EFFECT OF EXOGENOUS ENZYMES ON THE UTILISATION OF TWO VARIETIES OF SORGHUM (*Sorghum bicolor*) BY BROILER CHICKENS

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JUNE, 2015

DECLARATION

I declare that the work in this Thesis entitled **"**EFFECT OF EXOGENOUS ENZYMES ON THE UTILISATION OF TWO VARIETIES OF SORGHUM (*Sorghum bicolor*) BY BROILER CHICKENS**"** was carried out by me in the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria under the supervision of Dr. P.A. Onimisi and Prof. G.S. Bawa. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this Thesis was previously presented for another Degree or Diploma at this or any Institution.

OLADIPO Foluke Mary	_____	_____
Name of student	Signature	Date

CERTIFICATION

This thesis "EFFECT OF EXOGENOUS ENZYMES ON THE UTILISATION OF TWO VARIETIES OF SORGHUM (*Sorghum bicolor*) BY BROILER CHICKENS" by Oladipo, Foluke Mary meets the regulation governing the award of Masters of Science (ANIMAL SCIENCE) Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This Thesis is dedicated to the “LORD of hosts” my refuge and my strength whom through it all made me to be still and know that He is God.

This work is also dedicated to my anchor and dear husband, Dr. Olusola Oladipo whose support, encouragement and love kept me going through this time and to our beloved daughter, Oladipo Oluwatomiwa who has always remained strong and cope with my absence at a very tender age.

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ABSTRACT

Two feeding trials were carried out to determine the effect of exogenous enzyme inclusion in sorghum (*Sorghum bicolor*) based diets for broiler chickens. The first feeding trial was conducted to determine the effect of feeding broiler chickens with two varieties of sorghum namely, Jandawa (Red sorghum) and Kaura (Samsorg 17) with or without phytase inclusion using five (5) Treatment 1 was maize based diet; Treatment 2, Jandawa based diet; Treatment 3, Kaura based diet; Treatment 4, Jandawa based diet with phytase; and Treatment 5, Kaura based diet with phytase. The second trial was conducted with five (5) diets to evaluate the response of broiler chickens fed Kaura based diets supplemented with Ronozyme® phytase, Ronozyme® protease and Roxazyme® G2 G and a combination of protease and G2 G. Treatment 1 was maize based diet; Treatment 2, Kaura based diet with phytase; Treatment 3, Kaura based diet with protease; Treatment 4, Kaura based diet with G2 G; and Treatment 5, Kaura based diet with a combination of Ronozyme® protease and Roxazyme® G2 G. Three hundred and seventy-five (375) broiler chicks were used in each trial and each allotted in a completely randomized design (CRD) to five (5) dietary treatments replicated thrice and having (twenty-five) 25 chicks per replicate. Diets were formulated to meet standard requirements. Water and the various treatment diets were offered to the birds *ad libitum* for the period of each experiment. Data were subjected to Analysis of Variance (ANOVA) and significant differences among treatment means were compared using the Duncan Multiple Range Test. In the first trial, the starter phase showed that chicks fed Kaura with phytase inclusion had significantly ($P < 0.05$) higher final body weight and weight gain. Finisher broiler chickens showed no significant ($P > 0.05$) differences in the final body weight, weight gain, feed intake and feed to gain ratio across all treatments. Feed cost/Kg gain for the eight (8) weeks period showed that birds fed Kaura with phytase had significantly ($P < 0.05$) lower cost of production. The dressing percentage was significantly ($P < 0.05$) highest for birds fed

Kaura diets (T3 and T5). Diets with phytase inclusion had significantly ($P < 0.05$) higher tibia calcium and phosphorus contents. There were no significant ($P > 0.05$) difference in blood calcium and phosphorus. Results of the second trial showed that chicks fed Kaura with protease and G2 G inclusion had significantly ($P<0.05$) highest final body weight and weight gain. Feed/gain ratio, feed cost and feed cost/Kg gain were not significantly ($P > 0.05$) different. Finisher broiler chickens showed significant ($P<0.05$) differences in the final body weight. Kaura diet with phytase had significantly lower final body weight and dressing percentage than all the other treatments. Parameters measured for blood quality were not significantly ($P > 0.05$) different for all the treatments. It is concluded that phytase in Kaura based diets significantly improved growth performance of broiler chicks, enhanced bone mineralization and significantly reduced cost of production than Jandawa and maize based diets, also Roxazyme® G2 G and the combination with Protease in Kaura based diets improved growth performance for broiler starter chicks and also resulted into comparable performance with maize based diets for broiler finisher. Blood quality was not compromised. The Kaura variety of sorghum with Ronozyme® phytase, Ronozyme® protease combined with Roxazyme® G2 G inclusion can be used for effective nutrient utilization and improved growth performance by broiler chickens.

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CHAPTER ONE

1.0 INTRODUCTION

The poultry industry has become an important economic activity in many countries. The biggest single expense in any system of poultry production is feed, which accounts for about 70% of total production cost per bird (Khattak, 2006). The frequent collapse of many commercial poultry farms in Nigeria have been attributed to high cost of poultry feeds (Ogundipe, 2002) and feed cost is expected to continue in the upward swing (Conolly, 2012).

