{a}	3.34^{d}	22.62^{f}	32.08^{g}	8.68^{a}	20.07^{g}
Ash pretreated maizecob									
	0	39.25^{a}	2.96^{b}	2.98^{g}	3.01^{g}	23.86^{g}	36.44^{a}	0.00^{g}	20.74^{a}
	4	37.03^{b}	2.92^{c}	3.29^{f}	3.29^{f}	23.77^{f}	34.20^{d}	4.40^{f}	20.46^{b}
	7	36.23^{d}	2.87^{d}	3.53^{e}	3.75^{e}	23.66^{c}	34.76^{b}	4.61^{e}	19.88^{c}
	14	35.72^{f}	3.05^{a}	3.84^{d}	3.53^{d}	23.42^{d}	34.54^{c}	5.21^{d}	19.78^{d}
	21	35.98^{e}	2.62^{f}	4.21^{c}	3.35^{c}	23.38^{e}	33.36^{e}	7.08^{c}	19.28^{e}
	28	35.23^{g}	2.87^{d}	4.96^{b}	3.81^{b}	22.96^{b}	33.17^{f}	8.97^{b}	19.22^{f}
	35	36.64^{c}	2.77^{e}	6.32^{a}	4.12^{a}	21.46^{a}	31.20^{g}	14.38^{a}	19.02^{g}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

3.8 Effect of bacterial fermentation, nutrient supplementation and alkaline pretreatment on the mineral composition of maize cob

The mineral content in untreated com cob was found to be influenced by the treatment. All the mineral contents of the com cob increased throughout the period of fermentation in nutrient supplemented and bacteria inoculated substrates, as seen in Tables 18-24. In Table 18 the untreated maize cob showed increase in calcium content from 230.8 mg/100g on day 0 to 286.0 mg/100g on day 35; Iron increased from 3.2mg to 5.1mg/100g; Potassium 22.6 mg/100g to 25.6mg/100g; Magnesium from 22.9 mg/100g to 31.6 mg/100g. Only phosphorus decreased slightly from 231.7 mg/100g to 230.5 mg/100g.

The NaOH pretreated and ash pretreated maizecob also follow the same trend except the fact that phosphorus increases slightly during fermentation. The ash pretreated maize cob had higher content of all the minerals than all the other treatment for example calcium content in day 0 was 360.8 mg/100g compared to 230.8 mg/100g and 233.5 mg/100g in untreated and NaOH pretreated Maize cob (Table 18). In NaOH pretreated maize cob, calcium content increased from 233.5 mg/1`00g to 238.5 mg/100g on day 35. Iron increased from 3.3 to 3.9 mg/100g, potassium, 23.3 to 23.1 mg/100g; Magnesium, 23.3 to 23.6 mg/100g and phosphate, 232.7 to 248.9 mg/100g (Table 18). In ash pretreated, calcium content increased from 360.8 mg/100g on day 0 to 377.4 mg/100g on day 35. Iron, 5.1 to 6.1 mg/100g potassium, 52.3 mg/100g to 54.1 mg/100g magnesium, 33.6 to 34.9 mg/100g and phosphate, 411.6 mg/100g to 425.0 mg/100g.

In Table 19 where maize cob were supplemented with chicken extract and inoculated with *Bacillus subtilis*. There was a slight increase in all the mineral content compared to Table 18 where there was peptone supplement except in NaOH pretreated maize cob where there was a decrease in the mineral content. In untreated corn cob with chicken extract supplementation and *B. subtilis* as inoculum (Table 19). The intial calcium content on day 0 was 246.5mg/100g, iron 4.0mg/100g, potassium, 23.2mg./100g, magnesium, 23.4mg/100g and phosphorus 237.3mg/100g compared to where peptone was supplement in Table 18 where calcium was 230.8mg/100g, Iron, 3.3 potassium 22.6, magnesium 22.9 and phosphorus 231.7mg/100g. These trends can also be seen in Table 20 and 21.

The unsupplemented Maize cob (Table 22 and 23) and the control of Table 24 showed a decrease in the initial value of the mineral content of the untreated Maize cob compared with the supplemented and inoculated Maize cob. A slight decrease was also observed in the magnesium content of water soaked corn cob (Table 24) during fermentation. This decrease was significant at $P \leq 0.05$.

In Table 23 where maize cob was inoculated with *B lichenformis* with no supplementation, the calcium value of 214.1mg/100g; iron, 3.0 mg/100g; potassium, 21.3 mg/100g; Magnesium 28.9mg/100g and phosphorus 189.8mg/100g were lower than the chicken extract supplemented maize cob inoculated with *B lichenformis* in Table 21 where calcium was 247.2 mg/100g; Iron 3.9 mg./100g; potassium, 23.2 mg/100g; magnesium, 24.1 mg/100g and phosphorus 233.0 mg/100g.

A slight decrease was observed in magnesium of untreated maize cob (Table 24) from 29.1 mg/100g to 29.0 mg/100g. NaOH pretreated maize cob magnesium decreases form 24.2 mg/100g to 23.7 mg/100g and ash pretreated magnesium decreases from 33.4 mg/100g to 35.2 mg/100g.

Table 18: Mineral content of maize cob supplemented with peptone and inoculated with *Bacillus subtilis*

Treatments	Days	mg/100g dry weight Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	230.78g	3.29^{e}	22.57^{f}	22.90^{d}	231.72^{c}
	4	231.04^{f}	3.26^{f}	22.60^{e}	23.00^{c}	231.04^{d}
	7	231.78^{e}	3.45^{d}	22.68^{e}	23.10^{c}	231.04^{d}
	14	237.73^{d}	3.48^{c}	23.34^{d}	23.77^{b}	236.71^{b}
	21	239.44^{c}	3.48^{c}	23.55^{c}	23.70^{b}	238.80^{a}
	28	281.76^{b}	4.94^{b}	25.28^{b}	31.58^{a}	230.53^{e}
	35	286.03^{a}	5.05^{a}	25.61^{a}	31.58^{a}	230.53^{e}
NaOH pretreated maizecob						
	0	233.49^{e}	3.29^{d}	22.34^{d}	23.27^{d}	232.73^{g}
	4	233.13^{f}	3.35^{c}	22.23^{d}	23.24^{d}	234.14^{e}
	7	233.30^{e}	3.25^{d}	22.01^{e}	23.16^{e}	233.31^{f}
	14	237.71^{c}	3.31^{c}	22.32^{d}	23.69^{c}	237.71^{d}
	21	240.53^{a}	3.48^{b}	22.52^{e}	23.87^{b}	241.30^{b}
	28	237.39^{d}	3.84^{a}	22.81^{b}	23.99^{b}	240.06^{c}
	35	238.54^{b}	3.85^{a}	23.09^{a}	24.63^{a}	248.91^{a}
Ash pretreated maizecob						
	0	360.75^{g}	5.09^{f}	52.25^{g}	33.64^{f}	411.63^{g}
	4	363.76^{e}	5.34^{e}	52.95^{f}	34.35^{e}	417.15^{f}
	7	363.44^{f}	5.53^{d}	53.02^{e}	34.50^{d}	417.70^{e}
	14	366.64^{d}	5.76^{c}	53.82^{d}	35.07^{b}	436.98^{a}
	21	367.01^{c}	5.88^{b}	53.89^{c}	35.18^{a}	433.84^{b}
	28	376.42^{b}	5.70^{c}	54.32^{a}	35.05^{b}	427.88^{c}
	35	377.43^{a}	6.10^{a}	54.18^{b}	34.90^{c}	425.01^{d}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 19: Mineral content of maize cob supplemented with chicken extract and inoculated with *Bacillus subtilis*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	246.52^{g}	4.04^{b}	23.22^{e}	23.47^{f}	237.25^{e}
	4	246.73^{c}	4.03^{b}	23.41^{d}	23.74^{e}	239.17^{c}
	7	245.74^{f}	4.26^{a}	23.40^{d}	23.47^{f}	238.97^{d}
	14	249.87^{g}	4.26^{a}	24.04^{c}	24.04^{cd}	242.99^{a}
	21	250.89^{c}	4.26^{a}	24.14^{b}	24.14^{c}	242.47^{b}
	28	251.93^{b}	3.66^{d}	24.34^{a}	26.39^{a}	196.44^{f}
	35	252.65^{a}	3.71^{c}	24.17^{b}	26.28^{b}	200.69^{f}
NaOH pretreated maizecob						
	0	214.75^{e}	3.02^{f}	21.73^{d}	28.70^{cd}	188.82^{e}
	4	214.16^{f}	3.08^{e}	21.59^{e}	28.60^{d}	188.39^{f}
	7	212.24^{g}	3.11^{d}	21.30^{f}	28.60^{d}	187.56^{g}
	14	218.15^{d}	3.11^{d}	21.81^{e}	28.82^{ed}	192.11^{d}
	21	219.36^{c}	3.29^{e}	21.77^{cd}	28.85^{bc}	192.36^{c}
	28	219.98^{b}	3.31^{b}	22.36^{a}	29.57^{ab}	195.43^{b}
	35	220.27^{a}	3.65^{a}	22.97^{b}	30.04^{a}	195.69^{a}
Ash pretreated maizecob						
	0	403.44^{g}	6.85^{e}	54.65^{g}	34.70^{f}	434.67^{g}
	4	411.89^{f}	6.92^{d}	55.65^{f}	35.06^{e}	441.87^{f}
	7	414.35^{e}	6.94^{c}	55.77^{e}	35.18^{d}	443.39^{e}
	14	419.56^{d}	6.84^{e}	56.55^{d}	35.62^{c}	450.54^{b}
	21	421.48^{c}	6.79^{f}	56.99^{b}	35.62^{c}	448.32^{d}
	28	422.06^{b}	7.34^{b}	56.87^{c}	36.32^{b}	449.28^{c}
	35	427.11^{a}	7.63^{a}	57.19^{a}	36.50^{a}	455.00^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 20: Mineral content of com cob supplemented with peptone and inoculated with *Bacillus licheniformis*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	229.91^{g}	3.28^{f}	22.64^{f}	23.14^{f}	231.61^{f}
	4	231.52^{f}	3.41^{e}	22.64^{f}	22.97^{g}	229.86^{g}
	7	232.75^{e}	3.43^{de}	22.76^{e}	23.27^{e}	232.67^{b}
	14	238.77^{d}	3.49^{d}	23.44^{d}	23.70^{d}	236.97^{d}
	21	239.19^{c}	3.60^{c}	23.56^{c}	23.82^{c}	238.17^{c}
	28	262.17^{b}	4.46^{b}	24.68^{b}	25.27^{a}	240.69^{b}
	35	275.56^{a}	4.57^{a}	24.88^{a}	24.88^{b}	241.13^{a}
NaOH pretreated maizecob						
	0	244.34^{g}	4.02^{c}	23.18^{f}	23.43^{g}	236.82^{g}
	4	248.79^{f}	4.08^{bc}	23.60^{e}	23.96^{f}	242.16^{c}
	7	251.99^{c}	4.26^{a}	23.99^{c}	24.25^{c}	242.50^{b}
	14	251.28^{e}	4.24^{a}	24.11^{b}	24.11^{e}	241.82^{d}
	21	252.37^{b}	4.10^{b}	24.14^{b}	24.14^{d}	241.36^{e}
	28	251.80^{d}	3.93^{d}	23.84^{d}	26.59^{b}	241.05^{f}
	35	260.89^{a}	4.25^{a}	24.79^{a}	27.11^{a}	252.39^{a}
Ash pretreated maizecob						
	0	354.13^{g}	5.16^{f}	51.91^{g}	32.39^{g}	409.15^{g}
	4	359.86^{f}	5.25^{f}	52.88^{f}	32.99^{f}	416.78^{f}
	7	363.31^{e}	5.45^{e}	53.25^{e}	33.60^{e}	419.76^{e}
	14	367.98^{d}	5.63^{d}	54.21^{d}	33.57^{d}	426.05^{e}
	21	368.78^{c}	5.73^{c}	54.45^{c}	33.73^{c}	425.30^{d}
	28	374.94^{b}	5.83^{b}	54.68^{b}	34.34^{b}	432.61^{b}
	35	380.37^{a}	6.11^{a}	54.81^{a}	34.61^{a}	438.07^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 21: Mineral content of maize cob supplemented with chicken extract and inoculated with *b. licheniformis*

Treatments	Days	mg/100g dry weight Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	247.18^{f}	3.85^{f}	23.20^{e}	24.13^{d}	232.96^{f}
	4	246.98^{g}	4.01^{d}	23.10^{f}	23.60^{f}	236.97^{e}
	7	249.48^{e}	3.94^{e}	23.34^{d}	24.02^{e}	239.37^{c}
	14	252.68^{c}	4.17^{c}	23.82^{c}	24.48^{b}	244.05^{b}
	21	251.92^{d}	4.18^{c}	23.99^{b}	24.42^{bc}	246.79^{a}
	28	272.73^{a}	5.46^{a}	24.74^{a}	25.17^{a}	237.51^{d}
	35	259.80^{b}	4.72^{b}	22.84^{g}	24.39^{c}	230.68^{g}
NaOH pretreated maizecob						
	0	247.41^{g}	3.86^{d}	23.55^{g}	23.48^{g}	234.79^{g}
	4	253.59^{f}	4.10^{b}	24.08^{f}	23.98^{f}	239.93^{f}
	7	253.98^{e}	4.00^{c}	24.11^{e}	24.11^{e}	241.92^{e}
	14	257.73^{c}	4.09^{bc}	24.54^{c}	24.54^{d}	249.08^{d}
	21	256.50^{d}	3.76^{e}	24.78^{a}	24.78^{c}	249.67^{e}
	28	258.75^{b}	4.15^{b}	24.56^{b}	26.48^{a}	249.81^{b}
	35	267.39^{a}	4.34^{a}	24.19^{d}	25.07^{b}	254.61^{a}
Ash pretreated maizecob						
	0	413.29^{g}	7.06^{e}	55.45^{g}	34.79^{g}	436.83^{g}
	4	416.01^{f}	7.45^{d}	55.90^{f}	35.18^{f}	439.71^{f}
	7	417.77^{e}	7.67^{c}	56.13^{e}	35.43^{e}	441.57^{e}
	14	424.16^{d}	7.79^{b}	56.99^{d}	35.87^{d}	449.21^{d}
	21	425.33^{c}	7.82^{a}	57.19^{c}	36.04^{c}	452.31^{c}
	28	432.94^{d}	7.67^{c}	57.94^{b}	36.06^{b}	457.35^{b}
	35	431.05^{b}	7.80^{a}	58.22^{a}	36.29^{a}	462.73^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 22: Mineral content of com cob inoculated with *Bacillus subtilis*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	215.10^{g}	3.02^{e}	21.17^{f}	28.99^{c}	189.82$_{g}$
	4	216.75^{f}	3.01^{e}	21.31^{e}	29.08^{b}	190.52^{f}
	7	216.85^{e}	3.24^{d}	21.19^{f}	28.91^{d}	193.59^{e}
	14	218.03^{d}	3.27^{c}	21.46^{c}	29.11^{a}	195.40^{c}
	21	216.83^{c}	3.27^{c}	21.43^{d}	28.79^{e}	197.66^{b}
	28	221.74^{b}	3.64^{b}	24.13^{a}	26.07^{f}	203.92^{a}
	35	211.19^{a}	3.57^{a}	22.79^{b}	24.53^{g}	194.05^{d}
NaOH pretreated maizecob						
	0	244.06^{d}	3.94^{f}	23.71^{f}	23.57^{f}	238.25^{f}
	4	246.42^{e}	3.98^{e}	23.78^{e}	23.71^{g}	237.06^{g}
	7	249.42^{d}	4.11^{d}	24.07^{d}	24.07^{e}	244.28^{e}
	14	250.59^{c}	3.98^{e}	24.54^{b}	24.28-d	248.06^{d}
	21	250.59^{c}	4.18^{c}	24.54^{b}	24.35^{c}	249.72^{c}
	28	257.86^{b}	4.35^{b}	24.48^{c}	24.90^{b}	252.62^{b}
	35	262.60^{a}	4.62^{a}	24.77^{a}	25.19^{a}	256.45^{a}
Ash pretreated maizecob						
	0	408.83^{g}	6.90^{e}	54.83^{g}	36.38^{a}	430.45^{g}
	4	416.80^{f}	6.99^{d}	55.81^{e}	35.48^{e}	437.81^{f}
	7	418.09^{e}	7.10^{e}	55.09^{f}	35.95^{c}	440.89^{e}
	14	419.97^{d}	7.13^{c}	56.42^{d}	35.39^{d}	442.87^{d}
	21	422.78^{c}	7.19c	56.83^{c}	35.70^{f}	449.24$_{c}$
	28	429.65^{b}	7.44^{b}	57.48^{b}	35.15^{g}	452.16^{b}
	35	434.72^{a}	7.64^{a}	57.75^{a}	36.12^{b}	457.24^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 23: Mineral content of maize cob inoculated with *Bacillus lichenifomis*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	214.06^{d}	3.04^{c}	21.33^{c}	28.92^{d}	189.77^{e}
	4	213.51^{f}	3.00^{c}	21.10^{e}	28.86^{e}	189.24^{f}
	7	213.73^{e}	3.07^{bc}	21.05^{d}	28.86^{e}	188.76^{g}
	14	217.39$_{b}$	3.29^{a}	21.49^{b}	29.32^{c}	191.10^{c}
	21	214.06^{d}	3.29^{a}	21.15^{c}	29.35^{b}	191.30^{b}
	28	219.29^{a}	3.12^{b}	21.50^{a}	29.64^{a}	194.05^{a}
	35	216.20^{c}	3.12^{b}	21.22^{c}	28.84^{f}	190.97^{d}
NaOH pretreated maizecob						
	0	238.38^{g}	3.25^{e}	22.80^{e}	23.92^{f}	238.38^{g}
	4	240.62^{f}	3.18^{e}	23.10^{d}	24.14^{e}	240.62^{f}
	7	244.83^{e}	3.30^{d}	23.54^{c}	24.33^{d}	244.05^{e}
	14	247.89^{d}	3.69^{c}	24.00^{b}	24.68^{c}	246.04^{d}
	21	250.00^{b}	3.71^{c}	24.39^{a}	12.57^{g}	247.35^{c}
	28	249.25^{c}	3.99^{b}	23.95^{b}	25.09^{b}	250.93^{ab}
	35	251.41^{a}	4.08^{a}	24.44^{a}	25.40^{a}	255.86^{a}
Ash pretreated maizecob						
	0	417.77^{g}	6.92^{e}	55.90^{g}	35.10^{f}	439.71^{d}
	4	423.19^{f}	6.99^{e}	56.39^{f}	35.41^{e}	443.56^{c}
	7	424.91^{e}	7.14^{d}	56.81^{e}	35.81^{d}	447.17^{c}
	14	429.15^{d}	7.21^{c}	57.49^{d}	35.70^{d}	454.14^{b}
	21	430.66^{c}	7.12^{d}	57.69^{c}	36.21^{c}	454.14^{b}
	28	436.30^{b}	7.62^{b}	57.96^{b}	36.73^{b}	458.97^{ab}
	35	436.80^{a}	8.00^{a}	58.31^{a}	37.40^{a}	463.20^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 24: Mineral content of maize cob soaked in water

