FUNGAL BIODEGRADATION AND EXOGENOUS ENZYME SUPPLEMENTATION OF CASSAVA STARCH RESIDUE FOR BROILER PRODUCTION

BY

Akintunde Adedayo ADEDOYIN

BSc. (Agric) Special Honours in Animal Science

MSc. (Agricultural Biochemistry& Nutrition)

Matriculation Number: 88230

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ABSTRACT

Cassava Starch Residue (CSR) contains insoluble fibrous component that makes it unsuitable for monogastrics. Its digestibility can be enhanced through microbial biodegradation or probiotic-addition which had been used in ruminants feeding. Information on fungal-treated or enzyme-supplemented-CSR and its use as feedstuff for poultry is a contribution to the giving body of knowledge. Thus, fungal biodegradation and exogenous enzyme supplementation of CSR in broiler was investigated.

One hundred and eighty a-day old Hybro-chicks were allotted to six treatments (r=6) and fed for 49 days on diets containing CSR of 0(control), 5,10, 15, 20 and 25% for optimal utilisation determination. Feed Conversion Ratio (FCR), Body Weight Gain (BWG g/day), Relative Weights (RW) of gizzard, kidney, liver, and heart were measured. Blood cholesterol (mg/100mL) was determined. Apparent Nutrient Digestibility (AND) of Crude Protein (CP) and Crude Fibre (CF) were evaluated. The CSR was biodegraded with *Aspergillus niger*, *Trichoderma viride* and their mixtures (ratio 1:3, w/w) for 0, 7, 14 and 21 days using solid state fermentation procedure for screening. Ether Extract(EE), CP, CF, Acid Detergent Fibre (ADF), Neutral Detergent Fibre(NDF), and Acid Detergent Lignin (ADL) of biodegraded-CSR were determined. Furthermore, 180 a-dayold chicks were randomly alloted to groups (n=36) and fed diets containing 0, 5, 10, 15, 20 and 25% of *A. niger* biodegraded-CSR for 49 days. Pure enzymes (Roxazyme G2G and cellulase) were added to CSR each at 10, 20 and 30% and fed to 210 1-day old chicks (n=42) for 49 days. FCR, BWG, RW; AND: CP, CF, EE, ADF, NDF, ADL, total protein and blood cholesterol were assessed. Data were analysed using the descriptive statistics and ANOVA of completely randomized design at $\alpha_{0.05}$.

The FCR and BWG of birds fed 5% CSR were 2.01±0.01 and 41.0±0.15, and at 0% CSR were 2.10±0.03 and 38.50±0.16 respectively. The RW of gizzard and liver were significantly higher in birds fed CSR compared with control. Blood cholesterol decreased significantly from 148.9±10.5 mg/100mL in control birds to 93.90±6.6 mg/100mL compared to birds fed 25% CSR , while AND of CP and CF were similar in CSR diets. Biodegradation increased CP of CSR by 72.8±0.31% and decreased CF by 33.9±0.09% after 14days. *Aspergillus niger* decreased the NDF from 68.0±0.28% to 53.7±0.16%, ADF 32.0±0.13% to 20.6±0.09%, and ADL 14.9±0.31% to 12.4±0.12% in CSR than *T. viride* and mixture of *A. niger and T. viride.* The FCR (1.94±0.02)

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and BWG (46.4±0.17) of birds fed 15% biodegraded-CSR was different when compared with 0% biodegraded-CSR. Addition of Roxazyme G2G or Cellulase each with 20% CSR diet improved FCR (1.89±0.01, 1.91±0.04) and BWG (47.2±0.12, 47.1±0.20) respectively compared with control. However, RW and AND of CP, CF, EE, NDF and ADF improved in birds fed *A. niger*-biodegraded and enzyme supplemented diets. Total serum proteins and blood cholesterol levels were however not affected.

Fungal biodegraded at 15% and enzyme supplemented at 20% cassava starch residue enhanced performance of broilers and also lowered the blood cholesterol.

Keywords: Enzyme supplemented diet, Improved feedstuff, Fibre biodegradation, Hybro-chicks

Word count: 493.

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CERTIFICATION

We certify that this study was carried out under our supervision by Akintunde Adedayo ADEDOYIN, in the Department of Animal Science, University of Ibadan, Ibadan.

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Professor O.O.Tewe
Agric Biochemistry and Nutrition Unit
Department of Animal Science
University of Ibadan, Ibadan.

………………………………………

Professor A.A. Onilude
Industrial Microbiology Unit
Department of Microbiology
University of Ibadan, Ibadan.

DEDICATION

This project work is dedicated to God,

And

My parents who did everything to see me through life,

And

To the entire academic moulders and builders of my life.

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Finally, I humble myself before God and vow to be in His presence all the days of my life, by His grace. It is Him who gave me this great horizon. I shall ever remain grateful. Amen

Akintunde Adedayo, **ADEDOYIN**

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CHAPTER ONE

1.0 INTRODUCTION

Agriculture is the leading non-oil sector to the total gross domestic products (GDP) yet, production of staple crops and livestock have been able to meet the needs of a rapidly growing population. As at April 1999, Nigeria was one of the eighty-sixth Nation of the world, defined as Low-Income-Food-Deficit Countries (LIFDC), forty-three of which were in Africa alone. These were countries which were nutritionally characterized by a supply gap, between countries' own production and physiological nutritional needs, in addition to a demand deficit occasioned by lack of purchasing power (FAO, 2009). This is because the feed cost is often implicated in the high costs of livestock products particularly poultry. Feed cost is estimated to represent over 70% the costs of producing poultry intensively. The energy part of the finished feed represents the largest single dietary requirement of animals in terms of the cost due to the amount required (Olomu, 2003).

Maize still constituted the main conventional energy force of livestock feeds in Nigeria, contributing about 60-80% of the total diets. In the last three decades there has been a gross inadequate supply of maize to the Nigerian livestock feed industry. This is mainly due to high cost of maize and the uncertainty about their sustainable supply as energy source for livestock necessitating the search for alternatives.

Cassava (*Manihot esculenta* Crantz) is an annual root crop grown widely in tropical and sub-tropical areas. It thrives in sandy-loam soil with low organic matter, receiving low rainfall and high temperatures. The cassava root contains up to 3,870kcal/kgME (Tion and Adeka, 2010). The wide acceptability of cassava plants in the tropics makes it a possible alternative to cereals. Tewe (2004) highlighted the tremendous potentials of cassava and its by-products for livestock feed in sub-Saharan Africa. The author reported that cassava has transformed from a famine security and subsistence crop to an industrial crop. To underline this fact, the African continent account for more than 90% of the global cassava production of which Nigeria is the world largest producer of cassava with about 37.6 million metric tonnes annually (FAO STAT, 2012). Toward this, the presidential initiatives on cassava in Nigeria has expanded the use of cassava root for product other than the traditional foods. These includes;

- Starch for textile, food and pharmaceutical industries
- Flour for flour milling industries and confectionaries

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- Ethanol for the petroleum, cosmetic and beverage industries
- Fructose syrup for beverage, bottling industries,

It is estimated that about one third of cassava root production is now used for such products (Tewe, 2004). It then becomes imperative that cheap available agro-industrial by-products such as cassava peel, cassava starch residue, cassava sieviate and other by-products produced from processing sheds or factories should be optimized for feeding monogastric animals. Cassava starch residue constitutes 10-20% of the processed roots. This led to the study on the use of root crop residues for feed formulation and broiler production. Besides getting benefits from selling cassava starch, flour, ethanol, and fructose, farmers could get more benefits from raising poultry based on using cassava starch residue as an available feed resource. This will enable agriculture and especially cassava play a pivotal role in the attainment of some of Millennium Development Goals (MDGs). Meanwhile, the potential value of cassava by-products and their maximum inclusion rates in livestock diet depend on their nutritional characteristics, their safety for human health and the alternative uses. In developing countries like Nigeria, greater proportion of abundance Agro-industrial by-products (AIBs) such as cassava peel, cassava starch residue, produced are grossly unexploited due to it inherently low protein and high Non-starch polysaccharides (NSPs). They are refractive to monograstic digestive enzymes because of lack of and or insufficiency of endogenous enzymes secretion. These components may lower the utilization of other diet nutrients, leading to a reduced bird performance. Examples of such NSPs components in cassava and its by-products include β-glucan, arabinoxylan and certain amylopectin (Caret *et al.,* 1997). With the increasing role of biotechnology, specifically fungal biotechnology with its inexpensive mode of application has been used as a tool for the effective conversion of these wastes into useful products. Fungi can increase the protein and soluble sugars and reduce the complex carbohydrate of the AIBs (Agro-industrial by-products) as posited by Onilude (1999); Iyayi and Losel (2001); Iyayi and Aderolu (2004); and Aderolu and Oyedokun (2009). Similarly, exogenous enzymes added to the feed or used during processing of feedstuffs have the potential to improve feed efficiency, reduce pollution associated with poultry manure and increase the use of low costs feed ingredients. (Bedford and Schulze, 1998; Bedford 2000; Acamovic, 2001, Cowieson and Adeola, 2005).

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Supplementation of fibrous diets with high quality protein and amino acids improved the utilization of such diet and spare the dilution effect on energy by fibrous feed ingredient (Delorme and Wojeik, 1992; Longe, 2006). Other methods employed in the improvement of dietary fibre utilization are moist heating, physical reduction and pelleting, radiation techniques, supplementation of diet with sand or grit, chemical treatment of fibrous diets, nutrient supplementation, and use of antibiotics (Onifade 1993; Longe 2006).

In furtherance, Vander Wal (1993) also outlined routes by which wastes could be turned to feed. These are:

- Physical and chemical processing: physical treatment involves mechanical, ultra filtration, precipitation and texturization while chemical treatment involves the use of sodium hydroxide, urea and alkali.
- Microbial conversion by growing micro-organisms on waste or by direct production of single cell protein (SCP).
- Conversion of wastes by animal through direct feeding to ruminants. The nutritive value should be fairly predictable and utilization should be economically viable.

The surge to integrate poultry production and root crop residue utilization would go a long way to ease farmers' efforts at producing feed for their poultry flocks. Production of poultry meat and eggs through increased proportion of improved residue in the diet would develop rural poultry production, improve nutrition, reduce protein deficiency and pollution hazard among the teaming population in developing countries. This approach tends to improve also the integration of root-crop and poultry production.

Indeed, the biological value of animal protein is clearly superior to that of plant protein and the fact that the ratio in human nutrition does not appear to be improving at least for the moment, it would be necessary to increase the availability of animal protein using cost effective but productive diets such as those based on root-crop residue. However, the paucity of information on the use of cassava starch residue in broiler diets also provided no room for comparative selection of differently treated cassava starch residue in broiler.

1.1 Justifications of the Study

- The cost of feed accounts for 60-80% of the total cost of producing poultry intensively.
- Production of less expensive energy ingredient for broiler production.

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- Use of locally available enzyme and safe microbial species to enhance the feeding value of Cassava Starch Residue.
- Greater reduction in by-products dumped around the processing sites
- Increase in the feed quality of fibrous by-products by the conversion of NSPs to single cell protein.
- Cassava starch residue is readily available all year-round.

1.2 General objectives of the Study

- The general objectives are to investigate changes in the physical, biochemical and nutritional status of cassava starch residue biodegraded with *A. niger* and *T. viride* and how these changes and enzyme supplementation influence their utilization by broilers.
- To compare the efficacy of Roxazyme G2G and cellulase enzymes as growth ameliorator in broilers fed high fiber diets.

1.3 The specific objectives of the study include:

- To determine the optimal level of inclusion of raw cassava starch residue as feed for broilers.
- To determine the optimum biodegradation period of cassava starch residue with *Aspergillus niger* and *Trichoderma viride.*
- To evaluate the effects of feeding biodegraded and enzymes supplemented cassava starch residue on the feed intake, feed to gain ratio, body weight and dress percentage of broilers.
- To evaluate the economic viability of inclusion of biodegraded and enzyme supplemented Cassava Starch Residue (CSR) in the diets of broiler.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of Dietary Fibre in Nutrition

Dietary fibre (DF) has been defined by various authors (Longe, 2006, and Hill, 2010,)as the reminant of vegetative cell walls which are not hydrolyzed by alimentary enzymes of humans. Ologhobo (2012) defined DF as unavailable carbohydrate and lignin , Souffrant, (2001) and Hill, (2010) defined it as plant material which is resistant to digestion by secretions of the human gastro-intestinal tract. Cellulose, hemicellulose, lignin and pectin are the plant constituents usually considered as part of dietary fibre while additional minor components include cutins, gums, mucillages, some proteins and oligosaccharides (Longe, 2006). Due to the complexity and variability of the fibre composition, therefore, each fibre is characterized by distinctly different functional or physical properties.

From the current understanding; fibre is not a single substance nor is it inert, indigestible unavailable material which simply passes through the human gut. Also, it is no more assumed to be of little or no benefit in the nutrition of the simple- stomached animals including humans. Thus, the recognition of the importance of DF in human nutrition in the last two decades has reopened the question for other simple-stomached animals that may be important in agricultural production (Van Soest and Demeyer, 1999; Hill, 2010). Similarly, Thomas, (1997) noted that the recent clinical interest in fibre digestion in humans has stimulated numerous studies with simple stomached animals. Inspite, its little significance as a nutrient source in comparison to other macro and micronutrients, DF has been shown to be a health requiring component of our diet today. This is more so because the use of DF is now considered as a routine treatment for constipation, coronary heart disease (Anderson, 1986), diverticular disease (Jenkins *et al*., 1986), obesity (Hagander *et al*., 1998), diabetes, hypercholesterolemia and cancer. In livestock production, research on AIBs utilization has recently gained more interest for various reasons. The most important reason is because studies in the recent past have showed that AIBs (fibrous ingredients) can be considerably utilized by monogastric livestock as energy yielding feedstuffs. The present study therefore tends to indicate the possibility of substantial inclusion of fibrous feedstuffs into monogastric animal diets. Microbial degradation of fibre in the caecum and colon of pigs generates volatile fatty acids which may provide from 5 to 30% of the energy requirements for growing pigs (Yen *et*

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al., 1991). Approximately, 10% value was estimated by McNeil, (1984) for humans. Yen *et al*., (1991) however indicated that this estimation may even be higher for adult animals, because the numbers of celluloytic bacteria in feacal samples of adult pigs are larger than those in growing pigs. Van soest and Demeyer (1999) also reported that the short chain fatty acids produced from fibre fermentation were efficiently utilized. Although, Austic and Neshiem (1999) indicated that poultry are less capable than most mammalian monogastric animals in the digestion of fibre.

With respect to animal feeds in the tropics, diets formulated from locally available AIBs often contain high fibre content. Although, diets high in DF can limit energy intake, bulky diets may be more appropriate in the tropics where high ambient temperatures determine energy requirement (Longe, 2006) and concluded that fibre rich ingredient may have a useful role to play in animal feeding in the tropics provided the optimum nutrient intake is not impaired and that sufficient digestible energy is metabolized. Various authors (Pond 1989; Olubamiwa *et al*., 2002 and Longe 2006) asserted that as conventional high energy cereals and other food sources progressively increase in demand for direct human use in response to world population growth, alternative fibrous ingredients will be used increasingly for livestock production.

2.2 Significance of Dietary Fibre

The significance of dietary fibre thus includes.

1. High fibre diets, due to their consistency, encourage mastication and stimulate the secretion of digestive juices. The soluble components of dietary fibre cause an increase in the viscosity of the stomach contents, thereby retarding gastric emptying, this affecting the rate of digestion and the uptake of nutrients, and creating a feeling of satiety (gut fill) (IFST, 2001).
2. Dietary fibre is observed to have laxative effects and it seems to be achieved this via two different mechanism:

   Insoluble fibre is resistant to fermentation in the gut and is excreted in the faeces, thus it increases stool weight by its ability to hold water. Soluble fibre, though not excreted in the faeces, produces an increase in stool weight due to an increase in bacteria cells, which also hold water. By the mechanisms above, fibre improves bowel function and tends to reduce the risk of disease and disorders.

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3. Dietary fibre may also bind/dilute secondary bile acids, which are potential carcinogens. It may also reduce the toxic effects of heavy metals and pesticides (Dietary Fibre and Resistant starch, 2001; IFST, 2001).
4. Dietary fibre (NSP, resistant starch and oligosaccharides), on fermentation by bacterials in the gut produce short chain fatty acids (SCFA) and gases (acetate, propionate and butyrate) which are important sources of energy for the cells of the colon, maintaining colon welfare (Dietary fibre and resistant Starch, 2001).
5. Dietary Fibre serves as prebiotics (non-digestible food ingredients such as resistant starch and oligosaccharides used as a source of fuel for bacterial in the gut) (Hill, 2010).
6. Dietary Fibre influences the utilization of other nutrients in the diets due to it's absorptive properties (Longe, 2006), changes in gut transit time, digestion and absorption, therefore feed intake (Abdelsamie *et al*., 1983).
7. Dietary fibre has an abrasive action in the intestinal wall, which is believed to cause an increase in metabolic nitrogen excretion (Ologhobo, 2012).
8. High dietary fibre is known to adversely affect growth rate and feed conversion (Tewe, 2004). Annison *et al*., (1996) and Meeusen and Vallet (2001) explained that 5 to 8% of the dietary energy in poultry is derived from hind gut fermentation of carbohydrate, and thus high fibre diets, which undergo more hind gut fermentation, result in lower energy production, lower feed conversion rate (FCR) and decreased growth rate.
9. Dietary fibre is also reported to have effects on blood parameters such as blood cholesterol, total lipids and blood glucose (Hale *et al*., 1986, Dietary Fibre and resistant Starch, 2001, Requirement of non human primates, 2003).
10. Soluble fibre has been shown to improve glucose metabolism and insulin response (IFST, 2001).
11. Dietary fibre has an effect on enzymes activity (Patridge *et al*., 1982).

2.3 Properties of Dietary Fibre

The different materials that are classified share the characteristics resistance to endogenous digestive enzymes, their physical and biological effects are adverse (Whittemore *et al*, 2008) and this is based on their physiochemical properties. Van Soest (1982) and

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Whittemore, *et al*. (2008) asserted that DF demonstrate a wide range in their physical and chemical properties.

According to the method used for its measurement and depending on the medium used to determine the compounds (Van Soest and Wine, 1967), dietary fibre can be classified as crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and non starch polysaccharides (NSP).

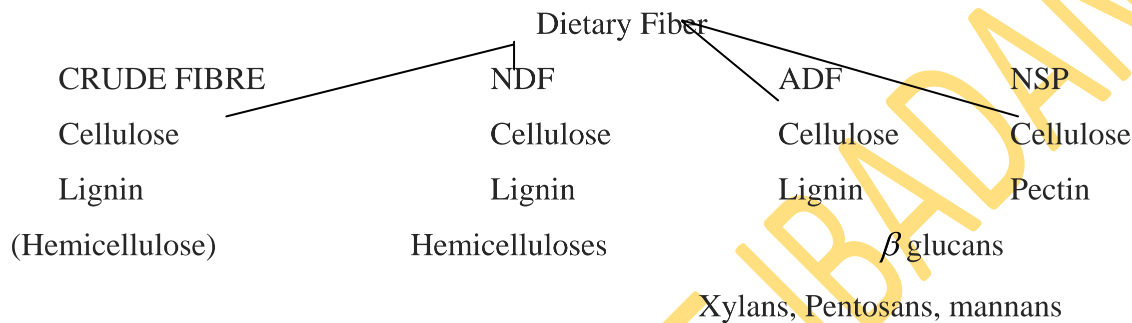

Fig 1: Properties of Dietary fibre
(Source; Souffrant, 2001)

Because of the different chemical composition, all dietary fibre compounds have special physical properties that affect the efficiency of the digestive processes of animals of different forms.

Mainly, the plant cell wall is composed of microfibrils of cellulose forming a strong frame work that gives rigidity to the plant. These microfibrils are embedded in a matrix composed of a lignin network (Phenylpropane unit) which cements other matrix polysaccharide such as hemicelluloses (arabinoxylan, xyloglucan) and Pectins (Longe, 2006).

Each source of fibre has an intrinsic property based on its chemical composition and physical characteristics determines the biological and its fermentative properties, the maximum limit for the rate and extent of microbial degradation of dietary fibre sources (Iyayi, 2004). Longe (2006) noted that the extent of fermentation of a particular substrate under optimal condition may differ depending upon the physical structure of the fibre, apart from its chemical structure particularly for fibre from natural sources which would be more complex in nature compared with purified fibre like cellulose derivatives of cellulose, pectins etc. this the distinctive nature of a fibre source is predicated on its physical chemical structural

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(morphological) properties. These varying properties largely elicit different and sometimes similar physiological actions as they transverse the gastrointestinal tract of animals.

Irrespective of the individual differences, some generalization can still be identified, hence, fibre chemistry and morphology are frequently discussed according to the most common macromolecules or macrocomponents such as cellulose, hemicellulose, lignin, pectin etc. or according to the type of monomerics units of the non-starch polysaccharides (NSP) such as hexosans, pentosans, hexuronic acids etc. (Onifade, 1999, Ologhobo, *et al*., 2007).

2.4 Physical Properties of Dietary Fibre

The main physical properties of DF are discussed in context of the features it exhibits both in a physiological environment and isolated state. It is not uncommon therefore for some physical characteristics to be discussed as physiological implications of DF in the gastro intestinal tract (GIT); since some of these properties are demonstrable in a physiological environment (Longe, 2006). Eastwood and Passmore (1983) asserted that DF is a biological unit, and not a chemical entity *per se,* and related to the age and anatomy of the fruit or plant eaten. The physical properties will be determined by the chemistry of the constituent polysaccharides and lignins. In plant, these macromolecules are systematically intermeshed for anatomical and physiological functions. The physical attribute of the fibres in plant cell wall determine the shape and so the function of the plant and also the effects of DF on digestion and absorption. The proportion of water soluble and insoluble fibre, fibre chemistry, particle size, and the extent to which cellular and pore structure of the fibre is affected by processing and cooking all affect digestion and absorption. The physical effects of fibre in the gut be different from those observed in vitro. Factors such as osmolality, pH, the presence of bacteria will profoundly influence the physiological effects of fibre (Anderson and Chen, 1979). Different fibres have been reported to have antagonistic or synergistic effects when fed together (Coffey, 2008).

Some properties of plant fibres can be discussed. Such as water holding capacity (WHC), caution exchange capacity (CEC), absorption properties and gel formation (Zhang *et al*., 2005). All these have been extensively discussed (Anderson and Clen, 1979: Roberson and Eastwood, 1981 and Eastwood and Passmore, 1983).

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2.5 Water Holding Capacity/H_2o Absorption

Ability of fibre to hold maximum volume of water which is known as fibre saturation point depends on the chemistry of the macromolecules (Aderolu, 2000, and Badal, 2003), the peculiarities of the electrolyte concentration and pH of the surrounding liquid. Water may pass into the capillary structure of a fibre without changing its volume. The lignins are less hygroscopic than polysaccharides i.e. water is a poor solvent for lignin. Dietary fibre has a water holding capacity which is a function of the fibre source and the amount of water each fibre can hold influences the stool weight. Water holding capacity is found to be more a function of fibre structure than chemical composition (Robertson and Eastwood, 1981; Aderolu, 2000) and there exists a close relationship between it and acid detergent fibre (ADF) but not lignin and the water holding capacity.

Water holding capacity seems to be greatest for vascular tissues such as roots stems and leaves and it is less marked in storage organs. It is not affected to a great extent by time of soaking or temperature neither is it affected cooking (MC connel *et al*, 1974). The main sources from variation in water holding capacity (WHC) arises from variation in the type of plant material considered. The role of DF appears to be retention of water within the lumen of GIT and more especially feaces (Coffey, 2008). Williams and Olmstedt (1936) showed that different vegetables could variably influence stool weight and suggested rational advice on what high fibre containing diet aimed at increasing stool weight should contain. Rasper (1979) observed decreased in WHC of soybean hull and peanut red skins due to heat treatment prior to hydration. Enzymes treatment was revived to cause increase polar-site such as carboxyl and amino groups which have high affinity for water binding. (Ogunlayi, 1995; Aderolu, 2000), observed that the WHC of yam bean was improved by cooking following soaking in all the test solution.

2.6 Particle Size Analysis

This refers to the size of the feed or individual particles of the feeding stuffs. According to Wadhwa *et al*. (1998) and Zakaria *et al*. (2008) increase in particle size irrespective of moisture level or soaking period, the release of water soluble protein and carbohydrate decreased and water holding capacity increased linearly. Water holding capacity increased with increase in particle size while water soluble carbohydrate and protein decreased with increase

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particle size. Only a few studies have focused on the nutritive value of legumes (Lacassagne *et al*; 1992). According to Coffey (2008) Starch and protein digestibility as observed in the excreta of chicken was affected by the accessibility in the coarse particles. Long-staff and McNab (1987) found reduced feacal starch digestibility when whole or split compared to ground peas were fed to adult cockerels. Decreasing particle size therefore could be a means of improving starch availability through disrupting cell wall and enhancing endogenous enzymes accessibility (Yegani and Korver, 2008). Coffey (2008) said particle size significantly influenced digestibility with more intact cell walls in the coarser particles, thereby restricting nutrient uptake.

2.7 Water and Ethanol Solubilities

According to Ogunlayi (1995) microbial enzymes in the various forms were found to influence the solubility of feeds between the cereals and legumes. This author found different ethanol solubility property when feed was treated with heat. The same author found that cereals fibre appeared very little affected by heat treatment when compared to legumes fibre products. Resper (1979) observed a reduction in hydration capacity of soyhull and peanut red skins due to heat treatment at $110^{o}c$ for 2 hours prior to hydration. Both ethanol and water solubilities were enhanced by the microbial treatment. The degree of solubilities of fibre had been extensively used in dietary fibre classification (Graham and Aman 1991). It is therefore concluded that solubility of protein and carbohydrate in water increase with increase in moisture level irrespective of particle size.

2.8 Cation exchange capacity

Acid polysaccharides with uronic acid moieties can act as cation exchanger i.e they can bind metals. Cation exchange properties are mainly related to free carboxyl groups in pectin and to a lesser extent in hemicellulose characteristics for a space. Dietary fibre might bind certain mineral as well as bile acids and ingestion of very large quantities would decreased the availability of minerals such as calcium, zinc, copper, iron and magnesium (Anon, 1977; Carret *et al,* 1998).

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2.9 Chemical Properties of Dietary fibre

The cell wall chemistry of both monocot and dicot plant along with their seeds have been properly discussed by Selvendran, (1984). The structure of plant cell (DF) changes with growth, stage of differentiation, specialist fractions of the cell, as well as the cell environment. Understanding the chemistry of dietary fibre enhances the appreciation of the extent of utilization and the physiological response it elicits in the consumer either animal or man. Dietary fibre macromolecules are cellulose, hemicellulose lignin, and pectins among others.

2.10 Physico-Chemical Properties of Dietary Fibre

The main physico-chemical properties of dietary fibre with nutritional significance are cation exchange capacity, hydration properties, viscosity and organic compound absorptive properties (Back Kunden, 2001). The physicochemical properties are linked to the type of polymers that make up of the cell wall and their intermolecular association (McDougall *et al*., 1996). The hydration properties are characterized by the swelling capacity, solubility, water holding capacity (WHC) and water binding capacity (WBC).

The solubilizaiton process of the polymers is the swelling in which incoming water spread the macromolecules until they are fully extended, dispersed and then solubilised (Thibault *et al*., 1992). Solubilizaiton is not possible in the case of polysaccharides that adopt regular ordered structure (e.g. Cellulose or linear arabinoxylan) where the linear structure increases the strength of the non-covalent bonds that stabilize the ordered conformation. Under these conditions only swelling can occur (Thibault *et al*., 1992).

Morris (1992) reported that majority of polysaccharides give viscous solution when dissolved in water. The viscosity is primarily dependent on the molecular weight of the polymer and the concentration. Large molecules increase viscosity of diluted solutions and their ability to do this depends primary on the volume they occupy (Bach Knudsen, 2001).

2.10.1 Cellulose

This is the major structural polysaccharide of the plant cell wall, and most widely distributed polymer on the earth (Rabinovich *et al.,* 2002a). It is an unbranched polymer of glucose or homogenous polymer (contrary to hemicellulose and pectin), formed from linear chain of β(1-4) linked D-glucopyranosyl unit (whereas starch is formed of α(1-4) linked and

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1-6 D glucopyranosyl chain). Cellulose molecules are arranged within the microfibrils in a highly ordered crystalline state in chain 4000-6000mm long and possibly.4nm in diameter each consisting of several thousands of glucose units (Eastwood and Passmore, 1983). Cellulose is soluble and partially hydrolysed in strong acid solution (i.e. 72% H_2S04).