Sorghum grain is the third most important cereal crop grown in the United States and the fifth most important cereal crop grown in the world (USDA, 2010). Maunder (2002) reported that sorghum is a conventional crop of Africa and Asia and an introduced and hybridized crop in the western hemisphere. Sorghum's nutritional outline includes several minerals which is unevenly distributed and is more concentrated in the germ and the seed coat.

Sorghum contains some anti-nutritional factors which inhibit the use of important nutrients like protein, energy and minerals (phosphorus, calcium, zinc and magnesium) in diets. To improve the utilization of sorghum and indeed dietary nutrients in poultry diet, enzymes are put into consideration (Bedford, 1996). Enzyme inclusion in poultry feeds has been of the major nutritional advances in the last fifty years. It is the culmination of something that nutritionists realized for a long time but until 1980's it remained beyond their reach (Khattak, 2006). Poultry naturally produces enzymes to aid the digestion of feed nutrients. However, they do not have enzyme to break down fibre completely and need exogenous enzymes in feed to aid digestion.

The benefits of using enzymes in poultry diets include not only enhanced performance and feed conversion but also less environmental problems due to reduced output of excreta. Feed enzymes have the potential to reduce effects of anti-nutritional factors, render nutrients more available for digestion and absorption, increase energy value of feed ingredients and allow for greater flexibility in feed formulation, thus reducing formulation costs and modulating or stabilising gut microflora. Some of the enzymes that have been used over the past several years and have potential for use in the feed industry include cellulase (β-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases (Khusheeba and Sajid, 2013).

One area that has received relatively little attention is the use of combinations of enzymes such as carbohydrases and proteases to improve nutrient retention and performance of growing chickens. Logic would suggest that if the use of one enzyme can improve body weight gain (BWG) and feed conversion ratio (FCR) of chickens compared with birds that have been fed a diet containing no enzyme, the use of 2 enzymes might improve the scale and consistency of the response (Ravindran *et al*., 1999). The reality is somewhat different. Antagonistic (Naveed *et al*., 1999; Saleh *et al*., 2004), sub-additive (Zyla *et al*., 2000; Wu *et al*., 2004; Kim *et al*., 2005; Leslie *et al*., 2005); additive (Zyla *et al*., 2000; Mulyantini *et al*., 2005), and synergistic (Ravindran et al.,1999) effects of enzyme combinations have been reported. Clearly, this variety of responses to enzymes and combinations of enzymes is unacceptable for producers of poultry products. Therefore, it is critical that more information need to be generated that will allow tools to be developed, based on whole analysis of animal data, to show when enzyme combinations can offer an additive, sub-additive, or synergistic effect on performance parameters.

Nigeria was positioned as the number one producer of sorghum followed by the United States and India and was the fifth exporter of sorghum in the world market (FAO, 2012). The

effective utilization of sorghum in poultry diets will therefore boost poultry production in Nigeria. It was however discovered that the performance of broiler chickens fed sorghum based diet were significantly lower compared to those fed maize based diet which could be a result of the presence of anti-nutrients (Selle *et al.*, 2010). Hence the need to improve the utilization of sorghum based diet with single or combined enzymes inclusion.

Objectives

The objectives of this study were:

1. To evaluate the effect of two varieties of sorghum with or without phytase enzyme on growth performance, nutrient digestibility and bone mineralization of broiler chickens.
2. Evaluation of the effect of Ronozyme® phytase, Ronozyme® protease and Roxazyme® G2 G as single enzymes and their combinations in Kaura based diet on growth performance, nutrient digestibility and haematology of broiler chickens.

Research Hypotheses

Experiment 1

H_O: Inclusion of phytase in two varieties of sorghum based diets has no effect on growth performance, nutrient digestibility and bone mineralization in broiler chickens.

H_A: Inclusion of phytase in two varieties of sorghum based diets has effect on growth performance, nutrient digestibility and bone mineralization in broiler chickens.

Experiment 2

H_O: Single enzyme and combination of enzymes inclusion in broiler chickens diet has no effect on growth performance, nutrient digestibility and haematology.

H_A: Single enzyme and combination of enzymes inclusion in broiler chickens diet has effect on growth performance, nutrient digestibility and haematology.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry

These are birds that have been domesticated (tamed) to serve the purpose of people, and were most often used to produce meat or eggs but they may be bred as show stocks or fighting cocks. Swans, guinea fowl, pea fowl, ostriches, chickens, ducks, turkeys, geese and quails are listed as poultry animals (free Encyclopedia). According to Adene and Oguntade, (2006) poultry are type of birds domesticated and managed by man for food and profit among which broiler chickens predominate.