Treatments	Days	mg/100g dry weight Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	215.02^{c}	3.02^{f}	21.75^{b}	29.06^{d}	189.06^{f}
	4	214.81^{a}	3.09^{e}	21.15^{e}	28.93^{e}	190.72^{d}
	7	212.30^{f}	3.13^{d}	20.90^{f}	28.67^{f}	186.75^{a}
	14	214.59^{e}	3.27^{c}	21.21^{e}	29.08^{c}	190.19^{e}
	21	214.81^{d}	3.42^{a}	21.41^{d}	29.21^{b}	192.14^{b}
	28	219.80^{a}	3.28^{c}	22.05^{a}	29.48^{a}	193.6^{a}
	35	216.60^{b}	3.34^{b}	21.48^{c}	28.94^{e}	192.07^{c}
NaOH pretreated maizecob						
	0	243.35^{b}	3.45^{c}	23.52^{a}	24.16^{b}	242.37^{b}
	4	244.06^{a}	3.46^{c}	23.51^{f}	24.49^{a}	244.06^{a}
	7	223.73^{f}	3.16^{f}	21.71^{e}	22.45^{g}	222.76^{g}
	14	232.55^{e}	3.26^{e}	22.48e	23.34^{f}	230.87^{f}
	21	235.77^{cd}	3.35^{d}	22.65^{d}	23.4^{e}	234.08^{e}
	28	235.68^{d}	3.79^{b}	23.13^{c}	23.56^{d}	235.68^{d}
	35	236.71^{c}	4.02^{a}	23.24^{b}	23.66^{c}	238.39^{c}
Ash pretreated maizecob						
	0	416.38^{g}	7.05^{e}	55.90^{e}	35.40^{e}	442.99^{f}
	4	418.80^{f}	7.34^{cd}	56.34^{d}	35.84^{c}	445.50^{d}
	7	425.65^{e}	7.35^{c}	57.27^{b}	35.99^{b}	450.78^{c}
	14	427.49^{d}	7.29^{d}	57.33^{b}	35.72^{d}	453.69^{b}
	21	430.12^{a}	7.32^{ce}	57.55^{a}	35.94^{b}	454.51^{a}
	28	427.80^{c}	7.60^{a}	57.10^{e}	36.18^{a}	438.93^{g}
	35	429.57^{b}	7.52^{b}	55.36^{f}	35.17^{f}	443.23^{e}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

3.9 Effect of fungal fermentation, nutrient supplementations and alkaline pretreatment on the mineral composition of maize cob

The mineral content in fungal fermented maize cob was found to be influenced by the treatment. All the mineral content of the maize cob, increased significantly throughout the period of fermentation in nutrient supplemented and fungal inoculated substrates, as seen in Tables 25 to 31. In Table 25 the untreated maize cob showed increase in calcium content from 228.2 mg/100g on day O to 282.0 mg/100g on day 35; iron increases from 3.0mg to 3.9 mg/100g; Potassium 23.3 to 31.0 mg/100g and Phosphorus from 212.6 mg/100g to 281.7 mg/100g. When NaOH pretreated was used there was significant increase in the mineral content of the maize cob throughout the period of fermentation in all the tanks. In Table 26 Calcium content increased from 206.1 mg/100g on day 0 to 274.2 mg/100g; Iron increased from 3.57 mg/100g to 5.23 mg/100g; Potassium 22.2 mg/100g to 29.9 mg/100g; Magnesium 24.4 mg/100g to 26.3 mg/100g and Phosphorus 225.6 to 299.7 mg/100g on day 35.

Table 25-31 show the mineral content of the ash pretreated maize cob. There was increase in the mineral content of the maize cob throughout the period of fermentation. It was also observed that the mineral content of the ash pretreated maize cob was about double the amount in the untreated and NaOH pretreated maize cob. For example in Table 27 where maize cob was supplemnted with peptone and inoculate with *A. oryzae*. Calcium content of ash pretreated maize cob was 433.7 mg/100g compared to 241.1 mg/100g and 187.4 mg/100g observed in untreated and NaOH pretreated maize cob respectively. Iron, potassium, magnesium and phosphorous value for ash pretreated were 7.3 mg/100g, 58.6 mg/100g, 36.2 mg/100g and 536.9 mg/100g respectively compared to their value in untreated 3.8mg/100g, 23.6 mg/100g, 29.7 mg/100g 277.7 mg/100g and that of NaOH pretreated value of 3.4 mg/100g; 21.3 mg/100g, 23.8 m/100g, 206.5 mg/100g respectively.

The unsuplemented maize cob (Tables 29-31) showed a decrease in the initial value of the mineral content of the untreated corn cob compared with the supplemented and inoculated corn cob. For examples in Table 29 which shows mineral content of maize cob inoculated with *A. niger*. The initial calcium content at day 0 of the untreated

cob was 208.0 mg/100g; iron, 3.0 mg/100g; potassium, 20.6 mg/100g, magnesium, 29.0 mg/100g and phosphorous, 185.6 mg/100g while in maize cob inoculated with *A. niger* and supplemented with chicken extract in Table 27, calcium content was 230.7 mg/100g, potassium, 26.1 mg.100g; magnesium, 29.3 mg/100g and phosphorous, 210.1 mg/100g, only iron had a slightly lower value of 3.0 mg/100g.

Table 25: Mineral content of maize cob supplemented with peptone and inoculated with *Aspergillus niger*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	228.18^{g}	2.96^{g}	23.30^{g}	29.35^{g}	212.56^{g}
	4	241.88^{f}	3.12^{f}	24.52^{f}	29.64^{f}	221.73^{f}
	7	252.29^{e}	3.47^{e}	26.59^{e}	29.89^{e}	239.26^{e}
	14	259.95^{d}	3.67^{d}	27.87^{c}	30.16^{d}	247.42^{d}
	21	262.51^{c}	3.82^{c}	27.58^{d}	30.43^{c}	252.67^{e}
	28	265.90^{b}	4.26^{b}	29.35^{b}	31.20^{b}	274.51^{b}
	35	282.03^{a}	4.87^{a}	30.98^{a}	31.97^{a}	281.67^{a}
NaOH pretreated maizecob						
	0	204.38^{g}	3.65^{g}	21.53^{g}	24.24^{f}	222.85^{g}
	4	225.09^{f}	3.92^{f}	22.12^{f}	24.64^{e}	236.54^{f}
	7	233.02^{e}	4.07^{e}	23.53^{e}	24.67^{e}	242.24^{e}
	14	249.77^{d}	4.42^{d}	25.07^{d}	25.33^{c}	248.82^{d}
	21	256.55^{c}	4.66^{c}	26.21^{c}	25.14^{d}	260.29^{c}
	28	270.42^{b}	5.06^{b}	28.84^{b}	25.57^{b}	281.85^{b}
	35	284.82^{a}	5.32^{a}	29.95^{a}	26.14^{a}	296.09^{a}
Ash pretreated maizecob						
	0	435,66^{g}	7.83^{g}	59.24^{g}	36.33^{g}	483.86^{g}
	4	452.19^{f}	8.01^{f}	59.61^{f}	36.68^{f}	491.78^{f}
	7	457.10^{e}	8.16^{e}	60.64^{e}	36.81^{e}	401.92^{e}
	14	466.36^{d}	8.27^{d}	62.92^{d}	36.99^{g}	515.28^{d}
	21	473.95^{c}	8.44^{c}	65.61^{a}	37.11^{c}	522.28^{c}
	28	486.23^{a}	8.79^{b}	65.25^{b}	37.80^{b}	546.92^{a}
	35	482.65^{b}	9.24^{a}	64.66^{c}	37.96^{a}	542.17^{b}

`Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 26: Mineral content of maize cob supplemented with chicken extract and inoculated with *Aspergillus niger*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	230.65^{g}	2.97^{g}	26.14^{g}	29.32^{g}	210.09^{g}
	4	246.53^{f}	3.29^{f}	26.19^{f}	29.65^{f}	218.19^{f}
	7	257.37^{e}	3.38^{e}	28.75^{e}	29.90^{e}	230.82^{e}
	14	269.30^{d}	3.68^{d}	29.74^{d}	30.27^{d}	248.72^{d}
	21	270.96^{c}	3.90^{c}	30.19^{c}	30.44^{c}	253.42^{e}
	28	278.97^{b}	4.31^{b}	31.05^{b}	31.55^{b}	274.58^{b}
	35	291.61^{a}	4.96^{a}	32.36^{a}	31.92^{a}	287.65^{a}
NaOH pretreated maizecob						
	0	206.06^{g}	3.57^{g}	22.18^{g}	24.38^{g}	225.55^{g}
	4	232.52^{f}	3.68^{f}	23.83^{f}	24.73^{f}	233.01^{f}
	7	236.99^{e}	3.89^{e}	24.35^{e}	24.84^{e}	246.24^{e}
	14	248.24^{d}	4.14^{d}	24.93^{d}	25.30^{d}	247.61^{d}
	21	249.02^{c}	4.46^{c}	26.95^{c}	25.52^{c}	259.03^{c}
	28	258.87^{b}	4.86^{b}	28.14^{b}	25.86^{b}	279.44^{b}
	35	274.15^{a}	5.23^{a}	29.87^{a}	26.28^{a}	299.65^{a}
Ash pretreated maizecob						
	0	436.02^{g}	7.41^{g}	58.68^{g}	36.13^{g}	483.90^{g}
	4	454.96^{e}	7.69^{f}	60.30^{f}	36.55^{e}	494.08^{f}
	7	465.22^{c}	7.94^{e}	61.54^{e}	36.52^{f}	501.83^{e}
	14	468.48^{e}	8.07^{d}	63.14^{a}	36.83^{d}	513.08^{d}
	21	472.44^{a}	8.50^{c}	63.00^{b}	37.47^{a}	521.53^{a}
	28	459.60^{d}	8.80^{b}	62.05^{c}	37.07^{c}	516.23^{b}
	35	453.30^{f}	8.95^{a}	61.85^{d}	37.17^{b}	514.41^{c}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 27: Mineral content of d maize cob supplemented with peptone and inoculated with *Aspergillus oryzae*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	241.14^{g}	3.75^{g}	23.61^{g}	29.68^{g}	217.72^{g}
	4	256.75^{f}	3.96^{f}	25.42^{f}	30.05^{f}	230.78^{f}
	7	273.79^{d}	4.19^{e}	27.47^{e}	30.49^{e}	243.19^{e}
	14	273.32^{e}	4.21^{d}	28.93^{d}	30.61^{d}	249.25^{d}
	21	276.68^{c}	4.47^{c}	29.87^{c}	30.73^{c}	249.94^{c}
	28	287.12^{b}	4.92^{b}	31.37^{b}	31.81^{b}	269.03^{b}
	35	298.05^{a}	5.49^{a}	33.68^{a}	32.66^{a}	289.33^{a}
NaOH pretreated maizecob						
	0	187.44^{g}	3.38^{g}	21.32^{g}	23.82^{g}	206.51^{g}
	4	215.85^{f}	3.66^{f}	23.37^{f}	24.30^{f}	213.74^{f}
	7	224.65^{e}	3.90^{e}	24.42^{e}	24.43^{e}	234.80^{e}
	14	226.67^{d}	4.30^{d}	26.88^{d}	25.00^{d}	249.96^{d}
	21	247.88^{c}	4.51^{c}	27.39^{c}	25.03^{c}	255.4^{c}
	28	266.7^{b}	4.82^{b}	27.84^{b}	25.53^{b}	272.33^{b}
	35	288.1^{a}	5.24^{a}	30.00^{a}	26.15^{a}	296.61^{a}
Ash pretreated maizecob						
	0	433.66^{g}	7.31^{g}	58.55^{g}	36.18^{g}	477.94^{g}
	4	453.99^{f}	7.55^{f}	59.61^{f}	36.31^{f}	491.78^{f}
	7	467.90^{e}	7.77^{e}	61.09^{e}	36.71^{e}	511.00^{a}
	14	468.64^{d}	8.06^{d}	62.54^{d}	37.00^{d}	511.63^{d}
	21	468.76^{c}	8.67^{c}	62.75^{c}	37.14^{c}	513.98^{c}
	28	478.81^{b}	9.17^{b}	64.85^{b}	37.55^{b}	525.98^{b}
	35	482.71^{a}	9.34^{a}	65.02^{a}	37.87^{a}	536.85^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 28: Mineral content maize cob supplemented with chicken extract and inoculated with *Aspergillus oryzae*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	235.97^{g}	3.75^{f}	23.06^{g}	29.63^{g}	209.69^{g}
	4	256.27^{f}	3.50^{g}	24.64^{f}	29.73^{f}	222.93^{f}
	7	267.68^{e}	4.01^{e}	25.03^{e}	30.07^{e}	235.16^{e}
	14	270.84^{d}	4.33^{d}	27.64^{d}	30.26^{d}	244.78^{d}
	21	274.04^{c}	4.49^{c}	28.75^{c}	30.40^{c}	247.52^{e}
	28	284.40^{b}	4.97^{b}	31.41^{b}	31.36b	265.57^{b}
	35	294.94^{e}	5.44^{a}	32.55^{a}	32.12^{a}	280.95^{a}
NaOH pretreated maizecob						
	0	201.98^{f}	3.04^{g}	22.99^{f}	23.70^{g}	211.10^{g}
	4	226.41^{e}	3.93^{f}	43.90^{e}	24.65^{f}	224.49^{f}
	7	245.35^{d}	4.13^{e}	26.23^{d}	24.94^{e}	238.10^{e}
	14	256.62^{c}	4.47^{d}	26.31^{d}	25.33^{d}	252.16^{d}
	21	256.88^{c}	4.60^{c}	27.32^{c}	25.51^{c}	257.60^{c}
	28	274.98^{b}	4.91^{b}	28.49^{b}	25.93^{b}	283.64^{b}
	35	291.91^{a}	5.42^{a}	30.00^{a}	26.33^{a}	297.56^{a}
Ash pretreated maizecob						
	0	432.73^{g}	7.30^{g}	57.53^{g}	35.72^{g}	488.31^{g}
	4	454.88^{f}	7.75^{f}	59.23^{f}	36.16^{f}	505.98^{f}
	7	465.17^{e}	7.84^{e}	60.47^{e}	36.48^{e}	518.68^{e}
	14	479.62^{c}	8.31^{d}	63.26^{c}	37.06^{d}	532.73^{c}
	21	473.42^{d}	8.74^{c}	63.15^{d}	37.13^{c}	531.63^{d}
	28	490.65^{a}	9.26^{b}	65.42^{a}	37.77^{b}	550.50^{a}
	35	487.66^{b}	9.39^{a}	64.95^{b}	37.95^{a}	549.60^{b}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 29: Mineral content of maize cob inoculated with *Aspergillus niger*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	208.04^{g}	3.00^{e}	20.57^{g}	28.95^{f}	185.55^{g}
	4	220.24^{f}	3.12^{d}	23.06^{e}	29.17^{e}	197.31^{f}
	7	228.04^{e}	3.20^{c}	23.02^{f}	29.27^{d}	205.35^{e}
	14	235.47^{c}	3.17^{cd}	24.07^{c}	29.40^{b}	212.53^{c}
	21	232.12^{d}	3.24^{b}	23.86^{d}	29.35^{c}	210.37^{d}
	28	236.31^{b}	3.35^{d}	25.86^{b}	27.49^{g}	218.37^{b}
	35	242.69^{a}	3.58^{a}	26.27^{a}	29.75^{a}	220.41^{a}
NaOH pretreated maizecob						
	0	205.32^{f}	3.05^{f}	19.66^{f}	23.72^{f}	211.84^{f}
	4	202.16^{g}	2.89^{g}	18.86^{g}	23.79^{e}	207.9^{g}
	7	230.45^{e}	3.11^{e}	21.18^{e}	24.21^{d}	222.83^{e}
	14	234.84^{c}	3.32^{d}	21.73^{d}	24.24^{c}	232.68^{d}
	21	233.35^{d}	3.44^{c}	21.49^{c}	24.26^{b}	234.5^{c}
	28	238.97^{b}	3.50^{b}	22.44^{b}	24.21^{d}	235.47^{b}
	35	241.81^{a}	3.62^{a}	23.39^{a}	24.29^{a}	249.23^{a}
Ash pretreated maizecob						
	0	418.56^{f}	7.00^{e}	54.87^{g}	35.45^{f}	450.50^{g}
	4	432.50^{e}	7.29^{d}	55.12^{f}	35.61^{e}	455.97^{f}
	7	440.77^{b}	7.37^{e}	56.29^{d}	35.84^{c}	463.08^{d}
	14	440.63^{b}	7.41^{c}	56.72^{c}	35.93^{b}	467.33^{c}
	21	432.77^{d}	7.26^{d}	56.09^{e}	35.72^{d}	461.12^{e}
	28	446.17^{a}	7.58^{a}	58.09^{a}	36.11^{a}	481.17^{a}
	35	435.31^{c}	7.45^{b}	57.91^{b}	36.07^{a}	479.22^{b}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 30: Mineral content of maize cob inoculated with *Aspergillus oryzae*

Treatments	Days	mg/100g dry weight				
		Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	214.38^{f}	3.08^{f}	22.03^{f}	28.96^{e}	190.96^{f}
	4	223.75^{e}	3.19^{e}	23.77^{e}	29.32^{d}	204.42^{e}
	7	236.42^{d}	3.30^{c}	24.08^{d}	29.39^{cd}	209.17^{c}
	14	239.09^{c}	3.25^{d}	24.38^{c}	29.45^{bcd}	212.14^{b}
	21	236.49^{d}	3.31^{c}	23.80^{e}	29.42^{bc}	208.96^{d}
	28	242.96^{b}	3.45^{b}	25.19^{b}	29.53^{b}	212.30^{b}
	35	253.52^{a}	3.79^{a}	26.77^{a}	29.85^{a}	222.84^{a}
NaOH pretreated maizecob						
	0	233.36^{g}	3.52^{b}	21.09^{g}	24.35^{e}	238.10^{f}
	4	259.77^{f}	3.41^{c}	22.75^{f}	24.56^{d}	256.72^{e}
	7	267.16^{d}	3.58^{b}	23.80^{e}	24.75^{e}	266.27^{c}
	14	271.94^{b}	3.62^{b}	24.62^{c}	24.93^{a}	266.34^{c}
	21	271.07^{c}	3.61^{b}	24.48^{d}	24.81^{b}	260.10^{d}
	28	276.71^{a}	3.83^{a}	25.41^{b}	24.82^{b}	274.29^{b}
	35	265.53^{e}	3.91^{a}	25.71^{a}	24.76^{e}	280.06^{a}
Ash pretreated maizecob						
	0	418.83^{g}	6.99^{f}	55.17^{a}	35.68^{f}	449.35^{g}
	4	438.44^{f}	7.22^{e}	55.76^{b}	35.81^{e}	457.79^{f}
	7	448.94^{d}	7.40^{c}	57.12^{b}	36.11^{c}	467.46^{d}
	14	454.37^{b}	7.38^{d}	57.20^{b}	36.12^{c}	472.19^{c}
	21	446.36^{e}	7.23^{a}	57.06^{c}	35.90^{d}	461.35^{e}
	28	454.79^{a}	7.48^{b}	58.74^{a}	36.14^{b}	478.02^{b}
	35	451.90^{c}	7.63^{a}	58.75^{a}	36.17^{a}	483.37^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