Quantitatively, cellulose represent 40-50% of dry matter in hull of legumes and oilseed, 10-30% in forage and beet pulps, 3-15% in oil seeds or legume seed (Annison and Choct, 1993). Most cereal grains contain small quantities of cellulose 1-5% DM except in oats (10%). Mares and Stones, (1973a) reported that cellulose in cereal grain cell wall can be recovered from the insoluble residue left after vigorous extraction of cell wall material matrix component with alkali. Modified or isolated cellulose such as ethyl and carboxymethyl derivative (used in food industry and ethyl cellulose (used as laxative or a appetite suppressor) have very different chemical properties from the pure cellulose. As a result of the substitute group which disrupt the hydrogen bond, these compound are soluble than the natural forms (Cummings, 1984).

Cellulases can be divided into three major enzymes activity classes (Goyal et al., 1991; Rabinovich *et al*., 2002 a,b).

These are:

(1) Endoglucanases or endo 1 -4, β glucanase (EC.3.21.4)

(2) Cellebiohydrolases (E.C 3.21.91)

(3) β-glucosidase (E.C.3.2.1.21)

Endoglucanases are often called Carboxymethyl cellulose (CMC). Cellulases are proposed to initiate attack randomly at multiple internal sites in the amorphous region of the cellulose fibre opening sites for subsequent attack by Cellobiohydrolases (Wood, 1991).

Cellobiohydrolases often called exoglucanases, are the major component of the fungal cellulose system accounting for 40-70% of the total crystalline cellulose (Esterbauer, *et al.*, 1991). Cellobiohydrolases remove mono and dimmers from the end of the glucose chain.

β-glucosidase hydrolyse glucose dimmers and in some cases cello-oligosaccharidses to glucose. Generally, endoglucanases and cellobiohydrolases work synergistically in the hydrolysis of cellulose but the details of the mechanism involved are still unclear (Rabinovich *et al*., 2001 b).

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2.10.2 Hemicellulose

Hemicellulases are branched heterogenous polymers (units linked in $\beta 1 \rightarrow 3$, $\beta 1 \rightarrow 6$, α $1 \rightarrow 4$, $\alpha 1 \rightarrow 3$) such as highly branched arabino-galactan (in soybean) galactomannans (seed of legumes or glucomannan), and their uronic acid derivatives.

Various workers used the term hemicellulose and pentosans interchangeably because the pentose containing polysaccharides make up the bulk of hemicellulose (Neukon *et al*., 1976). According to Eastwood and Passmore (1983), hemicelluloses are group of several polysaccharides, with lower degree of polymerization than cellulose. They have a β1-4 linked backbone of xylose, mannose or glucose residues that can form extensive hydrogen bond with cellulose. The authors also reported that xyloglucan is the major hemicellulose of primary cell wall in dicotyledonous plants while mixed linked glucan ($\beta 1 \rightarrow 3,4$) and arabinoxylans are the predominant hemicelluloses in the cereal seed.

Quantitatively, hemicellulose constitutes about 10 to 25%DM in forage and agro-industrial by-products (bran, oil seed and legume seed, hulls and pulps) and about 2-12% Dm of grain and roots. They are closely associated with lignin in the cell wall and along with cellulose they both encrust the cell wall and form the secondary thickened tissues (Van Soest and McQueen, 1973). Anderson and Chen (1979) concluded that hemicellulose exhibit water-holding capacity and can bind cations. The principal sugar components of these hemicellulose heteropolysaccharides (Howard *et al*., 2003) are: D-xylose, D-Mannose, D-glucose, D-galactose arabinose D-lucoronic acid, 4-0 methyl-D glucuronic acid, D-galacturonic acid and to a lesser extent, L-rhamnose, L-fucose and various O-methylated sugars.

Based on the amino acid or nucleic acid sequence of their catalytic modules, hemicellulases are either glycoside hydrolases (GHs) which hydrolyse glycosidic bonds or carbohydrate esterases (CEs) which hydrolyse ester linkages of acetate or ferulic acid side groups (Howard *et al*., 2003) and according to their primary sequence homology, they have been grouped into various families (Henrissat and Bairoch, 1996; Rabinovich *et al*., 2002 a,b).

Xylan is the most abundant hemicellulose and xylanases are one of the major hemicellulases which hydrolyse the β-1,4 bond in the xylan backbone yielding short dioligomers which are further hydrolysed into single xylose units by β-xylosidase. Other xylanases are α-D glucuronidases which hydrolyse the α, 1-2 glycosidic bond of the 4-0 methyl D-glucuronic acid aide chain of xylans (Howard *et a*l., 2003).

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2.10.3 Lignin

Lignin can be described as a branched network of phenylpropane units (Bach Knuden, 1997). Bhat, (2000) described lignin as an amorphous high molecular weight (between 1000 to 4500nm), aromatic polymer composed of phenylpropane residue. Lignins are partly linked to cell wall cellulose and non-cellulose polysaccharides (Liyama *et al.,* 1994). It is formed at the site of lignifications by the enzymatic dehydrogenation and subsequent polymerization of coniferyl, sinapyl and P-coumaric alcohols, the monomeric units derived from these alcohols are called guiacyl (3-methoxyl 4-hydroxy phenyl propane) and P-coumaryl (4-hydroxyl phenyl propane) residue respectively (Selvendran, 1984).

In live plants, lignification serves two main functions. It cements and anchors the cellulose microfibrills and other matrix polysaccharides and this may stiffen the wall thus preventing biochemical degradation and physical damage of the wall (Bach Knuden, 1997). These properties of lignified wall are important in dietary fibre because they minimize the bacterial degradation of the walls in the intestine of animals and humans. Also, lignin is generally regarded as the main non-carbohydrate fraction in plant cell wall and the source of such resistance to microbial degradation. Gordon *et al*., (1983) however, reported that lignin is not only component of the lignocellulose matrix, it also forms part of the lignin carbohydrate complex stabilized by phenolic acid (Ferulic, 4-coumaric acid) and acetyl constituent of cell wall. The lignin-carbohydrate complexes are usually chemically modified during microbial attack and are thus not recovered in acid detergent fibre (Gordon *et al*., 1983).

Bhat (2002) indicated that lignin is virtually insoluble in strong acid or alkali and it is not digested or absorbed in the colon. Lignin can bind bile salts and other organic materials and may delay or impair small intestinal absorption of associated nutrients. Identifying lignin degrading microorganisms has been hampered because of the lack of reliable assays, but significant progress has been made through the use of a 14 C-labelled lignin assay (Freer and Detroy, 1982).

Two families of lignolytic enzymes are widely considered to play a key role in the enzymatic degradation. Phenol oxidase (Laccase) and Peroxidases (lignin Peroxidase Lip) and Manganese peroxidase (MnP) (Krause *et al*., 2003, Malherbe and Cloete, 2003). Other enzymes whose roles have not been fully elucidated included H_20_2-producing enzymes: glyoxal oxidase (Kerstern and Kirk 1987) glucose oxidase (Bourbonnaise and Paice 1988),

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methanol oxidase (Nishida and Eriksson, 1987) and oxido-reductase (Bao and Renganathan, 1991).

2.10.4 Pectins

These are a complex mixture of colloidal polysaccharides, they are partly esterified rhamnogalacturonans with a α(1-4) linked D-galacturonan chain interspersed with L-rhamnopyranosyl residues with side chains which include D-glucuronic and galacturonic acid. Some acidic groups are methylated (Eastwood and Passmore, 1983). The basic structure is a polymer of galacturonic acid with variable degrees of esterification of the uronic acid with methyl or acetyl groups (Anderson and Chen. 1979). Pectic substances belong to the nitrogen free extract fractions of the feedstuffs (Wilde, 1980). Pectins are a group of heterogeneous polysaccharides found in the intracellular regions and the cell walls of most fruits and vegetables, with the greatest abundance in limes, lemons, grapefruits and oranges. Wilde (1980) reviewed the chemistry of pectins in a study on the utilization of citrus pectins in pigs. Ologhobo (2012) confirmed that pectins are not digested in the small intestine and therefore pass through the small intestine to be degraded almost completely in the large intestine of humans and pigs. The monomers of the pectic substances are however absorbable. Wilde (1980) states that pectic substances should be fully available to monogastric animals, since the galacturonic acid monomers are linked by α-1-4 bonds, which are comparable to the bonds in starch or glycogen. Rouse (1977) affirmed that pectin is composed of D-anhydrogalacturonic acid units linked through (1- 4) glucosidic bonds forming a polygalacturonic acid with some of the carboxyl groups esterified with methanol. Variations in methoxyl content exist among different plant pectins. Over 50% of the carboxyl groups of high methoxyl or high ester pectins are esterified, which correspond to a methoxyl content of 7-10g/100g pectin (Nelson, 1977). This degree of methoxylation is commonly present in citrus pectin. Ingestion of 55 -79g of methoxyl is usually fatal (Cooper and Kini 1962). However, studies involving the consumption of about 50g/day pectin in human subjects have not resulted in poisoning. Pectins readily hold water in an interconnecting network (Eastwood and Passmore, 1983), while Rotenberg and Anderson (1980) asserted that they have an absorbent effect in the alimentary canal and increases the feacal excretion of lipids, sterols, bile acids and nitrogen substances.

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2.10.5 Gums

Gums are water-soluble viscous polysaccharides of 10,000 - 30, 000 units, mainly glucose, galactose, mannose, arabinose and their uronic acids, which may be methoxylated and acetylated (Beg *et al.,* 2001). Prema, (2002) was of the view that they are not components of the cell wall, but that they have biochemical and physiological properties that resemble pectins and certain hemicelluloses. However, they are a complex group of highly branched polysaccharides. Gums commonly used in the food industry are gum arabic, tragacanth, karaya gum, carob gum and guar gum, which are obtained as exudates from stems or seeds of tropical and semi-tropical trees and shrubs.

2.10.6 Mucilages

Mucilages are polysaccharides from seeds and seaweeds used in small amount in food industry as thickening and stabilizing agents by virtue of their water holding and viscous properties. The mucilages of some seeds (e.g. ispaghula husks) are bulk laxatives made up of highly branched arabinoxylans. Alginic acid from sea weed is a polymer of (1-4) linked B-D mannuronic acid or of (1-4) linked –L guluronic acid or a mixture. There are also polysaccharides containing glucose in seaweed mucilages. Beg *et al.* (2001) opined that the mucilages may be sulphated into a variable degree.

2.10.7 Other plant cell wall substances

Longe (2006) and Ologhobo (2012) mentioned other compounds that are intimately linked to the plant cell wall. These include, phytic acid, silica, lipids, plant sterols, proteins saponins, tannins, cyanide etc. they are largely indigestible components.

2.10.8 The Non Starch Polysaccharides and Plant cell Wall Constituent

The carbohydrates which include the low molecular weight (LMW) sugar, starch and various cell wall and storage non-starch polysaccharides (NSPs) are the most important energy sources for non-ruminant and ruminant animals (Bach Knudsen, 1997). The NSP and lignin are the principal components of call wall and are commonly referred to as dietary fibre. (Theander *et al*., 1993, 1994). Dusterhoft *et al*., (1991) observed that the intracellular NSOs such as fructan and mannans may also be constituents of some plant materials.

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The plant cell walls consist of a series of polysaccharides often associated and/or substituted with protein and phenolic compounds in some cells together with the phenolic polymer lignin (Selvendran 1984, Thender *et al*., 1993). Back Kundsen (1997) reported the building blocks of the cell wall polysaccharide to include: Pentose; arabinose and xylose, the hexoses; glucose, galactose and mannose, the 6 deoxyhexoses; rhamnose and fucose and the uronic acid, glucoronic and galacturonic acids. The main polysaccharides of plant cell wall according to Selvendran (1984), and Theander *et al*., (1993) are cellulose, arabinoxylans, mixed linked (1-3, 1-4) D-glucan (β-glucans), Xyloglucan, Xylan, rhamnogalacturonans and arabinogalactan.

Among the numerous types of cell wall polymers (Chesson, 1995), five major classes of fibre can be selected according to their chemical structure and their properties: four classes of water insoluble polymers (Lignin, cellulose, hemicellulose, pectin substances) and one class of various water soluble NSP and oligosaccharide.

Oldale (1996) concluded that NSPs of plant cell; cellulose, hemicellulose, pectin, lignin and protein, are also found in the plant cell wall, however, the author observed that the NSPs fraction accounts for 70-90% of the cell wall.

2.10.9 Degradation of Lignocellulose

Lignocellulose consists of lignin, hemicellulose and cellulose. Due to the difficulty in dissolving lignin without destroying it and some of its subunits, its exact chemical structure is difficult to ascertain (Sun and Cheng, 2002). In general, lignin contains three aromatic alcohols (Coniferyl alcohol, Sinapyl and P-Coumaryl). In addition, grass and dicot lignin also contain large amount of phenolic acids such as P-coumaric and ferulic acid.

Lignin is further linked to both hemicellulose and cellulose forming a physical seal around the latter two components that is an impenetrable barrier preventing penetration of solution and enzymes.

Of the three components, lignin is the most recalcitrant to degradation whereas cellulose, because of its highly ordered crystalline structure, is more resistant to hydrolysis than hemicellulose. However, hydrolysis methods have been used to degrade lignocellulose. Weak acids tend to remove lignin but result in poor hydrolysis of cellulose whereas strong acid treatment occurs under relatively extreme corrosive conditions of high temperate and pH which

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necessitate the use of expensive equipment. Also, unspecific side reactions occurred which yield non-specific, by-products other than glucose, promote glucose degradation and therefore reduce its yield.

Some of the unspecific products can be deleterious to subsequent fermentation unless removed. There are also environmental concerns associated with the disposal of spent acid and alkaline. For many processes enzymes are preferred to acid or alkaline since they are specific biocatalysts and they can operate under much milder reaction conditions, do not produce undesirable products and are environmentally friendly.

In general the technology of bioprocessing of raw materials or their constituents into bio-products entails three steps; process design, system optimization and model development. Processing involved the use of biocatalysts, whole microorganisms or their enzymes or enzymes from other organisms to synthesize or bioconvert raw materials into new products; recover/ purify such bioproduct and subsequently any needed downstream modifications.

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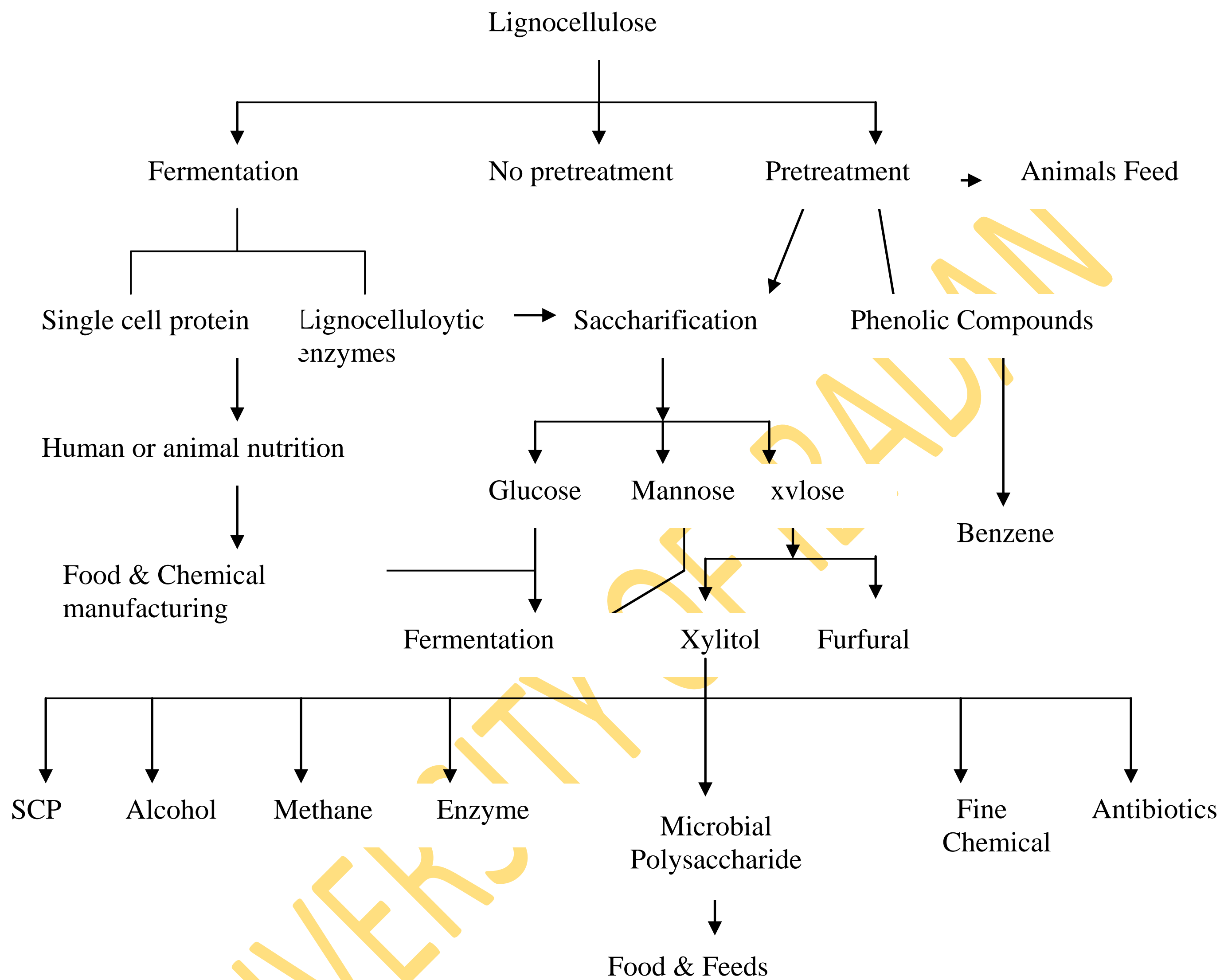

Fig 2: Generalized process stages in lignocellulose bioconversion into value added bioproduct

Sources: Howard *et al*; (2003)

2.10.10 Lignin Degradation under Aerobic Conditions

There are some debates and perhaps significant variability in the rate of lignin decomposition in aerobic system. Lynch and Wood (1985) stated that "little, if any, lignin degradation occurs during compositing" and Liyama *et al.* (1995) assumed constant lignin as the basis of their calculations of polysaccharide degradation. However, Hammounda and Adams (1989) measured lignin degradation ranging from 17% to 53% in grass, hay and straw during 100 days of composting and Tomati *et al* (1995) reported a 70% reduction in the lignin content of olive waste compost after 23 days under moisture (65-85%) thremophillic conditions. Interestingly, after this initially high decomposition rate under thermophillic conditions, Tomati *et al*., (1995) also found no further reduction in lignin content during the subsequent 67 days under mesophillic conditions. In contrast, in a laboratory incubation study, Horwath *et al*., (1995) measured 25% lignin degradation during mesophillic compositing and 39% during thermophillic composting of grass straw during a 45-day experiments.

Yang *et al.* (1988) observed that the addition of nitrogen to woody materials inoculated with *Phanerochaete chrysosporium* increased lignin degradation rate. Ladisch *et al.,* (1983) concluded that differences between plant species is likely related to differences in lignin structure. Gymnosperm plants have lignin composed of coniferyl alcohols while angiosperm lignin is composed of both coniferyl and sinapyl alcohols, whereas grass lignin is mainly of coniferyl, sinapyl, and p-coumaryl alcohols. While significant lignin degradation appears possible during aerobic composting, a number of factors are likely to affect the decomposition rate. Conditions which favour the growth of white rot fungi, including adequate nitrogen, moisture, and temperature, all appear to be important in encouraging decomposition, as does the composition of the lignocellulosic substrate itself.

2.10.11 Anti-Nutritive Effects of Soluble NSP

Grain legumes are used in monograstric diets mainly to supply protein. In addition to protein, they also contain substantial amount of NSPs. Cellulose and xylan which are the major NSPs in cereal grains are only found in the hulls or husk of most legumes (Choct, 1998; Choct and Kocher, 2001). The NSPs include a range of compounds possessing different physicochemical properties. Their nutritional effects in monogastric animals are diverse and in some cases extreme. It is, however, generally conceded that the major detrimental effects of

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NSP are associated with the viscous nature of these polysaccharides, their physiological and morphological effects on the digestive tract and interaction with the microflora of the gut (Yang and Choct, 2009). The mechanism includes altered intestinal transit time, modification of the intestinal mucosa and changes in hormonal regulation due to varied rate of nutrient absorption (Yegani and Korver, 2008).

2.10.12 Viscosity

The viscosity of the digesta associated with dietary carbohydrate has been given prominence as the major factor contributing to the adverse effects of ingredients in diets of poultry (Wang and Gu, 2010). According to Willis and Reid (2008) the viscosity of NSPs depends on their solubility and molecular weight, solubility in turn depends on the chemical structure of the NSPs and their association with the rest of the cell wall components. Viscosity is a result of the dissolution of large-molecular weight non starch polysaccharide complexes from the endosperm cell walls of the cereals concerned. By their own right, these compounds increase the viscosity of the solution, but at critically high concentrations they can aggregate, forming macro molecular complexes (Yang and Choct, 2009) that massively increase solution viscosity. These soluble compounds are primarily arabinoxylans and - β glucans.

Choct and Kocher (2001) reported that inclusion of various pectins in poultry diets as well as mixture of pentosans, β-glucan and other viscous carbohydrates increase viscosity of the digest and reduce the apparent and morphology of the intestinal villi. The physical effects of viscosity on nutrient digestion and absorption also appear to be similar regardless of the NSP sources. Ikegami *et al.*, (1990) and Coffey (2008) reported that hind guts viscosity decreases the rate of diffusion of substrates and digestives enzymes and hinders their effective interaction at the mucosal surface.

A reduction in the digesta flow rate results in increase in microbial number in the small intestine, de-conjugation of the bile salt (Coffey, 2008) and production of toxins are some of the possible deleterious effects of increased micro-organims number. This might also increase feed passage time which will ultimately influence feed intake.

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2.10.12.1 Viscosity in the intestine may pose a problem through two direct mechanisms:

1. As viscosity increases, the rate of diffusion of solutes is reduced (Bedford, 1996). This effect evidently slows the rate of digestion and hence the rate of nutrient extraction from the diet. The larger the molecule, the greater the impact of highly viscous gels on the rate of diffusion.
2. As viscosity increases, the ability of the gut to physically mix the contents is severely compromised (Edward *et al*., 1988). These have severe implications for fat digestion because vigorous mixing is required for good emulsification, which is a prerequisite for efficient fat digestion. This is certainly borne out by the results of Danick *et al*., (1995), who found that the digestion of tallow (which is more dependent on emulsification for digestion) was far more severely compromised than that of soyabean oil in a highly viscous rye-based diet.

Nevertheless, there are indications that high digesta viscosity affects performance by altering gut-enterocyte turnover rates, endogenous-enzyme synthesis rates, microflora and coccidian populations, and litter quality (Choct *et al*., 1995; Bedford and Morgan 1996; Smithard and Silva, 1996).

2.10.13 Modification of Gut Physiology

The soluble NSP cannot only act as a physical barrier to nutrient digestion and absorption by increasing gut viscosity, but also change gut function by modifying endogenous secretion of water, protein, electrolytes and lipids (Johnson and Gee, 1998; Angkenaporn *et al.* (1994). Ikegami *et al*., (1990) observed significant adaptive changes in the digestive system of rat when prolonged consumption of soluble NSP was administered. The changes in the guts were enlargement of the digestive organs and increased secretion of digestive juices, accompanied by a decreased of nutrient digestion.

Ide *et al*., (1989) and Yang and Choct (2009) observed that viscous NSP can enhance bile acid secretion and subsequently result in significant loss of this acid in the feaces. Kay and Truswell (1977) implicated gel-forming soluble dietary fibres in the binding of bile acids in the small intestine leading to a drain on the enterohepatic system. Yegani and Korver (2008)Thus is order to replenish the bile acid pool, cholesterol is said to be mobilized from the general body circulation for the purpose. The continued drain of bile acid and lipid by

sequestration, and increased elimination of feacal acidic and neutral sterol, may ultimately influence the absorption of lipids and cholesterol in the intestine. These effects could lead to major changes in the digestive and absorptive dynamics of the gut with consequent poor overall efficiency in nutrient assimilation by the animal.

2.10.14 Process of NSP Digestion and Its Effects on Poultry

Due to the type of digestive system present in poultry and the absence of appropriate endogenous enzymes, poultry is unable to digest NSPs and certain oligosaccharides, thus the digestibility of those carbohydrates is achieved by chemical (in crop of poultry) and microbial degradation (in lower parts of the small intestine and entire large intestine).A small amount of physical digestion may occur as fibrous materials go through the gizzard where they are ground into fine particles. (Choct and Kocher, 2001).

Reports by Oldale (1996), Oderkirk (1996) and Chock and Kocher (2001) agreed that the insoluble NSP fraction of the diet is virtually undigested in the alimentary canal, while the soluble NSP fraction which contains mixed-linked β-D- glucans and pentosans, which poultry cannot digest axiomatically, are only party broken down by fermentation processes. This process of fermentation gives rise to the formation of a viscous gel resulting in viscous digesta. As viscosity increase, the rate of diffusion decreases and causes decreased digestibility of all substrates.

Viscous digesta, according to Odekirk, (1996) causes:

- Decrease in gut ability to stir content
- Prevent enzyme from getting to cell for breakdown
- Decrease contact of nutrient with gut wall
- Decrease absorption of nutrient into the blood
- Undigested feed and water absorbed by NSP exist the bird
- Poorer growth
- Decreased feed conversion
- Wet litter resulting in down grading due to hock damage in broiler and dirty eggs in layers.

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Contrary to the above opinion, Meeusen and Vallet (2001) reported that high fibre diets decreased the water/ feed intake ratio resulting in higher faecal dry matter and better litter quality.

2.11 Effects of Fibre on Nutrient Utilization

From the clinical and nutritional significance of dietary fibre; increased intake of fibre has been widely encouraged. The nutritional, physiological and metabolic effects of fibre have been consistently observed to vary with fibre types. Thus, specific fibre types have been shown to be more effective in modulating carbohydrate, lipids and mineral utilization and/or metabolism.

Generally, many authors have reported inverse relationship between dietary fibre and digestibility coefficients and/or bioavailability of nutrients (Laplace and Lebas; 1981; Mitaru and Blair, 1985; Longe, 1985; Verel *et al.*, 1988; Onifade,1999; Longe, 2006, and Ologhobo, 2012).

2.12 Effects of Fibre on Protein Utilization

Dietary fibre from natural sources is much more complex completely containing other kinds of fibre component in different physical structures. This could enrich the diet in other nutrients, and declining the amounts of available protein.Therefore, when DF is increased adequate protein nutrition becomes critical (Delorme and Wojcik, 1982). The suggestion of the above authors is especially relevant because natural fibre sources are widely reported to have negative influence on protein and amino acids digestibilities. The decrease in amino acid digestibility observed (Sauer *et al.*, 1991) might result from the absorption of amino acid and peptides by fibre, withholding these from absorption in the small intestine (Mitaru and Blair, 1984). Longe (2006) showed that the nature of fibre influences the effects of fibre on apparent digestibility of nitrogen.

These authors opined that the negative influence of DF on nitrogen digestibility can in many cases be attributed to the lower availability of the protein added in the fibre source. Also, further investigation by Ologhobo (2012) reported that cellulose levels affected true protein digestibility, biological value and net protein utilization in a rat study where cellulose was varied between 0 and 40% in the diet. At 15% inclusion; the effect of cellulose on true

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digestibility became significantly negative, while BV and NPU became more effected with highly significant differences from the control group; though the difference in BV between the control group and the group with 40% cellulose was less than 3% units.

The effects of cellulose on protein digestibility is probably not due to the energy levels in the diet, but due to a specific effect of fibre on protein digestibility (Szelenyi –Galantai *et al*., 1981). However the reduction in BV at 40% dietary cellulose might be due to protein being used as an energy source, as rats on it hardly gained weight. In contract with ileal digestibility, systematic negative interactions between fibre sources on overall digestibility of amino acids were observed in most cases at the end of the small intestine (Laplace *et al.,* 1989).