Chickens are one of the most common and widespread domestic animals, and with a population of 19 billion in 2011 (The Economist, 2011), they are well dominated in the world than any other species of bird. Broiler chickens (*Gallus gallus domesticus*) are a domesticated fowl, a sub-species of the Red Jungle fowl, bred and raised purposely for meat production (Kruchten, 2002)

2.2 Nutrient Requirement of Broiler Chickens

The need to meet the nutrient requirement of poultry birds is of paramount importance in production. According to the reports of Atteh (2004), sustainable poultry production can only be attained with better understanding of bird's nutrient requirement. Nutrient requirement shows the amount of energy, protein, amino acid, minerals and vitamins needed for the optimum growth and production of animal.

2.2.1 Energy requirement of broiler chickens

The most critical nutrient required by broiler chickens is known to be energy which is needed for growth, maintenance of body tissues, egg production, regulation of body temperature and activity (Pond *et al*., 1995). Major sources of energy are carbohydrate. Carbohydrates are food substance that provides energy for the birds. It forms the largest nutritional need of birds and makes up to 60-80% of poultry ration. In formulating poultry diets, the nutrient requirements of broiler chickens have frequently been expressed per unit of dietary metabolizable energy (Mbajiorgu *et al*., 2011). Metabolizable energy (ME) is the amount of energy in feed ingredients required by birds for normal body physiology. Most of the dietary energy comes from plant sources in the form of carbohydrates from cereal grains and cereal by-products such as maize, sorghum, millet, maize bran and offal others include starchy roots and tubers such as cassava flour, cassava chips and sweet potatoes (Aderemi *et al*., 2006). The performance of birds is closely associated with feed nutrient and energy utilization, which is primarily related to availability of more nutrients from the feed ingredients (Olukosi *et al*., 2007).

Feed intake (FI) has shown to be affected by dietary energy level in broiler chickens. Plavnik *et al*. (1997) and Nahashon *et al*. (2005) reported that as the level of dietary energy increased, birds satisfied their energy needs by reducing their feed intake. Decreases in feed intake with high energy levels in the diets of broiler chickens have also been reported by Leeson (2000) and Veldkamp *et al*. (2005). It is assumed that that broiler chickens adjust their FI according to the dietary energy content and the degree of changes approximately matches what would be expected based on dietary energy. Some studies have also found the same effect and agree with this concept (Leeson *et al*., 1996; Dozier III *et al*., 2006). However, a study has found that broiler chickens do not change their FI based on different energy level of the diet and

disagrees with this concept (Plumstead *et al*., 2007). This study speculates that broiler chickens may not change their FI with different dietary energy content due to the intense genetic selection for growth and will eat to physical capacity. Other studies suggested an age effect on the chicken's ability to regulate FI (Brickett *et a*l., 2007; Kamran *et al*., 2008). Young chickens may not regulate their FI due to limited gut capacity but are able to change their FI according to the dietary energy level when the birds are older. Consequently, in formulating diets for poultry, the nutrient requirements of broiler chickens have frequently been expressed per unit of dietary metabolisable energy (Gonzalez and Pesti, 1993). NRC (1994) summarized that this practice is based on the theory that birds will adjust their feed intake according to their metabolisable energy requirements.

In the tropics, ME of 2800Kcal/kg for chick starter is needed for optimum performance (Olomu, 1995). Leeson *et al*. (1996) researched that broilers at 25 and 49 days of age were able to adjust their feed intake to a constant energy intake over a range of dietary ME from 2700 to 3300 Kcal/kg. A significant higher feed intake was noticed in low ME diets (2700 Kcal/kg) than higher ME diets (2900 to 3300Kcal/kg) which indicated that broilers have an innate to eat to a fixed energy requirement rather than to physical capacity as was suggested by Newcombe and Summers (1984).

The gross energy of 4300Kcal is required by chickens, out of which only 3350 Kcal is digested. About 960 Kcal is excreted in faeces. Further breakdown shows that only 3110 Kcal digestible energy is metabolized, and 240 Kcal is lost in urine. In poultry research studies, Metabolizable Energy (ME) and Net Energy (NE) are usually used to measure energy availability and utilization by birds. Metabolizable energy can be accurately determined from the difference of the amount of energy present in food from energy lost in faeces, urine and combustible gases (NRC, 1994).

2.2.2 Protein and Amino Acid Requirement of Broiler Chickens

Proteins have been described as complex organic compounds of high molecular weight composed of 22 different amino acids or derivatives that are linked by peptide bonds to form a primary chain structure (Mbajiorgu *et al*., 2011). Amino acids are further classified into essential and non-essential amino acid based on their ability to be synthesised in the body.

Meeting the protein needs of broiler birds are more difficult compared to other nutrients. Protein can be sourced from both plants and animals. Sources of plant protein include soya beans meal, groundnut cake, sunflower meal e.t.c while, the animal sources are mostly fish meal, blood meal, meat meal and feather meal.