Table 31: Mineral content of maize cob soaked in water

Treatments	Days	mg/100g dry weight Ca^{++}	Fe^{++}	K^{+}	Mg^{++}	PO_4-
Untreated maizecob						
	0	215.02^{f}	3.02^{e}	21.73^{g}	29.06^{g}	189.06^{g}
	4	225.06^{e}	3.19^{d}	23.44^{f}	29.25^{f}	198.10^{f}
	7	233.79^{d}	3.27^{c}	24.26^{c}	29.51^{d}	206.11^{e}
	14	242.36^{b}	3.34^{b}	24.08^{d}	29.55^{c}	211.75^{c}
	21	239.54^{c}	3.39^{b}	23.85^{e}	29.43^{e}	209.41^{d}
	28	242.40^{b}	3.45^{b}	24.73^{b}	29.60^{b}	219.93^{b}
	35	257.0^{a}	3.73^{a}	26.92^{a}	29.96^{a}	237.55^{a}
NaOH pretreated maizecob						
	0	243.35^{g}	3.45^{g}	23.52^{e}	24.16^{e}	242.37^{g}
	4	272.69^{c}	3.49^{f}	23.39^{f}	24.31^{d}	253.81^{f}
	7	258.46^{f}	3.59^{e}	25.44^{b}	24.43^{c}	254.30^{e}
	14	272.9^{b}	3.71^{c}	25.11^{c}	24.84^{b}	266.34^{c}
	21	269.60^{d}	3.68^{d}	24.3^{d}	23.85^{f}	263.06^{d}
	28	277.95^{a}	3.92^{b}	25.13^{c}	24.93^{a}	271.32^{b}
	35	269.29^{e}	3.98^{a}	25.64^{a}	24.94^{a}	276.29^{a}
Ash pretreated maizecob						
	0	416.38^{g}	7.05^{e}	55.90^{g}	35.40^{f}	442.99^{g}
	4	436.19^{f}	7.30^{d}	56.18^{f}	35.64^{e}	452.91^{f}
	7	442.98^{c}	7.48^{b}	57.23^{c}	35.85^{d}	463.38^{e}
	14	448.08^{b}	7.41^{c}	56.24^{e}	36.01^{c}	472.00^{d}
	21	445.46^{c}	7.33^{d}	56.99^{d}	35.89^{d}	474.56^{c}
	28	455.07^{a}	7.57^{a}	59.01^{a}	36.21^{a}	480.67^{b}
	35	443.54^{d}	7.61^{a}	58.74^{b}	36.14^{b}	483.03^{a}

Values followed by the same letter(s) on the same column are not significantly different (P≤0.05) each value represents a mean of three readings.

3.10 Chemical composition of experimental diet

The composition of experimental diets is shown in Table 32. The control (Diet 1) is the broiler finisher diet while Diets 2 to 7 represent different percentage replacement of maize with Microbial Degraded Cob (MDC) designated as Bacteria Enriched Cob Product (BECP) and Fungi Enriched Cob Product (FECP). Diet 2 contain BECP (18%) i.e. 18% replacement of maize with BECP, Diet 3, BECP (82%), Diet 4, BECP (50%); Diet 5, FECP (18%); Diet 6, FECP (82%) and Diet 7, FECP (50%).

The Chemical composition of the experimental diets, BECP and FECP are presented in Table 2. The crude protein value was highest in the control i.e. D1 with a value of 9.5% and lowest in D7, 7.7%. The crude fibre was highest in diet D3 where there was 44% replacement of total feed with bacteria degraded maize cob with a value of 13.6%. The percentage of Ash was highest in D6 with a value of 9.2 while the Ether Extract was highest in D5 with a value of 4.4%.

Table 32: Composition of the experimental diets (broiler finisher) ingredient %

Ingredient %	Diets (Kg/100kg						
	1	2	3	4	5	6	7
Maize	54.00	44.00	10.00	27.00	44.00	10.00	27.00
Bacteria Enhanced cob Product (BECP)	-	10.00	44.00	27.00	-	-	-
Fungi Enhanced Cob Product (FECP)	-	-	-	-	10.00	44.00	27.00
Wheat offal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Groundnut cake	15.20	15.20	15.20	15.20	15.20	15.20	15.20
Soybean meal	12.50	12.50	12.50	12.50	12.50	12.50	12.50
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Broiler premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Salt (Nacl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100	100

Composition of premix supplying the following per kg of feed. Vit A= 5000IU Vit D3=888,000IU, E=12, 000 mg, Vit K=32,000 mg, Vit B= 1,000 mg.

Pantothenic acid = 200mg, biotin= 10,000mg; Vit B2=2,000 mg, Vit B6= 1,500mg Vit B12=3,000mg, folic acid = 15,000mg, chloride= 60,000mg, Manganese 10,000mg, Iron=15,000mg, Zinc=800mg, Copper=400mg; Iodine=80mg; Cobalt= 40mg, Selenium= 8,000mg.

Table 33: Chemical composition of the experimental diet and microbial degraded maize cob (BECD and FECD) (on dry matter basis)

Nutrients %	Treatments/Diets								
	1	2	3	4	5	6	7	BECP	FECP
Dry matter (DM)	91.37	91.53	91.30	90.77	91.80	91.23	92.20	91.30	91.23
Moisture Content%	8.13	8.47	8.70	9.23	8.20	8.77	7.80	8.70	8.77
Crude Protein (CP) %	9.47	9.03	8.50	7.90	8.63	8.43	7.67	9.05	6.71
Crude fibre (CF)%	12.27	13.30	13.60	12.10	11.50	12.83	13.33	23.77	4.52
Ether extract (EE)%	4.17	4.17	4.37	4.13	4.33	4.17	4.23	2.41	3.47
Ash%	7.47	5.67	4.97	8.10	7.23	9.27	8.57	3.51	2.74
Carbohydrate (By difference)	58.17	59.43	60.10	58.43	60.13	56.53	58.40	52.65	56.79
Nitrogen-free extract (NFE)%	66.62	67.83	68.56	67.77	68.31	65.30	66.20	61.26	65.56
Metabolisable energy (kcal/kg)	3053.17	3079.85	3102.35	3030.54	3095.04	2967.83	2976.52	2704.79	2856.72

Calculated according to the formula for Pauzenga (1985) and Igwebuike *et al.*, (2009).

M E (Kcal/kg)= 37 x % CP + 81 x % EE + 35.5 x % NFE

MDC= Microbial Degraded Con-cob

3.11 Performance of broiler chicken fed graded levels of microbial degraded maize cob (MDC)

Results of performance parameters are presented in Table 34. There were no significant differences in the mean feed intake, body weight gain and feed conversion ratio in diets 1, 2, 4, 5 and 7. These indicate up to 18% and 50% replacement of Maize with BECP and FECP in Broiler finisher diets. However, there were significant differences in the mean feed intake, body weight gain and feed conversion ratio of diets 3 and 6 where there was 82% replacement of maize with BECP and FECP in boiler diet compared to the control and other diets. A significant difference was observed between the chicken fed diet 1 and 3 as seen in Plate 1.

The economic performance of the Boiler chicken presented in Table 35 showed that cost per kilogram of the feed decreased steadily as the level of microbial degraded Cob (MDC) increased in the diet. Similarly, total feed cost followed the same trend. The best cost/kg gain of ₦185.7 was recorded in diet 5 with FECP (18%) in the broiler finisher feed. This was followed by diet 2 (₦208.4) with BECP (18%) and diet 7 (₦227.4) with FECP (50%). All these were lower than the control cost of ₦235.9 which was the commercial feed ingredient

Table 34: Performance of broiler chicken fed graded levels of microbial degraded maize cob (BECP and FECP)

	Treatments/Diets						
Parameters	G_1D_1	G_2D_2	G_3D_3	G_4D_4	G_5D_5	G_6D_6	G_7D_7
Initial weight (g)	600^{b}	580^{c}	645^{a}	600^{b}	540^{c}	530^{c}	570^{c}
Final weight (g)	2040^{a}	2160^{b}	1400^{f}	1900^{c}	2175^{b}	1588^{e}	1860^{d}
weight gain (g)	$1,440^{b}$	1580^{a}	755^{e}	1300^{c}	1635^{a}	1058^{d}	1290^{c}
Mean daily weight gain (g)	34.28^{c}	37.62^{b}	17.98^{g}	30.95^{d}	38.93^{a}	25.19^{f}	30.71^{e}
Feed intake (g)	5190^{d}	5310^{c}	6351^{a}	5370^{b}	4852^{g}	5020^{f}	5105^{e}
Mean daily feed intake (g)	123.57^{d}	126.43^{c}	151.21^{a}	127.85^{b}	115.52^{g}	119.52^{f}	121.54^{e}
Feed Conversion Ratio (FCR)	3.60^{e}	3.36^{f}	8.41^{a}	4.13^{c}	2.97^{g}	4.74^{b}	3.96^{d}
Feed Utilization (FU)	0.28^{ab}	0.30^{a}	0.12^{d}	0.24^{c}	0.34^{a}	0.21^{cd}	0.25^{bc}
Mortality	-	-	3	-	2	1	-

Table 35 **Economic performance of broilers chicken fed with graded levels of microbially degraded maize cob (BECP and FECP)**

	Treatments/Diets						
Parameters	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**
Levels of substitution							
BECP (%)	0	18	82	50			
FECP (%)					18	82	50
Initial weight (g)	600[b]	580[c]	645[a]	600[b]	540[d]	530[d]	570[c]
Final weight (g)	2040[a]	2160[b]	1400[f]	1900[c]	2175[b]	1588[e]	1860[d]
Total weight gain (kg)	1.44[b]	1.58[a]	0.76[e]	1.30[c]	1.64[a]	1.06[d]	1.29[c]
Total feed intake (kg)	5.19[d]	5.31[c]	6.35[a]	5.37[b]	4.85[g]	5.02[f]	5.11[e]
Cost/kg feed (₦/kg)	65.52[a]	62.52[b]	52.32[d]	57.42[c]	62.52[b]	52.32[d]	57.42[c]
Feed cost/kg gain (₦/kg)	235.87[d]	208.39[f]	440.07 [a]	237.14[c]	185.68[g]	248.00[b]	227.38[e]

Cost per kilogram of the various ingredient used to compound the experimental diets. Maize= ₦56.00, Wheat offal= ₦30.00;
Microbial degraded cob = ₦26.00. Groundnut cake = ₦60.00 Soybean meal = ₦80.00; Bone meal = ₦35.00; Salt = ₦60.00, Broiler premix = ₦600.00 and Methionine = ₦1,300.00 Fish meal= ₦150.00.

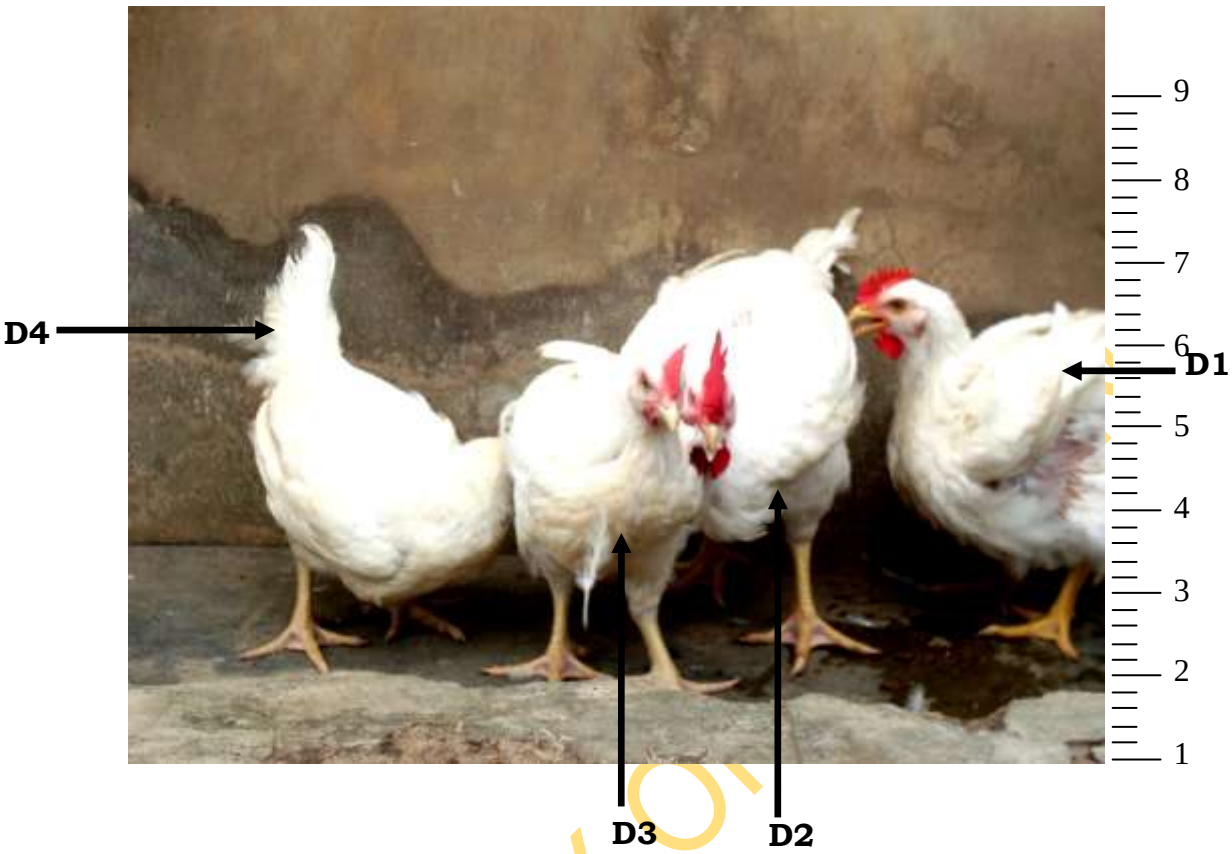


Plate 1: Experimental birds fed with 0%, 18%, 82% and 50% BECP.

D1 – Control Bacteria-enriched cob product (0%)

D2 – BECP (18%)

D3 – BECP (82%)

D4 – BECP (50%)

Photograph of experimental birds fed with Bacteria Enriched Cob Product (BECP)

3.12 Haematological indices and blood chemistry in broiler chicken fed diets containing graded levels of microbial degraded cob (MDC)

The haematological and differential counts are shown in Table 36. The PCV value rated below (20.0 – 33.0%), Hb (6.5 – 10.6g/dl), RBC (1.7 – 2.6 x $10^6/mm^3$) did not differ significantly (P>0.05) among the treatment. However, the value of the white blood cell (WBC) was much higher in D3 and D6 where there was BECP (82%) and FECP (82%) (27.2 and 20.4 x $10^3/mm^3$) compared to the other values D1-D7 which ranged between 11.2 and 16.2 x $10^3/mm^3$. The MCV, MCH and MCHC were similar (P>0.05) in all treatments.

The differential counts (%) of monocytes and eosinophils were also not significantly different (P>0.05). However, lymphocytes and heterophils were significantly different (P<0.05). The blood chemistry parameters in Table 37 showed that there were no significant difference between the total protein, globulin and A.G. Ratio albumin had a higher value in D3,BECP (82%); D6, FECP (82%) and D7 FECP (50%) i.e. 2.5, 2.0 and 2.1 respectively compared to 1.5 in D1, control; 1.6 in D5, FECP (18%) and 1.8 in both D2 and D4 i.e. BECP (18%) and BECP (50%). There were significant differences in Getinine, AST and ALT.

Table 36: Haematological indices in broiler chickens fed with diets containing graded levels of microbially degraded cob (MDC).

	Treatments/Diets							
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	
BECP		18	82	50				
FECP	0				18	82	50	
Indices	1	2	3	4	5	6	7	SEM
PCV%	23.00^{e}	20.00^{g}	33.00^{a}	25.00^{d}	28.00^{b}	26.00^{c}	22.00^{f}	0.05
Hb (g/d1)	6.80^{d}	6.50^{e}	10.60^{a}	7.80^{c}	8.50^{b}	8.40^{b}	6.60^{e}	0.05
RBC (x106/mm^3)	1.84^{d}	1.69^{f}	2.58^{a}	2.42^{b}	2.50^{a}	2.33^{c}	1.77^{e}	0.016
WBC (x103/mm^3)	13.40^{e}	13.35^{e}	27.20^{a}	14.80^{d}	11.20^{f}	20.35^{b}	16.15^{c}	0.03
MCHC (g/de)	29.56^{g}	32.50^{a}	32.12^{c}	31.20^{d}	30.36^{c}	32.31^{b}	30.00^{f}	0.027
MCH (Pg)	36.96^{d}	38.46^{b}	41.09^{a}	32.23^{g}	34.00^{f}	36.05^{e}	37.29^{c}	0.016
MCV (F1)	125^{b}	118.34^{d}	127.90^{a}	103.31^{g}	112.0^{e}	111.59^{f}	124.29^{c}	0.05
Differential counts (%)								
Monocycles	2.00^{b}	2.00^{b}	2.00^{b}	0.00^{d}	1.00^{c}	6.00^{a}	2.00^{b}	0.042
Lymphocytes	69.00^{d}	72.00^{b}	70.00^{c}	79.00^{a}	65.00^{e}	25.00^{g}	58.00^{f}	0.05
Heterophils	25.00^{e}	23.00^{f}	27.00^{d}	18.00^{g}	29.00^{c}	65.00^{a}	33.00^{b}	0.05
Eosinophils	4.00^{c}	3.00^{d}	1.00^{e}	3.00^{d}	5.00^{b}	4.00^{c}	7.00^{a}	0.05

Values with different superscript are significantly different at (P<0.05) SEM=Standard Error of Mean abc= means in the same row bearing different superscripts differ significantly (P<0.05), PCV= Packed cell volume, Hb= Haemoglobin concentration, RBC= Red Blood Cell counts, WBC=White Blood Cell counts, MCV=Mean corpuscular volume, MCH=Mean Corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration.