In an attempt to determine the interplay between fibre and environmental temperature on nitrogen digestibility; Schoenherr *et al*., (1989) used primiparous sows housed in warm or hot environment, and they found that fibre addition significantly depressed digestibility of dietary nitrogen independent of the thermal environment. Just (1982) indicated that apparent nitrogen digestibility decreased on fibrous diet due to changes in microbial fermentation in the hindgut. The study by Bchoenherr *et al*., (1989) illuminated the assertion of Just (1982) in that a greater proportion of the dietary nitrogen disappeared in the hindgut as the DF level increased.

As DF levels increased, greater quantities of feedstuffs (Cell wall constituents mainly) were transported to the caecum and colon, which resulted in elevated levels of microbial metabolism including synthesis of protein and amino acids, most Microbial protein are subsequently excreted in feaces which decreases the apparent digestible nitrogen expressed as a percentage of nitrogen intake (Schoenherr *et al.,* 1989). A similar conclusion was drawn by Sauer *et al* (1991) in their study on the effect of two sources of fibre; purified cellulose and natural fibre on ileal and feacal digestibility of amino acids and bacterial nitrogen excretion in pigs. The authors showed that results based on feacal analysis method may provide an erroneous interpretation of the effect of fibre on amino acid digestibility. However, the inclusion of fibre affected amino acid digestibility only slightly when determined according to ileal analysis method. Nonetheless, the result of Aderolu (2000) did not imply that sources of fibre other than purified and natural fibres have little or no effect on ileal amino acid digestibility. When feacal analysis method was used, there was a considerable decrease in the

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digestibilities of most of the amino acids. The decrease in feacal amino acid digestibilities resulted largely from an increase in the synthesis of bacterial protein in the large intestine. Ologhobo and Saucer (1992); Ologhobo (2012) affirmed the contribution of large intestinal microbial proteins to the feacal nitrogenous matter; which erroneously increase facal crude protein and hence lower digestibility. They concluded that feacal analysis method underestimates protein and amino acid digestibilities, and the use of ileal digestibility method was recommended. Pont *et al*., 1989 and Pond 1989 reasoned that the digestible energy (DE) and its efficiency of utilization for tissue synthesis in pig is depressed by dietary inclusion of fibrous feedstuffs.

Addition of fibre depressed significantly the proportion of energy digested and retained for metabolism (Schoenherr *et al*., 1989). As energy concentration decreased, more of the dietary nutrients were transferred to the lower intestine, the information of volatile fatty acids was increased, and a larger part of digestible energy disappearing in the caecum.

Eggum *et al*., (1982) observed that the greater the proportion of total digestion that occurs in the large intestine, the poorer will be the utilization of energy apparently digested by the host which will result in a lower live weight gain. This is because the energy values of nutrients absorbed in the hindgut is lower than the foregut.

Ewan, (1993) opined that the additional heat increment needed when fibrous feeds are fed is contingent upon the increased metabolic energy expenditure to digest the unreadily digestible ingredients.

Stahly and Cromwell (1986) showed that in a cold environment, the additional heat released during the digestion and metabolism of fibre may be used to meet the animal's elevated maintenance need thus sparing other nutrients for tissue synthesis. Although, the heat released during the digestion and metabolism of fibre is likely lost in a warm environment, and might actually increase the burden of heat dissipation in pigs housed in a hot environment, Schoenherr *et al* (1989) observed that the digestibility or the metabolizability of the dietary energy was not affected either in warm or hot environment.

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2.13 Effects of Fibre on the Utilization of Fats and Oils

Dietary fibre affects lipid digestibility and/or absorption in animals. Many a fibre type especially the viscous soluble polysaccharides have an absorbent effect in the alimentary canal and increase the feacal excretion of lipids, sterols, bile acids, and nitrogenous substances. In this way, fibre affects the absorption of fatty acids, and indirectly their content pattern in different tissues (Rotenberg and Andersen, 1980).

Dietary pectin increased the excretion of various fatty acids in the feaces and decreased the amount apparently digested in different degrees. The excretion of longer chain fatty acids increased more than those with shorter chains (C18> C17> C16> C14), and the excretion of the more saturated acids more than the less saturated (C16: 0 > C16: 1: C17: 0>C17:1).

Cherry and Jones (1982) observed that hens fed cellulose supplemented diets exhibited significant decrease in liver weights, serum lipids and liver lipids. The increase in total lipid excreted by the hens was considerably more than that for the hen fed basal diet and this was consequent upon high dietary cellulose which induced significant increase in quantity of excreta. Some dietary fibres such as pectin, lignin, etc, in bran and alfalfa conjugate with bile acids resulting into increase bile acid excretion, and an associated malabsorption of cholesterol and other lipids.

2.14 Effects of Fibre on Vitamins Utilization

In as much as fibre has been implicated to affect most nutrient availability in negative way, it will not be out of place to consider the influence of dietary fibre on vitamins in same perspective. However, unlike other dietary nutrients little is known on the effect of fibre on vitamins.

The incidence of megaloblastic anaemia (of pregnancy) associated with folate deficiency is most frequently observed in developed countries where diets that are traditionally high in fibre are consumed. Onifade and Tewe (1992) also observed megaloblastic anaemia suspected to be caused by folic acid/or vitamin B complex deficiencies in rabbits fed fibrous diet upon haematological screening. Similarly, Abad and Gregory (1985) studied the absorption of radiolabeled, folates in rats. They reported that cabbage, orange juice and wheat bran weakly reduce the bioavailabilityof extrinsically added folates, and that cabbage and cooked beans excreted a stronger inhibitory effect on the availability of polyglutamates than

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the monoglutamyl form. Wheat bran had little effect on folic acid but reduced utilization of heptaglutamyl folate in rats (Keagy, 1985). Dietary fibre had a cation exchange capacity (Allen *et al*., 1985) and decrease intragastric concentration of hydrogen ion (H^+), pepsin and bile acids (Rydning *et al.,* 1984). Thus it has potential for direct interaction with folate compounds,; may interfere with folate binding to other diet components; may alter $_pH$ of the medium, and may alter the rate and extent of digestion of other diet components.

2.15 Effects of Fibres on Mineral Utilization

A fibre source may be beneficial or detrimental to the mineral nutrition status of the animal (Ologhobo, 2012). Moore *et al*., (1986) had previously concluded from their study on the effect of oat and wheat bran on mineral utilization in growing pigs fed with and without antibiotics that the difference in chemical and physical properties and susceptibility to degradation, may be as important as level in the diet in determining the impact of dietary fibre on mineral balance.

Generally, the consumption of diets containing high levels of fibre has been shown to exert a negative effect on mineral absorption in humans and animals. Linderman *et al*. (1986) equally reported that net retention of minerals like calcium, phosphorus, sodium, manganese and zinc were reduced with increasing levels of fibre from peanut hulls in swine ration.

Moore *et al.* (1986) were of the opinion that the ability of fibre source to decrease mineral balance is partially related to the number of cation bindings sites available on the fibre matrix, and the extent to which a fibre source is degradable within the digestive tract.

Various researchers (Karland 1989, Devendra 1998, southgate 1998) observed most of the effects of DF on mineral binding are apparently due to the presence of phytate, oxalates and other tannins. Furthermore, it was shown that phytates are the major inhibitors, but that some residual effects of fibre on zinc were evident. Dietary phosphates, oxalates, tannins, fatty acids and pectins are capable of binding calcium, iron and zinc; calcium and iron; calcium and magnesium; and calcium respectively (Southgate, 1999). Harmuth-Home and Schelenz (1988) suggested a number of different mechanisms that could be envisaged for the interference of these neutral polysaccharides with mineral absorption.

(i) ion exchange involving hydroxyl groups only

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(ii) unspecific reduction of diffusibility due to the viscous consistency of the polysaccharide gel;

(iii) retention of metal ions in the pores of the polysaccharide gel as in gel filtration. This was similar to the observation by Richard and Cronath (1983) and Van Soest (1984).

(iv) a shortened transit time, which does not permit optimal absorption of dietary minerals or reabsorption of endogenous mineral secreted with digestive fluids

(v) an increase in the total amount of endogenous mineral excretion or

(vi) an increased loss of microbial cell mass containing trace mineral which were taken up from gut wall secretion.

In as much as greater weight of evidence implicate fibrous with low mineral bioavailability proper care should be taken to provide sufficient mineral supplement in such diets.

2.16 Physiological Implications of Dietary Fibre

The interaction of dietary fibre with the digestive and absorptive environment in the gastro-intestinal tract of animals facilitates its influence on the physiology of the consuming matter. Hitherto, the very essence of adding fibre to diets of monogastric was to allow for adequate intestinal motility (Racynski *et al.* 1982; and Fonty and Gouet, 1989). Meanwhile, Pond (1989) identified some physiological effects of dietary fibre to include: gastric emptying, rate of transit of digesta; gut motility; digestive and secretory responses; absorption of specific nutrients and gut hypertrophy.

2.17 Effects of Fibre on Intestinal Functions

The alimentary tract is the anatomical channel through which exogenous nutrients come into contact with the metabolic environment of the animal. Austic and Neshiem (1999) defined the digestive system as the passage connecting the outside environment to the metabolic world of the animal. Although, the many different materials that are classified as dietary fibre all share the characteristic resistance to endogenous digestive enzymes, their physical properties and biological effects are exceedingly diverse (Johnson *et al.,* 1988). The lumen of the gastro-intestinal tract is bathed with a host of digestive enzymes which aid the transformation of food into readily absorbable and utilizable forms. The versatility of the

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intestinal pathway to adjust to different dietary regimes has play a role in the control of structure and function at several sites in the gastro-intestinal tract (Institute of Food Research, 1989).

2.18 Effects of Fibre on secretory Response of Animals

Low (1989) had presented evidence that raising the dietary non-starch polysaccharide (fibre) increases secretions from the salivary glands, stomach, liver, exocrine pancreas and intestinal walls. These increase include not only water, but electrolytes, proteins and lipids as well as shed epithelia cells, and can be accompanied by increase in the rate of protein synthesis in gut tissue. All may play important roles in the effects found in the gastroduodenal region.

Generally, enzymes are rapidly induced or repressed by changes in the metabolic environment, and a wide rage of substances can be hydrolyzed, reduced, degraded or synthesized. Partridge *et al*. (1982) found the concentration of trypsin (Ec 3,4,21,4), chymotrypsin (EC3,4,21,1) carboxypeptidases A (EC. 3,4, 17,1) carboxypeptidases B (EC. 3,4,17,2), -amylase (EC 3,2,1,1,) and lipase (EC. 3,1,1,3) to be significantly higher the more fibrous barley based diets than cornstarch based diets for growing pigs. Also, the *in vitro* studies by Schneeiman (1988) showed absorption of proteolytic enzymes to fibre and a decrease in the activity of these enzymes.

The mechanism by which fibre may influence enzyme activity variable, however both *in vitro* and *in vivo* studies showed that fibre alters digestive enzymes action (Gagne and Action, 1983).Apart from the exocrine secretory response, large amount of water are frequently found within the gut when the content of dietary fibre is increased. It is apparent that this is due not only to the hydrophilic nature of many forms of non starch polysaccharides, but also to increase endogenous secretions (Low, 1989).

2.19 Effects of fibre on Gut and Visceral Organs

Effects of fibre on gut and visceral organs of livestock animals to diet intake and composition have been redressed by Coffey (2008) for pigs (Abdelsamie *et al.,* 1983; Summers and Leeson, 1986) for poultry and (Wyatt et al., 1988) for rats. The digestive system of birds which consumed fibrous diets were found to be larger than those on low fibres diets (Savory and Gentle, 1976). Gizzard weight was reported to be increased on fibrous diets

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(Kubena *et al*., 1974; Savory and Gentle, 1976 and Summers and Leeson, 1986). Abdelsamie *et al* (1983) also found that at similar feed intakes, high fibre contents increased the weight and length of the gastro-intestinal tract of broilers. The aforementioned increase in gizzard weight was an hypertrophic response to high fibre diet. Brainion (1963) pointed out that gizzard size is affected by the amount of work required of the muscular walls of the organ to breakdown the feed particles. Also, this relationship between visceral organ size and basal heat production has important implications for livestock production, because nutrients are diverted from the growth of edible carcass when visceral organ weights are increased (Pond *et al*., 1989).

Dietary fibre has also been implicated with intestinal cells turnover and functional damage (Patterson and Burkholder, 2008). It was shown in rats (Johnson and Gee, 1986) that the prolonged consumption of fibre stimulates the proliferation of mucosa cells both mucosa morphology and the physiological activity of the epithelia. Cassidy *et al,* (1982) reported the scanning electron micrographs of the jejunum of fibre-fed animals which showed loss of microvilli, and more actively secreting goblet cells, a groove at the tips of the villi, distorted and damaged cells, cellular debris entangled with mucous materials, and in some instances of haemorrhagic debris on the surface. The authors found the severity of morphological changes to be in the order of bran <cellulose< glycan< pectin < alfalfa. The morphological damage of the intestine may be partly responsible for the increase in excretion of nitrogen, fat minerals and electrolytes when fibre content of the diet was increased. Varel *et al* (1987) reasoned that with the increased turnover of intestinal epithelia cells, the animals was forced to expend greater energy for maintenance of the intestinal tract, and less energy will be available for growth or weight gain.

2.20 Effects of fibre on Intestinal Transit Time and Faecal Weight

High fibre diets increase the bulk of the faeces and rate of passage of material through the large intestine (Eastwood and Passmore, 1983). Similar finding has been reported (Kass *et al*., 1980; Ravidram *et al*., 1984; Varel *et al.,* 1988; and Fahey *et al*., 1990). As dietary fibre levels increased greater quantities of feedstuffs (especially cell walls) were transported to the caecum and colon (Schoenherr *et al*., 1989). The extent of digestion in vivo is related to the competition between rate of passage and rate of digestion. Thus, the dietary sources of fibre with a longer retention time would be expected to show a greater extent of proximate nutrients

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and cell wall digestion (Ehle *et al*., 1982). This is because fermentation of fibre is strongly correlated with the residence time of ingesta in the adult pig (and perhaps some other monogastric animals) to be longer because of the larger size of the intestinal tract; and this would support more extensive degradation of fibre.

Low (1989) and Walter *et al*., (1888) noted that increasing dietary fibre in diets leads to greater fecal output of water and of solid matter. Longe (1985) attributed high faecal weights or rats fed on fibrous diet to be partly due to the presence of fibre fractions of dietary origin in faeces, while Stephen and Cummings (1980) also ascribed increased bacterial proliferation during fermentation of fibre.

The solubility and fermentability of the dietary fibre apparently affect the faecal consistency and water content. Easily fermentable types of fibre such as pectin have poor bulking capacity, while the very good bulking capacity of wheat bran can be explained by its relative resistance to fermentation (Nyman and Asp, 1992).

2.21 Effects of fibre on Haematological and Serum Metabolities

Blood with its myriad of constituents provides a valuable medium both for clinical investigations and nutritional evaluation of the individual. The ingestion of numerous dietary components have measurable effects on the blood constituents (Church *et al.,* 1984). Kerr *et al.,* (1982) indicated that nutrient levels in the blood and body fluids might not be valid indications of nutrient function at cellular level; they are considered to be proximate measure of long term nutritional status.

Rotenberg (1981) found a reduction of packed cell volume in rats fed pectin based diet. This observation was attributed to the transfer of some interstitial fluid into the blood vessels. Also Onifade (1993) suggested nomocytic iron deficiency anaemia in chickens fed on maize offal diets, and 30% and 40% of Brewer dried grains. Meanwhile Rotenberg and Agergaard (1993) reported that the haematological indices were within normal ranges in rats fed cellulose up to 14% of the diet, while those fed on 10% pectin had values closer to the lower limits of the biochemical ranges. Soluble fibre has also been shown to selectively lower serum LDL cholesterol and improves glucose metabolism and insulin response (IFST, 2001; Dietary fibre and Resistant Starch, 2001; and Requirement of non human primates, 2003).

This phenomenon has been hypothesized to be due to several mechanisms.

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1. Soluble fibre may bind acids or cholesterol in the intestine, preventing their re-absorption into the body. The liver responds by taking up more LDL-cholesterol in the blood.
2. Short chain fatty acids (SCFA) products of fermentation of soluble fibre in the gut may inhibit synthesis of cholesterol by the liver, reducing the concentration of blood cholesterol.
3. The high viscosity of soluble fibre may slow the rate of digestion and absorption of carbohydrate affecting insulin activity, which is implicated in the removal of LDL-cholesterol in the blood.
4. The low saturated, high poly unsaturated fatty acid profile, vitamin E contents, and presence of physiochemical such as plant sterols, saponins, phytic acid and tannins in fibre have also been shown to contribute to a cholesterol lowering effect.

Dietary fibre, (especially soluble dietary fibre β-glucans) may slow digestion and absorption of carbohydrates, hence lower blood glucose and insulin responses.

Resistant starch has also been implicated in the reduction/delay in rise of blood glucose and insulin following a meal by slowing the rate and extent of digestion and absorption of carbohydrate (Dietary Fibre and Resistant Starch, 2001; Spellberg and Edwards, 2011).

2.22 Concept of Enhancing the Nutritive Value of Agricultural By-Products

Longe (1988) and Dierick *et al.* (1989) advocated the increase utilization of non-conventional feed resources in non-numinant feed. They suggested processing techniques which are simple and inexpensive, and so do not significantly increase costs, but still make it worthwhile in term of nutrient availability. Processing techniques widely document in literature could be grouped into physical, chemical and biological treatments.

2.22.1 The physical treatments

Which include the following methods, moist heating, physical reduction and pelleting, and radiation techniques among others is not without its own disadvantages. Cooking and autoclaving is expensive and it can lead to irreversible swelling which may affect the digestibility of cellulose whose crystalinity is responsible for its resistance to microbial degradation. Although Campbell *et al* (1983) reported that body weight, feed utilization, fat

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retention and bone ash of chicks were substantially improved by gamma radiation. However, the cost of irradiation and the technicality involve in the process make it not readily available.

2.22.2 Chemical Methods

This have also be tried to improve the nutritional quality of fibrous feed stuff (Akhtar *et al*;1993, Longe, 2006). The advantages of this approach although numerous but the following disadvantage equally worth consideration. Chemicals are expensive, potentially harmful, and result in unacceptable pollutants in the environment, besides it requires competent hands to handle. A less expressive and more ecological benign procedure is therefore required.

2.22.3 Biological Treatments

Biological treatment include the usefulness of microbial proteins, antibiotics, probiotics, enzymes, ensiling etc. These constitute the most recent methods of enrichment of non digestible feedstuffs or those imbued with the well known anti-nutrients. Dierick (1989) emphasized that polyphenols such as tannins are not removed by physical or chemical treatment but by fermentation or germination. Even, the nutritive value of maize inform of lysine and tryptophan contents leading to improvement in biological value and utilizable protein was achieved through germination (Ram *et al.,* 1979). During which a large variety of plant endogenous enzymes are produced or activated, all having the biological functions of mobilizing the reserve polymers by degrading them to easily available water soluble products (Nout, 1991). The following authors found improved nutritional qualities in these feedstuff hydrolysis of phytic acid in rice bran, reduction of sapponin content in soyabeans and lowering of trypsin inhibitor content of cowpeas and chick peas (Tangendjaja *et al*., 1988; Dierick 1989). Besides ensiling, the most recent additive for improving silage quality is the biological aid. This involves microbial inoculants and cellulolytic enzymes with easy and safer handling and application to its credit. It is neither volatile nor corrosive, which is aimed at break down cell walls to provide a wealth of readily available substrates (Dutton 1987) Hristov (1993) observed that the enzyme treatment had a positive effect on alfafa spp silage fermentation. In addition to the endogenous phytases in cereals and legumes, the use of exogenous enzymes e.g. hydrolytic enzymes have been used to enhance the nutritive value of non-digestible feed stuffs. Cowan *et al* (1996) advocated that addition of enzymes to feed

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ingredients results in an improve energy availability that reduces the difference between gross and metabolisable energy or raw material. The level of improvement seen was related to energy types and to dosage and correlates well with the substrates specificity of the various enzymes present. Enzyme according to Classen (1996) containing carbohydrate hydrolytic activities are the most effective in improving starch utilization in cereal grains. Apart from improving the nutritional qualities of the feedstuffs, exogenous supplementation of cereal based diets can significantly improve chick performance. By increasing rate of gain, efficiency of feed utilization the apparent metabolizable energy of dry matter, fat and protein, with excellent improvements being obtained with diets containing rye, oat and barley (Pettersson and Aman, 1988; Ronald et al, 1996). The commercially available enzyme preparations include proteinases, amylases, cellulases, glucanases, pentosanes, xylanases and lipases (Rotter *et al.*, 1989). These have the following advantages, water solubility, nutrient accessibility, digestibility, fermentability, reduction in viscosity, reduction in anti nutritional factors and taste and aroma improvement. The microbial enzymes sources, which account for 90% of those, use around the world (Under-Koffler, 1972) is more advantageous than the commercial prepared enzyme Edward (1972). The following advantages are found in the microbial enzymes;

i. Microbial enzyme does not compete for glandular tissues of animals with other more expensive products made from a limited supply of the same glandular tissue.
ii. There is scanty of non microbial sources.
iii. There is irregularity and non predictability of supplied from non microbial sources which may be subject to seasonal, climatic and other agriculturally related uncontrollable variables.
iv. Production from non-microbial sources cannot be expended at will in response to increased demand microorganisms both aerobic and anaerobic are able to produce extracellular enzymes to degrade macromolecules like starch, cellulose, hemicellulose, lignin and pectin of the plant cell (Priest, 1984) as well as proteins and other membrane constituents.

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2.23 Biomass Breakdown

Hundreds of species of fungi and bacteria are able to degrade holo-cellulose and lignin to lower molecular weight products. Some of which are then further, metabolized by facultative and obligate soil bacterial and actinomycetes (Maccubin Hodson, 1980) into cellulose and components by organisms include aerobes, mesophiles and themophiles. They are both widespread and abundant in natural environment. Although many microorganism can grow on cellulose or produce enzymes that can degrade amorphous cellulose relatively few produce the entire complement of extracellular cellulasses able to degrade crystalline cellulose *in vitro* fungal are usually faster lignin degraders, and among the fungi, *Trichoderma ssp* are the best known source of extracellular cellulose capable of solubilizing crystalline cellulose.

2.24 Biodegradation of Plant Cell Wall Polysaccharides.

Aspergillus

The genus *Aspergillus* is a group of filamentous fungi with a large number of species. The first record of this fungus can be found in Micheli's *Noval Plantarum Genera* (1729), but a more detailed description of the aspergilli did not appear until the middle of the 19th century. In 1926, the first classification of these fungi was proposed describing 11 groups within the genus (Tom and Church, 1926). A re-examination of the genus was published by Thom and Raper (1945), identifying 14 distinct groups. Some of these groups consist of pathogenic fungi (e.g. *A. fumigatus, A. flavus,* and *A. parasticus*), but most important for industrial applications are some members of the group of black aspergilli (*A. niger* and *A. tubingensis*). In addition to the morphological techniques traditionally applied, new molecular and biochemical techniques have been used in the reclassification of this group of aspergilli (Nikkuni *et al*., 1996). These analyses resulted in the clear distinction of eight groups of black aspergilli (*A. niger, A. tubingensis, A.fortidus, A. carbonarius, A. joponicus, A. aculeatus, A. heteromorphus,* and *A. ellipticus*) (Parenicova, 1991). Products of several of these species have obtains a GRAS (Generally regarded as safe) status, which allows them to be used in food and feed applications. The black aspergilli have a number of characteristics which make them ideal organism for industrial applications, such as good fermentation capabilities and high level of protein secretion. In particular, the wide range of enzymes produced by Aspergillus for the degradation of plant cell wall polysaccharides is of major importance to the food and feed

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industry. Recently, several *Aspergillus spp.* have received increased interest as hosts for heterologous protein production (Davies, 1994). Major enzymes activities found in *Aspergillus niger* include cellulose, hemicellulase, xylanase and β glucanase (Halymn, 1998).

2.24 2 Trichoderma

Trichoderma sp. is a fungus that is present in nearly all soils and other diverse habitats. In soil, they are frequently the most prevalent culturable fungi. *Trichoderma* was introduced into mycological literature by Pearson in 1974 and the first occurrence of the specie in the soil was documented by Quademans and Koning (1902).

They are favoured by the presence of high levels of plant roots, which they colonize readily. Some strains are highly rhizosphere,. i.e., able to colonize and grow on roots as they develop (Harman, 1998). *Trichoderma* spp. attack, parasitize and otherwise gain nutrition from other fungi. Since *Trichoderma* spp. grow and proliferate best when there are abundant healthy roots, they have evolved numerous mechanisms for both attack of other fungi and for enhancing plant and root growth. A recent list of mechanisms as outline by Harman (1998) follows.

- Mycoparasitism
- Antibiosis
- Competition for nutrients or space
- Tolerance to stress through enhanced root and plant development
- Solubilization and sequestration of inorganic nutrients
- Induced resistance
- Inactivation of the pathogen's enzymes

According to Onilude (1994), the following factors affect the efficiency of *Trichoderma* spp as a source of celluulolytic enzymes. Increase in substrate concentration, temperature (enzymes production increases from 15^0C to 30^0C but after this the activity of the enzyme fall and around 55^0C it falls to zero), increases in agitation will increase the mycelia dry weight and medial protein will also increase, the P^H (maximum pH of 5.5).

Major enzyme activities found in *Trichoderma* spp. include cellulase, xylanase and endo-β-2, 3: 1,4-glucanase. It also contains amylase, pectinase and protease. Godfrey and West (1996b)

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and Uhlig, (1998), both reported that 20% of the world enzyme markets are from *Trichoderma* and *Aspergillus*. With all the activity of its enzyme production, *Trichoderma* has the ability to complement the birds own digestive enzymes.

2.24.3 Degradation of Cellulose and the Xyloglucan Backbone

Four classes of enzymes are involved in the biodegradation of cellulose

1. Endoglucanases (EC 3.2.1.4) hydrolyze cellulose to glucooligosaccharides.
2. Cellobiohydrolases (EC3.2.1.19) release cellobiose from crystalline cellulose.
3. Glucosidases ((EC 3.2.1.21) degrade the oligosaccharides to glucose.
4. exoglucanases release glucose from cellulose and glucooligosaccharides.

The distinction between exoglucanases and cellobiohydrolases is not always clear due to differences in the methods used to study these enzymes. All four classes of enzymes have been identified in aspergilli, although the number of isozymes produced by different species or even strains of the same species can differ (de Vries and Visser, 2001).

Production of celluloytic enzymes by Aspergilli has been observed using the following carbon sources: cellulose (Pushalkar *et al*. 1995), sophorose and 2-0- -D-glucopyranosyl-D-xylose (Hrmova, 1991) and cellobiose, glucose and xylose (Ali, and Sayes. 1992). However, other factors are importance as well. Production of both endo- and exoglucanases in *A. fumigatus* was much higher when ammonia was used as a nitrogen source instead of nitrate (Stewart, and Parry, 1981), whereas the production of β- glucosidase in *A. terreus* was higher on nitrate as nitrogen source than on ammonia (Pushalkar, *et al*., 1995).

2.24.4 Synergy between Polysaccharide-Degrading Enzymes

Efficient degradation of polysaccharides requires cooperative or synergistic interactions between the enzymes responsible for cleaving the different linkages. Synergy has been reported for many enzymes from *Aspergillus* involved in xylan degradation, usually between a main-chain-cleaving enzyme and one or more accessory enzymes.

Synergistic action has been observed between endoxylanase, β-xylosidase, arabinoxylan, arabinofuranohydrolase, and acetylxylan esterase in the degradation of different (glucuronoarabino) xylans (Kormelink and Verogen, 1993).

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Synergy has also been observed between these enzymes and some of the other xylanolytic enzymes. The release of ferulic acid from xylan by a feruloyl esterase from *A. niger* was strongly enhanced by the addition of endoxylanases (Bartolome, *et al*., 1995, and de Vries, *et al*., 1997). Similarly, both endoxylanase and β-xylosidase positively influenced the relase of 4-O- methylglucuronic acid from birchwood xylan by A. tubingensis (de Vries, *et al.,* 1998). The latter enzyme enhanced the activity of endoxylanase and β-xylosidase on this substrate.