Dietary crude protein (CP) requirements are somewhat of a misnomer as the requirement is based on the amino acids content of the protein. Once digested and absorbed, amino acids are used as the building blocks of structural proteins (muscle, skin, ligaments), metabolic proteins, enzymes, and precursors of several body components. Body proteins are constantly being synthesized and degraded, an adequate amino acid supply is critical to support growth or egg production. Protein in poultry ration is usually based on the crude protein content. Broiler starter ration should contain from 21-24% protein and a typical laying ration should contain 15-17% protein (Ensminger *et al*., 1990). Aduku (1992) reported 23% and 20% for broiler starter and finisher, respectively. For optimum production, protein and energy feeds should be at corresponding levels for instance, if a high level of energy feed is used in production of feed, the protein content of the feed must also be high if the birds must ingest adequate amount of protein (Mander, 1980).

In poultry, 22 amino acids are needed to form body protein, some of which can be synthesized by the bird (non-essential), whereas, others cannot be made at all, or in sufficient quantities to meet metabolic needs (essential). Essential amino acids must be supplied by the diet, and a

sufficient amount of non-essential amino acids must also be supplied to prevent the conversion of essential amino acids into non-essential amino acid. Additionally, if the amino acids supplied are not in the proper, or ideal ratio in relation to the needs of the animal, then amino acids in excess of the least limiting amino acid will be deaminated and likely used as a source of energy rather than toward body protein synthesis.

The cost of protein and its importance in growth and meat yield, formulating diets that match the amino acid (AA) requirements of the bird avoids costly losses due to deficiency and avoids high costs due to over formulation (Emmert and Baker, 1997; Khaksar and Golian, 2009). The ideal protein concept of formulating diets ensures that AA levels not only meet the bird's minimum requirements but are also balanced to avoid catabolism of excessive AA.

2.2.2.1 *Ideal protein*

The ideal protein notion is based on formulating diets that have a balanced proportion and appropriate amount of indispensable and dispensable AA. Lysine serves as the reference AA and other indispensible AA are formulated to be proportional to the lysine level (Emmert and Baker, 1997; Baker, 2003). Regardless of being the second limiting AA in poultry, lysine is chosen as the reference AA because of its importance in protein synthesis for meat production and the fact that it is easier to analyze than methionine (Emmert and Baker, 1997). Thus, in most cases, the ratios of other AA to lysine are used for diet formulation. This result in formulation of balanced diets that minimize AA catabolism associated with AA levels that are above the bird's requirement.

The NRC (1994) amino acid recommendations for broilers were based on peer-reviewed research published between 1947 and 1991. However, the present commercial birds are very different from commercial birds available prior to 1991, due in part to genetic selection as

well as management practice and feed related changes (Havenstein *et al*., 1994; Williams *et al*., 2000).

2.2.3 Fat Requirement of Broilers

Fats and oils are required in poultry diet formulations to enhance the palatability of the diet, the absorption of fat-soluble vitamins, and to regulate the passage rate of the digesta in the gastrointestinal tract (Fouad *et al*., 2014). They are also the most concentrated sources of energy (Baião and Lara, 2005). Fats are insoluble in water but soluble in organic solvent such as chloroform. Fats provide the body with energy approximately twice the amount of protein. Poultry birds require 5-6% of fat (Olomu, 1995). The following foods are rich in fat and oil: groundnut oil, palm oil, oil sludge, and full fat soyabeans. A small amount of fat in poultry ration helps to make feed less dusty, more palatable and of high energy (Kekeocha *et al*., 1985).

Aduku (2004) reported that fat is a major storage and most efficient form of metabolic fuel which carries fat soluble vitamins (A, D, E and K). It is also a structural component of membranes, that provides insulation to keep the body warm, form protective coat under the skin and improve egg size and feathering.

Simple lipids are esters of fatty acids and certain alcohols, particularly glycerol and cholesterol. Birds are not able to synthesize all fatty acids and thus, some are considered essential fatty acids. Linoleic (18:2, n-6) and linolenic (18:3, n-3) fatty acids are recognized as metabolically essential. However, the linoleic acid is the only essential fatty acid which its diet requirement has been demonstrated (NRC, 1994). The profile of fatty acids is of importance to the quality of the utilized lipid, absorption of these lipids by the bird, and also because it influences the quality of the fat deposited on the broiler carcass.

2.2.4 Vitamin and Mineral Requirement of Broilers

Oluyemi and Roberts (2000) reported that broiler birds require ample amount of minerals and vitamins in their diets for proper functioning of the animal. Poultry require supplemental dietary vitamins since common feed ingredients used in poultry production do not provide adequate quantities to meet minimum requirements. The vitamin requirement of poultry is usually affected by several factors such as age, body size, breed/strain, diet composition, diseases, endogenous or exogenous toxins, environment, feather coverage, feed form, feed intake, management conditions, minerals-vitamin bioavailability, other nutrient concentration, physiological condition, sex, stress, water intake etc.