Table 37: Blood chemistry in broiler chicken fed with diets containing graded levels of microbially degraded maize cobs

	Treatments/Diets						
	D1	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**
BECP	0	18	82	50			
FECP					18	82	50
Diet	1	2	3	4	5	6	7
Total protein	3.20^{c}	2.90^{e}	3.40^{b}	2.70^{e}	3.00^{d}	3.60^{a}	2.50^{f}
Albumin	1.50^{d}	1.80^{c}	2.50^{a}	1.80^{c}	1.60^{d}	2.00^{b}	2.10^{b}
Globulin	1.70^{bc}	1.10^{e}	0.90^{f}	1.90^{a}	1.40^{d}	1.60^{c}	0.40^{g}
A.G. Ratio	1.10^{a}	0.60^{c}	0.40^{d}	0.50^{cd}	0.90^{b}	0.80^{b}	0.10^{e}
Getinine	0.20^{e}	0.60^{c}	0.40^{d}	1.00^{b}	1.20^{a}	0.20^{e}	0.60^{c}
AST	157.00^{a}	140.00^{d}	152.00^{b}	140.00^{d}	138.00^{c}	133.00^{f}	145.00^{c}
ALT	64.00^{c}	64.00^{c}	62.00^{d}	74.00^{a}	56.00^{e}	52.00^{f}	72.00^{b}

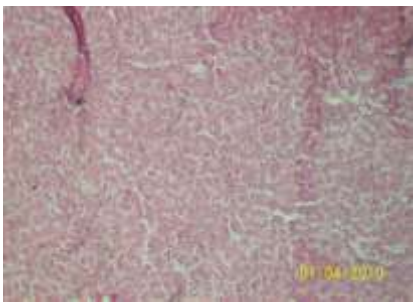
1 – 7 refers to Diet formulation

3.13 Histopathological studies of experimental birds

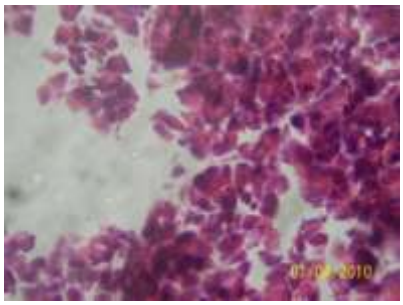
The Liver, Kidney, Lungs and Pancreas of experimental birds were examined for pathological symptoms (Table 38). There were no visible lesion seen in the liver of all the Birds except in diet 7 where a few foci of necrosis and cellular infiltration by mononuclear cells were seen (Plate 4). The kidney showed marked congestion in chicken fed diet 3 and 5 (Plate 2). Generalized and severe congestion of the lungs was observed in chicken fed Diet 3 while the others shows no visible lesion or very mild congestions (Plate 3). There were no visible lesion in the pancreas of all the birds in the different diets as seen in plate 5.

Table 38: Histopathological studies of experimental birds organs

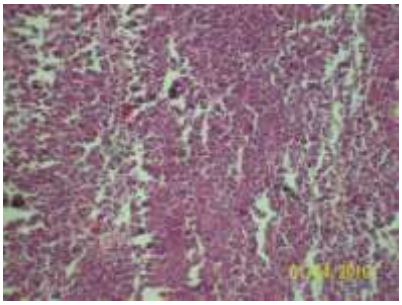
Treatment/ Diet	Liver	Kidney	Lungs	Pancreas
D1	No visible lesions seen	The Interstitial (lymphoid) compartment is reduced in density	Very mild congestion	No visible lesions seen
D2	Tissues scattered	The interstitial (lymphoid) compartment is reduced in density	No visible lesions seen	No visible lesions seen
D3	No visible lesions seen	Marked congestion	Generalized and severe congestion	No visible lesions seen
D4	Mild congestion	No visible lesions seen	No visible lesions seen	No visible lesions seen
D5	No visible lesions seen	Marked haemorhage in the intestitium few tubular cells are necrotic	Generalized congestion	No visible lesions seen
D6	No visible lesions seen	Interstitial congestion and haemorhage	No visible lesions seen	No visible lesions seen
D7	There are few foci of necrosis and cellular infiltration by mononuclear cells.	Mild congestion	No visible lesions seen	No visible lesions seen



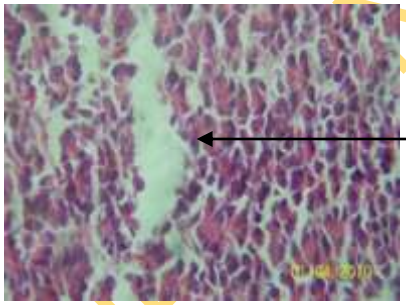
D1 LV



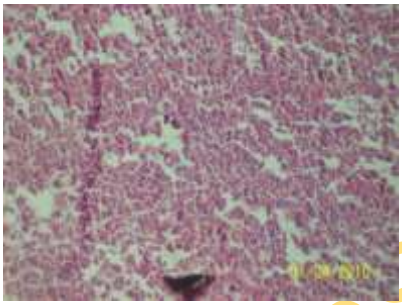
D2 LV



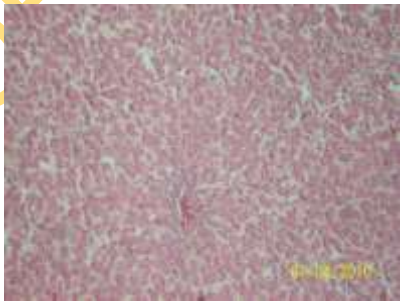
D3 LV



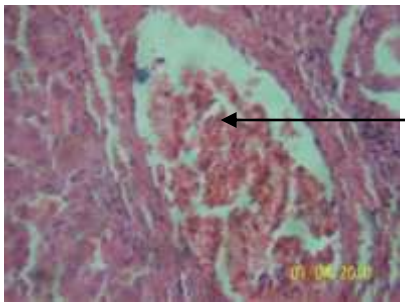
D4 LV



D5 LV



D6 LV



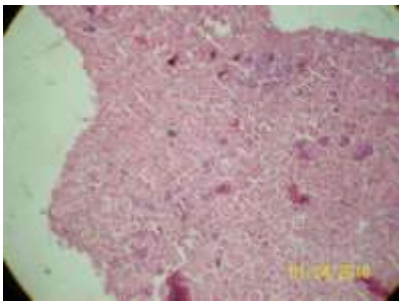
D7 LV

Plate2: Photomicrograph of the liver of experimental birds showing the tissues of the liver. congestion of blood vessels is shown in the liver of diets 4 and 7

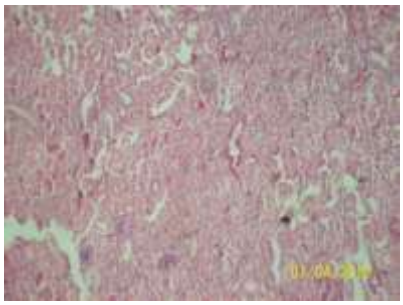
D – Diets

1-7 – 1 = control, 2 = Bacteria –enriched cob product 18%, 3 = BECP 82%, 4 = BECP 50%, 5 = Fungi-enriched cob product 18%, 6 = FECP 82%, 7 = FECP 50%

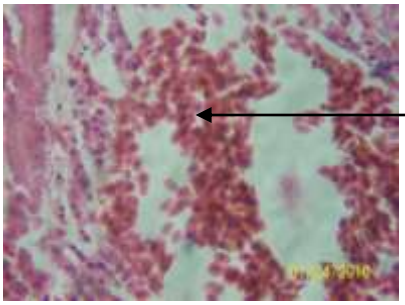
LV – Liver



D1 KD

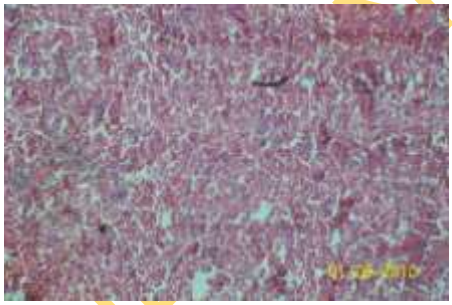


D2 KD

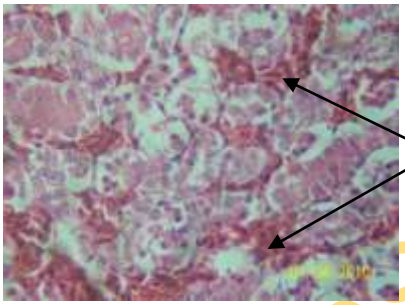


Marked Congestion

D3 KD

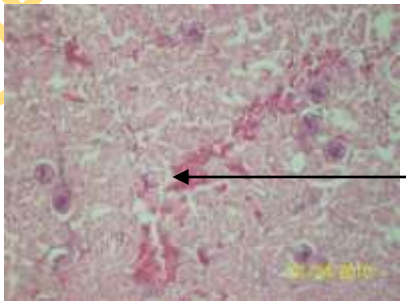


D4 KD



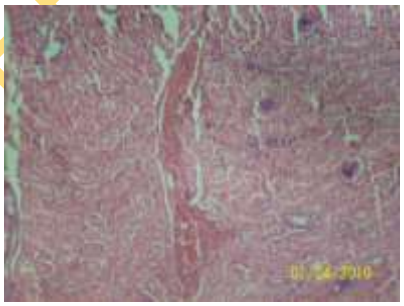
Marked Haemorhage

D5 KD



Haemorhage

D6 KD



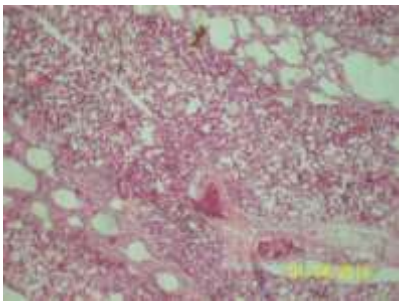
D7 KD

Plate3: Photomicrograph of the kidneys of experimental birds showing the tissues of the kidney. Tissues of diets 5 and 6 shows haemoragic cells while marked congestion of interstitial cells is seen in diet 3.

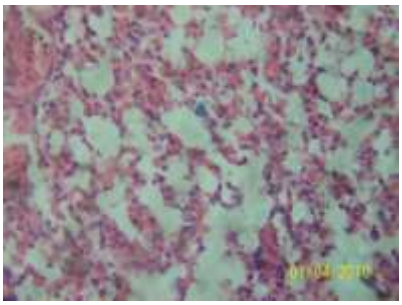
D – Diets

1-7 – 1 = control, 2 = Bacteria –enriched cob product 18%, 3 = BECP 82%, 4 = BECP 50%, 5 = Fungi-enriched cob product 18%, 6 = FECP 82%, 7 = FECP 50%

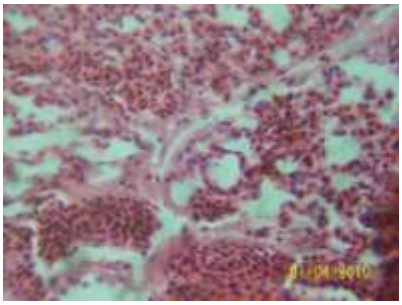
KD – Kidney



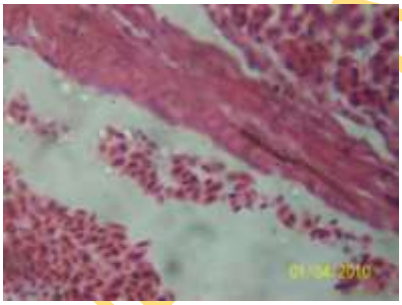
D1 ET



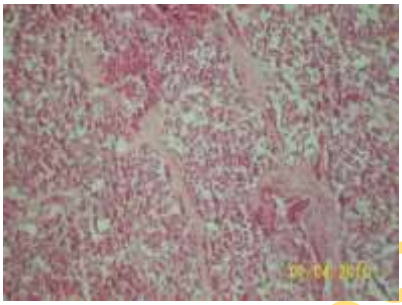
D2 ET



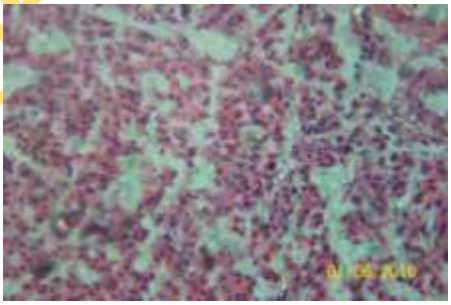
D3 ET



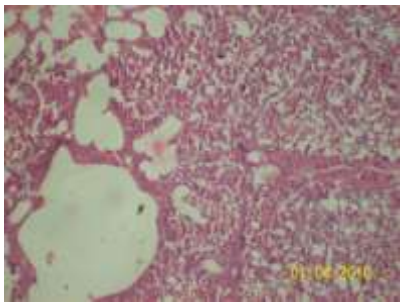
D4 ET



D5 ET



D6 ET



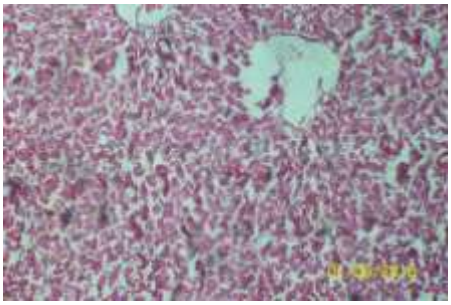
D7 ET

Plate4: Photomicrograph of the lungs of experimental birds showing the tissues of the lungs.

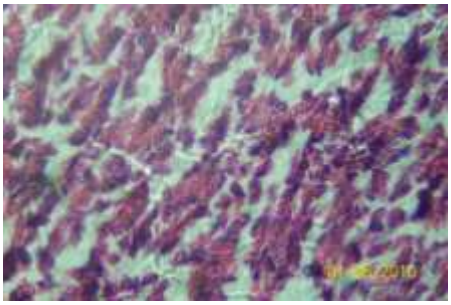
D – Diets

1-7 – 1 = control, 2 = Bacteria –enriched cob product 18%, 3 = BECP 82%, 4 = BECP 50%, 5 = Fungi-enriched cob product 18%, 6 = FECP 82%, 7 = FECP 50%

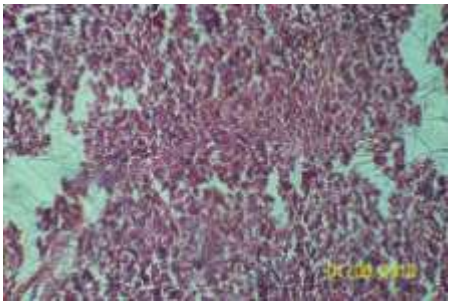
ET – Exchange Tissue (Lungs)



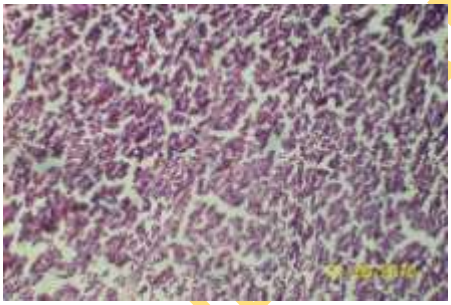
D1 PANCREAS



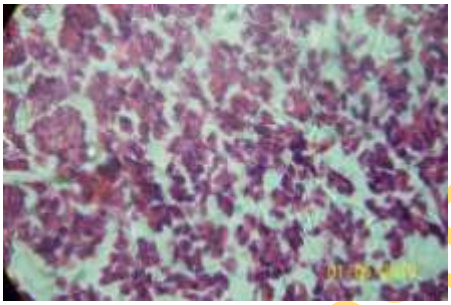
D2 PANCREAS



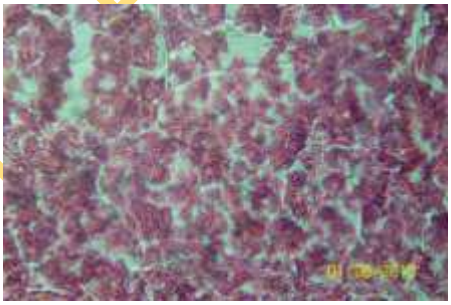
D3 PANCREAS



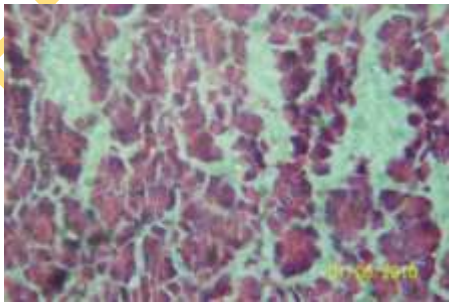
D4 PANCREAS



D5 PANCREAS



D6 PANCREAS



D7 PANCREAS

Plate5: Photomicrograph of the pancreas of experimental birds

D – Diets

1-7 – 1 = control, 2 = Bacteria –enriched cob product 18%, 3 = BECP 82%, 4 = BECP 50%, 5 = Fungi-enriched cob product 18%, 6 = FECP 82%, 7 = FECP 50%

CHAPTER FOUR

DISCUSSION

The occurrence of bacterial and fungal species in degrading maize cob has been confirmed in this study. The bacteria isolates were found to show cellulolytic activity. This is consistent with the findings of Emtiazi *et al.* (2007) who reported that most microorganism associated with plant might have cellulase activity for adoption on establishment of a plant microbe interaction. The highest cellulolytic activity was observed in *Bacillus subtilis* with reducing sugar value of 0.7mg/ml on day 7 (Fig. 1). Cellulolytic property of bacteria species like *Pseudomonas, Cellulomonas, Bacillus* and *Micrococcus* has been reported (Immanuel *et al.,* 2006). Carboxylmethyl cellulase (CMcase) activities were shown in *Bacillus pumilus,* (Graw and Wilson, 1961, Poorma and Prema, 2007) *Bacillus sphaericus,* (Singh *et al.,*2004); *Bacilus circulans* (Barel, 1990) and *Bacillus subtilis* (Amritkar *et al.,* 2004). For fungal isolates, the highest reducing sugar of 1.7mg/ml was found in *Aspergillus niger* on day14 (Fig. 2). Fungi of the genera *Aspergillus* and *Penicilluim* have been reported as good cellulase producers (Hoffman and Wood, 1985, Milala *et al.,* 2005, Abo State *et al.,* 2010).