2.25 Solid State Fermentation

Aerobic microbial transformation of solid materials or "Solid Substrate Fermentation" (SSF) can be defined as the application of living organisms and their components to industrial products and the process not an industrial in itself, but an improvement technology that will have a large impact on many different sector (Ologhobo, 2012). Adeolu (2000) considered SSF as a process in which solid substrate are decomposed by known mono or mixed cultures of microorganisms under controlled environmental conditions, with the aim of producing high quality product. The substrate is characterized by relatively low water content.

It has been reported that solid state fermentation (SSF) is an alternative process to produce fungal microbial enzymes using lignocellulosic materials from agricultural wastes due to its lower capital investment and lower operating costs (Jecu, 2010). The SSF process, for the reasons stated, will be ideal for developing countries. Solid-state fermentations (SSF) are characterized by the complete or almost complete absence of free liquid. Water, which is essential for microbial activities, is present in an absorbed or in complexed-form within the solid matrix and the substrate (Cannel and Moo-Young, 1980). These cultivation conditions are especially suitable for the growth of fungi, known to grow at relatively low water activities. As the microorganisms in SSF grow under conditions closer to their natural habitats they are more capable of producing enzymes and metabolites which will not be produced or will be produced only in low yield in submerge conditions (Jecu, 2010). SSFs are practical for complex substrates including agricultural, forestry and food-processing residues and wastes which are used as carbon sources for the production of lignocellulolytic enzymes (Haltrich et al, 1996, and Longe, 2006). Compared with the two-stage hydrolysis-fermentation process during ethanol production from lignocellulosics, Sun and Cheng (2002) reported that SSF has

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the following advantages: (1) increase in hydrolysis rate by conversion of sugars that inhabit the enzyme (cellulose) activity; (2) lower enzyme requirements; (3) higher product yield; (4) lower requirement for sterile conditions since glucose is removed immediately and ethanol is produced; (5) shorter process time; and (6) less reactor volume. Malherbe and Cloete, (2003) reiterated that the primary objective of lignocellulose treatment by the various industries is to access the potential of the cellulose encrusted by lignin within the lignocellulose matrix. They expressed the opinion that a combination of SSF technology with the ability of an appropriate fungus to selectively degrade lignin will make possible industrial-scale implementation of lignocellulose based biotechnologies.

New applications of SSF have been suggested for the production of antibiotics (Barrios *et al.,* 1988), secondaries metabolites (Trejo-Hernandez *et al.,* 1993) or enriched foodstuffs (Senez *et al.,* 1980). SSF is a batch process using natural heterogeneous materials (Raimbault, 1981 and Tengerdy, 1985), containing complex polymers like lignin (Agosin *et al,* 1989), pectin (Kumar, 1987; Oriol, *et al.,* 1988a) and lignocellulose (Roussos, 1985). SSF has been focused mainly to the production of feed, hydrolytic enzymes, organic acids, gibberellins, flavours and biopesticides.

Bacteria, yeasts and fungi can grow on solid substrates and final application in SSF processes. Bacteria are mainly involved in composting, ensiling and some other food processed (Doelle *et al.,* 1992). Yeasts can be used for ethanol and food or feed production (Saudedo Castaneda *et al.,* 1992a, 1992b, Denev, 2006 and Denev, *et al.* 2007, and Chevaux and Fabre, 2007).

But filamentous fungi are the most important group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties. The hyphal mode of fungal growth and their good tolerance to low water activity (A_w) and high osmotic pressure conditions make fungi efficient and competitive in natural microflora for bioconversion of solid subtrates (Denev, 2006).

Microorganisms are currently the primary source of industrial enzymes: 50% originates from fungi and yeast, 35% from bacterial, while the remaining 15% is either from plant and animals origin (Boopathy, 1994). Microbial enzymes are either produced through submerged fermentation (SMF), or solid substrate fermentation (SSF) techniques. According to the central food technological research Institute (CFTRI) in India, enzymes production by SSF

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accomplishes high productivity per unit volume of fermentor space than SMF techniques. Processing waste such as soyabean hulls (Jha *et al*, 1995) and Cassava peels (Ofuya and Nwajuba, 1990) has been upgraded through production of enzymes by SSF technique. The work of authors like Yang *et al.,* (1993), Onilude (1994), Balagopalan (1996), Belewu and Banjo, (1999), Iyayi and Losel (2001), Iyayi and Aderolu (2004) among others clearly showed the use of microorganism for upgrading lignocelluloses into animal feeds.Like all technologies, SSF has its disadvantage and these have received the attention by Mudgett (1986). Problems commonly associates with SSF are heat build-up, bacterial contamination, scale-up, biomass growth estimation and control of substrate content.

2.25.1 Pretreatment to Enhance Biodegradability

Biodegradability can be enhanced by pretreatment of lignocellulosic materials, including acid (Grethelin, 1985) or alkali treatment (Jackson, 1977; Van Soest, 1994), ammonia and urea (Basaflia *et al.,* 1992; Van Soest, 1994), physical grinding and miling (Ladisch *et al.,* 1993; Fahet *et al.,* 1992), fungal degradation and steam explosion (Sawada *et al,* 1995) and combined alkali and heat treatment (Gossett *et al*., 1976). Gharpuray *et al.,* (1983) examined several of these pretreatment options individually and in combination and found that those treatments which enhanced specific surface area were most effective at increasing enzymatic hydrolysis. While pretreatment may be uneconomical when considered as a separate process in compost feedstock preparation, in some cases it may be incorporated in other preprocessing operations at little additional costs. However, because many lignocellulosic ingredients in composting serve dual roles as energy sources and porosity enhancers, treatments which reduce porosity and pore size distributions may prove counter-productive to maintaining an aerobic process.

2.25.2 Benefits from the use of feed enzymes on fibre feed stuffs

The typical formulation of animal diets is quite variable and is dependent upon the costs of ingredients, which in turn is dependent upon their availability. The efficiency of utilization of any fibrous feedstuffs is often limited by the presence of anti nutritional factors (ANFs). Such anti nutritional factors are commonly referred to as non-starch polysaccharides (NSP) classified as β-glucans, arabinoxylans and certain anylopectin (Caret *et al*., 1997).

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This in order for a non-ruminant to access the starch contents of a cassava, it must be able to penetrate the tough fibrous parenchyma and sclerenchyma layers before getting to the xylem layers. 'The main problem with root tubers is endosperm cell wall not the thin cellulose component however but the soluble NSPs which encrust it. The bulk of these NSP is arabinoxylan (Sun and Cheng, 2002). This is a linear polymer of variable length, which is constructred of D xylose linked β,1-4 with arabinose substituted along the backbone. Researcher like Bada (2003), Howard *et al*., (2003) identified soluble β-glucans and arabinose as being the fractions responsible for impending digestion through causing a viscous intestinal environment. This rate of digestion of a feed and the absorption of the product of digestion relies on the formation of a complex between the digestive enzymes and its substrate and subsequent release of its products (e.g. amylase and starch-releasing maltase), Gilbert (2003) asserted that the structures of hemicellulose is more complex than cellulose and requires several different enzymes with different specifities for complete hydrolysis, the polysaccharide does not form tightly packed crystalline structures like cellulose and is, thus more accessible to enzymatic hydrolysis. Elimination of these anti-nutritive components increase the productivity of the diet and in doing so reduces manure output. Growth rate and feed conversion efficiency is improved and the moisture contents of the litter is significantly reduced (Bedford, 2000). The increased intestinal viscosity reduces digestion rate and nutrient assimilation rate. Thus despite the increase in gut motility associated with vicious intestinal environment, the feed transit is slowed, the flushing effect of the feed is reduced and intestinal bacterial are able to multiply and migrate to the upper part of the small intestine. Since the micro flora will also digest and utilized the starch and protein in the digester they compete effectively with the host nutrients. This further increases the situation for the 'hosts'. In addition, some intestinal bacterial produce bile acid degrading enzymes which further reduce the lipid digesting capabilities of the host (Feighner and Dashkeviz, 1988). The solution to these NSPs is the use of feed enzymes, which must fulfil these conditions; meaningful rate of hydrolysis, and ability to function at the temperature and pH ranges encountered in the digestive tracts. It is worth noting that the intestinal viscosity encountered in birds is much reduced in older birds due to increase digestive capacity and gut maturity motility of the older birds, Bedford (2007) concluded that the use of commercial enzyme significantly reduced intestinal viscosity and improved weight gain and feed conversion efficiency (FCE).

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CHAPTER THREE

3.0 OPTIMAL LEVEL OF CASSAVA STARCH RESIDUE (CSR) ON BROILER PERFORMANCE

3.1 INTRODUCTION

Various researchers, Tewe and Bonkaga (2001), and Iyayi and Aderolu (2004) have documented the nutritive value of cassava and it by- products. However, studies designed for evaluating the nutritional potential of cassava starch residue in broiler diets are lacking. Tewe (2004) observed that a restricted inclusion of cassava and its by-products such as cassava sieviate, cassava grits, in monogastric diets were recommended by most authors because of its low protein, presence of cyanide, and high non-starch polysaccharides (NSPs). Experimentation on optimal level of cassava starch residue would not only enrich nutritional information on the chosen feed resource, but also serve a veritable guide in the economic choice of ingredient. The latter advantage is more so desirable in the face of rising production costs of monogastric animals and their products.

Also, suggested possibilities concerning the subsistence of broiler chicken on dietary fibre were passed in the body of the previous experiments, with a note revoking the sparing inclusion of the dietary fibre sources as currently practiced.

Therefore, this initial study was conducted to determine the performance of broiler birds fed graded levels of raw cassava starch residue as suggested for other cassava by-products starting from the recommended optimal inclusion rate between 10-20% (Aderemi *et al.*; (2003), Tewe (2004); Adedoyin *et al.,* (2009). Another implicit motive of these investigations was the aim to define a phased graded use of CSR in practical nutritional husbandry of broiler chickens.

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3.2 MATERIALS AND METHODS

3.2.1 SOURCES OF DRIED CASSAVA STARCH RESIDUE

Dried CSR (TME 419) used for this study was obtained in large quantity from cassava processing factory Matna, Akure, Ondo State, Nigeria. This was to safeguard the fluctuating quality of this ingredient with different batches as noted by Omole and Tewe (1989).

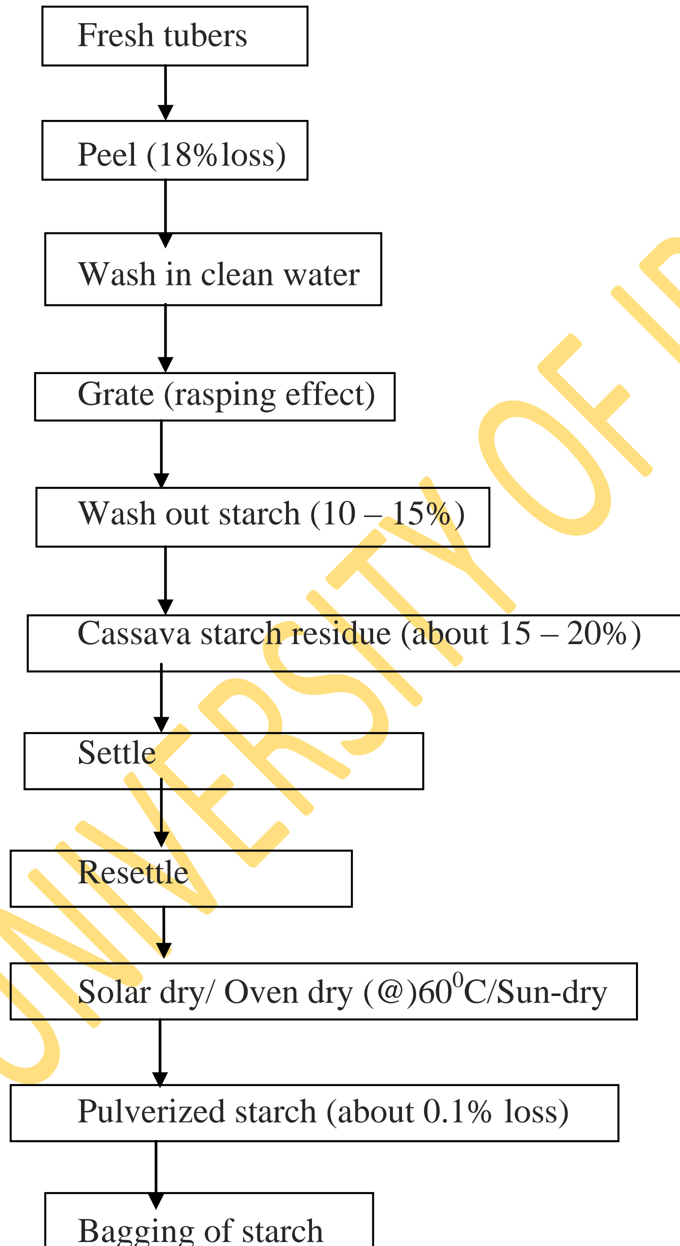

Fig 3: Process flow chat for manufacturing cassava starch residue and cassava starch.

Source: Matna Processing Factory, Akure.

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3.2.2 EXPERIMENTAL DIETS

Six dietary treatments were evaluated in each study. The diets were made isonitrogenous 23% and 21% crude protein for starter and finisher, respectively with provision for at least the minimum calorie requirement (2950kcal/kg) of the chickens as recommended by Olomu (2003). Cassava starch residue, was included in the diet at graded levels of 0,5,10,15,20,and 25% for both the starter and finisher diets.

3.3.4 MANAGEMENT OF EXPERIMENTAL BIRDS

One hundred and eighty day-old hybro broiler chicks were used for the study. Thirty chicks per dietary treatment of five replicates each. The chicks were reared on a deep litter system with feed and water supplied *ad-libitum*. Prior to the arrival of the day old chicks, the brooder house, feeders and drinkers were properly cleaned and disinfected with Morigad. The house was partitioned into pens according to the design of the experiment. Wood shaving used as litter materials was spread on the floor of the pen at the height of 2.5cm. at the starter phase, the feeders were flat trays and fountain drinkers. Electric bulbs (200E/Y/watts) provided source of heat for brooding. Ventilation was adequate, the brooding temperature of 32 – 35°c was followed as suggested by Olomu (2003). Routine medication (vaccine and drugs) were administered as when due (Table 3).

At the end of the 7 days of brooding, the birds were weighed and allotted to their respective treatment diets and reared for 49 days. Records of feed consumption and body weight were kept on weekly basis while, body weight gain and feed to gain ration (feed conversion ratio) were estimated from the data collected.

3.3.4 METABOLIC TRIAL

Digestibility study was carried out during the seventh to eight week of the experiment. Two birds from each replicate whose weights were close to the respective means were selected for metabolic trial in cages fitted with facilities for feeding, water supply and collection of droppings. The birds were allowed to adjust for 4 days before droppings collected for three days. Diets were served to the birds and the left over were properly recorded.

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The droppings were later separated from feathers and spillover feed particles and oven dried at 60^0C for 48 hours. The dried samples were milled and stored for subsequent chemical analysis to determine the nutrient digestibility in the diets

3.3.4.1 Dry matter Digestibility: After oven dry, the dry matter contents of the samples were determined as a percentage of initial weight.

$$\% \text{ D. M.} = \frac{\text{Weight after oven drying gm}}{\text{Weight before oven drying gm}} \times 100/1$$

$$\text{D. M. digestibility} = \frac{F0 - A0}{F0} \times \frac{100}{1}$$

F_0= DM Intake = %DM of Feed x Total amount of feed intake

A_0= DM Output = % DM of faeces x Total amount of faeces voided

3.3.4.2 Nutrient Digestibility: Digestibility of nutrients were calculated using the following formular.

$$\text{Digestibility} = \frac{(F_0 \times F_i) - (A_0 \times Ai)}{F_0 \times F_i}$$

Fi = % Nutrient (ash, NFE, CF, EE and E^0) in the feed

$F_{0= \text{Dry matter}}$ weight of feed consumed (Dry matter intake)

Ai=% Nutrient (ash, CF, EE, - NFE, E^0) in the faeces

A_0= Dry matter weight of faeces voided (Dry matter output)

3.3.5 Experimental design: The experimental design was a complete randomized design

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TABLE 1: Medications (Drugs and Vaccination)

Age of birds (days)	Vaccine and Drugs Used
1	New castle disease vaccine i/o
10	New castle disease vaccine and infectious Bursa disease vaccine (IBDV)
24	New castle disease vaccine (Lasota) oral-administration
38	2nd IBDV

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3.3.6 SERUM COLLECTION

Blood was collected from two birds per replicate at the 8th week of the experiment into sterile sample tubes without anticoagulant. The tubes were kept in a slanting wooden rack at 45^0C and the blood was allowed to clot. The clotted blood was centrifuged for 15 minutes at 3,500 Revolutions Per Minute (rpm). A clear fluid which is the serum was pipetted out into a clean and sterilized bottle and taken for analysis.

The serum protein and albumin were analyzed using sigma assay kits, glucose and cholesterol with randox kits. The buiret method was utilized in the determination of the total protein fraction while the serum was subjected to the direct colorimetric method for albumin with Bromocresol green (BCG) as the dye as described by Toro and Ackerman (1975). The globulin concentration was obtained by subtracting albumin from the total protein.

Albumin/globulin ratio was obtained by dividing the albumin value by the calculated globulin value as described by Toro and Ackerman (1975) (Appendix 1).

3.3.7 CARCASS QUALITY EVALUATION

At the end of the eight week, two birds per replicate were slaughtered after they were starved overnight. The live weight, and bled weight were taken. The abdominal fat was also weighted and recorded. The weights of the internal organs (liver, heart, gizzard, lung, kidneys and spleen) were also taken.

3.3.8 CHEMICAL ANALYSIS

Sample of the test diets and feacal outputs were analysed for proximate constituents using the procedure of A.O.A.C. (1999). While the nitrogen free extract (NFE) was estimated as follows: NFE=100- (% crude protein + % crude fibre +ether extract + Ash).

CELL WALL COMPONENTS

Cell wall components consisting of Neutral Detergent Fibre (NDF) and Acid detergent Fibre (ADF) were determined by the method of Van Soest *et al*. (1992) using acetic acid. Hemicelluloses content was estimated as the difference between NDF and ADF while cellulose content was estimated as the difference between ADF and ADL.

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ENERGY DETERMINATION

METABOLISABLE ENERGY CALCULATION OF CASSAVA STARCH RESIDUE

This was calculated according to the procedure of Pauzenga (1985) as follows: ME (kcal/kg DM) =37 x % protein +81.8 x %fat +35.5 x % nitrogen free extracts

3.3.9 STATISTICAL ANALYSIS

All data generated were subjected to analysis of variance using SAS statistical package (SAS, 1999), the design employed was completely randomized and significant treatment means were separated using Duncan Multiple Range Test (1955) of the same software.

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TABLE 2: Percentage Composition of the Experimental Starter Diets

INGREDIENTS(%)	DIETS					
	A	B	C	D	E	F
	0% (control)	05%	10%	15%	20%	25%
Maize	50.00	45.00	40	35.00	30.00	25.00
Cassava starch residue	0.00	5.00	10.00	15.00	20.00	25.00
Groundnut cake	13.00	14.00	14.50	15.00	15.25	15.25
Wheat-offal	4.00	2.50	2.00	1.00	0.25	0.00
Palm kernel oil	0.00	0.50	0.50	1.00	1.50	1.50
Full Fat Soya	25.00	25.00	25.00	25.00	25.00	25.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster-shell	1.25	1.25	1.25	1.25	1.25	1.25
Broiler Premix	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Table-salt	0.25	0.25	0.25	0.25	0.25	0.25
Total (kg)	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Nutrient						
Crude Protein	23.64	23.70	23.62	23.37	23.09	22.82
Crude Fibre	3.30	3.92	4.12	4.62	5.96	6.68
ME Kcal/kg	3009.10	3018.00	3006.12	3008.40	3016.23	2993.24

Premix provide per kg of diet Vit A, 10,000 i.u; vit D3, 2000 i,u; vit. E 23mg; vit K, 2.mg; calcium pantothenate, 7.5mg; B12, 0.015mg; folic acid, 0.75mg; choline chloride, 300mg; vit B1, 1.8mg; vit B2, 5mg; vit B6, 3mg; manganese, 40mg; iron, 20mg; zinc, 53.34mg; copper, 3mg; iodine, 1mg;cobalt, 0.2mg; selenium, 0.2mg; zinc, 30mg.

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TABLE 3: Percentage Composition of the Experimental Finisher Diets

INGREDIENTS(%)	DIETS					
	A 0% (control)	B 05%	C 10%	D 15%	E 20%	F 25%
Maize	50.00	45.00	40.00	35.00	30.00	25.00
Cassava starch residue	0.00	5.00	10.00	15.00	20.00	25.00
Groundnut cake	16.00	17.50	18.50	19.00	20.50	21.00
Wheat-offal	7.00	4.50	3.00	2.00	0.50	0.00
Palm kernel Oil	0.00	1.00	1.50	2.00	2.00	2.00
Full Fat Soya	19.00	19.00	19.00	19.00	19.00	19.00
Fish Meal	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50
Oyster-shell	2.50	2.50	2.50	2.50	2.50	2.50
Broiler Premix	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Table-salt	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Nutrient						
Crude Protein	21.34	21.30	21.21	21.30	21.15	21.01
Crude Fibre	3.31	3.93	4.62	5.26	5.94	6.69
ME kcal/kg	2950.5	2921.2	2919.2	2940.3	2925.8	2902.4

Premix provide per kg of diet Vit A, 10,000 i.u; vit D3, 2000 i,u; vit. E 23mg; vit K, 2.mg; calcium pantothenate, 7.5mg; B12, 0.015mg; folic acid, 0.75mg; choline chloride, 300mg; vit B1, 1.8mg; vit B2, 5mg; vit B6, 3mg; manganese, 40mg; iron, 20mg; zinc, 53.34mg; copper, 3mg; iodine, 1mg;cobalt, 0.2mg; selenium, 0.2mg; zinc, 30mg.

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RESULTS

3.4.1 CHEMICAL COMPOSITION OF THE TEST INGREDIENT (CASSAVA STARCH RESIDUE)

The result of the chemical composition of cassava starch residue was shown in Table 4. The crude protein content (4.50%) and metabolizable energy (2616.6Kcal/kg) observed for cassava starch residue were lower. However , cassava starch residue has high percentage of crude fibre (16.70%).

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TABLE 4. Proximate and Detergent Fibre Composition of Test Ingredient

Chemical composition	Cassava Starch Residue CSR (%^{DM})
Proximate	
Dry matter	88.59
Crude protein	4.05
Crude fibre	16.70
Ether extract	2.85
Ash	7.90
Nitrogen free extract	62.45
ME kcal/kg	2616,6
Detergent fibre fraction	
Cellulose	17.07
Hemicelluloses	36.03

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TABLE 5: Nutrient Composition of Experimental Diets Fed To Broiler Birds

Parameters	STARTER DIET					
	A 0% (control)	B 05%	C 10%	D 15%	E 20%	F 25%
Dry matter (%)	91.37	91.08	90.3	89.99	88.9	89.97
Crude protein (%)	24.16	24.47	24.19	24.12	24.06	24.01
Crude fibre (%)	2.91	3.18	3.84	4.14	4.31	4.89
Ether extract (%)	3.11	3.68	3.91	4.15	3.72	4.09
Ash (%)	4.91	5.11	5.11	4.44	4.29	4.44
MEKcal/Kg	3009.1	3018.1	3006.3	3008.4	3016.21	2999.24
	FINISHER DIET					
Dry matter (%)	90.01	89.7	88.9	89.8	87.99	88.96
Crude protein (%)	21.65	21.94	21.82	21.36	21.60	21.46
Crude fibre (%)	3.12	3.80	3.89	4.41	4.11	4.81
Ether extract (%)	3.41	3.99	4.16	4.88	4.31	4.71
Ash (%)	4.13	4.39	4.83	5.10	4.88	4.99
MEKcal/Kg	2950.6	2921.3	2919.2	2940.3	2925.8	2902.4

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3.4.2 PERFORMANCE CHARACTERISTICS AND NUTRIENT DIGESTIBILITY

The performance characteristics and nutrient digestibility of broiler birds fed graded levels of cassava starch residue diets are shown in Table 6. The average weight gain of birds fed 5% CSR (diet B) was significantly (P<0.05) higher than other diets. No significant (P>0.05) difference was observed in feed to gain ratio at the starter phase.

Birds fed 5% CSR (diet B) had the least feed to gain ratio value of (2.01±0.03), followed by birds on diet (A) control (2.10±0.03) while those fed diets C (2.37±0.05), D (2.49±0.04) and E (3.03±0.7) were not significant (P<0.05) to one another. The crude fibre digestibility of the birds ranged between (49.5±0.22%) for diet (F) (25% CSR) and (64.27±0.45%) for control diet (A). Analysis of variance showed significant (P<0.05) differences among the dietary treatment means. The crude protein (CP) digestibility value for the control diet (A) was also significantly (P<0.05) higher (71.08±0.5%) than values reported for birds fed diets B (68.66±0.45%), C (67.88±0.44%), D (68.07±0.6%), while the least value was obtained in diet E (66.44±0.61%)

The same trend explained above was also observed for dry matter digestibility (DMD); for birds on the control diet A (69.3±0.51%), followed by birds fed diet B (69.96±0.49%), C (67.08±0.5%), D (67.01±0.39%) whereas the least was observed with birds on diet F (64.09±0.48%).

3.4.3 ORGANS CHARACTERISTICS

The organs characteristics of broiler birds fed graded levels of cassava starch residue are presented in Table 7. Dressed weight of broiler chickens fed control diet (A) (1708.5 ±0 .5kg) was significantly higher (P<0.05) than those birds fed graded levels of cassava starch residue; diets (B) (1692±0.8kg), (C) (1570±1.5kg), (D) (1503±0.8kg), (E) (1541±0.9kg) and (F) (1491.0±07.kg). The trend of the weights of spleens were similar to that of the heart. Relative gizzard weights were significantly (P<0.05) higher correspondingly with the increased levels of cassava starch residue in the diets. Birds fed diets A (1.38±0.04) control and diet B (1.31±0.05) (5% CSR) showed no significant (P>0.05) difference in the abdominal fat. However, the abdominal fat values were (P<0.05) significantly lower in diets C (1.21±0.03), D (1.10±0.6), E (1.04±0.03) and F (1.01±0.06). The length of the intestine also showed

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significant (P<0.05) differences among the different diets values ranging between 189cm and 204cm.

3.4.4 SERUM METABOLITES

The results are shown in Table 8. Serum thiocyanates were similar across the cassava starch residue based diets B (11.26±0.11), C (11.27±0.14), D (11.38±0.21), E (11.61±0.15) and significantly higher in F (12.01±0.12) while no value of (0.00) was recorded for diet (A) control. Meanwhile, cholesterol levels were significantly (P>0.05) affected, ranged between (103.8±0.52mg/100ml) diet F and (146.1±0.60mg/100ml) diet (A) control.

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TABLE 6: Performance Characteristics and Nutrient Digestibility of Broilers Fed Graded Levels of Cassava Starch Residue

Parameters	DIETS						
PERFORMANCE CHARACTERISTICS							
Starter Phase	**A 0%(control)**	**B 05%**	**C 10%**	**D 15%**	**E 20%**	**F 25%**	**SEM ±**
Average weight gain/bird/day(g)	0.32±0.11	0.32±0.12	0.32±0.11	0.31±0.13	0.31±0.12	0.31±0.13	0.03
Feed to gain ratio	2.40±0.02	2.46±0.03	2.47±0.04	2.46±0.07	2.52±0.06	2.52±0.07	0.50
finisher Phase							
Average weight gain/bird/day(g)	$385±0.16^{b}$	$410±0.15^{a}$	$381±0.11^{C}$	$380±0.14^{C}$	$353±0.20^{d}$	$351±0.19^{d}$	0.23
Feed to gain ratio	$2.10±0.01^{ef}$	$2.01±0.03^{f}$	$2.37±0.03^{d}$	$2.49±0.02^{C}$	$3.03±0.03^{ab}$	$3.30±0.03^{a}$	0.04
Cost/kg wt. gain (#)	105.30	102.60	110.50	116.00	129.20	136.90	-
NUTRIENT DIGESTIBILITY							
Crude protein (%)	$71.08±0.17^{a}$	$68.66±0.11^{b}$	$67.85±0.20^{C}$	$68.07±0.13^{C}$	$66.41±0.12^{d}$	$67.00±0.11^{de}$	1.70
Crude fibre (%)	$64.27±0.10^{a}$	$62.98±0.14^{b}$	$60.17±0.20^{C}$	$57.98±0.15^{d}$	$55.40±0.13^{e}$	$49.55±0.18^{f}$	2.15
Dry matter (%)	$69.31±0.19^{ab}$	$69.96±0.20^{a}$	$67.08±0.19^{C}$	$67.01±0.16^{C}$	$66.60±0.21^{d}$	$64.09±0.20^{e}$	2.15

abcdef: means on the same row with different superscripts are significant (P<0.05)

±SEM: Standard error of the means

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TABLE 7: Organs Weight of Broiler Chickens Fed Diets Containing Graded Levels of Cassava Starch Residue.