Oluyemi and Roberts (2000) reported that vitamins are organic substances required by animals in very small amounts which help in regulating various body processes to maintain normal health. Vitamins are classified into two groups, namely, fat-soluble vitamins and water-soluble vitamins. The fat-soluble are vitamin A, D, E and K. The water-soluble vitamins are the B complex group of vitamins, and vitamin C. Vitamins in the B complex group are thiamine (B1), riboflavin (B2), niacin, pantothenic acid, pyridoxine (B6), biotin, choline, folic acid and cobalamine (B12). Vitamins, A, B2 and D3 are called "critical vitamins" for poultry, as any deficiency in their availability severely impairs the growth and egg production.

Minerals are not organic but chemical elements. Those required in small but significant amounts (calcium, phosphorus, sodium, potassium, magnesium and chlorine) are macro minerals and the micro minerals or trace minerals are iodine, cobalt (as cobolamin), iron, copper, zinc, selenium and molybdenum (NRC, 1994). Most minerals must be added to the diet for good growth and egg production. Laying hens need a ration of about 3-4 % calcium to make egg shells. This can be supplied free choice as small pieces of bone-meal, coral, sea-shell or limestone. Phosphorus in plants is present as phytate phosphorus and is only partially

available unless phytase enzyme is added to the feed (Canadian Poultry, 2008). Growing birds require about 0.4% phosphorus, adults about 0.3%. Calcium and phosphorus levels must be in balance since high levels of one cause deficiency of the other. Sodium and chlorine are usually added as salt (NaCl) at about 0.25% of the ration (2.5 kg NaCl/1000 kg)

2.3 Sorghum (*Sorghum bicolor*)

Maize compared to other cereal crops have been widely used in production of food for man and feed for farm animals in general. It enormous use and demand has now made farmers look for alternate sources of grain with similar nutritive value and availability such as sorghum (*Sorghum bicolor*), millet (*Pennisetum glaucum),* wheat e.t.c. Its benefits are ability to tolerate drought, soil toxicities and temperature extremes effectively than other cereals. In terms of the nutritive value, cost and availability, sorghum grain is the next alternative to maize in poultry feed (Subramanian and Metta, 2000).

2.3.1 World Sorghum Production and Trade

In the year 2010, United States was positioned as the second largest producing country of sorghum grain and the top exporter of grain sorghum, accounting for almost 75 percent of the global trade. Its production is primarily focused in a stretch of land beginning in southern Nebraska and ending at the southern tip of Texas (USDA, 2010). However, 90% of sorghum produced by United States and India is destined to animal feed, making Nigeria the world leading country for food grain sorghum production (MAFAP, 2013)

According to the USDA (2010), Nigeria was positioned as the number one producer and number five exporter of sorghum in the world market (Table 2.1). Sorghum is the third cereal

in terms of production after maize and millet, with more than 4.5 million tonnes harvested in 2010, representing 25% of the total cereal production (FAOSTAT, 2012). In virtually all the north of the country, it is the primary food crop (USAID, 2011) and as at the year 2014, sorghum was produced in virtually all the states in Nigeria, though some states produce more than others some of the states that sorghum is produced in large quantity include Plateau, Kano, Kaduna, Sokoto, Gombe, Bauchi, Zamfara, Benue, Kogi, Nassarawa and Taraba. (Annonymous 4, 2015).

Sorghum is not only termed as the third cereal in terms of production but also compares well in nutrient composition with other major cereal grains (Table 2.2). The crude protein content of sorghum is higher than that of maize but about equal to wheat. In terms of energy value, sorghum is rated as high as 90 – 100% of maize depending on the livestock specie. However, sorghum is lower than maize but higher than wheat in fat content. The calcium and phosphorus level of sorghum is higher than that of maize though they are equal to wheat.

The major portion of sorghum produce in Nigeria is currently utilized mainly by the larger beer sector and the non-alcoholic malt beverage sector. Sorghum is increasingly being used as a substitute for more expensive and imported raw materials like malted barley and because of this, the local demand is more than the current local supply owning to the increasing demand from local industries in Nigeria, though there is no official figure on the national production. The international market is also witnessing increasing demands (MAFAP, 2013).

Table 2.1: World Sorghum Production and Exports in 2010 (Metric tons)

Country	Production		Export	
Nigeria	11,500	19.3%	50	0.8%
US	9,728	16.3%	4,084	65.6%
India	6,980	11.7%	75	1.2%
Argentina	3,629	6.1%	1,507	24.2%
Brazil	1,825	3.0%	25	0.4%
China	1,650	2.8%	41	0.7%
Australia	1,600	2.7%	350	5.6%
Others	11,637	19.6%	50	0.8%

Source: USDA, 2010

Table 2.2: Nutrient composition of sorghum and other major staple foods in their raw form.

	Maize	Rice	Wheat	Sorghum
Water (g)	10	12	13	9
Energy (kJ)	1528	1528	1369	1419
Protein (g)	9.4	7.1	12.6	11.3
Fat (g)	4.74	0.66	1.54	3.3
Carbohydrates (g)	74	80	71	75
Fibre (g)	7.3	1.3	12.2	6.3
Sugar (g)	0.64	0.12	0.41	0
Calcium (mg)	7	28	29	28
Iron (mg)	2.71	0.8	3.19	4.4
Magnesium (mg)	127	25	126	0
Phosphorus (mg)	210	115	288	287
Potassium (mg)	287	115	363	350
Sodium (mg)	35	5	2	6
Zinc (mg)	2.21	1.09	2.65	0
Copper (mg)	0.31	0.22	0.43	-

Source: Nutrient Data Laboratory. United States Department of Agriculture. Retrieved June 2014.