The production of cellulase under different physiological conditions by *Pseudomonas putida, Bacillus subtilis, Bacillus licheniformis I* and *Bacillus licheniformis II* in liquid medium was studied. The result of cellulase activity at different temperatures by the bacterial isolates revealed that cellulase activity was highest for *Bacillus subtilis* at 28^{o}C with a value of 0.5mg/ml. This differed from the result of Shabeh *et al.* (2010) who reported optimum temperature for celulase production by *Bacillus subtilis* at 45^{0}C. *B. licheniformis I* and *II* had the highest production at 40^{o}C with a value of 0.5mg/ml and 0.4mg/ml respectively. *B subtilis* also had the highest cellulase production on day 4 at 40^{o}C. This agreed with the findings of Kahen and Husaini (2006) who reported 40^{o}C as the optimum temperature for maximum cellulase activity by *Bacillus.* Cellulase production was observed to be highest at pH 7.0 on day 7 in *Bacillus subtilis* with a value of 0.5mg/ml (Fig. 4). Other Bacterial species also had the highest production at pH 7.0 except *Bacillus licheniformis I* whose highest production of 0.4mg/ml at pH 5.5. This agreed with Ugwuanyi *et al.,* (2008) who reported aerobic digestion of corn cob waste by *Bacillus stearothermophillus* at pH 7.0. Krairitthichai and Thongwai (2008) also found

the optimum cellulase activity of *Bacillus sp* isolated from the soil of Northern Thailand to be at 45^{o}C and pH 7.0. When different nitrogen sources were used, peptone was observed to induce the highest cellulase activity with value of reducing sugar of 0.8mg/ml on day 4 in *B. licheniformis II.* *B. licheniformis I* had its highest reducing sugar value of 0.7mg/ml on day 4 in NH_4Cl while *B. subtilis* had its highest value of 0.7mg/ml in urea on day 4 (Fig. 5a and 5b). *Bacillus subtilis* and *B. licheniformis I* had the highest reducing sugar production in yeast extract, peptone and urea. This agreed with the result of Deka *et al.* (2011) who reported that peptone and yeast extract significantly and positively affected the cellulose enzyme production by *Bacillus subtilis* showing six fold increase as comapred to unoptimized medium. Li *et al.* (2008) also reported that yeast extract and peptone have significant effect on cellulase production.

The fungal isolates had their highest reducing sugar production at 40^{o}C except *Penicillium expansum* and *Rhizopus oryzae* (Fig. 6). This agreed with Immanuel *et al.* (2007) who reported the optimum temperature for cellulase enzyme production by *Aspergillus niger* and *Aspergillus fumigatus* at 40^{o}C. The optimum pH for *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus oryzae* was pH 4.0 while *Penicillum expansum* and *Aspergillus oryzae* was at pH 5.5. Akiba *et al.*,(1995) reported that CMCase production was high at pH 4 and 4.5 by *Aspergillus niger*. Optimum activity for *Aspergillus niger*, *Aspergillus oryzae* and *Penicillum expansum* occurred in peptone. This agreed with the result of Guatam *et al.*, (2011) who reported that peptone enhanced the production of cellulase by *A niger* on solid municipal waste residue.

The crude protein production increased significantly throughout the period of fermentation. The observed enhancement of maize cob in terms of the crude protein has been reported earlier by several workers (Adeyemi and Adeyemi, 2000; Adeyemi and Familade, 2003 and Oduguwa *et al.*, 2007). The increased crude protein in fermented maize residue may be due to proliferation of microorganism (Familade and Babayemi, 2009) and utilization of the substrate for microbial cell biomass (Oduguwa *et al.*, 2007). The highest crude protein value of 9.5% was observed in untreated maize cob supplemented with chicken extract and inoculated with *Bacillus licheniformis* compared with that of NaOH pretreated and ash pretreated maize cobs with 7.5% and 8.0% units respectively. This showed that pretreatment of the Maize cob did not have any effect on

the crude protein production. This was contrary to the result obtained by Pece *et al.* (1990) in which pretreatment of maize cob with NaOH led to biomass yield and protein production. The highest percentage of cellulose degraded was also observed in untreated maize cob supplemented with chicken extract and inoculated with *Bacillus subtilis.* The value of 6.6% crude protein was higher than 4.5% and 4.9% obtained in NaOH pretreated and Ash pretreated maize cob respectively. This was contrary to the result obtained by Familade and Babayemi, 2009 who observed that cellulose and hemicelluloses were significantly ($P<0.05$) reduced in treated maize cob when compared with control.

Fungal fermentation of maize cob with nutrient supplementation and alkali pretreatment was also observed with reference to its chemical composition. (Tables 11-17). Crude protein value of the maize cob at the beginning of the experiment was within the range of 2.4 – 3.4% reported by Fetuga *et al.* (1975), Longe and Tona, (1988) and Adeyemi and Familade (2003) but much higher than the value of 1.8% crude protein and 0.7% ether extract reported by Isikwenu *et al.* (2000). Discrepancies such as these may be due to varietal differences and post harvest management of maize cob (Adeyemi and Familade, 2003). There were significant differences in crude protein production from day 0 to day 35 and crude protein increased throughout the period of fermentation. The highest crude protein value of 7.6% was observed in ash pretreated maize cob supplemented with chicken extract and inoculated with *Aspergillus niger*. This was higher than in the untreated and NaOH pretreated Maize cob which were 7.05% and 7.2% respectively.

The highest percentage (%) of cellulose degraded 17.3% was also found in the same table where Ash pretreated maize cob was inoculated with *Aspergillus niger* and supplemented with chicken extract. Microbes are able to synthesis beta glucose (Babayemi *et al.* 2009) which is used by the microbes to break down cellulose, hemicelluloses and phenolic polymers. The highest percentage of cellulose degraded in bacteria fermented cob was 6.6% which was far lower than the 17.3% degraded in fungal fermented maize cob. The result showed that fungi were better cellulose degraders than bacteria. The hyphae mode of growth of fungi which penetrates the substrate coupled with their ability to grow at low water activity and high osmotic pressure condition makes fungi efficient and competitive in natural microflora for bioconversion of solid substrate

(Faniyi, 2006). The crude fiber also decreased significantly during the maize cob fermentation. This was also supported by the work of Oduguwa *et al.* (2007) who also recorded a decrease in the crude fiber during maize cob fermentation with fungi. Fermentation generally reduced crude fibre content in crop residues especially when fermented with fungi (Belewu, 2003).

In the study to determine the effect of bacteria fermentation on the mineral content of the maize cob, the mineral present in the maize cob include calcium, potassium, magnesium, phosphorus and iron. These are essential macroelements required to facilitate catalytic actions in enzyme systems for efficient metabolic processes. Studies on the mineral composition of the substrate revealed the presence of calcium, potassium, magnesium and phosphorus in higher concentration than the other elements (Table 18-24). This agrees with Mullen *et al.* (2010) who reported that important plant mineral nutrients such as potassium, phosphorous, calcium and magnesium are concentrated in the maize cob ash. Singh and Moore (1982) in their analysis of the starter mash also observed that the calcium content was highest i.e. 1% followed by Phosphorus 0.6%, Nacl was 0.50% while Potassium was 0.2% per 0.45kg of ration. There were increase in the amount of minerals present in the fermentation media throughout the period of fermentation. The mineral content of the maize cob after fermentation conform to the mineral requirement of poultry which is about 3kg in 100kg of feed (Singh and Moore, 1982). This study revealed that the fermentation of maize cob improves the essential mineral content of the cob thereby making it more acceptable as a replacement for maize in poultry feed. In his work on fermented poultry liter as chicks feed, Onilude (1999) also reported a significant increase in the amount of potassium, calcium, and phosphorus.

The effect of fungal fermentation on the mineral content of the maize cob was also studied (Tables 25-31). The calcium, magnesium and phosphorus concentrations during and after fermentation was found to be higher than that of the bacteria fermented maize cob. Adejoye (2007) also observed that samples fermented by *P. giganties* recorded a higher concentration of these elements than in other fermented samples. The high concentration of calcium, magnesium and phosphorus in the substrate after fermentation may be attributed to nutrient composition of the substrate which may

determine the availability of these elements. This agreed with the result Oseni and Ekperigin (2007) who reported high level of magnesium, potassium and calcium in maize cob and shaft fermented with *Aspergillus niger*. However, increase in calcium content after fermentation was contrary to the findings of Cooke and Whips (1993) and Adejoye (2009).

The chemical composition of the experimental diets, Bacteria-enriched cob product (BECP) and Fungi enriched cob product (FECP) gave crude protein values 7.7-9.5% which were lower than the recommended range (20-22%) for broiler finisher diets (Olomu, 1995). The crude fiber ranged between 11.5 to 13.3% and it increased with increasing level of BECP and FECP in the broiler finisher diets. These were higher than the recommended values (Olomu, 1978). The crude fiber value was markedly lower in FECP when compared to BECP. Fungi are the main cellulase producing microorganisms though a few bacteria and actinomycetes have also been reported to yield cellulase activity (Immanuel *et al*., 2007). The Ether extract (2.4 to 4.4%) was adequate to meet the fatty acid requirement for broiler finishers. A slightly lower value was obtained for Nitrogen Free Extract (NFE) in diet 6 and 7, the reduction in NFE in feed compounded with FECP may be linked with the activities of the microflora i.e. the utilization of the soluble portions of the carbohydrate (which constitute the NFE) by microbes (Anupama, 2000).

Result of performance parameters (Table 34) showed that there was no significant difference in mean feed intake, body weight gain and feed conversion ratio in Diets 1 (control), 2 (BECP 18%), 4 (BECP 50%), 5 (FECP 18%) and 7 (FECP 50%). This indicates up to 50% replacement of Maize with BECP or FECP in broiler finisher diet had no adverse effect on the performance characteristics of the broiler. Adeyemi and Familade, (2003) also observed that the average feed intake was similar across dietary treatment and up to 20% fermented maizecob replacement of maize also shows little difference in the observed weight gain of the chicken. However, there were significant differences in the mean feed intake, body weight gain and feed conversion ratio of the diet 3 (BECP 82%) and 6 (FECP 82%) where there was 82% replacement of maize in broiler diet. The higher level of fibre may have led to depressed growth. This result agreed with that of Opara (1996) and Iyaji (2001) who observed that additional level of

fibre in diets of animal depressed growth. Moreover, feeds that are high in fibre content lead to higher feed intake as birds will eat to satisfy their energy requirement. The lower weight gained by chicken fed diet 3 and 6 can also be observed Table 34 especially in diet 3 where we have the highest mortality of 3. A significant difference was observed between chicken fed diet 1 and 3 as seen in Plate 1. Higher fiber level have been implicated in lowering digestive efficiency leading to lower dry matter digestibility (Akpe *et al.*, 2007).

The Economic performance (Table 35) showed that cost per kilogram of the feed decreased steadily as the level of BECP and FECP increased in the diets. Similarly the total feed cost followed the same trend. Igwebuike *et al.* (2009) and Ekeniyen *et al.*,(2006) also observed that the cost per kilogram of the feed decreased steadily as the level of YPM used to replace maize in the diet increased. The cost per kilogram gain of Diet 3 (₦440.0) which represents BECP 82% replacement of maize in the total feed compared with the cost/kg gain of Diet 6 (₦248.0) FECP (82%) suggest that poultry utilization of fungal degraded cob was better than the bacteria degraded maize cob. Diet 5 FECP (18%) recorded the best cost per kilogram gain (₦185.7) followed by Diet 2 BECP (18%) with a cost of ₦208.4. In Diet 7 FECP (50%) the total cost per kilogram gain (₦227.4) was still lower than that of the control (₦235.9). Bioconversion of agricultural and industrial wastes to protein rich food has an additional benefit of making the final product cheaper. (Anupama, 2000) This would affect the negative cost value of waste used as substrate to yield SCP.

The haematological indices in broiler chickens fed diet containing graded levels of BECP and FECP and differential counts revealed that there were no significant difference between Packed Cell Volume (PCV) the Haemoglobin concentration (Hb) and Red Blood Cell (RBC) (Table 36). These values fall within the normal range for healthy broiler chickens as reported by Anon (1980) and Mohammed *et al.* (2009). However, the White Blood Cell counts (WBC) was significantly different. The WBC counts was much higher representing 27.2 and $20.4x10^3$ mm^3 in broiler chicken fed with diet 3 BECP (82%) and diet 6 FECP (82%). This may have been synthesised upon immunological indications (Fasina *et al.*, 1999) of disease condition and the readiness of the immune system for defence (Kehinde *et al.*, 2009). Hackbath *et al.* (1983) reported that diets have

very strong influence on haematological traits. Robert *et al*. (1993) opined that the higher the value of WBC the better the ability of birds to fight against diseases. The differential counts of Monocytes, Lymphocytes, Heterophils and Eosinophils were also significantly different ($P<0.05$). Alteration in the quantitative and qualitative composition of the haematology as well as biochemical constituents of the blood are warning signals on and an indication of impared functions (Oloyede *et al.*,2004). Babatude and Pond (1987) showed that performance traits and haematological traits are strongly correlated and this assertion was demonstrated in the present study.

The blood chemistry (Table 37) showed that there was no significant difference between total protein, Albumin, Globulin and A. G. ratio, Getinine, AST and ALT. Total protein was higher 3.6 and 3.4 in diets 3 and 6 (in which there was 82% replacement of maize with BECP and FECP) than that of the control and other diet which ranges between 2.5 to 3.2. Kehinde *et al.,* (2009) explains that enhanced blood protein level is associated with stress on the kidney, liver and spleen. These increased Serum ALT of birds fed diet 4 and 7, 74 and 72 compared to 52 to 64 obtained in the other birds fed diet 1 – 3 and 6 may be implicated in the necrosis observed in the liver of birds fed diet 7. Coles (1986) observed that increase in serum ALT is specific for hepatic disorder especially in dog and cat.

The organs of the experimental birds which include the liver, kidney, lungs and pancreas were examined for pathological symptoms. There was no visible lesion seen in the liver and pancreas of all the groups except in Diet 7 where there were a few foci of necrosis (plate 4) in the liver. This also correlates with the result of the blood indices in which the PCV, HB and RBC blood count falls within the normal range for healthy broiler chickens (Anon, 1980). The necrosis seen in the liver of birds in diet 7 may be due to the increase in total blood protein observed (Table 37). According to Kaneko (1989) increase in total protein and globulin concentration in serum has been associated with liver disease. The kidney however showed marked congestion in chicken fed diet 3 and 5 (plate 2) High level of blood protein (Table 37) observed in Diet 3 and 5 may cause stress on the kidney (Kehinde *et al.*, 2009). Generalized and severe congestion of the lungs was also observed in Chicken fed diet 3 large number of lymphocyte produced

by chicken in diet 3 (Table 36) may have immunological implicating to suggest readiness to fight a disease condition (Fasina *et al.* 1999).

These results showed that up to 50% replacement of maize broiler finisher feed with BECP or FECP was tolerable for broiler chicken. Fungi degraded cob was also more acceptable than acteria degraded cob.

CONCLUSION AND RECOMMENDATIONS

Two bacterial isolates *Bacillus subtilis* and *Bacillus licheniformis* and two fungal isolates *Aspergillus niger* and *Aspergillus oryzae* were selected for solid state fermentation of the maize cob using alkali pretreatment and nutrient supplementation. The bacterial fermentation increased the percentage of crude protein more than fungal fermentation while the percentage of cellulose degraded in bacterial fermentation was very low compared to that of fungal fermentation. Supplementation with chicken extract enhances the organism's utilization of the maize cob for protein production and cellulose degradation. It is recommended that fungal fermentation with maize cob using chicken extract as supplement could enhance cellulose degradation.

Alkali pretreatment of the maize cob did not affect bacterial fermentation positively whereas ash pretreatment enhanced fungal fermentation hence ash pretreatment is therefore recommended for fungal degradation of maize cob.

Fermentation of the maize cob with both bacteria and fungi increased the essential mineral content of the substrate. However ash pretreated maize cob increased the level of the minerals in the substrate more than in the NaOH pretreated and untreated. It is proposed that ash pretreatment of maize cob for batch fermentation should be carried out to determine its effect on animal feed production.

Batch fermentation using *Bacilus subtili*s and *Aspergillus niger* solely with chicken extract supplementation was done and poultry feed were compounded using the resulting bacteria-enriched cob product (BECP) and fungi enriched cob product (FECP) to replace maize at different concentrations/percentages. The resultant diets when fed to the broiler chicken as finisher feed showed no lethal effect on the chicken. This showed that BECP and FECP can be used to replace Maize at 50% and can be acceptable to the poultry. Diet 3 (D_3) which represent BECP (82%) replacement of Maize in the broiler finisher feed had highest record of mortality i.e. 3 out of 10 birds. The weight gain by the birds was very poor and the economic performance was too low. This showed that up to 50% replacement of maize with degrade cob was acceptable.

When BECP (18%), FECP (18%) and FECP (50%) were fed the chicken, the economic performance was better than that of chicken fed with the commercial feed.

This is highly recommended to poultry farmers as this will greatly increase their profit margin while also helping to reduce pollution in the country.

The blood chemistry and histopathological studies revealed that no serious damage was caused to the organs of the birds. This will alleviate the fear of the farmers as to the effect of BECP and FECP on their poultry. However, BECP (82%) in feed which caused severe congestion in the kidney and lungs should be avoided.

Large scale maize cultivation with poultry farming should be encouraged by the government, as this will help plough back the waste into the economy better. Since the chicken carcass and maize cob, which represent waste in both industry is being used to compound poultry feed and thus recycled back into profitable economy.

REFERENCES

Abo-State, M.A.M., Hammad, A.E., Selin M. and Gannam R.B. 2010. Enhanced production of cellulases by *Aspergillus sp* on agricultural wastes by solid state fermentation. *American – Eurasian Journal of Agricultural & Environmental Science.* 402 – 410.

Abu O.A, Anjuwon, I.A., Adetunji, V.A. and Tewe, O.O. 2009. Response of Broilers Fed cassava Grit, Palm kernel cake and De-oiled cake with or without B Mannase. In *Proceedings of 14th Annual Conference of Animal Science Association of Nigeria* (ASAN) Sept 14 – 17 2009. Akinlade, J.A. Olayeni, T.B., Rafiu, T.A., Akinwumi, A.O., Aderinola, O.A., Ojebiyi, O.O. and Odunsi, A.A. Eds. LAUTECH Ogbomoso Nigeria. 131 - 133.

Adebowale, E.A. 1985. Organic waste ash as possible source of alkali for animal feed treatment. *Animal Feed Science and Technology* 13: 237 – 248.

Adebowale, E.A. 1985b. Response of sheep and goats fed maize straw treated with local alkali. *Nigeria Journal of Animal Production* 12: 137 – 140.

Adebowale, E.A. 1988. An overview of recent trends and development in the use of unconventional feed ingredients for ruminant animals: applicability to the *Niger*ian conditions. *Proceedings of National Workshop on Alternative Formulations of Livestock Feeds in Nigeria, organized* by the Economic Affairs Office, The Presidency, 21 – 25 November 1988 Babatunde, G.M. Ed. Ilorin: ARMTI 544-578

Adebowale, E.A. 1992. Maize residue as ruminant feed resources in Nigeria. *World Animal Review* 73:24-30.