PARAMETER	DIET						
	A 0%(control)	B 05%	C 10%	D 15%	E 20%	F 25%	SEM ±
Live weight(g)	2218.01±8.9[a]	22111.0±8.8[ab]	2020.02±7.9[c]	2001.02±9.9[e]	2010.04±8.7[cd]	19681.0±9.8[f]	84.3
Dressed weight(g)	1708.50±7.6[a]	1692.10±8.3[ab]	1570.00±7.2[c]	1503.50±6.8[d]	1541.01±7.7[cd]	1491.01±9.1[d]	39.8
Liver weight(g)	1.11±0.02[ef]	1.23±0.01[e]	1.36±0.3[de]	1.73±0.01[c]	1.85±0..3[b]	1.98±0.02[a]	1.28
Kidney weight(g)	0.61±0.00[ef]	0.63±0.02[e]	0.69±0.01[de]	0.84±0.03[bc]	0.83±0.02[ab]	1.01±0.01[a]	0.03
Heart weight(g)	0.30±0.01[c]	0.36±0.02[e]	0.35±0.01[c]	0.45±0.02[b]	0.470±0.03[ab]	0.51±0.03[a]	0.05
Spleen weight(g)	0.38±0.00[bc]	0.48±0.01[b]	0.51±0.00[a]	1.51±0.01[a]	0.51±0.02[a]	0.52±0.01[a]	0.01
Gizzard weight(g)	1.38±0.05[ef]	1.50±0.06[de]	1.58±0.05[cd]	1.67±0.06[c]	1.80±0.07[b]	2.00±0.07[a]	0.14
Length of intestine(cm)	188±10.1[d]	199.1±10.1[d]	201.1±9.2[b]	203±8.8[b]	201.0±8.9[bc]	204.0±8.7[a]	22.04
Abdominal fat(%)	1.38±0.02[a]	1.31±0.03[ab]	1.21±0.02[c]	1.10±0.01[d]	1.04±0.02[de]	1.01±0.01[ef]	0.07

abc…: Means on the same row with different superscripts are significant ($P<0.05$)

±SEM: Standard error of the means

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TABLE 8: Serum Thiocyanate and Cholesterol of Broiler Chickens Fed Graded Levels of Cassava Starch Residue

PARAMETER	DIET						±SEM
	A 0%(control)	**B 05%**	**C 10%**	**D 15%**	**E 20%**	**F 25%**	
Serum thiocyanate level (mg/100ml)	0.00^{d}	$11.26{\pm}0.11^{bc}$	$11.27{\pm}0.14^{bc}$	$11.38{\pm}0.1^{b}$	$11.64{\pm}0.10^{b}$	$12.01{\pm}0.14^{a}$	0.08
Cholesterol (mg/100ml)	$146.1{\pm}0.6^{a}$	$139.1{\pm}0.59^{ab}$	$129.70{\pm}0.61^{c}$	$118.90{\pm}0.56^{d}$	$110.10{\pm}0.6^{d}$	$103.90{\pm}0.52^{de}$	20.05

abcde: Means on the same row with different superscripts are significant ($P<0.05$)

±SEM: Standard error of the means

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3.5 DISCUSSION

3.5.1 PROXIMATE COMPOSITION OF CASSAVA STARCH RESIDUE

The proximate composition of the test ingredient revealed that CSR had low protein, metabolisable energy but high crude fibre content. This could have resulted from the complex matrix of polymers in CSR produced are considered to be a lignin-like compound and are poorly degradable (Longe, 2006).

3.5.2 PERFORMANCE CHARACTERISTICS AND DIGESTIBILITY STUDIES

The performance characteristics of broilers fed graded levels of cassava starch residue was similar with pervious studies on broilers and other simple-stomached livestock (Ademosun, 1973; Abdelsamie *et al;,* 1982; Panigrahi and Powell, 1992; Tewe ,2004, Longe, 2006, and Coffey, 2008). The feed intake of broiler birds fed test diets demonstrated a clear increase in feed intake and feed to gain ratio proportionately to the increasing dietary levels of the fiber. It was indicated by Tewe (1975); Tewe *et al*, (2002); Tewe,(2004) that with increasing dilution of the diets with fibre, greater weights and much greater volume of feed were necessary to meet requirements for energy and other nutrients. This perhaps explained the noticed increase in feed intake of broiler birds in the study. Also, that the specific weights of the diets reduce with increased dietary fibre levels as well as nutrient density. The average weight gain of (410±0.15g/day) of broiler birds fed diet (B), 5% CSR and the control diet (A) (385±0.16g/day) were significantly better than those birds fed other diets. The depressed action of increase in fibre on growth was especially manifested at the highest inclusion rates, as shown by the relative performance characteristics in Table 6.

The findings (McDonald et al., 1999; Onifade,2000) permits the inclusion that between 5 - 20% inclusion rate of the dietary fibre both energy and other nutrient availabilities are not severely depressed; this was facilitated by the capability of the broiler birds to adjust their intake compensatorily although restrictedly. Therefore, feed to gain ratio became poorer at a level above 5% CSR correspondingly with incremental levels of the dietary fibre.

A nutrient digestibility is the ratio of the nutrient retained to the total intake expressed in percentage (Longe, 2006). Digestibility is one of the most essential tools with which one can assess the nutritive value of a particular feedstuff. However, components of feed nutrients

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cannot be said to beneficial unless such nutrients are capable of being properly utilized or digested and assimilated.

The result obtained from this study showed that there were significant differences in the digestibilities of the experimental diets. Birds on the control diet had the highest digestibility values of 71.08±0.17%, 64.27±0.11% and 69.31±0.13% for crude protein, crude fibre and dry matter digestibilities respectively. This might be due to the effect of the fibre components as the compensatory augmentation of feed intake on high fibre diets circumstantially increase fibre intake, and this would tend to exert more marked depression on the nutrient utilization of all dietary components as shown in (Table 6).

3.5.3 ORGANS CHARACTERISTICS

The gizzard weight was significantly affected by the diets, although, values obtained for the birds fed diet (B) 5% CSR was similar to that of control. This was expected as reported in various studies Tewe (2004), and Longe (2006) that the maximum dietary fibre level is 4.5% for broilers. Longe (2006); and Ologhobo (2012) reported that a prolonged consumption of soluble NSP (from high fibre) led to significant adaptive changes in the digestive system of poultry. Some of the changes include enlargement of the digestive organs probably caused by increased secretion of digestive juices, and accompanied by decreased in nutrients digestion. The result obtained for gizzard weight in this study was similar to that of Kubena *et al*., (1980); Summer and Leeson (1996), Yang and Choct (2009), and Wang and Gu (2010) who reported that gizzard weight was higher in birds fed more fibrous diets. The increase in size might have been due to increase muscular activity, which led to the increase in weight and size observed in the liver and kidney of birds fed diet containing more than (15% CSR).

The length of the intestine also increased with increased fibre, (Wang and Gu, 2010) also reported that at similar feed intake, fibrous diets increased the weight and length of the GIT in broilers. Longe and Ogedengbe, (1989) reported the implication of the effect of dietary fibre on performance was a function of the source and concentration of the fibre.

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3.5.4 SERUM METABOLITES

The result obtained for serum metabolites showed significant dietary (P<0.05) differences for serum thiocyanate with 25% CSR diet giving highest values than the control and other CSR based diets. This is an indication that cassava starch residue at high level of inclusion was accompanied by increasing thiocyanate levels possibly results from breaking down of cyanide in liver (Coffey, 2008).

The total cholesterol values obtained were significantly different(P<0.05). The cholesterol values for birds fed diet (A) control (146.1±0.61mg/dl) were significantly higher than other diets B,C,D,E and F, although fell within the normal range values of 54 to 159.00mg/dl as reported by Mitruka and Rawnsley, (1977). Comparing the other treatments with the control, it could be observed that the birds fed with diets B (139.1±0.59mg/dl), C (129.7±0.61mg/dl), D (118.9±0.56mg/dl), E 109.9±0.6mg/dl) and F (103.8±0.52mg/dl) had valued lower than the control (146.1±0.61mg/dl) and this could be due to the fibre content of the diets. Also, it might be due to a slight reduction in lipogenesis brought about by a concomitant reduction in regulatory activity of acetyl CoA carboxylase- an enzyme which mediates in the rate-limiting step of carboxylation of acetyl CoA to malonyl CoA in the fatty acid synthesis (Onilude, 1999).

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CHAPTER FOUR

4.0 ENHANCEMENT OF NUTRITIONAL PROPERTIES OF CASSAVA STARCH RESIDUE BIODEGRADED WITH *ASPERGILLUS NIGER* AND *TRICHODERMA VIRIDE*.

4.1 INTRODUCTION

The major ingredients in feeds for poultry in Nigeria are maize and soybean or groundnut cake to supply energy and protein respectively. Fish meal is a good source of animal protein which is used to complement the amino acids of vegetable protein sources. However, the cost of the main ingredient may limit the maximum quantity of fishmeal used, giving way to alternatives. Despite the potential of low-grade feedstuffs as ingredients in the tropics, their utilization is dependent on their chemical composition. Their low protein and high crude fibre contents limit their use in poultry feeds. The crude fibre content is high in non-starch polysaccharide (NSPs) (Longe, 2006) both water-soluble (pectins, gums and mucilages) and insoluble (other hemicelluloses, cellulose and lignin). The cell wall content has been shown to be a good predictor of ME value for poultry feedstuffs (Carre *et al*. 1984). Bulk density of these by-products is related to gut fill while the constituents cannot be optimally digested by the endogenous enzymes of poultry due to anti-nutritive effects.

As regards the poor nutritive values and non-suitability of Agro-industrial by products (AIBs) for animals use, research results have shown that pretreatment to digest rigid cell walls of plant origin prior to its use in feed enhances their digestibility by making possible the release of readily digestible monomers.

Nigeria is still leading the production of cassava in the world with about 37.54million metric tonnes per annum (FAO 2012). Cassava starch residue constitutes 10-20% of the processed roots. Its characterized by low protein content and metabolisable energy and low digestibility. Upgrading these residues by biological means to produce a utilizable product from a substance of little initial feedings value might be needed. Fungi, a saprophyte capable of extracellular digestion of fibre components (NSPs) through the enzymes secreted by it, has been found to be promising in enhancing the nutritional profile of the choice feedstock. This experiment was designed to evaluate the changes in CSR after degradation with *Aspergillus niger* and *Trichorderma viride*, in a bid to harness low quality CSR to resolve the shortage of livestock feeds and reduce environmental hazards.

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4.2 MATERIALS AND METHODS

4.2.1 Experimental site

The degradation process was carried out at the Department of Microbiology laboratory, University of Ibadan, Nigeria.

4.2.2 Experimental Feedstuff

The unconventional feedstuff used for this study was dried cassava starch residue.

4.2.3 Source of Fungi

The fungi (*Aspergillus niger* and *Trichoderma viride*) were isolated from cassava by – products contaminated soil, at the Department of Microbiology, University of Ibadan. The fungi were kept on potato dextrose agar (PDA) at 30°C and sub-cultured every two weeks to ensure viability and active growth.

4.2.4 Preparation of Sample and Inoculation

One weekold slants of the fungi were used for the fermentation process. About 10ml of distilled water was used to harvest the spores of the fungi and the spore count was done using haemocytometer to obtain 10^6 spores/ml which was used for the Solid State Fermentation.

4.2.5 Solid state Fermentation

Solid state fermentation was carried out in 250ml Erlenmeyer flasks, in a controlled temperature chamber of 30°±2°C. The solid substrates contained 30g of dried milled cassava starch residue in separate flaks. Sterilization (autoclaving) was done at 121°C for 20 minutes before inoculation. About 15 mls of sterile distilled water was added to each flask prior inoculation to enhance fungal growth. Three mililitres of each of the inoculum was used to inoculate the solid substrate in their respective flasks and the mixture at ratio 1:3 of *A. niger x T. viride* respectively. The flask for each treatment was replicated three times for each period of 0,7, 14 and 21 days of fermentation. At the end of each fermentation period, fungal activity was terminated by introducing the flasks of growing cultures into a water-bath set at 80°C for 15 minutes. The recovered materials were oven-dried at 60°C for 72 hours and stored at room temperature (32°±5°C) pending chemical analyses.

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4.2.6 Biomas loss: The biomass loss was monitored according to the method of Aderolu (2000) for the number of days the fermentation exercise lasted. The initial weight at day zero and the final weight on the last day of the experiment were noted. The final weight was then subtracted from the initial weight and the difference was then divided by the value of day zero and the percentage found.

4.2.7 Chemical Analysis: The samples were analyzed for Dry matter, Crude Protein, Crude fibre, Ether Extract, Ash, (A.O.A.C 1990), Acid detergent Fibre (ADF), Acid Detergent Lignin (ADL) and Neutral Detergent Fibre (NDF) were estimated as outlined by Van Soest 1999, using acetic acid). Hemicellulose of the samples were estimated by difference between NDF and ADF, while Cellulose was estimated by the difference between ADL and hemicellulose. Metabolizable energy was calculated using the equation of Pauzenga, (1985).

4.2.8 Statistical Analysis: The data obtained were subjected to statistical analysis of variance (ANOVA) of SAS (1999) while significant means were separated using Duncan Multiple Range test (1955)of the same package.

4.2.9 Experimental Design: The experiment was design as a 3 x 4 factorial arrangement of treatment with three replicates. The two factors are, effect of the different organisms and the effect of days on the fermentation.

4.3.0 RESULTS

4.3.1 Effects due to interactions of days of fermentation and organisms on Biomass loss (%)

The biomass loss of fungi-degraded cassava starch residue for 7, 14 and 21 days is shown in Table 9. Results showed that for each of the fungi treatment, there were significant differences between biomass loss observed in 7, 14 and 21days. A significant increase in biomass loss from 23.80±1.0% (day 7) to 33.11±0.9% (day 21) in *A. niger* while losses for *T. viride* and combination of inoculum of *A.niger + T. viride* were 15.86±0.8% to 27.44±1.1% and 17.65±1.2% to 30.01±1.4% respectively. Between the three treatments (*A.niger, T.viride,* and *A. niger + T.viride) A. niger* degradation of CSR substrate was significantly (P<0.05) higher than *T. viride* degradation of CSR and combination of inoculum of *A. niger + T.viride* degraded CSR throughout periods.

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TABLE 9: Effects Due to Interactions of Days of Fermentation and Organisms on Biomass Loss (%) of Biodegraded Cassava Starch Residue.

Organisms	DAYS			
	7	14	21	±SEM
A. niger	23.80 ± 1.0^{cx}	30.20 ± 0.7^{abx}	33.11 ± 0.9^{ax}	0.60
T. viride	15.86 ± 0.8^{cz}	21.42 ± 0.9^{by}	27.44 ± 1.1^{ay}	0.80
A.niger +T.viride	17.65 ± 1.7^{bcy}	19.89 ± 1.8^{by}	30.01 ± 1.4^{ax}	0.50

A,b,c… Means on the same row with different superscripts are significantly different (P<0.05)
x,y,z… Means on the same column with different superscripts are significantly different (P<0.05)
±SEM: Standard error of the means

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4.3.2 Effects due to days of fermentation and organisms on the Nutrient composition of Biodegraded CSR

The effects of days of fermentation and organisms on the nutrient compositions cassava starch residue are shown in Tables 10 and 11respectively. Table 10 shows higher significant contents of CP, (7.16±0.07%), ME (2785.4±24.2Kcal/Kg) and least CF (11.67±0.3%), cellulose, hemicellulose and ADL were observed in biodegraded CSR substrate on day 14 of fermentation.

In table 11, effects of *A. niger*, on CP and ME contents of cassava starch residue were higher ($P<0.05$) than that of *T.viride* and combination of *A.niger + T.viride* inoculum, although, the cellulose (12.07±0.03%) and hemicellulose (34.00±0.4%) contents of *T. viride* fermented substrate were similar ($P>0.05$) to that of *A. niger*.

4.3.3 Effects due to Interactions of days of Fermentation and Organisms on the Nutrients Composition of Biodegraded CSR

The interactions of days of fermentation and organisms on the crude protein, crude fibre and ME contents of cassava starch residue were shown in table 12, 13 and 14. On day 14th of fermentation a significant ($P<0.05$) increase in CP contents of biodegraded CSR with *A. niger* inoculum with mean value of (7.16±0.07%) compared to *T.viride* (6.84±0.03%) and combination of *A. niger and T.viride* inoculum (6.88±0.03%) biodegraded CSR respectively.

The CF decreased significantly ($P>0.05$) with increase in CP contents in all the three treatments (*A. niger*, *T.viride* and combination of *A. niger + T.viride*), the least CF values was observed in CSR biodegraded with *A. niger* inoculum (10.31±0.05%) followed by CSR biodegraded with combination of *A. niger + T.viride* inoculum (11.89±0.05%).

The ME for CSR biodegraded with *A. niger* had the highest mean value of 2897.1±23.1Kcal/kg DM. compared to 2739.1±21.2kcal/kg Dm for the CSR inoculated with combination of *A. niger + T.viride*. The same trend was observed for other periods.

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TABLE 10: Effects Due to Days of Fermentation on the Nutrient Composition of Biodegraded Cassava Starch Residue

Parameters	DAYS				
	0	7	14	21	±SEM
Crude Protein(%)	4.05±0.00^{y}	6.03±0.03^{x}	7.16±0.07^{w}	6.93±0.05^{w}	0.15
Crude Fibre(%)	17.00±0.01^{w}	12.86±0.04^{x}	11.67±0.03^{y}	11.50±0.03^{y}	0.14
ME Kcal/kgDM	2589.3±20.5^{y}	2718.8±22.3^{x}	2785.4±24.4^{w}	2757.6±22.5^{w}	66.5
Neutral Detergent fibre(%)	68.03±0.33^{w}	62.20±0.41^{x}	54.55±0.32^{y}	53.65±0.33^{z}	22.06
Acid Detergent fibre	32.00±0.04^{w}	27.45±0.04^{x}	20.60±0.06^{y}	20.38±0.07^{y}	0.27
Acid Detergent lignin (%)	14.93±0.07^{w}	13.50±0.09^{x}	12.44±0.08^{y}	12.37±0.09^{y}	0.37
Cellulose (%)	17.06±0.07^{w}	13.95±0.10^{x}	8.06±0.08^{y}	8.00±0.06^{y}	0.01
Hemicellulose (%)	36.03±0.06^{w}	35.73±0.07^{w}	33.93±0.06^{x}	33.28±0.07^{x}	0.24

wxy: Means on the same row with different superscripts are significant ($P<0.05$)

±SEM: Standard error of the means

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TABLE 11: Effects Due to Organisms on the Nutrient Composition of Biodegraded Cassava Starch Residue

Parameter	Organisms			± SEM
	Aspergillus niger	*Trichoderma viride*	*Aspergillus niger +Trichoderma viride*	
Crude Protein (%)	7.17±0.07[a]	5.88±0.03[b]	5.93±0.03[b]	0.10
Crude fibre (%)	12.29±0.03[c]	14.14±0.04[a]	13.34±0.3[b]	0.03
ME(Kcal/kgDM)	2764.2±6.8[a]	2675.6±7.8[b]	2698.20±8.2[b]	87.4
NDF (%)	58.76±0.02[c]	59.58±0.01[b]	60.48±0.02[a]	0.09
ADF(%)	24.48±0.03[b]	25.58±0.09[a]	25.26±0.06[a]	0.05
ADL(%)	12.98±0.01[b]	13.51±0.02[a]	13.44±0.02[a]	0.08
Cellulose (%)	11.49±0.03[b]	12.07±0.03[a]	11.75±0.03[b]	0.07
Hemicellulose (%)	34.28±0.04[b]	34.00±0.04[b]	35.22±0.05[a]	0.18

abc…. Means on the same row with different superscripts are significant ($P<0.05$)

ME: Metabolisable Energy

± SEM: Standard error of the means

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TABLE 12: Effects Due to Interactions of Days of Fermentation and Organisms on the Crude Protein (%) Composition of Biodegraded Cassava Starch Residue

ORGANISMS	DAYS				
	0	7	14	21	±SEM
Aspergillus niger	4.05 ± 0.01^{z}	6.40 ± 0.04^{ay}	7.17 ± 0.07^{aw}	7.07 ± 0.05^{ax}	0.25
Trichoderma viride	4.05 ± 0.01^{z}	5.72 ± 0.03^{cy}	6.84 ± 0.06^{bx}	6.90 ± 0.04^{bw}	0.03
Aspergillus niger + *Trichoderma viride*	4.05 ± 0.01^{z}	5.97 ± 0.05^{by}	6.88 ± 0.06^{bw}	6.81 ± 0.05^{cx}	0.01
±SEM	0.01	0.02	0.03	0.03	

abc: Means on the same column with different superscripts are significant ($P<0.05$)

wxyz: Means on the same row with different superscripts are significant ($P<0.05$)

±SEM: Standard error of the means

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TABLE 13: Effects Due to Interactions of Days of Fermentation and Organisms on the Crude Fibre (%) Composition of Biodegraded Cassava Starch Residue

ORGANISMS	DAYS				
	0	**7**	**14**	**21**	**±SEM**
Aspergillus niger	17.07 ± 0.02^{w}	11.63 ± 0.04^{cx}	10.31 ± 0.03^{cy}	10.24 ± 0.04^{cy}	0.16
Trichoderma viride	17.07 ± 0.02^{w}	13.97 ± 0.05^{ax}	12.83 ± 0.04^{ay}	12.78 ± 0.04^{ay}	0.17
Aspergillus niger + Trichoderma viride	17.07 ± 0.02^{w}	12.99 ± 0.05^{bx}	11.89 ± 0.04^{by}	11.48 ± 0.05^{by}	0.16
±SEM	0.27	0.56	0.42	0.55	

abc: Means on the same column with different superscripts are significant (P<0.05)

wxyz: Means on the same row with different superscripts are significant (P<0.05)

±SEM: Standard error of the means

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TABLE 14: Effects Due to Interactions of Days of Fermentation and Organisms on the Metabolisable Energy Kcal/Kg (Dm) content of Biodegraded Cassava Starch Residue

ORGANISMS	DAYS				±SEM
	0	7	14	21	
Aspergillus niger	2589.3±22.6^{z}	2772.3±23.0^{ay}	2897.1±22.8^{aw}	2798.3±22.8^{ax}	30.12
Trichoderma viride	2589.3±22.6^{y}	2682.8±23.1^{cx}	2719.2±24.0^{cw}	2711.3±23.1^{cw}	45.28
Aspergillus niger + Trichoderma viride	2589.3±22.6^{z}	2701.4±23.3^{by}	2739.1±24.3^{bx}	2763.1±23.8^{bw}	40.05
±SEM	28.02	27.81	30.97	30.29	

abc: Means on the same column with different superscripts are significant ($P<0.05$)

wxyz: Means on the same row with different superscripts are significant ($P<0.05$)

±SEM: Standard error of the means

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4.4 DISCUSSION

4.4.1 Effect due to interactions of days of fermentation and organisms on biomass loss (%)

The different treatments to which cassava starch residue were subjected resulted in different rate of biomass loss. The CSR substrate treatment which had the least biomass loss might due to the resistant starch formed that affects microbial activities. This must have resulted to the low biomass loss observed in *T. viride* degraded CSR substrate. Since the degradation of fibre proceeds stepwise with the breakdown of polysaccharide into oligosaccharides, these can be hydrolyzed by glucosidase into their components monomers which are not as bulky and crystalline as the original polysaccharide (Aderolu, 2000) . Sharma (1987) and Karunananda *et al* (1991) proposed the possibility of the different strains microbes producing different amount of polysaccharide degrading enzymes which may result in variation in biomass loss within the same fibre feedstuff. The same author opined that maximum activities of polysaccharides degrading enzymes were correlated with the significant increase in biomass loss. This observation was also in line with the substrate-enzyme specificity as suggested by Bertin and Andrieu (2005). Zadrazil and Brunnet (1980) reported a degradation of (60.30%) in sunflower and (21.60%) in rice husk using *Pleurotus florida* and with *P. cornucopiae*, degradation of (68.10%) and (59.60%) were observed for sunflower hull and rice husk, respectively.

4.4.2 Effect due to Days of Fermentation on the Nutrient Composition of CSR

The effects of fermentation periods of fungi incubation on the nutritional compositing of cassava starch residue showed that *A. niger* resulted in increase crude protein content and decrease cellulose content (Table 11) of cassava starch residue significant better than *T.viride* and the combination of *T.viride* and *A. niger*. This might be due to the multienzyme production ability of Aspergilli organisms as recognized by de Vries and Visser (2001), and Bhat (2002). However, fourteen days of fermentation produced better nutritional status than seven days and 21 days, and this might have been connected with the active growth of microorganisms which was asserted by Iyayi and Aderolu (2004). Thus, the ME Kcal/kg of *A. niger* degraded CSR substrates were significantly improved than other treatments on 14th day of fermentation.

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4.4.3 Effects Due to Interactions of Days of Fermentation and Organisms on The Nutrient Composition of Cassava Starch Residue

In the interactions of days of fermentation × organisms, *A. niger* inoculated substrates in all the periods produce significantly higher degradation percentage above 20.00% than *T. viride* and combination of *T. viride* + *A. niger* inoculated substrate. Badal (2003) concluded that degradative changes in fibrous crop residues after fungal incubation were caused by a complex interactions of many factors including cell wall phenolic acid. As the days increased, mycelia growth increased, spore formation and germination took place leading to increase the enzyme secreted by the fungi for extracellular digestion, thus percentage degradation increased.

The species of fungi used on the CSR showed significant ($P<0.05$) differences in the substrate studied. The crude protein increased by 76.80% (4.05±0.01% to 7.16±0.07%) on the CSR when fermented with *A. niger* for 14 days. The change in the protein of fermented product could have resulted from slight protein synthesis by the proliferation of the micro-organisms used and a synthesis of enzyme protein from a rearrangement of the different proportion following the degradation of other constituents. Iyayi and Losel (1999) obtained 152.00% increase in crude protein of cassava peel after 20 days using *A. niger* and 161.10% increase in pulp. Abu *et al.,* (1997) obtained increase of 134.98% (4.95% to 11.83%) using *A. niger* on sweet potato and 35.15% (4.97% to 6.69%) using *A. oryzae*. Meanwhile, variations among microbe strains were well documented in literature (Sharma, (1987); Onilude (1999), Karunananda *et al*., (1992). The increase in *A. niger* inoculated CSR substrate could be due to the production of α-amylase which was able to break the α-glycosidic bonds present in root tuber residue. The CP content of the degraded substrates increased with period of fermentation up to day (14) because the set period in this study (zero to 21 days) and the active period of the fungi when enzyme production and biodegradation are at 14th day. Various results documented corroborated this study. Iyayi and Losel (1999), Onilude (1999), Adebiyi (2008). Also, Aderolu (2000) submitted that at the initial stage day 0 to 14 days, none of the growth limiting factors of microbes, like oxygen, moisture and heat requirement were lacking. McCaskey and Anthony, (1979) reported that fermentation brings about an improvement in nutrient composition and palatability of feed, resulted in conversion of nitrogen and carbon to single cell protein (SCP) and reduced P^H. The “turnover” effect of proteins and amino acids as the

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quantity of free amino acids had been shown to increased as fermentation progresses (Murata *et al.,* 1967).