2.3.2 Varieties of Sorghum

Sorghum varieties are classified into two common categories i.e. the non-bird-resistant (or yellow grain sorghum), and bird-resistant (brown grain sorghum). The yellow and brown refer to seed coat color; though non-bird-resistant grain can have seed coat colors ranging from white to brown, with variations of yellow, tan and brown while, bird-resistant grain usually has seed coat colors between tan and dark red-brown. Thus, seed coat color is not a completely accurate method of differentiating between the two types of sorghum. (Myer *et al*., 2006). The major difference between the two sorghum varieties is the presence of tannins in the bird-resistant grain. Tannins have a bitter taste that birds do not like, hence the term bird-resistant. Futhermore, tannins interfere with the utilization of some of the nutrients, particularly protein, in grain sorghum, thus decreasing its nutritional value for birds and other monogastric animals.

2.3.3 Anti-nutritional Factors in Sorghum

Many plant components have the potential to hasten adverse effects on the productivity of farm livestock (D'Mello, 2000). As with some other foodstuffs, certain nutritional inhibitors and toxic substances are associated with sorghum grains.

2.3.3.1 *Phytic Acid*

The nutrient composition of sorghum shows that it is a good source of energy, protein, carbohydrate, vitamins and minerals including the trace elements. Sorghum grain contains 1.3 to 3.3% of ash and minerals such as phosphorus and potassium in varying amount. Sorghum nutritional quality is dictated by its chemical composition and the presence of anti-nutritional factors, such as phytate. Phytate or Phytic acid is a key storage form of phosphate, it is widely

distributed in plants, particularly in cereal grains and in legumes. The effects of phytate in human and animal nutrition are related to the interaction of phytic acid with proteins, vitamins and several minerals, thereby restricting their bioavailability in sorghum (Elkhalil *et al*., 2001). Amongst cereal grains, sorghum contains relatively high phytate concentrations (Selle *et al*., 2003) and it follows that broilers on sorghum-based diets should be advantaged by phytate-degrading enzymes. Nevertheless, there are indications that, in poultry, responses to phytase with sorghum are less than with other cereals (Wu *et al*., 2004; Pourreza and Ebadi, 2006). In disparity to tannins, sorghum phytate content has not been reduced through breeding research. Results of six surveys conducted between 1968 and 2003 showed that total phosphorus content in sorghum ranged from 3 to 4 g/kg and phytate-P content ranged from 2.1 to 2.4 g/kg (Nelson *et al*., 1968; Selle *et al*., 2003).

2.3.3.2 *Tannins*

Tannins are secondary compounds present in plants and consist of polyphenols of great variety (Hoste *et al*., 2006) which can be both beneficial and deleterious to animals. Animal nutritionists are interested in tannins because they bind protein, cellulose, hemicelluloses, pectin, phytate, and minerals to form indigestible complexes (Van Soest, 1994). The presence of tannin in poultry feeds is well known to suppress growth and performance of all types of poultry (Nyachoti et al., 1996). Tannins bind proteins and render them less available for metabolism, although tannin reduces bird damage in fields of sorghum, poultry birds can also be affected by the anti-nutritional properties of tannin since they are also classified as birds.

Etuk and Ukaejiofo (2007) reported 0.42% tannin content in brown coat coloured sorghum while, Subramanian and Metta (2000) reported that the local Indian sorghum variety and ICSV 112 variety developed by ICRISAT and grown in India contain no tannins. A level of 0.40% tannin was reported for samsorg 17, a variety previously coded (SSV) -3 (SK5912) and

developed from local collections of Kaura through mutation breeding at the Institute of Agricultural Research (IAR), Samaru, Nigeria. ICSV 400, released by ICRISAT in 1996 recorded tannin value of 0.69% (IAR, 1995; NCGRB, 2004, Etuk, 2008). Red sorghum on the other hand contains about 23g/kg tannins which when reconstituted reduced to 16g/kg (Kumar *et al.*, 2007). Studies have shown that tannin in sorghum affect feed intake and feed efficiency in broilers (Nyachoti *et al*., 1996; Hancock, 2000). Nyanmambi *et al*. (2007) argued that tannins reduced crypt depth, intestinal wall thickness, and sucrase activity with increased mucus production. Donald *et al*. (2008) reported similar intestinal morphology for broilers chicks fed corn- sorghum and wheat-based diets although birds fed corn- and wheat-based diets had better growth performance compared to those fed the sorghum-based diet.