Adejoye, O.A. 2007. Degradation of Agro-Industrial waste by white rot fungi. M.Phil thesis, University of Ibadan 134 pp.

Adeleye, E.O.A. 1988. Treatment of low quality roughages and agronomic wastes for livestock feeding. Their technology, applicability and adaptability to the local conditions. Invited paper presented at the workshop on alternative formulation of livestock seeds in *Niger*ia, Nov. 21 – 25, 1988.

Ademosun A.A. (1973) Nutritive evaluation of *Niger*ia forages. The effect of stage of maturity on the nutritive value of *Panicum maximum* (Guinea grass). *Nigeria Agricultural Journal* 10: 170-17

Adeyemi O.A. and Adeyemi A.A. 2000. Replacement of soybean meal with fermented Theratia cake in layers diet; Effects on performance, egg quality and nutrient retention, *Nigerian Journal of Animal Production* 27:24 – 28.

Adeyemi, O.A. and Familade, F.O. 2003. Replacement of maize by rumen filtrate fermented com cob in layer diets. *Bioresource Technology* 90:221 – 224.

Akiba, S., Kimura, K. and Kumugal, H. 1995. Purification and characterization of protein resistant cellulase from *Aspergillus niger*. *Journal of Fermentation Bioengineering* 79: 125 – 130

Akin, D.E. 1986. Chemical and biological structure in plants as related to microbial degradation of forage cell wall. In control of Digestion and Metabolism in Ruminants, ed. by Milligan, L.P; Grown, W.L and Robson, A., Prentice Hall Englewood CilTN-3 PP 134-15.

Akinola, S.O. and Abiola, S.S. 1999. Blood chemistry and carcass yield of cockerels fed melon husk diets. *Tropical Journal of Animal Science* 2.2: 39-44.

Akpe, A.F, Ibom, L.A., Ayuk, E.A. and Egbah, J.A. 2007. Comparative growth study of weaner pig fed cassava and cocoyam root meals. *Proceedings. 33rd Annual Conference of Nigeria Society for Animal Production (NSAP).* March 18 – 21, 2007. Agiang E.A., Afwunobi, I.N. and Olawoyin, O.O. Ed. Calabar: ASAN Abeokuta NSAP

Aletor, I.A., Agbele, J.O. and Shobayo R.A. 1982. The proceeding of the *Niger*ia *Society for animal production* held at gateway hotel Abeokuta pp 157 – 160. Cited by Kehinde, A.S., Meduna, A.J., Bobadoye A.O. and Babatunde T.O. (2009. In: Haematological and Serum Chemistry of guinea fowl fed cassava peel based diets. Proc of 34th annal conf ot ASSAN. Sept 14-17, 2009. Akinlade, J.A. Olayeni, T.B., Rafiu, T.A., Akinwumi, A.O., Aderinola, O.A., Ojebiyi, O.O. and Odunsi, A.A. Eds. LAUTECH, Ogbomoso. 68-69.

Alokan, J.A. (1988). A note on Maize cob in sheep diet, *Nigeria Journal of Animal Produciton* 15: 227 – 323.

Amakiri, A.O., Owero, J. and Jack, D.O. 2009. Effect of refined petroleum product (Kerosene) flame and fumes on haematological characteristics of broiler chickens. Proce. of 34th National Conference of Animal Science Association of Nigeira. (ASAN). Umoh B.J.; Udedibie, A.B.I.; Solomong T.P.; Obasi O.L.; Okon B.I. and Udoh E.J. Ed. Uyo: ASAN. 41 – 45.

Amritkar, N., Kamat, M. and Lali, A. 2004. Expanded bed affinity purification of bacterial α – Amylase and cellulose on composite substrate analogue – cellulose matrices. *Process Biochemistry* 39: 565 – 570.

Andriani, D., Praselya, B. and Park, D. 2011. Cellulase production by *Bacillus subtilis*. TD6 cultured on agricultural wastes. 33rd Symposium of Biotechnology, May 25, 2011 A Conference of the Society for Industrial Microbiology. Seattle, W.A. USA. http:/sim,antx/com/sim/33rd/webprogram/paper18352html.

Ani, A.O., Ugwuowo, L.C. and Omeje, O.D. 1999. The effect of graded levels of raw bambara nut (*Vigna subterrania (L*) Verde) waste and supplementary enzyme on the conformational characteristics of broiler finishes in Proceedings for 34th Annual Conference of Animal Science Association of Nigeria (ASAN). Umoh B.J.; Udedibie, A.B.I.; Solomong T.P.; Obasi O.L.; Okon B.I. and Udoh E.J. Ed. Uyo: ASAN. 324-327.

Aniket, R. 2005. Medicine plus medical encyclopedia. Total protein division of endocrinology and metabolism. John Hopkin University Baltmore M.D.

Anon, 1980. Guide to the care and use of experimental animal vol 9, Canadian council of Animal Care Offawa, Ontairo Canada. 85 – 90.

Anupama, R.P. 2000. Value added food: Single cell protein. *Biotechnology Advances.* 18. 6: 459 – 479.

AOAC, 1980. Official Methods of anlaysis (13th edition). Association of Official Analytical Chemists, Washington DC, USA.

Ariffin, H., Abdullah, N., UmiKalson, M.S., Shirai, Y. and Hassan, M.A. 2006. Production and Characterization of Cellulase by *Bacillus pumilus* EB3. *International Journal of Engineering and Technology* 3: 47 – 53.

Arora, D.S., Chender, M. and Gill, P.K. 2002. Involvement of lignin peroxidase, managenese peroxidase and lactase in the degradation and selective ligninolysis of wheat straw. *Internatinal Biodeterioration Biodegradation* 50: 145 – 120.

Aust, S.D. 1990. Degradation of environmental pollutants by *Phanerochaete – chrysosporium. Microbial Ecology* 208: 197-204.

Aust, S.D. and Benson, J.T. 1993 The fungus among us: Use of white rot fungi to biodegrade environmental pollutants. *Environmental health Perspective* 10. 3: 232-233

Babatunde, G.M. 1980. The Tottering *Niger*ia Livestock sector. Problems and possibilities. University of Ibadan Inaugural lecture, University of Ibadan, *Niger*ia. 42pp.

Babatunde, G.M. and Pond, E.R. 1987. Nutritive value of the *Niger*ia rubber seed (Hevea brasiliensis). *Journal of Animal Science* 368: 617 – 630.

Babayemi, O.J., Otukoya, F.K., Familade, F.O. and Daodu, M.O. 2009. Assessment of nutritive value of bovine liquor and urea treated corn-straw and Maize cobs as feed for the West African dwarf sheep and goats. *Nigeria Journal of Animal Production* 36: 213 – 324.

Baird, D.S., Johnson, D.A. and Seligy, V.I. 1990. Molecular cloning, expression and characterization of endo - 1, 4 - glucanase genes from *Bacillus polymyxa* and *Bacillus circulans*. *Journal of Bacteriology* 172: 1576 - 1586.

Baker, F.J. and Silverton, R.E. 1985. Introduction to medical laboratory technology. 6th ed. Butterworth, England. 408pp

Baldrian, P. and Valaskova, V. 2008. Degradation of cellulose by Basidiomycetes fungi. *FEMS Microbiology Reviews* 32. 3: 501-21.

Belewu, M.A. 2003. Nutritional qualities of maize cob and waste paper incubated with edible mushroom. *Nigeria Journal of Animal Production* 39: 1-6.

Bhat, M. A. and Bhat, S. 1997. Cellulose degrading enzymes and their potential industrial applications. *Biotechnology Advances* 15: 583 – 620

Bhat, M.K. 2000. Celluloses and related enzymes in biotechnology. *Biotechnology Advances* 18: 355-289.

Braun, A., Woltter, M. and Zadrazil, F. (2000). Bioconversion of wheat straw by *L. tuber* and its potential utilization as food, medicine and animal feeds, science and cultivation of edible fungi (ed. Van Grensven). A.A. Balkemal Potterdam 549-558.

Bumpus, J.A. and Aust, S.D. 1981. Biodegradation of environmental pollutants by the while rot fungus phanerochaete chrysosporium; involvement of the lignin degrading system. *Bio Essays* 6: 166-170.

Camassola, M. and Dillon, A.J.P. 2007. Production of cellulases and hemicellulases by *Penicillium echinulatumn* grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation. *Journal of Applied Microbiology* 103: 2196 – 2204.

Chen, P.J., Wei T.C., Chang, T.T. and Lin L.P. 2004. Purification and characterization of carboxymetyl celulase from *Sinorhizobium fredii. BotanicalBulletin of Academic sinica* 45: 111-118.

Chesson, A. 1987. Supplementary enzymes to improve the utilization of pigs and poultry diets. In Haresign W. and Cole DJA (eds.) *Recent Advances in Animal Nutrition* London, Bullerworths. 71 – 89.

Clawson, W.J., Garret, W. N and Richards, S. 1990. Rice strans utilization by livestock: A literature review. Univ. of California. Agri. Txt. Publ. MAL.

Coblay, L.S. and Steele W.M. 1976. An introduction to the botany of tropical crops. 2nd edition, Longman London. 34-38.

Coles, E.H. 1986. Veterinary Clinical Pathology 4th ed. WB Sanders & Co. Philadelphia. 10 – 97.

Cooke, R.C. & Whips J.M. 1993. Resource acquisition and utilization ecophysiology of fungi. Blackwell Scientiific productions. University Press Cambridge Great Britain. Pp 347

Coral, G., Arikon, B., Unaldi M.N. and Guvenmez, H. 2001. Some properties of crude carboxymethyl cellulose of *Aspergillus niger* 210 wild-type strain, *Turkey Journal of Biology* 6: 209 – 213.

Crawford, D.L. 1986. The role of actinomycetes in the deem position of lignoeellulases. *FEMS symposium* pp 34 – 728.

Crawford, R.L. 1981. Lignin biodegradation and transformation. New York: Wiley. Pp. 134

Damisa, D., Ameh, J.B. and Umoh, V.J. 2008. Effect of chemical pretreatment of some Lignocellosic wastes on the recovery of cellulose from *Aspergillus niger* AH_3 mutant. *African Journal of Biotechnology* 7: 2444 – 2450.

Deka, D., Bhargavi, P., Sharma, A., Goyal, D., Jawed, M. and Goyal A. 2011. Enhancement of cellulose activity from a new strain of *Bacillus subtilis* by optimization and analysis with various cellulosic substrates, *Enzyme Research* 2011:1 – 8

Dirar, H.A. 1992. Traditional fermentation technology and food policy in Africa. *Appropriate Technology* 19. 3: 21-23.

Dzowela, B.H. 1987. Effort to enhance maize stover utilization for smallholders livestock producer in Malawi. *In Proceedings of ARNAB works*hop on the utilization of Agricultural by-products as Livestock feeds in Africa. Little D.A. and Shid A.N. Ed. Malawi: ARNAB. pp 27-36.

Ekeniyen, B.V., Madubuike, F.N. and Dike, O.F. 2006. Effect of partial replacement of yam peel meal (Discorea spp.) for Maize meal (zea mays) on performance and carcass characteristics of finisher broiler chicks. *International Journal of Poultry Science* 5. 10: 142 – 945.

Ekperigin, M.M. 2007. Preliminary studies of cellulose production by *Acinetobactes anitratus* and *Branhamella* sp. *African Journal of Biotechnology* 6: 28 – 33.

Emert, G., Gum E. Lang. J., Liu, T. and Brown, R. 1974. Celluloses in food related enzyme (Mlutake J. ed) *Advances in Chemsitry series* 136 America. Washington D.C.

Emtiazi, G. Etemadifar, Z. and Tavassoli, M. 2003. A novel nitrogen fixing celluloytic bacterirum associated with root of Maize is a candidate for production of single cell protein. *Biomass Bioenergy* 25:423-426.

Emtiazi, G., Pooyn, G. and Shamalnasab, W. 2007. Cellulose Activities in Nitrogen Fixing PaeniBacillus isolated from soil in N-free Media. *World Journal of Agricultural Science* 3. 5: 602 – 608.

Encyclopedia Britannica 2003. Maize. Micropedia Knowledge in depth J. Harace Mc Farland Company. United State of America. 980 pp.

Erickson, K.E.L., Blanchette, R.A. and Ander, P. 1990. Microbial and enzymatic degradation of wood and word components. *Wood decomposition*. Springer verlag, New York. 41 – 62.

Eruvbetive, D. and Adegboyega, S.A 1995. Effects of aerobic fermentation of cassava nutritive composition of its product. Dept. of Animal Nutrition. University of Agriculture. Abeokuta

Fahey, G.C, Jr., Bourguin, L.D., Titgcr naycr, E.G. and Atwell, D.G. 1993. Post harvest treatment of fibrous feed stuffs to improve their nutritive value. In forage cell wall structure and digestibility Ed. by Jung H.cr., Buxtin, D.R., Halficd R.D., and Ralah, J., Pore symp. Maclison WL 715-760.

Fahy, P.C. and Hayward, A.C. 1983. Media and methods for isolation and diagnostic guide. Eds. P.C. Fahy and G.J. Persley. Academic Press Australia. 351 – 378.

Familade, F.O. and Babayemi O.J. 2009. Nutritive evaluation of ten day solid fermentation of maize cobs and straw with rumen liquor and urea. Proceedings of 14th Ann conf of ASAN. Sept 14-17 2009. Akinlade, J.A. Olayeni, T.B., Rafiu, T.A., Akinwumi, A.O., Aderinola, O.A., Ojebiyi, O.O. and Odunsi, A.A. Eds. LAUTECH, Ogbomoso, Nigeria. Annual Conference of Animal Science Association of Nigeria. 212-214

Fan, L.T. Gharpuray M.M. and Lee, Y.N. 1987. Cellulose hydrolysis, Berlin, Germany. Springs – Verlag, 3: 1 – 68.

Faniiyi, G.F. 1996. Replacement of untreated and lye-treated groundnut pods in broiler diets. *Wonder hands.* Accepted for Publication.

Faniyi, G.F. 1998. Biochemical characterization and utilization of cowpea (*Vigna unguiculanta)* and sorghum (*sorghum biocolor*) seedhulls upgraded with non-proteinous Nitrogen in broiler rations, Ph.D. Thesis, University of Ibadan, Ibadan, *Niger*ia.

Faniyi, G.F. 2002. Replacing groundnut cake with discarded cashew waste in the diets of pullet chickens, *Proceedings of 7th Annual Conference of Animal Science Association of Nigeria*, University of Agriculture, Abeokuta Sept 16-21 2002. Fanimo A. O. and Olanike J.A. Eds. Abeokuta: ASAN. 106-108.

Faniyi, G.F. 2006. Optimizing the usefulness of poultry faeces and crop residue for meat, fish and egg production. First distinguished staff lecture, Oyo State College of Education, Oyo Feb. 2006. 62 pp.

Faniyi, G.F. and Ologhobo, A.D. 1999. Effects of replacing brewers dried grains with lye and urea – treated cowpea and sorghum seedhuls in broiler diets. *Tropical Animal Investigatio*ns (TAP1) 2. 1: 69-82

Faniyi, G.P. 2006. Optimizing the usefulness of poultry faeces and crop residue for meat, fish and egg production, Inaugural lecture at Oyo State College of Education, Oyo, Pp 1 – 30.

Fasina O.E., Ologhobo A.D., Ayoade G.O., Adeniyi, G.A. and Adeyemi O.A. 1999. Nutritional and Toxicological assessments of Verrnonia amygdalina leaves (Bitter leaf) in nutrition of broiler chicks 2 – Effect on Performance haematological and biochemical indices. *In Proceedings of 4th Annual Conference of Animal Science Association of Nigeria*. September 14 – 16, 1999. Ologhobo, A.D.; Bamgbose A.M.; Egbunike, G.N., Iyayi, E.A.; Adewumi, M.K. and Adesehinwa A.O.K. Ed. Ibadan: IITA. 19-22.

Fetuga B.I., Babatunde G.M, Olusanya, A.O. and Oyenuga V.A. 1975. The composition, nutrient digestibility and energy value of maize cobs, yam peels, plantain peels for three weight groups of pigs. *Nigeria Journal of Animal Production* 2:95 - 99

Gautam, S.P., Bundela, R.S., Pandey, A.K., Khan, J. Awashi, M.K. and Sarsaiya, S. 2011. Optimization for the production of cellulose enzyme from municipal solid waste residue by two novel cellulolytic Fungi *Biotechnology Research Tnternational* 2011: 8.

Giekins, M.M.C., Dekkens, E., Viser, J. and Graaf, L.H. 1999. Two carbohydrates – encoding genes from *Aspergillus niger* requires D – Xylose and Xylamolytic transcriptional activator XluR for their expression. *Applied Environmental Microbiology* 65: 4340 – 4345.

Graw, F.H and Wilson, P.W. 1961. Physiology of nitrogen fixation by *Bacillus polmyxa*. Department of Bacteriology, University of Wisconsin, Madison. Wisconsin, 83:490-496

Guedeon, E., Desvaux, M. and Petitdemange, H. (2002). Improvement of cellulolytic properties of *Clostridium celullolyticum* metabolic engineering. *Applied. Environmental Microbiology* 68. 1: 53-58.

Hackbath, H, Buron, K and Schimansley, G. 1983. Strain differences in bred rator influence of strains and diets on haematological traits. *Laboratory Animal* 17: 1-12.

Hammer, R.J. 1989. Aspects of improving the nutritional value of cereal grains for monogastric. In forum feed symposium on feed enzyme and Animal Production Proceedings. Solihill England.

Harrigan, W.F. and MCcance, M.E. 1966. Laboratory Methods. In Microbiology Academic Press London, New York. pp 342

Hartley, R.D. 1985. Chemistry of Lignocellulosic plant materials and on-microbial processes for increasing their feed value for the ruminant. In improved utilization of lignocellulosic materials in animal feed. Paris, OECD. 10 – 30.

Hay, J. 2010. *Crop Residues*. *Cropwatch, Bioenergy*. A seminar presented at the WJAG farm show, Merfolk, Nebraska. January 14 2010., University of Nebraska – Lincoln.

Hayward, A.C. and Hodgkins, W. 1961. Taxonomic relationships of *Xanthomonas Uredovorus*. *Journal of General Microbiology* 06:133-139.

Hoffman, R. M and Wood, T.M. 1985. Isolation and partial characterization of a mutant *Penicillium* for the saccharification of straw. *Biotechnology. Bioengineering* 27: 81-85.