4.4.4 EFFECT ON INTERACTION OF DAYS OF FERMENTATION AND ORGANISMS ON CRUDE FIBRE COMPOSITION OF CSR

The reduction in CF content of all the fungi-degraded CSR substrate in this study could be due to the activity of the fungal enzymes which degraded the non starch polysaccharide (NSP). Campbell *et al* (1986), Howard *et al.,* (2003) recognized the disruption of the large molecules of fibre unit, reduction in their viscosity and total encapsulation of fibrous feed by microbes. The primary sources of industrial enzymes are about 53% from fungi and yeast, 35.00% for bacteria and 12.00% to 15.00% from plant or animal origin. The most efficient lignin degrading micro-organisms are fungi (Falcon *etal.*, 1995; Badal, 2003) and are known to have a multi-enzyme activities which brings about synergistic actions in degrading NSP. The CSR degrading with spores of *A. niger* gave the highest crude fibre loss (33.96%) compared to *T.viride* degraded CSR and combination of *A. niger + T.viride* degraded substrate. This might be due to efficient degradation of CSR by *A. niger* enzymes being produced during fermentation. In the report of Afolabi (1999), CF reductions of 23.94% (day 4), 26.63% (day 8) and 52.39 (day 10) in cassava peel were observed when inoculated with *A. niger* and with *R.stolonifer* 23.81% (day 4), 38. 36% (day 8) and 54.47% (day 10) were observed. The organisms used for the degradation of SCR (*A. niger, T. viride* and combination of *A.niger and T .viride*) were able to reduce the CF further even up to 14 days of the fungi incubation because the microbes were still within their active growth stage and growth limiting factors had not set in as suggested by Iyayi and Aderolu (2004). The enzymes produced by these microbes, cellulase by *A. niger* (Badal, 2003), and lignase by *T. viride* (Howard, *et a*l., 2003) resulted in varying degree of CF reduction.

4.4.5 EFFECTS OF INTERACTION OF DAYS OF FERMENTATION AND ORGANISMS ON METABOLISABLE ENERGY CONTENTS OF CSR

Low metabolisable energy (ME), according to Mollah *et. al*, (1983), Onifade (1992), Iyayi and Losel, (1999), and Longe, (2006) is due to reduce starch digestibility . The low ME observed in day O for the entire treatments may be due to the increased level of soluble NSP as suggested by Choct *et.al* (1999) and Iyayi and Aderolu (2004). Fermentation of this substrate

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by fungi specie produced enzymes which led to the increase observed in ME Kurulak (1999) reported varying degrees of increase in apparent ME of sunflower with the addition of enzyme. Iyayi and Aderolu (2004) working with *T. viride* on agro-industrial by-products brewer dried grain, rice bran, palm kernel meal and corn bran obtained 5.0, 6.3, 9.0 and 18.5% increases in energy, respectively. The increment in ME of fungi degraded CSR was below the values reported by Iyayi and Aderolu (2004) on agro-industrial by products. This might be due to the variation in nutrient composition of choice substrate, and also physical treatment CSR was subjected. The increased available energy content of fibre feedstuff subjected to microbial treatment may result into their better utilization by monogastric (Onilude, 1999).

4.4.6 EFFECTS OF INTERACTION OF DAYS OF FERMENTATION AND ORGANISMS ON CRUDE FIBRE COMPOSITION OF CSR

Cellulose is an homogenous polymer formed from linear chain of β(1-4) linked D-glucopyranosyl unit (Choct and Kocher, 2001). It represents about 35-50% DM of root crops. The highest cellulose degradation was observed when *A. niger* was inoculated on CSR substrate, for 14 day from 17.07±0.07 to 11.49±0.03% giving 32.68% degradation. This could be as a result of the enzymes produced by Aspergillus as reported by Badal (2003). Combination of *A. niger* and *T.viride* were able to degrade cellulose from 17.07±0.07% to 11.75±0.03% resulting in 31.16% degradation of cellulose in CSR. This could be because of synergistic actions of both fungi. (Balagopalan, 1996). Cellulose degradation in CSR substarte inoculate with *A. niger* is better than in combination *of A.niger + T.viride* and *T.viride* degraded CSR substrates, this could be a function of variability in the quantity and quality of complete cellulase enzyme complex being produce by various organisms.

Hemicellulose on the other hand is an heterogeneous polymer having β(1-4) linked backbone of xylose, mannose or glucose residue that can form extensive H^+ bonding with cellulose. It requires several different enzymes with different specificities for complete hydrolysis, the polysaccharides does not form tightly, packed crystalline structures like cellulose and is, thus, more accessible to enzymatic hydrolysis (Gilbert and Hazlewood, 2003). Hemicellulose consists of about 20 to 35% DM of root tuber. Badal, (2003), and Howard *et al* (2003) recognized endoxylanases, and βxylosidase, and several accessory enzymes, such as α-ι -arabionofuranosi-dase, α -glucuronidase, ferulic acid esterase, and P-

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coumaric acid esterase, as the enzyme necessary for hydrolyzing various substituted xylans. *A. niger* fermentation brought about (4.88%) loss in hemicellulose content of CSR followed by *T. viride* fermentation (5.55%) at 14 days. A similar trend was also observed by Adebiyi, (2008) using *A. niger, T. viride* and *R.Stolonifer* on cowpea seed hull. The author observed 42.30 and 32.00% decrease on 14th day of incubation with *A. niger* and *T. viride* respectively. The faster growth of *A. niger over T.viride* could have resulted in the better performance of *A. niger* in degrading the CSR.

Generally, the fibre components of the fermented CSR decreased over the periods of fermentation. During biodegradation process the hydrolytic enzymes of the fungus broke down polysaccharide content of the CSR into various sugars. *A. niger* had the highest (68.03±0.03 to 58.76±0.02%) NDF and ADF (32.00±0.04 to 24.48±0.03%) degradation percentages. This is contrary to the assertion by Onilude (1994) that *Trichodema spp* had the highest celluloytic enzymes secretion in solid state fermentation. The enzyme-substrate specificity could have resulted in better performance of *A. niger* on the CSR. In term of ADL degradation, for all the treatments *A. niger* however, gave a significant loss (14.93±0.07 to 12.98±0.01%) in lignin content in CSR; whereas *T. viride* fermentation resulted in total loss of (14.93±0.07 to 13.51±0.02%) likewise combination of *T. viride* + *A. niger* (14.93±0.07 to 13.44±0.02%) yielded loss of (9.97 %). This suggested the inability of *T.viride to* produce lignase which ought to have degrade part of lignin in CSR. *A. niger* , however, was the most efficient and extensive lignin degraders in this study.

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CHAPTER FIVE

5.0 EFFECT OF SUPPLEMENTING *ASPERGILLUS NIGER* DEGRADED CASSAVA STARCH RESIDUE IN BROILER DIETS

5.1 INTRODUCTION

The scarcity and fluctuating quantity and quality of the food and feed reserves in the world-over have increased interest in the exploitation of enormous by-products that are wasted in the traditional and industrial processing of root crops. There include products like cassava peels, cassava sievate, foofoo sievate and starch residue among others. . Cassava starch residue alone forms about 20% of the processed root. Within the past decade, a redirection of efforts to the use of agro-byproducts have been advocated as a way of solving this problem through microbial fermentation of these agro-industrial by-products (AIBs) into useful products. These wastes from the agricultural related small and large scale processes are extremely valuable to quantify, characterize and develop into wholesome feed for livestock. This will in turn, be a possible major revenue spinning project for the agricultural sector in developing countries

In Africa, particularly Nigeria, there are increasing needs for protein sources. Protein supply from livestock products (to include contributions from fish and wildlife) was estimated to be only 30g/caput/ day in 1993 and a projection of 53.2g/caput/day has been made for the year 2010 (Longe, 2006). This shows a short-fall of 15g/caput/day from the 45g recommendation of FAO (1993). For economic growth, therefore, research had been conducted in recent times and is currently being intensified to reduce the competition between humans and livestock for the same feedstuff. This suggests an opportunity for exploiting more of various AIBs that largely go to waste and pollution hazard if not properly disposed off.

5.2 MATERIALS AND METHODS

5.2.1 EXPERIMENTAL SITE

The degradation process was carried out partly at the Department of Animal Science, University of Ibadan within the Biotechnology Laboratory and partly at the Department of Microbiology, University Ibadan, Ibadan.

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5.2.4 Experimental Feedstuff

The dried cassava starch residue (CSR) used for this study was obtained in large quantity from a cassava processing factory (Matna), Akure, Ondo State. The test ingredient was later milled and autoclaved at 121°C for 30 minutes for sterilization.

5.2.5 Source of Fungus

Aspergillus niger inoculums were used in this experiment for the degradation process. The fungus was isolated from cassava waste soil in the culture bank of the Department of Botany and Microbiology, University of Ibadan, Nigeria. The isolation was made by the pour-plate method of Harrigan and McCance (1976) see Appendix 2. The pure culture of *A. niger* was routinely maintained on Potato dextrose agar (PDA) at 30ºC and sub-cultured every two weeks to ensure viability and active growth.

5.2.4 Inoculation and Incubation Procedure

The *A. niger* spores for this experiment were harvested and then adjusted to 10^6 spores per/ml with sterile water. About 30g of milled was put into 250ml Erlenmeyer flasks. The flasks with their contents were autoclaved at 121°c for 30minutes and allowed to cool then liquefied pineapple-pomace in form of syrup was introduced to adjust the moisture content to about 25%. The pineapple-pomace was added to provide more carbon for the organism to feed on and grow. After autoclaving the set of flasks were aseptically inoculated with the organism and placed in an incubator at 30°±2°C after about 4 - 5 days the microorganisms were fully grow. The larger samples of CSR with water to be used were later autoclaved and allowed to cool. Inoculations of the lager sample with the fully-grown *A. niger* on CSR, as a substrate was done. It was moistened with 500ml distile water to create a conducive condition for growth of the fungus left for 10days inside polythene that was tightened to prevent housefly contamination with occasional turning. At the end of the 10th day the larger sample was steamed at 80°C for 15minutes in order to terminate the activity of the fungus and later this sample was oven-dried for 72hours at 60°c, and was bagged and tagged biodegraded CSR as suggested by Onilude (1999), and Aderemi and Nworgu (2007).

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5.2.5 Management of Experimental birds and formulation of Experimental Diets

One hundred and eighty day-old hybro broiler chicks were used for the study. Thirty chicks per dietary treatment, of five replicates each. The chicks were reared in deep litter with feed and water supplied *ad-libitum*. The management of the birds was as outlined by Olomu (2003).

Six dietary treatments were formulated including the control and different levels of degraded cassava starch residue supplementation (5, 10, 15, 20 and 25%). The diets were made isonitrogenous (23 and 21% crude protein for the starter and finisher phases respectively) with the provision for at least the minimum calorie requirement (2950kcal/kg) of the chicken as recommended by Olomu (2003). The test ingredient was incorporated as stated above. At the end of the seven days of brooding, the birds were weighed and allocated to their respective treatments. Records of feed consumption and body weight were taken on weekly basis whereas, body weight gain and feed to gain ration were calculated from the data collected.

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TABLE 15: Nutrient Composition of Undegraded And Degraded Cassava Starch Residue (% On Dry Matter Basis)

NUTRIENT	UNDEGRADED-CSR (%)	DEGRADED-CSR (%)
Crude protein	4.05	7.01
Crude fibre	16.79	11.07
Ether extract	1.85	1.45
Ash	4.90	2.01
Nitrogen free extract	60.01	71.22
Cellulose	17.07	8.01
Hemicellulose	36.03	33.08

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TABLE 16: Percentage Composition of Broiler Starter Diet Containing Different Levels of *A. niger* Degraded Cassava Starch Residue

INGREDIENT (%)	DIETS					
	A	B	C	D	E	F
	0%(control)	5%	10%	15%	20%	25%
Maize	50.00	45.00	40.00	35.00	30.00	25.00
Cassava starch residue	0.00	5.00	10.00	15.00	20.00	25.00
Groundnut cake	12.00	14.00	14.50	15.00	15.25	15.25
Wheat-offal	5.00	2.50	2.00	1.00	0.25	0.00
Palm kernel oil	0.00	0.50	0.50	1.00	1.50	1.50
Full Fat Soya	25.00	25.00	25.00	25.00	25.00	25.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50
Broiler Premix	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Table-salt	0.25	0.25	0.25	0.25	0.25	0.25
Total(kg)	**100**	**100**	**100**	**100**	**100**	**100**
Calculted nutrient						
Crude protein	23.60	23.70	23.62	23.37	23.09	22.82
Crude Fibre	3.30	3.63	4.10	4.32	4.89	5.38
ME (kcal/kg)	3009	3018	3006	3006	3016	3000

Premix provide per kg of diet Vit A, 10,000 i.u; vit D3, 2000 i,u; vit. E 23mg; vit K, 2.mg; calcium pantothenate, 7.5mg; B12, 0.015mg; folic acid, 0.75mg; choline chloride, 300mg; vit B1, 1.8mg; vit B2, 5mg; vit B6, 3mg; manganese, 40mg; iron, 20mg; zinc, 53.34mg; copper, 3mg; iodine, 1mg;cobalt, 0.2mg; selenium, 0.2mg; zinc, 30mg.

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TABLE 17: Percentage Composition of Broiler Finisher Diets Containing Different Levels of A. niger Degraded Cassava Starch Residue

INGREDIENT (%)	DIETS					
	A 0% (control)	B 5%	C 10%	D 15%	E 20%	F 25%
Maize	50.00	45.00	40.00	35.00	30.00	25.00
Cassava starch residue	0.00	5.00	10.00	15.00	20.00	25.00
Groundnut cake	15.00	17.50	18.50	19.00	20.50	21.00
Wheat-offal	8.00	4.50	3.00	2.00	0.50	0.00
Palm Kernel Oil	0.00	1.0	1.50	2.00	2.00	2.00
Full Fat Soya	29.00	29.00	20.00	20.00	20.00	20.00
Fish Meal	2.00	2.00	2.00	2.00	2.00	2.00
Bone Meal	1.50	1.50	1.50	1.50	1.50	1.50
Oyster shell	2.50	2.50	2.50	2.50	2.50	2.50
Broiler Premix	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Table-Salt	0.25	0.25	0.25	0.25	0.25	0.25
Total (kg)	**100**	**100**	**100**	**100**	**100**	**100**
Caculated nutrient						
Crude protein	21.10	21.30	21.21	21.30	21.15	21.69
Crude Fibre	3.36	3.61	3.78	4.18	5.11	5.69
ME (Kcal/kg)	3001	3101	3061	3034	3011	3000

Premix provide per kg of diet Vit A, 10,000 i.u; vit D3, 2000 i,u; vit. E 23mg; vit K, 2.mg; calcium pantothenate, 7.5mg; B12, 0.015mg; folic acid, 0.75mg; choline chloride, 300mg; vit B1, 1.8mg; vit B2, 5mg; vit B6, 3mg; manganese, 40mg; iron, 20mg; zinc, 53.34mg; copper, 3mg; iodine, 1mg;cobalt, 0.2mg; selenium, 0.2mg; zinc, 30mg.

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5.2.6 METABOLIC TRIAL

Digestibility study was carried out between the seventh and eight week of the experiment. Two birds from each replicate whose weights were close to the mean were selected for metabolic trial with facilities for feeding, water supply and collection of droppings. The birds were allowed to adjust for 4 days before the faeces were collected for three other consecutive days. Diet was served to the birds and the left over was properly accounted for.

The droppings were later separated from feathers and spillover feed particles and oven dried at 60ºC for 48 hours. The dried samples were milled and stored for subsequent chemical analysis.

5.2.6.1 Dry matter Digestibility: After oven dry, the dry matter contents of the samples were determined as a percentage of initial weight.

$$\% \text{ D. M.} = \frac{\text{Weight after oven drying gm}}{\text{Weight before oven drying gm}} \times 100/1$$

$$\text{D. M. digestibility} = \frac{F_0 - A_0}{F_0} \times 100/1$$

F0= DM Intake = %DM &Feed x Total amount of feed intake

A0= DM Output = % DM of faeces x Total amount of faeces voided

5.2.6.2 Nutrient Digestibility: Digestibility of nutrients were calculated using the following formular.

$$\text{Digestibility} = \frac{(F_0 \times F_i) - (A_0 \times i)}{F_0 \times F_i}$$

Fi = % Nutrient (ash, NFE, CF, EE and E^0) in the feed

$F_{0= \text{Dry matter}}$ weight of feed consumed (Dry matter intake)

Ai=% Nutrient (ash, CF, EE, - NFE, E^0) in the faeces

A_0= Dry matter weight of faeces voided (Dry matter output)

5.2.7 SERUM COLLECTION AND DETERMINATION

Blood was samples from four birds per treatment at the starter and finisher phases and collected into sterile sample tubes without anticoagulant. The tubes were kept in a slanted wooden rack at $36^{O}C$ and the blood allowed to clot. The clotted blood was centrifuged and spun for 15 minutes at 3,500 revolutions per minute. A clear fluid which is the serum was pipetted out into a clean and sterilized bottle and then deep frozen.

The serum protein and albumin were analyzed using sigma assay kits, glucose and cholesterol with randox kits. The buiret method was utilized in the determination of the total protein fraction while the serum was subjected to the direct colorimetric method for albumin with Bromocresol green (BCG) as the dye as described by Toro and Ackerman (1975). The globulin concentration was obtained by subtracting albumin from the total protein.

Albumin/globulin ratio was obtained by dividing the albumin value by the calculated globulin value as described by Toro and Ackerman (1975).

5.2.8 ORGANS QUALITY EVALUATION

At the end of eight weeks, two birds per replicate were slaughtered after they were starved overnight. The Live weight and dressed weight were taken. The abdominal fat was also weighed and recorded. The weights of the organs (liver, heat gizzard kidney and spleen) were also taken.

5.2.9 HISTOPATHOLOGICAL EVALUATION

Kidney and liver of two birds per treatment were removed and after weighing they were preserved with 10% formalin for histopathological evaluation.

5.2.10 COST OF PRODUCTION

Feed cost per kg live weight gain was calculated by multiplying the cost of each kg of diet by the feed to gain ratio (FCR). The existing market price of each feedstuff at the time of feeding trial was used to calculate the cost per kg of each diet.

$$\text{Relative cost benefit (\%)} = \frac{\text{Cost differential}}{\text{Cost/kg Weight Gain of Control diet}} \times \underline{100}$$

5.2.11 CHEMICAL ANALYSIS

Samples of diet and droppings were used for proximate analysis according to the procedure of Association official of Analytical Chemist (A.O.A.C., 1990).

5.2.12 STATISTICAL ANALYSIS

All data collected were subjected to analysis of variance of completely randomized design using the SAS (1999) package and the means were separated using Duncan (1955) multiple Range test of the same software.

5.3 RESULTS

5.3.2 PROXIMATE COMPOSITION OF DIET

The proximate composition of the diets fed to broilers in this study at starter and finisher phases are presented in Tables 31 and 32 respectively.

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TABLE 18: Proximate Composition (G/100 Dm) of Broiler Starter Diets Containing Different Levels Of *Aspergillus niger* Degraded Cassava Starch Residue

Nutrients %	DIETS					
	A (0%)control	B (5%)	C (10%)	D (15%)	E (20%)	F (25%)
Crude Protein	23.44	23.44	23.60	23.71	23.82	23.00
Crude fibre	3.13	3.44	3.89	4.41	5.05	5.21
Ether Extract	4.13	5.46	5.86	6.11	4.86	4.91
Ash	7.49	8.45	8.15	7.99	7.84	7.84
Nitrogen free Extract	59.11	58.97	56.61	56.43	54.9	56.11
Metabolisable Energy (Kcal/kg)	3211.10	3256.4	3266.6	3217.8	3222	3274

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TABLE 19: Proximate Composition (G/100 Dm) Of Broiler Finisher Diets Containing Different Levels of *Aspergillus niger* Degraded Cassava Starch Residue

Nutrients %	DIETS					
	A (0%)control	B (5%)	C (10%)	D (15%)	E (20%)	F (25%)
Crude protein	21.57	21.81	21.98	21.66	21.34	21.51
Crude fibre	3.26	3.98	3.89	4.82	5.16	5.21
Ether extract	4.44	5.11	5.61	4.89	4.76	5.11
Ash	6.11	6.81	6.03	6.41	8.11	7.01
Nitrogen free extract	61.21	60.81	59.99	60.03	59.73	58.81
Metabolisable Energy (Kcal/kg)	3334	3299	3287.4	3277.1	3298	3274

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TABLE 20: Performance Characteristics of Starter Broilers Fed Supplemented Diets Containing Different Levels of Aspergillus Niger Degraded Cassava Starch Residue

Parameters	DIETS						
	A 0%(control)	B 5%	C 10%	D 15%	E 20%	F 25%	±SEM
Feed intake g/bird/day	33.6±0.7[e]	34.15±0.8[d]	35.14±0.7[c]	35.84±0.7[c]	36.01±0.8[ab]	36.49±0.8[a]	0.23
Weight gain g/bird/day	15.40±0.2[c]	16.01±0.3[ab]	16.08±0.3[ab]	16.32±0.2[a]	15.84±0.2[c]	15.61±0.2[c]	0.09
Feed: gain	2.17±0.01	2.13±0.02	2.19±0.02	2.19±0.02	2.27±0.03	2.34±0.03	0.03
Cost/kg feed (₦)	57.11	56.88	56.82	56.55	56.30	56.18	
Cost/feed consumed	40.29	40.79	41.92	42.56	42.57	43.05	
Cost/kg wt gain (₦)	261.62	254.77	260.61	260.70	269.40	275.78	

abc… means on the same row with the different superscript are significantly different ($P<0.05$)
±SEM: Standard error of the means

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TABLE 21: Performance of Finisher Broiler Fed Supplemented Diets Containing Different Levels of *A. niger* Degraded Cassava Starch Residue

Parameters	DIETS						±SEM
	A 0%(control)	B 5%	C 10%	D 15%	E 20%	F 25%	
Feed intake g/bird/day	76.6±0.15[f]	84.07±0.11[e]	86.86±0.12[C]	89.46±0.10[ab]	86.6±0.13[ab]	89.74±0.10[a]	14.25
Weight gain g/bird/day	39.96±0.4[f]	43.70±0.5[e]	45.36±0.4[b]	46.38±0.4[a]	45.01±0.4[bc]	44.88±0.3[d]	6.24
Feed: gain	1.92±0.03[C]	1.92±0.04[C]	1.93±0.02[C]	1.94±0.03[C]	1.99±0.04[bC]	2.06±0.03[a]	0.11
Cost/kg feed (₦)	57.11	56.88	56.82	56.55	56.30	56.18	
Cost/feed consumed	244.9	267.78	276.4	283.3	282.4	282.3	
Cost/kg weight gain (₦)	613.0	612.78	609.3	610.82	627.4	628.9	

a,b,c…..: Means in the same row with different superscripts are significantly different ($P<0.05$)

±SEM: Standard error of the means

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5.3.3 PERFORMANCE CHARACTERISTICS (starter phase)

Performance data of broilers fed diets containing different levels of *A. niger* degraded CSR at the starter phase (Table 20). The highest daily weight gain (16.32±0.2g/bird/day)was observed with birds fed diet D (15% inclusion) and this was significantly different from birds on the control diet, (15.40±0.2g/bird/day). There was significant ($P<0.05$) differences in the daily feed intake with the higher values being observed with birds fed *A. niger* degraded CSR supplemented diets B, C, D, E, and F. The valued ranged between 33.61±0.7g/bird/day (diet A) and 36.49±0.8g/bird/day (diet F).

No significant difference was observed in the feed to gain ratio in all the dietary treatments at the starter phase.

5.3.4 PERFORMANCE CHARACTERISTICS (finisher phase)

Performance characteristics of broiler birds fed diets containing different levels of *A. niger* degraded CSR at the finisher phase is shown in Table 21. Birds on diet D (15% inclusion) had the highest weight gain (46.38±0.4g/bird/day), followed by birds fed diet C (45.36±0.4/bird/day) while those fed diet A (control diet) gave least (39.96±0.3g/bird/day). The feed consumption pattern significantly ($P<0.05$) increased as levels of *A. niger* degraded CSR increase in the diets. The highest feed intake was observed with birds fed diet 6 (89.74±0.10/bird/day), while the least feed intake was recorded for birds fed diet 1 (76.60±0.15g/bird/day) control. Feed to gain ratio were similar across the dietary treatments.

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TABLE 22: Apparent Nutrients Digestibility of Broilers Fed Supplemented Diets Containing Different Levels of *A. niger* Degraded Cassava Starch Residue

Parameters	DIETS						
	A 0%(control)	B 5%	C 10%	D 15%	E 20%	F 25%	±SEM
Dry matter	68.43±0.07	69.76±0.9	68.07±0.10	67.89±0.09	68.02±0.08	69.18±0.09	1.03
Crude protein	71.03±0.11^{d}	78.14±0.10^{a}	77.68±0.09^{ab}	77.85±0.09^{ab}	75.01±0.11^{C}	69.88±0.09^{d}	1.11
Crude fibre	53.66±0.04^{C}	62.01±0.03^{a}	64.03±0.04^{a}	63.33±0.04^{a}	60.01±0.05^{b}	59.03±0.04^{b}	0.28
Ether extract	70.04±012^{C}	74.66±0.11^{ab}	76.84±0.12^{ab}	78.31±0.11^{a}	69.78±.12^{d}	71.11±0.10^{C}	1.14
Ash	76.32±0.11^{C}	81.41±0.10^{a}	82.09±0.11^{a}	80.94±0.13^{ab}	79.89±0.11^{C}	80.01±0.09^{ab}	1.10

abcd; Means on the same row with the different superscript are significantly different (P<0.05)

±SEM: Standard error of the means

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TABLE 23: Serum Biochemical Indices of Broiler Fed Supplemented Diets Containing Different Levels Of *A. niger* Degraded Cassava Starch Residue (Starter Phase)

Parameters mg/100ml	DIETS						±SEM
	A 0%(control)	B 5%	C 10%	D 15%	E 20%	F 25%	
Total protein	4.01±0.01	3.89±0.02	4.03±0.02	4.00±0.01	3.78±0.02	3.66±0.01	0.09
Albumin	1.68±0.00	1.58±0.02	1.71±0.02	1.81±0.02	1.62±0.01	1.54±0.02	0.10
Globulin	2.12±0.02	2.31±0.01	2.41±0.02	2.21±0.01	2.00±0.02	2.01±0.02	1.00
Albumin: globulin	0.79±0.01	0.68±0.02	0.70±0.02	0.81±0.01	0.81±0.01	0.76±0.02	1.11
Glucose	1.24±0.03	1.14±0.04	1.31±0.03	1.28±0.02	1.09±0.01	1.14±0.01	1.10
Cholesterol	$84.41±0.17^{a}$	$80.20±0.18^{b}$	$79.60±0.19^{bc}$	$71.41±0.10^{e}$	$77.11±0.12^{d}$	$68.12±0.12^{f}$	0.91

abcdef; Means on the same row with the different superscript are significantly different (P<0.05).

±SEM: Standard error of the means

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TABLE 24: Serum Biochemical Indices of Broilers Fed Supplemented Diets Containing Different Levels of *A. niger* Degraded Cassava Starch Residue (finisher phase)

Parameters mg/100ml	DIETS						±SEM
	A 0%control	B 5%	C 10%	D 15%	E 20%	F 25%	
Total protein	4.43±0.03	4.61±0.05	5.04±0.05	4.99±0.03	4.12±0.02	3.98±0.03	0.21
Albumin	2.39±0.01^{b}	2.27±0.02^{b}	2.65±0.01^{a}	2.41±0.02^{b}	2.01±0.01^{c}	1.99±0.02^{c}	0.14
Globulin	2.74±0.03	2.34±0.04	2.19±0.04	2.58±0.03	2.11±0.02	2.37±0.03	0.22
Albumin: globulin	0.62±0.00	0.97±.01	0.76±0.02	0.93±0.03	0.59±0.02	0.67±0.02	0.17
Glucose	142.4±9.12^{a}	141.1±10.0^{a}	143.7±11.1^{a}	145.0±8.9^{a}	130.0±9.02^{b}	128.9±10.01^{c}	12.66
Cholesterol	146.9±11.2^{a}	132.3±11.3^{b}	132.77±10.1^{b}	114.3±8.3^{c}	117.4±9.3^{c}	110.98±11.1^{d}	11.74

abcd; Means on the same row with the different superscript are significantly different ($P<0.05$)
±SEM: Standard error of the means

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TABLES 25: Organ Weights of Broiler Chickens Fed Diets Containing Different Levels of *A. niger* Degraded Cassava Starch Residue

Parameters(g)	DIETS						
	A 0%(control)	B 5%	C 10%	D 15%	E 20%	F 25%	±SEM
Crop	1.20±0.02[d]	1.30±0.01[c]	1.39±0.02[ab]	1.41±0.01[a]	1.38±0.02[ab]	1.44±0.01[a]	0.00
Gizzard-empty	3.08±0.03[e]	3.41±0.02[d]	3.58±0.03[bc]	3.69±0.02[bc]	3.88±0.03[b]	4.01±0.02[a]	0.18
Kidney	0.66±0.01	0.64±0.01	0.61±0.01	0.68±0.01	0.64±0.01	0.66±0.01	0.03
Liver	2.24±0.09	2.18±0.11	2.25±0.12	2.31±0.11	2.28±0.07	2.32±0.06	0.04
Heart	0.33±0.02	0.36±0.01	0.32±0.01	0.33±0.02	0.34±0.02	0.41±0.01	0.03
Spleen	0.13±0.01	0.10±0.01	0.13±0.02	0.11±0.01	0.09±0.01	0.12±0.00	0.01
Lung	0.78±0.03[a]	0.66±0.04[a]	0.59±0.3[b]	0.58±0.04[b]	0.60±0.03[b]	0.60±0.03[b]	0.03
Bile	0.13±0.01[b]	0.12±0.01[b]	0.14±0.01[b]	0.18±0.01[a]	0.12±0.02[b]	0.11±0.01[c]	0.03
Abdominal fat	1.69±0.02	1.58±0.02	1.64±0.03	1.55±0.02	1.41±0.02	1.32±0.02	0.10

abc.: Means on the same row with the different superscripts are significantly different ($P<0.05$).
±SEM: Standard error of the means

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5.3.5 NUTRIENT DIGESTIBILITY

The nutrient digestibility of broilers fed degraded CSR supplementation in the diets is presented in Table 22. Crude protein was significantly (P<0.05) highest in birds fed diets B (78.14±0.10%) and D (77.85±0.09%), while birds on diet A(control) gave the least crude protein (71.03±0.11%) value. Birds on diets A and F however, showed no significant differences in their crude protein digestibility. Crude fibre digestibility improved with birds on diets B (62.01±0.3%), C (64.03±0.4%), D (63.33±0.4%) and E (60.07±0.5%) compared to birds on diet A control (53.66±0.4%). Ether extract digestibility ranged from 69.78±0.12% and 78.31±0.11%. There was significant (P<0.05) differences in the Ash digestibility of birds fed with different levels of the degraded CSR in the diets.