2.3.3.3 *Oxalate*

Oxalates are naturally occurring anti-nutritional factors that form insoluble complexes with calcium, magnesium and iron which interfere with utilization of these mineral elements (Onimawo and Akubor, 2012). Oxalate is common constituent of plants and depending upon the species oxalate accumulates primarily as soluble oxalate or insoluble oxalates or in combination. Oxalate is an anti-nutrient which under normal conditions is confined to separate compartments. However, when it is processed and/or digested, it comes into contact with the nutrients in the gastrointestinal tract (Noonan and Savage, 1999). When released, oxalic acid binds with nutrients, rendering them inaccessible to the body. If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut. (Habtamu and Negussie, 2014).

2.3.3.4 *Dhurrin*

Dhurrin is a cyanogenic glucoside which when in reaction with enzyme readily yields hydrogen cyanide (HCN). It is located mainly in the aerial shoot of sorghum plant (D’Mello,

2000). In Nigeria, HCN level of 15.18g/kg has been reported for malted sorghum sprouts (Oduguwa and Fafiolu, 2004). The quantity of HCN in sorghum varies with cultivar and the growth condition but diminishes with age. Making fodder into hay or silage destroys the poison. Cyanide intake of up to 75.96mg/100g reduces apparent nutrient digestibility of broilers.

2.3.4 Consequences of anti-nutritional factors in Sorghum

Livestock and poultry require their diet contain a large percentage of cereal grains to provide necessary nutrient such as protein and energy in their ration. The primary cereal grains used in poultry rations include corn, wheat, barley, rice and sorghum. Sorghum however, is still the second most used cereal grain for commercial growers of broilers, turkeys and egg layers in regions where both are grown (Anonymous1, 2014). Among the five major cereals produced worldwide, sorghum is unique for its physical characteristics, proteins, high proportion of phytate, and ability to produce tannins. During the last five decades, the effects of tannins, phytate, kafirins, and starch characteristics have been evaluated in sorghum-based feeds and foods. Most of the presently cultivated sorghums are tannin free. However, less progress towards phytate-free sorghums has been made, and tannins sometimes still are found in sorghum.

Phytate which is present in plant sources of feed is poorly digested by monogastric animals particularly poultry. This is one constraint that phytic acid has caused to poultry. Nutrients affected by phytates include minerals and protein (Selle *et al*., 2000), and phytase has been shown to positively affect the release of energy (Scott *et al*., 2001).

Phytic acid does not only affect feed nutrients but also has a negative effect on the environment. According to Singh *et al*. (2005), some amounts of phosphorus is voided in the

faeces of poultry due to phytic acid occurrence and in the findings of Usayran and Balnave (1995) it was reported that this excreted phytate has led to pollution of areas of intensive poultry production, Powell *et al* (2008), further stated that this excreted phytate phosphorus could also lead to eutrophication of water bodies.

2.4 Enzymes

Enzymes are biological catalyst composed of amino acids. They bring about biochemical reactions without themselves undergoing any change. Enzymes are added to animal ration with the goal of increasing its digestibility, removing anti-nutritional factors, improving nutrient availability, as well as for environmental issues. A large number of carbohydrases, proteases, phytases and lipases were used for this purpose (McCleary, 2001). Most cereal and poultry benefit to some extent by the addition of enzymes that either augment nutrient availability or reduce the impact of anti-nutritional factors. For instance, the use of glucanase enzymes to lessen the negative effect of viscosity in wheat and barley-based diets (Anonymous 2). Therefore, sorghum with anti-nutritional factors and difficult to digest protein layers would seem a good feedstuff for enzymes to improve feeding values.

Researches with enzymes have demonstrated the importance of these substances to reduce the negative effect of anti-nutritional factors and improve feed efficiency. A research group studied the effect of adding a commercial mixture of pectinases, glucanases and hemicellulases to sorghum-soy feed rations for broilers, and found that ileal amino acid digestibility increased 3% while the ME was increased by over 6% when used in rations that were marginal in nutrients (Dominguez *et al*., 2009). This demonstrates that enzymes can be used to get more nutrients from sorghum. Cadogan *et al.*, (2005) tested phytase enzyme on

sorghum based diets and determined that the enzyme improved weight gain, amino acid digestibility, starch digestibility and performance of broilers. Limited studies do indicate that there are opportunities to use enzyme to improve bird performance.

2.4.1 Sources of Enzymes

Enzymes were used in the preparation of foods long before there was any knowledge of enzymes, possibly as long ago as 10,000 years ago. The industrial use of microbial enzymes in the western world started 100 years ago with the patenting of a process for the production of alpha-amylase from the fungus *Aspergillus oryzae* (Khattak *et al* 2006)**.** Enzymes are produced in every living organism from the highest developed animals and plants to the simplest unicellular forms of life, as they are essential for metabolic process. Most of the enzymes currently used in the food and beverage industry are from *Aspergillus*, but hemicellulases and cellulases are derived from *Trichoderma*. Recently, genes encoding for different enzymes, including phytases, ß-glucanases, and xylanases, have been cloned and expressed in different commercial systems (microorganisms and plants) according to Khattak *et al.* (2006)**.** It is possible to produce large amounts of cheap enzyme by continually selecting favorable microbes, growing them in advanced fermentation systems and by streamlining the extraction and purification of the enzyme (Wallis, 1996).