Igwebuike, J.O., Mohammed, G., Asheikh, L.G. Ubosi, C.O. and Mohammed, B. 2009. Efect of partial replacement of maize with yam peal meal on the growth and economic performance of broiler chicken in Proceeding of 14th Annual Conference of Animal Science Association of *Niger*ia (ASAN) Sept 14th – 17th 2009. Akinlade, J.A. Olayeni, T.B., Rafiu, T.A., Akinwumi, A.O., Aderinola, O.A., Ojebiyi, O.O. and Odunsi, A.A. Eds. LAUTECH, Ogbomoso, *Niger*ia. 508 – 510.

Immanuel, G., Akila Bhagavath, C. M. Iyapppa Raj, P., Essakking, P. and Palavessam A. 2007. Production and partial purification of cellulase by *Aspergillus niger* and *A. fumigatus* fermented in coir waste and saw dust. *Internet Journal for Microbiology* 3:1 – 17.

Immanuel, G., Dhanusa, R., Prema, P. and Palavesam A. 2006. Effect of different growth parametes on endogluconase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *International Journal of Environmental Science and Technology* 3. 1: 25-34.

Isikwenu, J.O., Akpodiek, O.J., Emegba I.O. and Bratte, L. 2000. Effect of dietary fibre (Maize cob) levels on growth performance of broiler birds in *Proceedings of 25th Annual Conference of Nigeria society of Animal production* 19 – 23rd March 2000 MOVA Umudike. 158-159.

Iyayi, E.A. 2001. Cassava leaves supplementation for feeding weaner swine. *Animal Production Investigation* 4: 141 – 150.

Jackson, M.G. 1977. The alkali treatment of straws. Review article; *Animal Feed Science Technology* 2: 105-130.

Jalc, D., Siroka, P. and Cercsnakova, Z. 1997. Effect of six species of white-rot basidiomycetes on the chemical composition and rumen degradability of wheat straw. *Journal of General Applied Microbiology* 43: 133-137.

Kahen, F.A.A. and Husaini, A.A.S. 2006. Enhancing α – amylase and Cellulase in vivo Enzyme Expressions on Sago Pith Resdue using *Bacillus amyloliquefaciens* froiens VMAS1002 *Journal of Biotechnology* 5: 391-403.

Kamakura, M. 1997. Preparation of immobilized cellulase bonds and their application to hydrolysis of cellulosic materials. *Process Biochemistry*. 325: 555-559.

Kaneko, J.J. 1989. Serum proteins and the disproteinemias. In clinical Biochemistry of Domestic Animals. Ed. Kaneko J.J. San Diego. California. Academic Press Inc. 142 – 165.

Kanosh, A.L., Essant S.A. and Zeinat A.M. 1999. Biodegradation and utilization of baggase with *Trichoderma ressei* *Polymer Degradation Stabilization* 62: 273-276.

Kashen, M.A., Manchur, M.A., Rahman, M.S. and Anvar, M.N. 2004. Effect of carbon and Nitrogen services on the production of reducing sugars, extracellular protein and cellulolytic enzymes by two celllolytic bacteria isolates. *Pakistan Jounral of Biological Sciences* 7:1660 – 1663.

Kategile, J.A. and Fresdenkson J.H. 1979. Effect of level of sodium hydroxide treatment and volume of sodium on the nutritive value of maize cobs. *Animal feed. Science Research* 4: 1-15.

Kehinde A.S., Meduna A.J. Bobadoye A.O. and Babatunde T.O. 2009. Haematological and serum chemistry of gunea fowl fed cassava peel based diets. In proceeding of 34th Annual Conference on animal agriculture in Nigeria and the global food challenges. Umoh B.I., Udedebie A.B.I., Solomon I.P., Obasi, O.L., Okon, B.I. and Udoh, E.J. Eds. Uyo: ASSAN. 68-69.

Koomnok, C. 2005. Selection of cellulose producing thermophilic fungi. 31st congress on Science and Technology of Thailand.

Koshyukousky, V., InamoloT., Ando, T., Nata, Y. and Ogimoto, K. 1985. Degradation of hay by rumen fungi in artificial rumen (RUSTITEC.) *Journal of General Applied Microbiology* 83-86.

Kossila, V.L. 1984. Location and potential feed use: In straw and other fibrous by-product as Feeds (Editors: F Sundstol and E. Owen). Elsevier, Smsterdam 2: 4-24.

Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 178:103.

Krairitthchai, S. and Thongwai, N. 2008. Isolation and Screening for Cellulase Producing Bacteria. 34th Congress on Science and Technology of Thailand. 1 – 6.

Krishna, G. 1985. Nylon bag dry matter digestibility in agro-industry byproducts and wastes of the tropics. *Agricultural wastes* 13 : 155-158.

Lakshimikent, K. and Mathur, S.N. 1990. Cellulolytic activities of *Chaetomium globossim* on different cellulosic substrates. *World Journal of Microbiology Biotechnology* 11: 23-26.

Lennox, J.A., Abriba, C., Alabi, B.N and Akubuenyi, F.C. 2010. Comparative degradation of Sawdust by microorgnaisms isolated from it. *African Journal Microbial Research* 4. 17: 1804 – 18097.

Li, X. amd Gao, P. 1996. Isolation and Partial characterization of cellulose degrading strain of *streptomyces sp* Lx from soil. *Letters in Applied Microbiology* 22:209-213.

Ll, W., Zhang, W.W., Yang, M.M. and Chen, Y.L. 2008. Cloning of the Thermostable Cellulase Gene from Newly Isolated Bacillus subtilis and its Expression in *Escherichia coli*, *Molecular Biotechnology* 40. 2: 195 – 201.

Longe, O.G and Tona G.O. 1988. Metabolsable energy values of some tropical feed stuffs for poultry. *Tropical Agriculture (Trinidad)* 65. 4: 358 -360.

Mandels M. and Reese, E.T. 1988. Fungal cellulose and microbial decomposition of cellulosic fibres, *Development in Industiral Microbiiology*. 5:5-20.

Maurice, R. 2001. General and microbiological aspects of solid substrate fermentation. Laborative de Biotechnology Microbiene Troopicale Centre ORSTOM LB 911 an Angropolis. Frano. 5043 – 34053

Milala, M.A., Shugaba, A., Gidado, A., Ene, A.C. and Wafar, J.A. 2005. Studies on the use of aricultural wastes for cellulase enzyme production by *Aspergillus niger*. *Research Journal of Agriculture and biological Sciences* 1. 4: 323-325.

Mohammed, G., Igwebuike, J.O. Adamu S.B. Dibial, E. and AY 2009. Haamatological indices and carcass characteristics of broiler chicken fed yam peel meal as Partial replacement of Maize. In proceedings of 14th Annual Conference of Animal Science Association of Nigeria (ASAN). Sept 14 – 17 2009. Akinlade, J.A. Olayeni, T.B., Rafiu, T.A., Akinwumi, A.O., Aderinola, O.A., Ojebiyi, O.O. and Odunsi, A.A. Eds. LAUTECH Ogbomoso: ASAN. 475-477.

Mullen, C.A., Boateng, A.A., Goldberg, N.M., Lima, I.M., Lantel, D.A. and Hicks, K.B. 2010. Bio-oil and biocher production form maize cobs and stover by fast pyrolysis. *Biomass and Bioenergy* 34: 67 – 74.

Nagole, E.N. Koyongo M.H and Said A.N. 1985. Chemical composition and digestibility and feeding value of Maize cobs. *Animal Feed Science Technology* 9: 121-130.

Nahm, K.H. (1982. Practical Guide to feed, forage and water analysis. Yoo Publishing Inc. South Korea Republic. pp 269.

Nakamura, K, and Kappamura, K. 1982. Isolation and Identification of crystallize cellulose hydrolyzing bacterium and its enzymatic properties. *Jounal of Fermentation Technology* 60. 4: 343 – 348.

Nelson, M.L., Kloptcnstain, T.J. and Britton, R.A. 1984. Protein supplementation of ammoniated roughages. maize cobs supplemented with a blood meal-Maize gluten meal mixture-lamb studies. *Journal of Animal Science* 59: 1601-1609.

Nishida, Y., Suzuki K.I., Kumayai, Y., Tanaka, H., Inove, A. and Ojima, T. 2007. Isolation and primary structure of a cellulose from the Japanese sea urchin. *Strongylocentrotus nudus*. *Biochime*. 1-10.

Oduguwa, O.O., Edema, M.O. and Ayeni A.O. 2007. Physico-chemical and microbiological analysis of fermented maize cob, rice bran and cowpea husk for use in composite rabbit feed. *Bio Resource Technology* 99: 1816 – 1820.

Odulami, M.O. 1988. Energy supplementation of forage and browse based diets for West Africa dwarf goats. M. Phil Thesis Obafemi Awolowo University, Ile-Ifc, *Niger*ia.

Ogunbanjo, S.A., Alamide, I.C., Adama, J.Y. and Abdulahi, J. 2009. Haematological parameters of savannah brown Does fed varying dietary levels of flamboyant tree feed meal. Proceedings of 34th Annual Conference of Animal Science Association of Nigeria on animal agriculture in Nigeria and the global food challenges. Umoh B.I., Udedebie A.B.I., Solomon I.P., Obasi, O.L., Okon, B.I. and Udoh, E.J. Eds. Uyo: ASSAN. 127.

Okafor, N. 1988. Industrial Microbiology. University of Ife Press Ltd. Ile-Ife, Nigeria 32-33.

Oladeji, J.T. 2010. Fuel characterization of briquettes produced form corncob and rice husk residues. *The Pacific Journal of Science and Technology*. II. 1: 101 – 106.

Olomu, J.M. 1978. Protein and energy nutrition in poultry diets in Nigeria. Published by *World Poultry Science Association* (Nigeria Branch). 41 – 58.

Olomu, J.M. 1995. Monogastric animal nutrition. Principles and Practice. A Jackem Publication Benin – city Nigeria.

Oloyede, O.B., Otunola, G.A. and Apata, D.F. 2004. Assessment of protein quality of processed melon seed as a component of poultry feed. *Biochemistry* 16. 2: 80 – 87.

Olutiola, P.O., Famurcwa, O. and Sonntag, II.G. 1991. An introduction to general Microbiology. A practical approach. Hendelberger Yerlagsanstalt and Druckcrci GMbh Hendelberg pp 267.

Onilude, A.A. 1999. Fermented poultry litter and fungal enzyme supplementation of high fibre broiler diets, growth responses, carcass characteristics and blood lipids of feed chicks *Nahrung* 43: 54-60.

Onilude, A.A. and Oso, B.A. 1999. Effect of fungal enzyme mixture supplementation of various fibre-containing diets fed to Broiler chicks 1: Performance and carcass characteristics. *World Journal of Microbiology and Biotechnology* 15: 309-314.

Onyeyilc, P.A., Iwoha, L.F. and Akinniyi, J.A. 1988. Chronic toxicity study of Focus Platyphyla Blume in Rat. *West African Journal of Pharmacology Drug Research* 14. 1&2: 27 – 30.

Opara, C.C 1996. Studies on the use of Alchannia cordiforlia leaf meal as feed ingredients in poultry diets. M.Sc thesis Federal University of technology, Owena *Niger*ia.

Oseni, O.A. and Ekperigin, M. 2007. Studies on biochemical changes in maize wastes fermented with *Aspergillus niger*. *BIOKEMISTRI* 19. 2: 75 – 79

Owolade, B.F., Fawole, B. and Osikalu, Y.O.K. 2001. Fungi associated with maize seed discolouration and abnormalities in S.W *Niger*ia. *Afncan crop Science Journal* 9. 4: 693-697.

Pan, C., Zhag, S., Fan, Y. and Hon, H. 2009. Bioconversion of corn cob to hydrogen using anaerobic mixed mciroflora.

Pazenga, V. 1985. Feeding Parent Stock. *Zootech International* pp 22 – 25.

Pearson, D. (1975). Laboratory technique in food analysis. London: Bulterworth. 7-20.

Pece, N., Petolti, N. and Molina, O. 1990. Bacterial protein production from maize cob pre-treated with NaoH at room temperature. *Biological Wastes* 34. 2: 123 – 131.

Persley, G.J. 1980. Studies on bacterial blight of cassava in Africa Ph.D thesis. University of Queensland, Australia. 182pp.

Poorma C.A. and Prema P. 2007. Production of cellulasefree endoxylanase from novel alkalophilic thermostable. *Bacillus pumilus* by solid-state fermentation and its application in waste paper recycling. *Bioresource Technology* 98: 485-490.

Prescott, M.C., Harley, J.P. and Klein, D.A. 2008. *Microbiology*. Sixth Ed. New York: MGraw II Pub. 1088pp.

Rastogi, G., Bhalla, A. and Adhikan A. 2010. Characterization of themostable cellulases produced by *Bacillus* and *GeoBacillus* strains *Bioresource Technology* 101. 22: 8798 – 8806.

Reese, E.T. and Mandels, M. 1963. Enzymic hydrolysis of cellulose and its derivatives. In, Methods in carbohydrate Chemistry, Vol III. Cellulose. Ed. R.L. Winstler. Academic Press, New York. 139 – 143

Reids, I.D. 1989. Solid State Fermentations for biological delignification. *Enzyme Microbiology Technology* II: 786 – 803.

Robert, K.M., Oary, K.G., Peter, A.M. and Victor, W.R. 1993. *Harpers Biochemistry* 23rd Ed. 665 – 763.

Rosado, A.S., de Azevedo, F.S., de Cruz, D.W., Van Elsas, J.D. and Seldin, I. 1998. Phenolypic and genetic diversity of *PaeniBacillus azotofixans* strains isolated from the rhizolpane or rhizosphere soil of different grasses. *Journal of Applied Microbiology* 84: 216 – 226.

Ryu, D. and Mandels, M. 1980. Celluloses: Biosynthesis and Application. *Enzymes Microbiology Technology* 2 91.

Sarojini, T.R. 2000. Modern Biology (New edition). Introduction to Microorganism. Microorganism and health African Feb. Publishers Limited. Onitsha, Nigeria. 162 – 203.

Saxena, S. Bahadur, J. and Varma, A. 1993. Cellulose and hemicellulose degradation bacteria from termite gut and mound soils of India. *International Journal of Microbiology* 33. 1: 55 – 60.

Scherief, A.A., El-Tanash, A.B. and Atia, N. 2010. Cellulase production by Aspergillus *fumigatus* grown on mixed substrate of wheat bran. *Research Journal of Microbiology* 5. 3: 195 – 211.

Schwarz, W.H. 2001. The Cellulosome and cellulose degradation by anaerobic bacteria. *Applied Microbiology Biotechnology* 56. 5–6: 634 – 649

Shabeh, M.S.A., Younis, M.A.M., Hezayem, F.F. and Nour-Eldein, M.A. 2010. Production of cellulase in low-cost medium by *Bacillus subtilis* KO Strain. *World Applied Sciences Journal* 8. 1: 35-42.

Shin, C.S., Lee, J.P. Lee, I. S. and Park, S.C. 2000. Emyme production of *Trichoderma ressei*. Rut C-30 on various lignocelulosic substrate. *Applied Biochemiesty Biotechnology* 84 – 86. 1-9: 237 – 245.

Singh, H. and Moore, E.N. 1982. Livestock and poultry production. New Delhi publication. Pp 494.

Singh, J., Batra, N. and Sobti, R.C. 2004. Purification and Characterization of alkaline cellulose produced by a novel isolate *Bacillus sphaericus* JS1. *Journal of Industrial Microbiology and Biotechnology* 31. 2: 51-56.

Singh, M. and Jackson, M.G. 1971. The effect of different levels of sodium hydroxide spray treatment of wheat straw on consumption and digestibility by cattle *Journal of Agric Science* 77: 5-10.

Skerman, V.B.O. 1967. A guide to the identification of the genera of Bacteria. 2nd ed. Williams and Wilkins, Baltimore, U.S.A.

Smith, E.J. 1996. "An industrial application of cellulases" *Journal.of Biotechnology & Bioengineering.* Press syndicate of the University of Cambridge. Third Edition. 68-83.

Smith, O.B., Idomu, O.A., Asaolu, V.O. and Odulami, O. 1991. Comparative rumen degradability of forages, browse, crop residues and agricultural by-products. *In Livestock Research for Rural Development* 3 (2).

Solomon, B.O., Amigun, B. Betikue, T.V., Ojumu, T. and Layokun, S.K. 1988. Optimization of cellulose production by *Aspergillusflarus* linn, isolate NSPK 101 grown on baggase. JNSCHE 18:61-68.

Solomon, B.O., Layokun S.K., Nwesigwe, P.K. and Olutiola, P.O. 1999. Hyrdolysis of saw dust by celllase enzyeme derived from *Aspergillus flavus* Linn. Isolates NSPR 101 beyond the initial fast rate period. JNSCHE 9: 1-2.

Steenhoudt, O. and Vanderleyden, J. 2001. Azospirillum a free living nitrogen-fixing bacterium closely associated with grasses: Genetic biochemical and ecological aspects. *FEMS Microbial Revolution* 24: 705-785.

Sunstol, R., Coxworth, E. and Mowat, D.N. 1978. Improving the nutritive value of wheat straw and other low qualify roughages by treatment with ammonia. *World Animal Revolution* 26: 13-21.

Thoraley, M.N. 1960. The Differentiation of *Pseudomonas* from other gram negative bacteria on the basis of arginine metabolism. *Journal of Applied Bacteriology* 68: 5-16.

Tuah, A. K. and Qrskov, E.R. 1989. Degradation of untreated and treated maize cobs and cocoa pod husks in the rumen. FAO corporate Document Rencusillory.http//.www.Fao.Wairdocs.11RIX5490.

Ugwuanyi, J.O., Harvey, L.M. and McNeil, B. 2008. Protein enrichment of corn cob heteroxylan waste slurry by themophilic aerobic digestion using *Bacillus stearothemophilus*. *Bioresource Technology* 99. 15: 6974 – 6985.

Updegraff, D.M. 1971. Utilization of cellulose from waste paper by *Myrothecium verucaria. Biotechnology and Bioengineering* 1: 77 – 97.

Vahidi, H., Kobarfard F. and Nawjoyan, F. 2004. Effects of cultivation conditions on growth and antifungal activity of *Myana leptocephala: Africa Journal of Biotechnology* 3. ii: 606 – 609.

Van Glyswusk, N.O. 1990. Enumeration and presumptuous identification of some functional groups of bacteria in the rumen of dairy cow fed grass sillage based diets. *FEM microbial Ecology* 73: 243 – 254.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O and B Books Inc, Corvallis, Oregon. 112: 126-127.

Varel, V.H. and Pond W.G. 1985. Enumeration and activity of cellulolytic bacteria from swine fed various levels dietary fiber. *Applied and Environmental Microbiology.* 858-862.

Zeikus, J.G. 1981. Lignin metabolism and the carbon cycle; polymer biosynthesis, biodegradation and environmental recaltrance. *Advances in Microbial Ecology* 51: 211-245.