5.4.6 SERUM METABOLITES

Serum metabolites of broilers fed starter and finisher diets containing different levels of *A. niger* degraded CSR (Tables 23 and 24). An improvement in the total protein of finisher birds over the starter phased was observed in the entire treatment. No significant (P<0.05) difference was observed in the total protein content and values obtained ranged between 3.98±0.02mg/100ml and 5.04±0.05mg/100ml. The values obtained for albumin: globulin ratio were similar across the dietary treatments.

The highest cholesterol value was observed in birds fed diet A control (146.91±11.2mg/100ml) while the least was observed with birds fed diet F (110.98±11.1mg/100ml). serum glucose level was significantly increased (P<0.05) in diet A (142.4±9.12mg/100ml) to diet D (145.0±8.9mg/100m), the level for birds on diets E(130±9.02mg/100ml) and F (128.9±10.01mg/100ml) being significantly (P>0.05) lower compared to other diets. Similarly, cholesterol decreased significantly (P<0.05) as the inclusion of degraded CSR increased with birds on diet F having the lowers concentration of 110.98±11.1mg/100ml and birds on treatment A (control) had the highest concentration of 148.91±11.2mg/100ml.

5.3.7 ORGANS CHARACTERISTICS

The relative weights of organs of broilers fed diets containing different levels of supplementation of *A. niger* degraded CSR are shown in Table 25. From the result, the crop

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and gizzard of birds on degraded CSR diets were significantly ($P<0.05$) different from those on the control diet A (1.20±0.02g) and (3.08±0.03g), respectively.

Although, no significant differences were observed in the weights of kidney, liver, heart, spleen and abdominal fat of birds in all the dietary treatments including the control.

The weight of the lungs of birds on the control diet A (0.79±0.03%) was however, higher than the birds fed supplemented diets.

5.4 DISCUSSION

Cassava starch residue is the fibrous constituents of the sclerenchyma and xylem parts of the root tuber. For monogastrics like broiler, the insoluble NSP fraction (lignocellulose a physic- chemical binder is virtually undigested in the alimentary canal of birds. To effect a breakdown in the structure of the residue, they were milled and degraded with *A. niger* for 14 days before their inclusions in experimental diets. This approach is supported by Iyayi and Aderolu (2004), that biodegradation of agro industrial by-products will enhance their nutritional status and inclusion in poultry diets.

The increase in daily weight gain (diet B: 43.70±0.05g, C: 45.36±0.04g, D: 46.38±0.04g as well as better feed to gain ratio 1.92±0.03, 1.93±0.02 and 1.94±0.03, respectively) of degraded CSR up to 15% inclusion in diets attested to effect of enzymes being produced during fermentation that produced appreciable amounts of reducing sugars from raw CSR which eventually improve the body mass of the broilers fed those diets, while decrease in the daily weight gain (g/bird) observed in diets E and F as compared to the other diets could be as a result of the increase in the crude fibre content of the feed (5.16%) and (5.71%) respectively which were slightly above the NRC (1994) recommended value of 2-5% for broilers. Onifade (1993), Longe (2006), and Coffey (2008)reported that the inclusion level of agro-industrial by-products in animal diet is restricted by factors such as the quality and digestibility of the materials, the species concerned and age of the animals.

Soluble NSP, according to Choct (1998) are associated with the viscous nature of the polysaccharides, their physiological and morphological effects on the digestive tract and interaction with the micro-flora of the gut. The mechanism includes altered intestinal transit time, modification of the intestinal mucosa and changes in hormonal regulation due to varied rate of nutrient absorption; could probably caused the poor feed conversion efficiency

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observed in birds fed diets E and F. The β-glucan, xylans and amylopectn have been reported to be present in the cassava root. (Caret *et al*, 1997) which brings about very low viscous condition in the small intestine thereby leading to interference with nutrients absorption (Graham and Petterson, 1989; Onilude and Osho 1999; Iyayi and Aderolu 2004).

Meanwhile, no significant difference (P<0.05) was observed in FCR at the finisher phase of broilers, the performance of the birds was better than the starter phase. This could be due to better utilization of fibre with increase in the age of the birds as reported by Onilude and Osho (1999). The difference between the daily weight gain of birds on the different supplemented diets and control (diet A) could be attributed to the fact that the lignocelluloses contents of the diets were heterogeneous and dissimilar in compositional percentages and forms leading to the varying rate of degradation.

Nutrient digestibilities were better in most of diets supplemented with degraded CSR compared to the control. This was because during *A. niger* degradation, enzymes were produced which gave synergistic effect leading to better utilization of nutrient (Zylar *et al*., 1999; Adebiyi et al., 2008).

De Vries and Visser (2001) identified four classes of enzymes involved in the complete hydrolysis of cellulose. These are endoglucanase, cellobiohydrolase, β-glucosidase and exoglucanase, and all the four classes have been identified in Aspergillus species.

Biodegradation according to Onilude (1999) brings about improvement in carcass parameters as observed in this study. Increase in the inclusion levels of biodegraded CSR caused an increase in the fibre level of the diets which resulted in reduction of abdominal fat from 1.69±0.02% to 1.32±0.02%. This decrease might have been due to reduction in the rate of absorption of dietary fat through binding of the fibre to bile acid in the small intestine, leading to a mop-up of bile in the enterohepatic system (Peterson and Aman, 1991; Onilude 1999).

The increased musculature of gizzards of broilers of supplemented diets showed that the organ exerted itself greatly in grinding the diets as compared to the birds on control (diet A), thereby reducing pressure on the gizzard. As much as live weight was influenced by dietary nutrients, the effect of supplementation of degraded CSR in broiler diets (up to 15%) on carcass yield was positive.

There was need to determine serum parameters because it is used in assessing the nutritional status of birds (Church *et al,* 1984), the total serum protein functions in defense

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mechanism and maintenance of osmotic pressure (Diane and Schist, 1982). Although no significant difference was observed in the total protein of the broilers fed diets supplemented with degraded CSR and the control diet at the starter phase, there was a progressive increase in the total protein of the birds on the different diets at the finisher phase. This corroborate the work reported by Ross *et al*., (1978) and Onifade (1993) who also observed an increase in total protein as the bird aged. The increase in glucose with increase in degraded CSR supplementation could be due to an increase in fibre intake which in turn caused an increase in glucose metabolism and insulin response. As the inclusion levels of degraded CSR increased, more of the soluble polysaccharides which are potential energy sources were available in the birds thus increasing glucose concentration in the serum., the significant decrease of blood glucose with increasing degraded CSR above 15% inclusion level in diets having more cellulose contents, substantiated the findings of Wolevever (1999) and Wang and Gu (2010) that there was a strong relationship between dietary cellulose and glycaemic index. The implications of lower level of glucose in the blood of birds fed degraded CSR on 20% and 25% inclusion levels indicate that less energy were available to those birds.

The significant differences ($P<0.05$) observed in serum biochemical parameters with the supplementation of different levels of *A. niger* degraded CSR, were however, fell within the normal recommended values for broilers as reported by Mitruka and Rawnsley, (1977).

The kidney and liver of the birds fed the control and test diets showed no histopathological lesion in the selected organs. This is an indication that *A .niger* did not produce sufficient toxin that could impair selected organs which made it safe for degradation of crop residue to be used as a feedstock.

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CHAPTER SIX

6.0 COMPARATIVE EFFECT OF EXOGENOUS ENZYMES ON THE PERFORMANCE OF BROILERS

6.1 INTRODUCTION

The collective understanding of previous studies in this thesis has corroborated the common knowledge that chickens like other simple-stomached animals perform relatively poor on fibrous diets.

Growth depression in chickens fed 20% degraded CSR diet had been shown in the earlier study to be sensitive to crude enzymes, and it was anticipated that the growth promoting activity of purified exogenous enzymes may ameliorate the depression of production variables in broiler fed high CSR-based diets.

Cassava starch residue (CSR) which is abundant is grossly unexploited due to the presence of complex non-starch polysaccharides (NSPs). Major NSPs in root crops are β-glucan, arabinoxylan and certain amylopectin which possess chemical cross--linking (Phenolic coupling, ester bond and calcium-ion bridges) between them and therefore, are not well digested by poultry (Adams and Pough, 1993 and Caret *et al*, 1997). Badal, (2003) reported that the total biodegradation of lignocellulosic biomass requires endo-β-1, 4-xylanase, β-xylosidase, and several accessory enymes which are necessary for hydrolyzing various substituted xylans. The main problems is not the thin cellulose component however, but the soluble NSPs which encrust it. Various researchers had documented this concept Acamovic, (2004), Cowieson and Adeola, (2005). The total degradation of NSPs has been proposed as the underlying mechanism to improve birds' performance through an enhanced nutrients digestion and absorption.

This is part of the continuing objective to contrive effective, economic and adaptable means for expanding the utilization of CSR as feedstock without compromising productive efficiency. This study therefore examined the effect of roxazyme G2 G and cellulase enzymes in CSR-based diets on broilers.

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6.2 MATERIALS AND METHODS

6.2.1 Source of ingredients

Sun-dried CSR used was obtained in large quantity from cassava processing factory (Matna, Akure). The commercial enzymes: Cellulase and Roxazyme G_2 G were obtained from ILRI and a commercial source within Ibadan metropolis, respectively. Cellulase produced by *A. niger* contains endo-1-4 β-glucanase 26000units/g and α-amylase 26000 units/g. Roxazyme G_2 G contains endo-1-4 β-glucanase 8000 units/g, endo-1,3(4) β- glucanase. 18000 units/g, and 1-4 β-xylanase 26000 units/g.

6.2.2 Enzyme Assays

Endoglucanase, β-glucosidase and xylanase activities were measured using Carboxymethyl Cellulose and Xylan Salicin as substrates. Enzymes activities were read from a glucose and xylose standard curve respectively as outlined by Atev *et al* (1997). Using the Dinitrosalicylic (DNS) reagent.

6.2.3 Management of Experimental birds

Two hundred and ten day-old hybro broiler chicks were used for the study. Thirty chicks were assigned to each dietary treatment, which was replicated five times. The chicks were reared in deep litter with feed and water supplied *ad-libitum*. The management of the birds was as outlined by Olomu, (2003).

Seven dietary treatments were formulated including the control diet I (basal), while diets 2-4 had graded levels of CSR (10, 20 and 30% inclusion respectively supplemented with Roxazyme G2 G). The same graded of CSR were supplemented with Cellulase in diets 5-7. The diets were made isonitrogenous (23% and 22% Crude protein for the starter and finisher phases respectively) with the provision for at least the minimum calorie requirement of the chicken as recommended by Olomu (2003). At the end of the seven days of brooding, the birds were weighed and allocated to their respective diets. Records of feed consumption and body weight were taken on weekly basis whereas, body weight gain and feed to gain ratio (Feed Conversion Ratio) were estimated from the data collected.

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Table 26: Percentage Composition of Broiler Starter Diets containing different Enzymes of varying levels of Raw Cassava Starch Residue (RCSR) (g/100g)

Ingredients	Diet 1 0% (control)	A Diet 2 Roxazyme G2G	Diet 3 Roxazyme G2G	Diet 4 Roxazyme G2G	B Diet 5 Cellulase	Diet 6 Cellulase	Diet 7 Cellulase
Maize	50	40.00	30.00	20.00	40.00	30.00	20.00
RCSR	0.00	10.00	20.00	30.00	10.00	20.00	30.00
Groundnut cake	13.00	14.00	14.50	14.75	14.00	14.50	14.75
Wheat-offal	4.00	2.00	1.00	0.25	2.00	1.00	0.25
Palm kernel oil	0.00	1.00	1.50	2.00	1.00	1.00	2.00
Full Fat soya	26.00	26.00	26.00	26.00	26.00	26.00	26.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster Shell	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Broiler Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Table-Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Roxazyme G2G(g)	0.00	0.20	0.20	0.20	0.00	0.00	0.00
Cellulase (g)	0.00	0.00	0.00	0.00	0.20	0.20	0.20
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated nutrient							
Crude Protein	23.31	23.23	23.11	23.08	23.23	23.11	23.08
Crude Fibre	3.23	3.76	4.68	6.84	3.76	4.68	6.84
ME(kcal/kg)	3008	3094	3101	3000	3094	3101	3000

Premix provide per kg of diet Vit A, 10,000 i.u; vit D3, 2000 i,u; vit. E 23mg; vit K, 2.mg; calcium pantothenate, 7.5mg; B12, 0.015mg; folic acid, 0.75mg; choline chloride, 300mg; vit B1, 1.8mg; vit B2, 5mg; vit B6, 3mg; manganese, 40mg; iron, 20mg; zinc, 53.34mg; copper, 3mg; iodine, 1mg;cobalt, 0.2mg; selenium, 0.2mg; zinc, 30mg.

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Table 27: Percentage Composition of Broiler Finisher Diets Containing different Enzymes of varying levels of Raw Cassava Starch Residue (RCSR) (g/100g)

	A				B		
Ingredients	**Diet 1 0% (control)**	**Diet 2 Roxazyme G2G**	**Diet 3 Roxazyme G2G**	**Diet 4 RoxazymeG 2G**	**Diet 5 Cellulase**	**Diet 6 Cellulase**	**Diet 7 Cellulase**
Maize	50.00	40.00	30.00	20.00	40.00	30.00	20.00
Cassava starch residue	0.00	10.00	20.00	30.00	10.00	20.00	30.00
GNC	14.00	14.50	15.0	15.25	14.50	15.00	15.25
Wheat offal	4.00	2.00	1.00	0.25	2.00	1.00	0.25
PK oil	0.00	1.50	2.00	2.50	1.50	2.00	2.50
FF Soya	26.00	26.00	26.00	26.00	26.00	26.00	26.00
Fish	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster Shell	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Broiler Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methoionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Roxazyme G2G(g)	0.00	0.20	0.20	0.20	0.00	0.00	0.00
Cellulase (g)	0.00	0.00	0.00	0.00	0.20	0.20	0.20
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated nutrient							
Crude Protein	22.89	22.84	22.71	22.64	22.89	22.71	22.64
CrudeFibre	3.22	3.79	4.68	6.84	3.79	4.68	6.84
ME(kcal/kg)	3000	3101	3112	3009	3101	3112	3009

Premix provide per kg of diet Vit A, 10,000 i.u; vit D3, 2000 i,u; vit. E 23mg; vit K, 2.mg; calcium pantothenate, 7.5mg; B12, 0.015mg; folic acid, 0.75mg; choline chloride, 300mg; vit B1, 1.8mg; vit B2, 5mg; vit B6, 3mg; manganese, 40mg; iron, 20mg; zinc, 53.34mg; copper, 3mg; iodine, 1mg;cobalt, 0.2mg; selenium, 0.2mg; zinc, 30mg.

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6.2.4 Metabolic Trial

Digestibility study was carried out between the seventh and eight week of the experiment. Two birds from each replicate whose weights were close to the mean were selected for metabolic trial with facilities for feeding, water supply and collection of droppings. The birds were allowed to adjust for 4 days before the droppings were collected for three other consecutive days. Diet was served to the birds and the left over were properly accounted for.

The droppings were later separated from feathers and spillover feed particles and oven dried at $60^{O}C$ for 48 hours. The dried samples were milled and stored for subsequent chemical analysis.

6.2.4.1 Dry matter Digestibility: After oven dry, the dry matter contents of the samples were determined as a percentage of initial weight.

$$\% \text{ D. M.} = \frac{\text{Weight after oven drying gm}}{\text{Weight before oven drying gm}} \times \frac{100}{1}$$

$$\text{D. M. digestibility} = \frac{F_0 - A_0}{F_0} \times \frac{100}{1}$$

F0= DM Intake = %DM &Feed x Total amount of feed intake

A0= DM Output = % DM of faeces x Total amount of faeces voided

6.2.4.2 Nutrient Digestibility: Digestibility of nutrients were calculated using the following formular.

$$\text{Digestibility} = \frac{(F_0 \text{ X } F_i) - (A_0 \text{ X } i)}{F_0 \text{ X } F_i}$$

Fi = % Nutrient (ash, NFE, CF, EE and E^0) in the feed

$F_{0=\text{ Dry matter}}$ weight of feed consumed (Dry matter intake)

Ai=% Nutrient (ash, CF, EE, - NFE, E^0) in the faeces

A_0= Dry matter weight of faeces voided (Dry matter output)

6.2.5 Serum Collection and Determination

Blood was sampled from four birds per treatment at the starter and finisher phases and collected into sterile sample tubes without anticoagulant. The tubes were kept in a slanted wooden rack at 45^0C and the blood allowed to clot. The clotted blood was centrifuged and spun

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for 15 minutes at 3,500 revolution per minute. A clear fluid which is the serum was pipetted out into a clean and sterilized bottle and then deep frozen.

The serum protein and albumin were analyzed using sigma assay kits, while glucose and cholesterol were analyzed using randox kits. The buiret method was utilized in the determination of the total protein fraction while the serum was subjected to the direct colorimetric method for albumin with Bromocresol green (BCG) as the dye as described by Toro and Ackerman (1975). The globulin concentration was obtained by subtracting albumin from the total protein.

Albumin/globulin ratio was obtained by dividing the albumin value by the calculated globulin value as described by Toro and Ackerman (1975).

6.2.7 Carcass Quality Evaluation

At the end of eight weeks, two birds per replicate were slaughtered after they were starved overnight. The Live weight, and dressed weight were taken. The abdominal fat was also weighed and recorded. The weights of the internal organs (liver, heat gizzard kidney and spleen) were also taken.

6.2.7 Cost of Production

Feed cost per kg live weight gain was calculated by multiplying the cost of each kg of diet by the feed to gain ratio (FCR). Ruling market price of Roxazyme G2 G enzyme was used to calculate the cost per kg of each diet.

$$\text{Relative cost Benefit (\%)} = \frac{\text{Cost differential}}{\text{Cost/kg Weight Gain of Control diet}} \times \frac{100}{}$$

6.2.8 Chemical Analysis

Samples of diet and droppings were used for proximate analysis according to the procedure of Association of official Analytical Chemist (A.O.A.C., 1990).

6.2.9 Statistical Analysis

All data collected were subjected to analysis of variance of completely randomized design using the SAS (1999) package and the means were separated using Duncan (1955) multiple Range test of the same software.

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Table 28: Performance of Starter Broilers fed Enzyme Supplemented diets Containing Different Levels of Raw Cassava Starch Residue (RCSR)

Parameters	Control	Roxazyme G2G			Cellulase			±SEM
	Diet A 0%	Diet B 10%	Diet C 20%	Diet D 30%	Diet E 10%	Diet F 20%	Diet G 30%	
Feed intake g/bird/day	33.14±0.35^{d}	35.11±0.41^{C}	36.01±0.38^{b}	37.11±0.41^{a}	34.96±0.37^{C}	36.21±0.39^{b}	37.24±0.38^{a}	0.23
Weightgain g/bird/day	17.41±0.04^{C}	18.99±0.06^{ab}	18.94±0.07^{ab}	19.09±0.03^{a}	18.72±0.04^{ab}	19.03±0.05^{a}	18.98±0.03^{ab}	2.21
Feed: gain	1.90±0.02^{b}	1.85±0.02^{C}	1.90±0.01^{b}	1.94±0.03^{a}	1.86±0.02^{C}	1.91±0.02^{b}	1.96±0.03^{a}	0.13
Cost/kg Feed (#)	57.41	57.13	57.38	57.41	57.13	57.38	57.41	
Cost/feed consumed (#)	53.27	56.16	57.85	59.93	55.92	58.17	59.86	
Cost/kg weight gain	305.98	295.72	305.46	312.48	298.73	305.70	315.39	

abcdef: Means on the same row with different superscripts are significantly different (P<0.05).

±SEM: Standard error of the means

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Table 29: Performance of Finisher Broilers fed Enzyme Supplemented diets Containing Different Levels of Raw Cassava Starch Residue (RCSR)

Parameters	Control	Roxazyme G2G			Cellulase			±SEM
	Diet A 0%	Diet B 10%	Diet C 20%	Diet D 30%	Diet E 10%	Diet F 20%	Diet G 30%	
Feed intake g/bird/day	76.6±0.5^{C}	86.53±0.4^{b}	89.69±0.6^{a}	89.97±0.4^{a}	86.04±0.4^{b}	89.66±0.5^{a}	89.44±0.4^{a}	10.23
Weight gain g/bird/day	39.96±0.07^{C}	46.56±0.08^{b}	47.22±0.09^{a}	47.01±0.06^{a}	46.22±0.05^{b}	47.10±0.04^{a}	46.58±0.06^{b}	5.42
Feed: gain	1.92±0.02^{a}	1.85±0.01^{d}	1.89±0.02^{bc}	1.91±0.03^{b}	1.86±0.02^{d}	1.91±0.02^{b}	1.92±0.03^{a}	0.13
Cost/kg Feed (#)	57.11	57.53	57.58	57.68	57.53	57.58	57.68	
Cost/feed consumed (#)	244.9	278.77	289.20	290.60	277.19	289.10	288.89	
Cost/kg weight gain	612.88	598.73	612.45	618.18	599.72	612.77	620.20	

abdef: Means in the same row with different superscripts are significantly different (P<0.05)

±SEM: Standard error of the means

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Table 30: Apparent Nutrient Digestibility of Broilers fed Enzymes supplemented diets containing different levels of Raw Cassava Starch Residue.

Parameters		Roxazyme G G			Cellulase			±SEM
	Diet A 0% (control)	Diet B 10%	Diet C 20%	Diet D 30%	Diet E 10%	Diet F 20%	Diet G 30%	
Dry matter	68.43±0.11[e]	78.01±0.09[a]	76.66±0.08[bc]	76.41±0.08[d]	76.92±0.10[bc]	77.01±0.11[b]	76.98±0.12[d]	2.10
Crude protein	72.03±0.13[e]	78.61±0.14[a]	78.11±0.17[a]	74.78±0.16[c]	74.11±0.13[d]	76.98±0.13[b]	75.14±0.12[c]	2.11
Crude fibre	53.66±0.11[ab]	64.44±0.09[a]	63.45±0.11[b]	62.49±0.09[c]	62.90±0.09[c]	64.09±0.08[a]	61.11±0.12[d]	1.43
Ether extract	70.04±0.21[e]	74.61±0.23[a]	73.98±0.21[b]	71.02±0.21[d]	72.21±0.22[c]	72.81±0.23[c]	70.39±0.21[e]	1.41
Ash	76.32±0.22[cd]	81.88±0.23[b]	82.41±0.21[a]	80.13±0.32[c]	82.31±0.21[a]	81.49±0.23[b]	81.79±0.21[b]	1.76

abcde: Means on the same row with the different superscripts are significantly different . ($P<0.06$)

±SEM: Standard error of the means

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Table 31: Serum Biochemical indices of Broilers fed Enzymes supplemented diets containing different levels of Raw Cassava Starch Residue (Starter phase)

Parameters		**Roxazyme G2G**			**Cellulase**			**±SEM**
mg/100ml	Diet A 0%	Diet B 10%	Diet C 20%	Diet D 30%	Diet E 10%	Diet F 20%	Diet G 30%	
Total protein	4.43 ± 0.02^{C}	5.04 ± 0.04^{ab}	5.61 ± 0.04^{a}	4.68 ± 0.03^{b}	4.87 ± 0.03^{b}	5.01 ± 0.03^{ab}	4.77 ± 0.03^{b}	0.14
Albumin	1.69 ± 0.01^{d}	2.41 ± 0.01^{b}	2.62 ± 0.01^{a}	2.43 ± 0.01^{b}	2.61 ± 0.02^{a}	2.49 ± 0.02^{b}	2.19 ± 0.02^{c}	0.12
Globulin	2.74 ± 0.01^{a}	2.60 ± 0.02^{a}	2.50 ± 0.01^{b}	2.25 ± 0.02^{c}	2.26 ± 0.02^{c}	2.52 ± 0.01^{b}	1.58 ± 0.02^{d}	0.21
Albumin: globulin	0.62 ± 0.02^{e}	0.92 ± 0.01^{d}	1.04 ± 0.02^{c}	1.08 ± 0.01^{c}	1.15 ± 0.01^{b}	0.98 ± 0.02^{d}	1.38 ± 0.01^{a}	0.41
Glucose	140.4 ± 13.1^{e}	148.1 ± 12.2^{a}	150.0 ± 13.1^{a}	141.4 ± 12.2^{b}	139.9 ± 12.2^{b}	148.1 ± 12.1^{a}	140.8 ± 11.0^{e}	1.77
Cholesterol	148.1 ± 13.3^{a}	130.4 ± 12.1^{b}	131.4 ± 14.7^{b}	116.5 ± 12.3^{d}	130.1 ± 11.1^{b}	128.8 ± 12.1^{bc}	117.9 ± 12.1^{d}	2.41
Urea	24.38 ± 0.08^{e}	28.8 ± 0.09^{b}	29.41 ± 0.08^{a}	27.71 ± 0.08^{c}	28.4 ± 0.09^{b}	26.47 ± 0.09^{d}	27.8 ± 0.06^{c}	0.11

abcde: Means on the same row with different superscripts are significantly different $(P<0.05)$

±SEM: Standard error of the means

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Table 32: Serum Biochemical indices of Broilers fed Enzymes supplemented diets containing different levels of Raw Cassava Starch Residue (finisher phase)

Parameters		Roxazyme G2 G			Cellulase			±SEM
mg/100ml	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G	
	0%	10%	20%	30%	10%	20%	30%	
Total protein	4.86±0.03[c]	5.33±0.02[a]	5.61±0.03[a]	5.00±0.02[ab]	5.01±0.02[ab]	5.11±0.03[a]	4.98±0.02[c]	0.21
Albumin	1.93±0.01	2.53±0.02	2.78±0.02	2.55±0.01	2.60±0.02	2.48±0.03	2.11±0.04	0.10
Globulin	3.07±0.01	3.01±0.02	2.99±0.01	2.81±0.01	2.79±0.01	3.00±0.01	2.84±0.00	0.20
Albumin: globulin	0.62±0.02[c]	0.84±0.02[b]	0.92±0.02[a]	0.91±0.02[a]	0.93±0.02[a]	0.83±0.01[b]	0.74±0.01[c]	0.07
Glucose	141.4±12.1	1.43.1±12.3	139.3±13.1	136.3±12.1	139.4±10.2	135.0±12.0	137.7±11.1	2.16
Cholesterol	142.2±12.9	138.1±12.6	127.2±12.7	128.1±12.2	138.5±11.1	121.2±11.8	130.0±11.8	1.14
Urea	23.94±0.08	23.3±0.07	25.94±0.08	25.5±0.08	26.1±0.06	25.3±0.08	24.3±0.09	0.21

abcde: Means on the same row with different superscripts are significantly different ($P<0.05$)

±SEM: Standard error of the means

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Table 33: Organs weight of Broiler birds fed Enzymes supplemented diets containing different levels of Raw Cassava Starch Residue

Parameters		**Roxazyme G2 G**			**Cellulase**			**±SEM**
mg/100ml	Diet A 0%	Diet B 10%	Diet C 20%	Diet D 30%	Diet E 10%	Diet F 20%	Diet G 30%	
Crop	1.38±0.01	1.31±0.00	1.40±0.01	1.39±0.01	1.33±0.01	1.36±0.02	1.30±0.01	0.06
Gizzard	3.08±0.02[b]	3.09±0.03[b]	3.11±0.03[b]	3.18±0.02[a]	3.11±0.03[b]	3.0±0.02[c]	3.10±0.03[b]	0.17
Kidney	0.66±0.01	0.67±0.01	0.63±0.01	0.68±0.01	0.63±0.01	0.64±0.01	0.59±0.01	0.02
Liver	2.24±0.02	2.11±0.03	2.27±0.02	1.41±0.03	2.14±0.03	2.31±0.03	2.09±0.03	0.04
Heart	0.33±0.00	0.31±0.01	0.28±0.01	0.30±0.01	0.31±0.01	0.31±0.01	0.26±0.01	0.03
Spleen	0.13±0.01	0.11±0.01	0.12±0.01	0.14±0.01	0.11±0.01	0.09±0.01	0.10±0.01	0.02
Lungs	0.69±0.02	0.64±0.02	0.38±0.02	0.53±0.03	0.63±0.02	0.45±0.03	0.61±0.03	0.03
Bile	0.13±0.01	0.11±0.01	0.12±0.00	0.09±0.01	0.11±0.01	0.09±0.01	0.12±0.01	0.03
Abdominal fat	1.69±0.01[c]	1.78±0.04[a]	1.68±0.05[b]	1.71±0.04[b]	1.82±0.05[a]	1.60±0.05[d]	1.60±0.05[d]	0.12

abcde: Means on the same row with different superscripts are significantly different ($P<0.05$)

±SEM: Standard error of the means

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6.3 RESULTS

6.3.1 Performance Characteristics (Starter)

The performance data of broilers fed roxazyme G2 G or cellulase enzyme supplemented diets containing different levels of raw cassava starch residue are given in Table 28. Feed intake and body weight gain were similar in the dietary enzymes. However, broilers fed diets contained (Roxazyme G_2 G and Cellulase) supplemented CSR-based diets D (37.11±0.41g/day/bird (1.94±0.03) and E (37.24±0.38g/day/bird) (1.96±0.03) had a significantly (P<0.05) increase on feed intake as well feed: gain ratio in contrast to the control diet 1(33.14±0.35g/day/bird) (1.90±0.00) respectively.