Zhang, M., Wang, F., SyR. Qi, W. and He, Z. 2010. Ethanol production from high dry matter corncob using fed batch simultaneous saccharification and fermentation after combined pretreatment. *Bioresource Technology* 101. 13: 4959 – 4964.

APPENDICES

Appendix 1A

Fig. 1: Reducing sugar (mg/ml) produced by bacterial isolate

CODE		0	4	7	10	14	21	29
BA	*Pseudomonas putida I*	0.01	0.07	0.15	0.01	0.01	0.01	0.01
BB	*Pseudomonas aeruginosa I*	0.01	0.03	0.04	0.01	0.01	0.01	0.01
BC	*Bacilus cereus I*	0.01	0.03	0.03	0.01	0.02	0.01	0.01
BD	*Bacillus cereus II*	0.01	0.03	0.03	0.01	0.02	0.01	0.01
BE	*Bacillus cereus III*	0.01	0.05	0.06	0.01	0.02	0.01	0.01
BF	*Bacillus careus IV*	0.01	0.03	0.03	0.01	0.02	0.01	0.01
BG	*Bacillus licheniformis IV*	0.01	0.02	0.03	0.01	0.01	0.01	0.01
BH	*Pseudomonas putida II*	0.01	0.05	0.06	0.01	0.01	0.01	0.01
BI	*Pseudomonas aeruginosa II*	0.02	0.04	0.04	0.02	0.02	0.02	0.01
BJ	*Pseudomonas aeruginosa III*	0.02	0.02	0.03	0.01	0.01	0.01	0.01
BK	*Proteus mirabilis*	0.02	0.03	0.03	0.01	0.01	0.01	0.01
BL	*Bacillus megaterium*	0.01	0.04	0.05	0.02	0.01	0.01	0.01
BN	*Pseudomonas cepacia*	0.02	0.05	0.06	0.01	0.02	0.01	0.01
BN	*Bacillus alvei*	0.01	0.08	0.05	0.06	0.08	0.01	0.01
BD	*Bacillus subtilis I*	0.08	0.32	0.60	0.14	0.03	0.01	0.01
BD	*Bacillus subtilis II*	0.02	0.03	0.06	0.03	0.06	0.01	0.01
BQ	*Bacillus licheniformis I*	0.01	0.04	0.11	0.01	0.01	0.01	0.01
BR	*Bacillus licheniformis II*	0.02	0.06	0.10	0.01	0.02	0.01	0.01
BS	*Bacillus licheniformis III*	0.01	0.06	0.06	0.01	0.08	0.01	0.01

Appendix 1b

Table 2: Reducing sugar (mg/ml) produced by fungal isolate

CODE	FUNGI	0	7	10	14	17	21	28
FA	*Rhizopus oryzae*	0.01	0.07	0.68	0.70	0.01	0/01	0.01
FB	*Aspergillus flavus*	0.01	0.02	0.80	0.80	0.01	0.01	0.01
FC	*Mucor racemosus*	0.01	0.02	0.65	0.69	0.01	0.01	0.01
FD	*Aspergillus niger*	0.01	1.02	1.48	1.68	0.01	0.01	0.01
FE	*Aspergillus flavus*	0.01	0.90	1.20	1.24	0.01	0.33	0.01
FF	*Penicillium restrictum*	0.01	0.03	1.29	1.43	0.66	0.04	0.01
FG	*Penicillium atrovenetum*	0.01	0.53	1.00	1.40	0.03	0.07	0.02
FH	*Aspergillus flavus*	0.01	0.55	1.05	1.60	0.02	0.03	0.02
FI	*Aspergillus oryzae*	0.01	0.33	1.04	1.44	0.01	0.01	0.01
FJ	*Penicillum expansum*	0.01	0.38	1.02	1.08	0.02	0.30	0.03
FK	*Botryotrichum piluliferum*	0.01	0.01	0.62	0.76	0.01	0.02	0.01
FL	*Aspergillus oryzae*	0.01	0.01	0.64	0.78	0.01	0.01	0.01
FM	*Geotrichum candidum*	0.01	0.33	1.00	1.06	0.48	0.07	0.02
FN	*Penicillm chrysogenum*	0.01	0.01	0.66	0.81	0.01	0.01	0.01

Appendix 2a

Reducing sugar (mg/ml) produced at 28°C by bacterial isolates

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.04	0.31	0.30	0.22	0.15	0.02	0.01
BO	*Baallus subtilis*	0.05	0.30	0.49	0.03	0.03	0.02	0.01
BQ	*Baallus Licheniformis I*	0.05	0.32	0.30	0.05	0.05	0.03	0.01
BR	*Baallus Licheniformis II*	0.04	0.30	0.41	0.22	0.16	0.02	0.01

Appendix 2b

Reducing sugar (mg/ml) produced at 32°C by bacterial isolates

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.04	0.31	0.51	0.07	0.02	0.02	0.01
BO	*Baallus subtilis*	0.04	0.30	0.39	0.05	0.02	0.02	0.01
BQ	*Baallus Licheniformis I*	0.05	0.32	0.41	0.06	0.02	0.02	0.01
BR	*Baallus Licheniformis II*	0.04	0.30	0.40	0.07	0.05	0.02	0.01

Appendix 2c

Reducing sugar (mg/ml) produced at 40°C by bacterial isolates

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.05	0.29	0.41	0.06	0.03	0.03	0.01
BO	*Baallus subtilis*	0.05	0.44	0.31	0.04	0.04	0.03	0.01
BQ	*Baallus Licheniformis I*	0.04	0.28	0.52	0.06	0.03	0.04	0.01
BR	*Baallus Licheniformis II*	0.05	0.30	0.44	0.05	0.04	0.02	0.01

Appendix 3a

Reducing sugar (mg/ml) produced at pH 4 by bacterial isolates

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.03	0.30	0.31	0.03	0.02	0.03	0.01
BO	*Baallus subtilis*	0.02	0.28	0.31	0.03	0.04	0.02	0.01
BQ	*Baallus Licheniformis I*	0.04	0.29	0.40	0.03	0.02	0.02	0.01
BR	*Baallus Licheniformis II*	0.05	0.31	0.42	0.03	0.04	0.02	0.01

Appendix 3b

Reducing sugar (mg/ml) produced at pH 5.5 by bacterial isolates

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.04	0.30	0.33	0.07	0.15	0.02	0.01
BO	*Baallus subtilis*	0.04	0.325	0.32	0.06	0.06	0.02	0.01
BQ	*Baallus Licheniformis I*	0.03	0.40	0.43	0.31	0.05	0.03	0.01
BR	*Baallus Licheniformis II*	0.04	0.31	0.32	0.15	0.06	0.02	0.01

Appendix 3c

Reducing sugar (mg/ml) produced at pH 7.0 by bacterial isolates

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.03	0.29	0.50	0.07	0.04	0.04	0.01
BO	*Baallus subtilis*	0.04	0.29	0.51	0.44	0.05	0.02	0.01
BQ	*Baallus Licheniformis I*	0.04	0.29	0.31	0.08	0.05	0.02	0.01
BR	*Baallus Licheniformis II*	0.03	0.30	0.43	0.17	0.07	0.04	0.01

Appendix 4a

Reducing sugar (mg/ml) produced by bacterial isolates with yeast extract as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.03	0.42	0.43	0.05	0.03	0.03	0.01
BO	*Baallus subtilis*	0.05	0.31	0.53	0.05	0.04	0.02	0.01
BQ	*Baallus Licheniformis I*	0.06	0.48	0.51	0.07	0.04	0.04	0.01
BR	*Baallus Licheniformis II*	0.04	0.31	0.43	0.05	0.05	0.02	0.01

Appendix 4b

Reducing sugar (mg/ml) produced by bacterial isolates with peptone as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.04	0.42	0.43	0.04	0.03	0.03	0.01
BO	*Baallus subtilis*	0.03	0.42	0.42	0.05	0.04	0.02	0.01
BQ	*Baallus Licheniformis I*	0.05	0.53	0.51	0.29	0.05	0.04	0.01
BR	*Baallus Licheniformis II*	0.03	0.81	0.41	0.02	0.05	0.02	0.01

Appendix 4c

Reducing sugar (mg/ml) produced by bacterial isolates with NH4CL as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.02	0.28	0.42	0.26	0.26	0.03	0.01
BO	*Baallus subtilis*	0.02	0.29	0.30	0.07	0.06	0.02	0.01
BQ	*Baallus Licheniformis I*	0.03	0.31	0.70	0.29	0.01	0.02	0.01
BR	*Baallus Licheniformis II*	0.03	0.31	0.42	0.32	0.04	0.02	0.01

Appendix 4d

Reducing sugar (mg/ml) produced by bacterial isolates with urea as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.04	0.31	0.42	0.04	0.03	0.01	0.01
BO	*Baallus subtilis*	0.03	0.71	0.50	0.03	0.03	0.01	0.01
BQ	*Baallus Licheniformis I*	0.03	0.41	0.51	0.03	0.03	0.01	0.01
BR	*Baallus Licheniformis II*	0.04	0.31	0.48	0.03	0.03	0.01	0.01

Appendix 4e

Reducing sugar (mg/ml) produced by bacterial isolates $NaNO_2$ as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
BA	*Pseudomonas Putida*	0.02	0.31	0.44	0.03	0.03	0.03	0.01
BO	*Baallus subtilis*	0.03	0.31	0.50	0.25	0.15	0.02	0.01
BQ	*Baallus Licheniformis I*	0.02	0.41	0.31	0.03	0.02	0.02	0.01
BR	*Baallus Licheniformis II*	0.03	0.32	0.44	0.02	0.02	0.02	0.01

Appendix 5a

Reducing sugar (mg/ml) produced at 28°C by fungal isolates

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.52	0.60	0.81	0.70	0.50	0.01
FD	*Aspergillus niger*	0.01	0.50	0.58	0.88	1.16	0.40	0.01
FH	*Aspergillusflavus*	0.01	0.30	0.43	0.83	0.92	0.07	0.02
FJ	*Penicillium expansum*	0.01	0.42	0.51	0.88	1.06	0.30	0.02
FL	*Apergillus oryzae*	0.01	0.40	0.50	0.89	1.01	0.40	0.02

Appendix 5b

Reducing sugar (mg/ml) produced at 32°C by fungal isolates

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.51	0.54	0.94	0.50	0.43	0.01
FD	*Aspergillus niger*	0.01	0.54	0.56	1.00	1.16	0.32	0.01
FH	*Aspergillusflavus*	0.01	0.51	0.52	0.68	1.00	0.42	0.02
FJ	*Penicillium expansum*	0.01	0.31	0.42	0.86	1.28	0.42	0.02
FL	*Apergillus oryzae*	0.01	0.42	0.54	0.69	1.05	0.30	0.02

Appendix 5c

Reducing sugar (mg/ml) produced at 40°C by fungal isolates

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.54	0.62	0.81	0.70	0.23	0.01
FD	*Aspergillus niger*	0.01	0.82	0.94	0.80	1.32	0.07	0.01
FH	*Aspergillusflavus*	0.01	0.43	0.70	0.78	1.12	0.28	0.08
FJ	*Penicillium expansum*	0.01	0.53	0.68	0.63	1.15	0.33	0.07
FL	*Apergillus oryzae*	0.01	0.57	0.68	0.80	1.13	0.30	0.02

Appendix 6a

Reducing sugar (mg/ml) produced at pH 4.0 by fungal isolates

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.62	0.97	0.52	0.98	0.28	0.01
FD	*Aspergillus niger*	0.01	0.54	1.12	0.97	1.45	0.32	0.01
FH	*Aspergillusflavus*	0.01	0.32	0.68	0.68	1.15	0.30	0.02
FJ	*Penicillium expansum*	0.01	0.65	0.68	0.86	1.20	0.54	0.02
FL	*Apergillus oryzae*	0.01	0.53	0.56	0.69	1.05	0.55	0.02

Appendix 6b

Reducing sugar (mg/ml) produced at pH 5.5 by fungal isolates

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.58	0.60	0.68	0.75	0.30	0.01
FD	*Aspergillus niger*	0.01	0.52	0.55	0.94	1.16	0.53	0.01
FH	*Aspergillusflavus*	0.01	0.54	0.58	0.82	1.01	0.33	0.02
FJ	*Penicillium expansum*	0.01	0.52	0.67	0.83	1.21	0.43	0.02
FL	*Apergillus oryzae*	0.01	0.68	0.68	0.86	1.16	0.53	0.02

Appendix 6c

Reducing sugar (mg/ml) produced at pH 7.0 by fungal isolates

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.66	0.88	0.54	0.88	0.30	0.01
FD	*Aspergillus niger*	0.01	0.52	0.52	0.75	1.15	0.30	0.02
FH	*Aspergillusflavus*	0.01	0.55	0.55	0.74	1.00	0.40	0.02
FJ	*Penicillium expansum*	0.01	0.43	0.50	0.82	1.05	0.42	0.02
FL	*Apergillus oryzae*	0.01	0.51	0.50	0.82	1.04	0.29	0.02

Appendix 7a

Reducing sugar (mg/ml) produced by fungal isolates with yeast extract as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.52	0.69	0.87	0.75	0.30	0.07
FD	*Aspergillus niger*	0.01	0.40	0.52	1.12	1.05	0.52	0.01
FH	*Aspergillusflavus*	0.01	0.33	0.64	0.80	0.98	0.32	0.02
FJ	*Nenicillium expansum*	0.01	0.68	0.82	0.82	1.20	0.58	0.02
FL	*Apergillus oryzae*	0.01	0.54	0.64	0.80	1.00	0.39	0.02

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Appendix 7b

Reducing sugar (mg/ml) produced by fungal isolates with peptone as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.40	0.54	0.81	0.68	0.51	0.01
FD	*Aspergillus niger*	0.01	0.51	0.54	0.72	1.22	0.52	0.01
FH	*Aspergillusflavus*	0.01	0.68	0.30	0.96	1.12	0.58	0.02
FJ	*Nenicillium expansum*	0.01	0.52	0.83	1.40	1.20	0.82	0.02
FL	*Apergillus oryzae*	0.01	0.64	0.62	0.87	1.25	0.58	0.02

Appendix 7c

Reducing sugar (mg/ml) produced by fungal isolates with NH4CL as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.60	0.62	0.58	0.70	0.43	0.01
FD	*Aspergillus niger*	0.01	0.60	0.61	0.76	1.30	0.32	0.01
FH	*Aspergillusflavus*	0.01	0.54	0.97	0.78	1.25	0.41	0.02
FJ	*Nenicillium expansum*	0.01	0.68	0.75	0.77	1.22	0.42	0.02
FL	*Apergillus oryzae*	0.01	0.52	0.54	0.81	1.02	0.33	0.02

Appendix 7d

Reducing sugar (mg/ml) produced by fungal isolates with urea as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.50	0.52	0.84	0.68	0.51	0.01
FD	*Aspergillus niger*	0.01	0.31	0.54	1.68	1.12	0.25	0.01
FH	*Aspergillusflavus*	0.01	0.54	0.68	0.80	1.12	0.30	0.02
FJ	*Penicillium expansum*	0.01	0.40	0.42	0.86	1.02	0.27	0.02
FL	*Apergillus oryzae*	0.01	0.29	0.40	0.81	0.92	0.28	0.02

Appendix 7e

Reducing sugar (mg/ml) produced by fungal isolates with $NaNO_2$ as nitrogen source

CODE	ISOLATE	0	4	7	10	14	21	28
FA	*Rhizopus oryzae*	0.01	0.58	0.83	0.52	0.88	0.42	0.01
FD	*Aspergillus niger*	0.01	0.61	0.68	0.76	1.20	0.74	0.01
FH	*Aspergillusflavus*	0.01	0.8	1.42	0.78	1.50	0.31	0.02
FJ	*Nenicillium expansum*	0.01	0.40	0.42	0.80	1.15	0.27	0.02
FL	*Apergillus oryzae*	0.01	0.57	0.58	0.80	1.05	0.52	0.02

Appendix 8

Nutrient Agar (fluka chemical sigma – Aldrich chemical GMblt Riedstr. 2 Steinhein India).

Formulation	g/ℓ
Peptone	5.0
Beef extract	3.0
Sodium chloride	8.0
Agar no. 2	12.0

Potato Dextrose Agar (Lab M Limited, Topley House 52, Wash lane, Lancashire BL9, 6AS, U.K.)

Formulation	g/ℓ
Peptone extract	4.0
Dextrose	20.0
Agar no 1	15.0

pH 5.6 $\pm$2

Yeast Extract Agar (Lab M Limited, Topley House 52, Wash lane, Lancashire BL9, 6AS, U.K.)

Formulation		g/ℓ
Yeast extract	–	0.3g
Peptone	–	0.5g
Agar	–	1.5g
Distilled water	–	100ml
pH	–	7.2

Appendix 9

COST PER KILOGRAM OF THE VARIOUS INGREDIENTS USED TO COMPOUND THE EXPERIMENTAL DIETS

Maize= ₦56.00

Wheat offal= ₦30.00

Microbial degraded cob = ₦26.00

Groundnut cake = ₦60.00

Soybean meal = ₦80.00

Bone meal = ₦35.00; Salt = ₦60.00

Broiler premix = ₦600.00

Methionine = ₦1,300.00

Fish meal= ₦150.00.

Appendix 10

Plate 6: Experimental Birds fed with control commercial diet (control). G1D1 Birds showed balanced growth, no mortality.

Appendix 11

Plate 7: Experimental Birds fed with BECP (18%). G2D2

Birds showed balanced healthy growth

Mean weight gain of birds were higher than the control

No mortality.

Appendix 12

Plate 8: Experimental Birds fed with BECP (82%). G3D3

Birds showed low weight gain

Highest mortality of 3 was recorded.

Appendix 13

Plate 9: Experimental Birds fed with BECP (50%). G4D4

Birds showed balanced healthy growth

No mortality.

Appendix 14

Plate 10: Experimental Birds fed with FECP (18%). G5D5

Birds showed healthy growth

Mean weight gain was higher than the control.

Appendix 15

Plate 11: Experimental Birds fed with FECP (82%). G6D6

Birds showed low weight gain

One mortality was recorded.

Appendix 16

Plate 12: Experimental Birds fed FECP (50%). G7D7

Birds showed balanced healthy growth

No mortality.

Appendix 17

Plate 13: Experimental Birds fed with BECP (18%), 50% and 82%

A : D2 – BECP (18%)

B : D3 – BECP (82%)

C : D4 – BECP (50%)

Appendix 18

Plate 14: Experimental Birds fed with BECP (0%) and BECP (18%)

A : D2 – BECP (18%)

B : D1 – BECP (0%)

Bird fed with BECP (18%) showed a higher weight gain than bird fed with commercial poultry feed.