6.3.2 Performance Characteristics (Finisher)

Results of the performance characteristics of broilers fed roxazyme G2 G and cellulase enzymes supplemented diets containing different levels of unbiodegraded cassava starch residue (Finisher phase) is shown in Table 29.

Birds fed diet 3 (20% inclusion CSR with Roxazyme G_2G had the highest weight gain and better feed to gain ratio respectively (47.22±0.09g/day/bird) (1.89±0.02) followed by birds fed diet 6 (20% inclusion CSR with cellulase) (47.10±0.04g/day/bird) (1.90±0.02) while those fed diet 1 (control) gave least (39.96±0.07g/day/bird) (1.92±0.02). The feed intake significantly (P<0.05) increased as levels of CSR inclusion increased in the diets, but feed conversion ratio were similar across the dietary treatments. 1(1.92±0.02), 2(1.85±0.01), 3(1.89±0.02), 4(1.91±0.03), 5(1.86±0.02), 6(1.91±0.02), and 7 (1.92±0.03). Cost/kg feed decreased slightly with increase in the inclusion level of CSR in the diets. However, cost/kg weight gain increased (P<0.05) at 30% inclusion level .

6.3.3 Nutrient Digestibility

The digestibility results of broilers fed experimental diets is shown in Table 30. There were significant differences (P<0.05) in nutrient utilization among the dietary treatments. Enzymes supplemented diets 2, 3, 4, 5, 6, and 7 differ significantly (P<0.05) from the control (diet A) in dry matter, crude protein, Ash and Ether extract digestibility. Diet 2 has the highest dry matter (78.01±0.09) and crude protein (78.61±0.14) digestibility. Crude fibre digestibility ranged from 53.66±0.11% - 64.44±0.09%.

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6.3.4 Serum Metabolites

Serum biochemical indices of broilers fed enzymes (Roxazyme G_2 G and Cellulase) supplemented starter and finisher diets containing different levels of Raw cassava starch residue (RCSR) (Table 31 and 32).

An improvement in the total protein of finisher birds fed enzymes supplemented diets over the starter phase was observed in the entire dietary treatments. The total protein contents obtained ranged from 4.43±0.02mg/100ml and 5.61±0.04mg/100ml.

The values obtained for Albumin: globulin ratio was similar across the enzymic diets. At finisher phase broilers fed enzymes supplemented diets 2,3,4,5,6 and 7 had results of the glucose index improved significantly (P>0.05) and its cholesterol concentration decreased compared to diet 1 (control) (140.4±13.1mg/10ml) and (148.1±13.3mg/100ml) respectively.

6.3.5 Organs characteristics

The relative weights of organs of broilers fed enzymes supplemented diets containing different levels of undegraded CSR are shown in Table 33. In the present study no significant difference was observed in the relative weights of crop, gizzard, kidney, liver, heart and abdominal fat of birds in all the dietary treatments.

6.4 Discussion

The use of non-starch polysaccharide(NSP)-degrading enzymes as feed additives has consolidated around their application to agro-industrial by-products based diets such as cassava starch residue (CSR) for broiler birds. Only with the application has a good understanding of their mechanisms been developed and reasons for their effectiveness established (Chesson, 2001).

Increased at the starter phase (g/day/bird) both feed intake and feed to gain ratio observed in diets D and G compared to other diets could be that increase in the crude fibre content of the diets (6.84%) which were much above that recommended value of 2.5 - 4% for broilers, (Olomu, 2003). This result implied that a prolonged inclusion of fibre in the diet is associated with significant reduction in the enzymes cleavage of the large molecules of NSP into smaller polymers, thereby augments the thickness of the gut and decreasing the nutritive value of feed. Longe (2006) and Adetunji (2010) opined that the inclusion level of agro-

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industrial by-products in monogastric diets is restricted by factors such as the structure of the substrate, position, and the age of animals. Another possible reason for the result observed here might be an intestinal viscosity which appeared highest in youngest birds and diminished with age. Improvements in feed intake and daily weight gain are the usual effects of enzyme addition in younger broiler chicks (to 28 days) fed on high fibre diets. Thereafter, benefits are more likely to be seen as improved feed conversion ratio which implies that viscosity reduction is playing a lesser role and that other benefits become more important as the bird ages (Garcial *etal.*, 2007). In the virtual absence of any "restorative" function for NSP-enzyme addition, benefits come only from the release of entrapped nutrients. Choct *et al.,* (2006) observed that the presence in the diet of high polysaccharides able to increase solution viscosity is thought to increase the size and stability of the unstirred layer at the mucosal surface of the digestive tract. This reduces the contact between the feed and the enzymes and slows the uptake in the foregut of released sugars, amino acids and lipids resulting in the impaired digestibility of the major nutrients. There is also cumulative evidence that increasing the viscosity of the digesta promotes bacterial proliferation to the detriment of both overall digestive efficiency and bird's health(McDonald *et a*l., 2002; Ajala *et al.,* 2003).

Meanwhile, similarities($P<0.05$) across the enzyme supplemented diets observed in feed intake at the finisher phase of broilers was an indication that better performance of birds noted when measured over the starter phase. This confirms an increase digestibility of nutrients with age and or body weight of birds. Field reports confirm that the ability of birds and other simple-stomach to digest and utilize diets increases with age, particularly in the case of high fibrous diets (Whittemore *et al*., 2003; Esonu *et al.,* 2004 and Taverrnnari *et al.,* 2008). This indicates that the digestive system of the birds are fully matured with full functional digestion and absorption capacities and high secretion of pancreative enzymes.

However, the discrepancies noted between the daily weight gain and feed to gain ratio of birds fed roxazyme G2 G and cellulase enzyme supplemented diets and control (diet A) might be attributed to the release of varying soluble NSPs leached from CSR cell walls within the digestive tract. Officer (2007) and Ani *et al.,* (2010) reported that exogenous enzymes work in combination with endogenous enzymes to break up large molecules to sizes that can be utilized by birds. These symptoms could be reproduced by the addition of isolated NSPs to problem-free experimental diets and reversed by the addition of appropriate NSP degrading-enzymes.

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The underlying cause of depression in the ME content of the CSR appears to be the release of β-glucans, arabinoxylans and certain amylopectin. The improve weight gain and better feed to gain ratio obtained in birds fed diet C (20% inclusion CSR with Roxazyme G_2G) compared to diet F (20% inclusion CSR with cellulase) as well as control (diet A) might be that the lignocellulose (lignin, hemicellulose, and cellulose) of the diets were varied, leading to the different rate of hydrolysis. Roxazyme G_2G is thought to be efficient in delignification and increased reduction in CSR viscosity, indicating a better enzyme activities when compared with cellulase. The two enzyme samples showed some differences in their range and amounts of compositional products, thereby further different mechanisms of enzymes action be expected are; transitional state stabilization that exposes molecule of NSP into enormous rate of degradation; stimulation of entropy loss of enzyme-substrate complex and by binding of substrate to enzyme through covalent catalysis. Walton *et al.,* (2003), Zakaria et al., (2008) and Yang and Choct (2009) reported significant need in synergistic interaction among various enzymes in having a total depolymerization of NSPs. Endoglucanase, cellobiohydrolase/Exoglucanase and β-glycosidase work synergistically in the hydrolysis of cellulose. But total hydrolysis of hemicelluloses requires endo- β-1,4-xylanase, β-xylosidose and several accessory enzymes for cleaving various substituted xylan backbone. Other posssibility include synergistic action between depolymerizing and side-group cleaving enzymes has been verified using acetylated xylan as a substrate. These beneficial effects of 1-4 β-xylanase (26,000 units/g) found in Roxazyme G_2 G type might have a positive impact on the whole enzymatic saccharification of CSR. Alternatively, this could also indicate that the xylan degrading-enzyme composite reported in Roxazyme G2 G may thus possess both a cellulose-binding domain (CBD) and a xylan-binding domain (XBD). The presence of CBD in xylanase is not uncommon (Irwin *et al.,* 1994; Tsujibo *et al.,* 1997; Sun *et al.,* 2008). Cellulose proposed could act as a general receptor for plant cell-wall hydrolases, since it is the only structural polysaccharide that does not vary in composition between plant species (Kellett *et al.,* 1999; Bourne and Henrissat, 2001). This is also a potentially interesting property with regard to the use of this category of xylanase-based enzyme in the breaking down and the release xylooligosaccharides to their limit products. This strong binding capacity of the xylanase-based enzyme to lignocellulosic materials and its low molecular weight (granules form) increased its survivability in the GIT as this may also assure easy access to the pulp

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matrix. Consequently, the process of lignin release by xylan removal and the further increased swelling of the fiber walls could also explain the trophic effect noted on the performance of broilers fed diet 3 (20% inclusion CSR with Roxazyme G2G). Also, Roxazyme G2G complexity appeared to influence more sequentially hydrolysis of CSR lignocelluloses to monomeric sugars than cellulase preparation counterpart. Similarly, the transferase activity described for xylan-degrading enzymes may be of great importance in hydrolyzing covalent cross links and junction zones formed at various unsubstituted regions of the xylan backbones(Fincher and Stone, 2006). Thus, the enhanced enzymes activities and increased-cell permeability favoured those birds fed Roxazyme G_2 G based diets 3 than cellulase counterparts. In this study, it is evident that cost/kg weight gain being increased at 30% inclusion upward obviously was the direct consequence of poor feed utilization as observed with high feed to gain ratio as well as high cost per feed consumed by birds.

The numerical improvement in nutrient digestibility obtained with the 2-type enzymes supplementation could be due to change in microbial flora and better gut health. Banday and Risam (2008) and Hajati (2010) have suggested that probiotic supplementation improved performance of broilers. The different mechanism of probiotic action suggested are; nutritional effect by regulation of metabolic reactions that produces toxic substances, stimulation of endogenous enzymes and by production of vitamins or antimicrobial substance. Various authors (Partanen *et al.,* 2007; Onu *et al.,* 2010) reported that enzyme can break down some NSPs in feedstuffs and help promote growth of "useful" bacteria. Without the enzyme, indigestible fiber promotes the growth of "harmful" bacteria. With the addition of the enzyme the indigestible starch works like a pre-biotic (starch can be fermented by microbes in the gut of the bird, as pre-biotic many selectively enhanced beneficial bacteria population in the gut like *bifido* bacteria and *lactobacilli*). The observation could be in accordance with that mention; an oligosaccharide, a pre-biotic used to control pathogenic scours of all kinds of livestock caused by Salmonella, and E. coli etc. It is very likely that the level of challenge will influence the response to the enzyme addition. More specifically, Francis *et al.,* (2009) and Shareef and Dabbagh (2009) in their seperates studies reported a significant reduction in colony forming units (CFU) of Salmonella and Clostridia species measured in the caeca of broilers fed diets containing Avizyme 1500 and *Saccharomyces cerevisiae,* respectively.

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In the contrary, a very subjective increase in levels of glucose of birds fed supplemented diets with the 2-type enzymes may ameliorate insulin secretion and glucose absorption by hydrolyzing lignocelluloses and improve energy metabolism as reported by Zou *et al* (2006), and Spellberg and Edward 2010). However, the parameters fell within the normal values recommended by Mitruka and Rawnsley, (1977). Nevertheless, the reduction in levels of cholesterol suggested that the enzymes supplementation could have enhanced the *lactobacilli* count. Gilliland *et al*., (1995) and Shareef and Dabbagh (2006) hypothesized cholesterol into their cellular membrane, thus, reduced cholesterol absorption in the system of the host animals. Onilude (1999); Aderemi (2004) reported conditions that slight reduction in lipogenesis brought about by concomitant reduction in regulatory activity of acetyl CoA carboxylase- an enzyme which mediates in the rate-limiting step of carboxylation of acetyl-CoA to malonyl CoA in fatty acid synthesis might also play an important role.

The addition of Roxazyme G_2G and cellulase enzyme was responsible for a significant ($P<0.05$) increase of total serum protein (Caret *et al*., 1997). Increase in serum total protein and Albumin synthesis noted depend on availability of proteins and as protein intake increases, the rate of synthesis increases whereas catabolic rate does not change easily.

The similarities ($P>0.05$) observed in relative weight of organs of birds fed control (diet 1) and test diets showed that exogenous enzymes work effectively with endogenous enzymes in breaking up large molecules of NSPs to sizes that are absorbable by birds within a reasonable time. The lesser the grinding time of most polysaccharides by muscular organs of birds in the digestive tract as purported by Adeola and Olukosi, (2008) the lower the relative weight of most organs.

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CHAPTER SEVEN

7.1 SUMMARY AND CONCLUSION

Four experiments were conducted to determine the nutritional evaluation of cassava starch residue (CSR) and its upgraded products for broiler production;

(i) In the first study the tolerance limit of broiler starters and finishers for varying levels of cassava starch residue was determined.

(ii) The second experiment involved the studying of the nutritional qualities of cassava starch residue before and after biodegradation with fungi species.

(iii) In the third trial, the substitution values of *Aspergillus niger* degraded (CSR) as promoter of growth in broiler diets were studied.

(iv) In experiment four, the supplemental efficacy between roxazyme G2 G and cellulase enzyme added at 0.020% or 0.0002/kg in broiler diets was examined.

The objectives were accomplished in a Completely Randomized Design (CRD), and 3×4 factorial arrangement for studies i, ii, iii, and iv respectively.

In experiment one, CSR was included into the diets of broilers at 0, 5, 10, 15, 20 and 25% w/w. The result indicated that:

(1) Broilers fed CSR consumed more feed than their control counterparts on diet A.

(2) Feed to gain ratio became poorer with increasing levels of CSR in the diets.

(3) Digestibility of dietary nutrients were reduced as fibre levels increased in the diet.

(4) The performance of broiler finishers on higher levels of CSR were similar to those of the broiler starters, but more clearly improved at the finisher phase.

(5) Relative weights of both intestinal organs and length of intestine were increased in broilers with increasing fibre levels.

(6) Serum thiocyanates were significantly increased as levels of CSR increases in broiler diets.

(7) Cost/kg weight gain on birds fed 5% CSR inclusion in diet was better than the control diet A.

(8) Cholesterol concentration were found to decrease with the broilers fed higher levels of CSR.

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In experiment two, the CSR was inoculated with monoculture of *Aspergillus niger*, *Trichoderma viride* and mixture of *A. niger X T. viride* at different fermentation periods of 7, 14 and 21 days, the proximate and fibre components were determined, the interactions between the treatments periods, and the fungi were also determined. The result revealed that:

(i) Biomass loss increased across the period of inoculation and the great loss was felt on *A. niger* inoculated substrate.

(ii) As the days of fermentation increased from 7 to 21 days, the nutrients composition of the CSR increased except crude fibre.

(iii) *A. niger* inoculum significantly increased the most of nutrient compositions of the CSR more than *T. viride* and combination of *A. niger X T. viride* inocula for the periods of fermentation.

(iv) Biodegradation resulted in breakdown of the detergent fibre components neutral detergent fibre (NDF), Acid detergent fibre (ADF) and hemicelluloses

(v) Metabolisable energy (Kcal/kgDm) value was observed to increase significantly ($P<0.05$) with increase in the period of fermentation.

(vi) The crude fibre was observed to reduce (39.60%) significantly ($P<0.05$) after 14 days of fermentation with *A. niger*, while *T. viride* a reduction of (24.83%) in CSR.

(vii) Interactions between the different organisms *(A. niger, T. Viride* and mixture of *A. niger x T. viride)* treatment, periods of inoculation (7,14 and 21) were also determined and *A. niger* at 14 days of fermentation produce the best result both in chemical and nutrient compositions of the CSR.

In experiment three, *Aspergillus niger* degraded CSR was incorporated into the diets of broilers at 0,5, 10, 15, 20, and 25% inclusions. The result revealed:

(1) That broiler birds can utilize *A. niger* degraded CSR better during the last 4 to 8 weeks (finisher phase) of age than at the starter phase (1 to 4 weeks).

(2) Utilization of *A. niger* degraded CSR supplemented diet was better in 15% inclusion level than the control diet.

(3) That there was no significant ($P>0.05$) difference in the total serum protein of birds fed different levels of supplemented diets.

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(4) That the highest serum glucose concentration value observed was in the birds fed 15% inclusion level of the degraded CSR while cholesterol decreased with increase in the inclusion level of degraded CSR.

(5) That the kidney weight of the birds irrespective of the percentage level of inclusion of the degraded CSR showed no significant ($P<0.05$) difference.

(6) Relative weights of both crop and gizzard organs were increase correspondingly ($P<0.05$).

(7) That the histopathological evaluation of the organs of the birds on the different levels showed no visible lesion as a result of *A. niger* degraded CSR supplementation in the diets of broilers.

In experiment four, Roxazyme G2G and cellulase enzymes were added at a rate of 0.020%, to diets containing 10, 20 and 30% CSR respectively for both starter and finisher phases. The result showed.

(1) Feed to gain ratio of the broiler birds were sustained when raised on diets containing 30% CSR supplemented with Roxazyme G2G or cellulase enzyme.

(2) Roxazyme G2 G or cellulase significantly ($P>0.05$) increased digestibility of DM, CP, CF, EE and Ash in CSR based diets when compared with control diet A.

(3) Percentage hydrolysis was significantly ($P>0.05$) better in Roxazyme G2G (53%) compared to cellulase that recorded (52.35%).

(4) It was economical to feed 20% CSR inclusion diets with Roxazyme G2G or cellulase compared to control diet.

(5) Growth depression of chicks fed high CSR was effectively and economically alleviated by roxazyme G2G enzyme compared to cellulase enzyme.

(6) Addition of roxazyme G2G or cellulase enzymes increased significantly ($P>0.05$) the total serum protein and glucose concentration of all dietary treatments than control diet.

(7) Relative weights of organs were similar across all the dietary treatments.

Therefore, it can be concluded from these results that CSR can be economically used in this order between 5 and 20% inclusion levels, such as 5% raw cassava starch residue, 15% *Aspergillus niger* biodegraded cassava starch residue and 20% raw cassava starch residue supplemented with Roxazyme G2G or cellulase enzyme.

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Moreover, roxazyme G2G or cellulase enzyme can be included at 0.020% rate in the high fibre diets (HFD) to ameliorate growth depression in broiler birds fed such diets. Percentage hydrolysis of roxazyme G2G supplemented diets were better than those birds fed CSR diets supplemented with cellulase enzyme.

7.2 Recommendations

Further investigations on some physiological factors such as; p^H, Increase in temperature, Oxygen supply and carbon dioxide build-up involved on the rate of biodegradation should be addressed in future studies.

The release of some 'locked-up' nutrients including breakdown of ferulic acid, coumaric acid and cinnamic acid bonds through hydrogenation, addition reaction of concentrated acids (H_2SO_4 or H_3PO_4), oxidizing agents ($KmnO_4$ or H_2CrO_4) should be further investigated.

Furthermore, the production of thermostable cellulase enzyme complex by fungi spp via Isolation, extraction, purification, and (SDS-PAGE: sodium deodecyle sulphate-polyacrylamide gel electrophoresis and IEF: isoelecrofocusing) techniques should be further studied.

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APPENDIX I

SERUM TOTAL PROTEIN: by biuret reaction method

Serum were thawed/spunned

To a series of Appropriately labelled tubes, were added 5ml of the biuret reagent.

To the separate tubes were added exactly, 0.1ml of standard samples and water (for reagent Blank)

It was mixed and allow to stand at 30^0C for 10mins.

The standard samples were read against blank at 550nm.

Serum blanks were also set up using the same procedure except that the tartarate-iodide blank solution was used in place of biuret solution. This was read against water blank (that is, using water instead of serum) at 500nm.

The absorbance obtained for the blank was substracted from that obtained with the respective sera to obtain the corrected readings. (Toro and Ackerman, 1975)

Calculation

Conc of sample (g/dl)

$$= \frac{\text{Conc. Absorbance of sample}}{\text{Conc. Absorbance of standard}} \times \text{Conc. of standard.}$$

SERUM ALBUMIN: by Bromocresol Green Binding Method.

To 5ml of the working dye reagent (Bromocresol green) was added 24ul of the serum and mixed.

A standard was treated similarly.

The samples and standard were allowed to stand for 10mins and later read against blank at 628nm (Toro and Ackerman, 1975)

Calculation: Conc. of sample (g/dl)

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. of standard}$$

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Note: The binding of the indicator by albumin in the buffered solution changed the colour of the solution.

SERUM GLOBULIN BY DIFFERENCE METHOD

This was calculated as difference between the values of total protein content and albumin content of the sample.

Globulin content (g/dl) = Total protein content – Albumin content

SERUM CREATININE: by Folin-Wu Filtrate method

To 3ml of water in a test tube was adder 2ml of serum and 1ml of the folin-wu sodium tungstate solution.

Mixed well and 2ml of the sulphuric acid solution added.

It was stopped and mixed again.

It was then allowed to stand for a few minutes 10-15mins after which the mixture was centrifuged.

2 ml of the work standard was treated similarly, diluted with water and precipitating agents.

To one test tube was added 3ml of the sample filtrate.

To the other tubes were added 1,2,3ml of the diluted working standard and made up of 3ml distilled water.

3ml of water was poured into one tube as blank.

To each tube was added 1ml of picric acid solution mixed and added 1ml of sodium hydroxide solution.

It was then mixed and allowed to stand at room T^0 for 20mins.

The standards and samples were read against blank at 520nm

Calculation: Conc. of sample (mg/dl)

$$= \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard}$$

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SERUM UREA NITROGEN: by diacetyl monoxime method

Equal volumes of the colour reagent (1g of diacetyl monoxime, 0.2g of thiosemicarbazide and 9g of sodium chloride, dissolved in water and made up to 1litre).

Add acid solution (mixture of 60ml of conc. sulphuric acid and 10ml of 85% phosphoric acid added to about 800ml of water with 0.1g ferric chloride dissolved in the solution, cooled and diluted to 1litre) were mixed.

5ml of the mixed reagent was pipetted into separate tubes.

To the tubes were added 0.05ml of samples and standards and one tube was reserved as blank.

All the tubes were heated in boiling water bath for 15mins, cooled, and the standards and samples were real against the blank at 520nm.

Calculation: Conc. of sample (mg/dl)

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard}$$

Serum cholesterol: by direct method (acetic anhydride + H_2SO_4)

To separate test tubes labelled blank, standard and sample were added 6ml of reagent.

To the standard tube was added 0.1ml serum

To the blank tube was added 0.1ml water.

Each tube was carefully mixed and then incubated for 15-18mins at 37^0c

The standard and sample were read against the blank at 625nm.

Calculation: Conc. of sample (mg/dl)

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard (200mg/dl)}$$

SERUM GLUCOSE: by O-toluluidine method using acetic acid

To a series of labelled test tubes were added exactly 3ml of toluidine reagent. The toluidine reagent consisted of 1.5g thiourea dissolved in glacial acetic acid with 60ml of pure O-toluidine added and mixed (stored at room T^0).

To the separate tubes were added 0.05ml of standards, samples and blank (0.05ml of H_2O)

The content of each tube was then mixed and heated at 100^0c for 12mins

The tube were cooled in running water.

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The standard and samples were read against the blank at 630nm.

Calculation: Conc. of the sample (mg/dl)

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard}$$

DETERMINATION OF TOTAL PLASMA TRIGLYCERIDE

This determine was based on the method of Gottfried and Rosenberg (1973), a modification of the of levy (1972).

Plasma triglyceride is extracted in normal heptanes.

The triglyceride is hydrolysed to glycerol in then oxidized with periodate.

The resulting formaldehyde is condensed with acetylacetone to form a yellow dehydrotoluidine derivate.

The intensity of yellow colour is measured at 425nm in an S.P. 800A U.V. Spectrophotmeter.

Procedure

2ml normal heptanes was added to 0.5ml of plasma in a test tube,

3.5ml of isopropanol was then added, followed by 0.1ml of conc. H_2SO_4

The test tube was mixed for about 30secs' and the phases were allowed to separate without centrifuging.

A standard triolein (100mg/dl) solution and a blank containing distill H_2O were simultaneously treated as above. 0.4ml of the heptanes was saponfied by adding 2ml of isopropanol and one drop of potassium hydroxide (6.25g/liter).

Mixture was well mixed, stopped and incubated at 70^0c was H_2O with for 10mins.

0.2ml of the sodium metaperiodate reagent and 1ml of the acetyl acetone reagent were added.

Mixture was well mixed, stopped and incubated for another 10minutes at 70^0c.

Allowed to cool for about 3mins of room T^0

Optical density (O.D) was react at 425mm against a blank.

Calculation: Conc. of triglyceride

$$\frac{\text{O.D of test}}{\text{O.D of standard}} = \frac{\text{Conc. of sample}}{\text{Conc. of standard}} \times \text{standard}$$

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APPENDIX II

ISOLATION OF FUNGI SPP

1. **Collection of Samples**: Collect about 10 soil samples from different Agricultural fields/forest on campus or from decayed/decaying wood and transport to the laboratory. Collection is into clean, STERILE containers, well labelled.

2. **Isolation:** *Prepare 250ml each of Potato Dextrose Agar (PDA) and Malt extract Agar (MEA);

* Sterilize in the autoclave at 121^{0}c, 15psi (Pounds per square inch),
  Leave in the MOLTEN state.

* Weigh out aseptically 1g of each soil sample.

* Incubate the 1g aseptically into 10-15ml of your molten agar in a petri dish;
  ALLOW TO SOLIDIFY

* Incubate at 28^{0}c (or 32^{0}c ± 4^{0}c) for 5 days.

* After incubation, check for:

  - Clamydospore production
  - Size of conidia and other features according to Watts *et al* (1988) and Rifai (1969).

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