Microorganisms that are generally involved in production of enzymes are; Bacteria (*Bacillus subtilis, Bacillus lentus, Bacillus amyloliquifaciens* and *Bacillus stearothermophils*), Fungus (*Triochoderma longibrachiatum, Asperigillus oryzae* and *Asperigillus niger*) and Yeast (*S. cerevisiae*).

2.4.2 Phytase

In recent times, literature has focused on the topic of amino acid digestibility and the effect of phytases, with some authors showing significant benefits (Onyango *et al*., 2005; Cadogan *et al*., 2009; Agbede *et al*., 2010; Pirgozliev *et al*., 2011). However, other papers have shown no benefit of phytase on this parameter (Boling-Frankenbach *et al*., 2001; Augspurger and Baker, 2004), although Boling-Frankenbach *et al.* (2001) suggested the effects of phytase were ingredient specific. Therefore, the application of a phytase under the right circumstances will reduce endogenous inputs for protein digestion which results in an improvement in digestibility of those amino acids which is more important than the endogenous losses, specifically cysteine, aspartate, threonine, glycine and serine (Peter and Baker, 2001; Cowieson *et al*., 2006; Selle *et al*., 2012).

2.4.2.1 *Importance of Phytase Enzyme*

The beneficial effect of exogenous phytases in poultry feed has been believed to be due to the direct hydrolytic effects on phytate and the subsequent improvement in the availability of minerals, amino acids, and energy (Selle and Ravindran, 2007). It had also been suggested that phytase in the poultry diets improved gut health as indicated by reduced secretions from the gastrointestinal tract (GIT), which consequently improved the efficiency of utilization of energy (Pirgozliev *et al.*, 2011). Phytase presents effective capacity to improve phytic phosphorus usage, mainly from feedstuffs presenting high phytate levels and therefore, reduces the pollutant potential of excreta, making poultry husbandry a more ecologically friendly activity. Furthermore, phytase enzyme also allows the use of non-conventional ingredients without reduction or impairment to birds' performance, with consequent reduction on production costs. Phytase inclusion in feed represents a possible economy, by making the phytic phosphorus available thereby making inorganic phosphorus supplementation,

unnecessary resulting in reduced diet formulation cost. Studies performed by Plumstead *et al.* (2008) evaluated phosphorus reduction in the diet associated with phytase supplementation, achieved interesting results on chick production by housed breeder fed those diets, allowing the reduction of inorganic phosphorus levels of the diets, decreasing expenses without affecting negatively broiler breeder performance.

2.4.3 Protease

Proteases are the most recent innovative additions to the range of enzymes available to the animal feed industry. Already, they provide profitable benefit to the livestock industry internationally through their positive effect on animal performance, litter quality, animal welfare and the environment. The supplementation of a protease in a ration requires formulation advice which resulted in no loss in performance trials and this had been the case in the studies of Angel *et al*., 2011 and Yan *et al*., 2012 negative performance reported by Tempra, (2013). In the work of Yan *et al* (2012) it was clear that the benefit of the protease was greater in the starter phase compared with the finisher phase which suggested that the young animal may be more responsive to such products. If correct then age specific recommendations would refine the protease offering considerably.

2.4.3.1 *Importance of Protease Enzyme*

Protease enzymes improve the digestibility and availability of proteins, optimising diet costs by allowing lower quality protein alternatives to be used in the diet. This helps save on feed costs, as grain and oilseed meal prices continue to fluctuate. Researchers have also been digging into the additional benefits of the protease enzyme and its link to gut health and bacteria balance in poultry and swine.

2.4.4 Roxazyme G

This is a specific blend of beta-glucanases, cellulases and xylanases which improves the efficiency of energy utilization in cereals and cereal by-products. It has been shown to increase the metabolic energy of cereals by as much as 8%. *Roxazyme-G* is an enzyme produced by fungus (*Trichoderma spp*). Its potential in reducing the viscosity of intestinal content with an improved feed utilization after consumption of non-starch polysaccharides (NSP) by monogastric animals has been reported by Pitson *et al*. (1993) and Agbede *et al*. (2002).

Roxazyme G2 is available as Roxazyme G2 G a light brown, non-dusty granulate and as Roxazyme G2 liquid, a brown aqueous liquid.

2.4.4.1 *Importance of Roxazyme G2 G*

Roxazyme® G2 G improves the condition of the litter, creating a better environment for birds. It improves performance in broiler chickens, improves live weight gain and superior feed conversion. In turkeys it shows faster growth and more efficient birds. In laying hens it shows increased rate of lay from less feed. In ducks it shows efficient feed use and faster growth. In piglets it shows improved performance. The free-flowing, low-dust and heat stable formulation of roxazyme® G2 makes it suitable for premix, basemix or finished feeds (Anonymous 3, 2015).

2.4.5 Combinations of Enzymes

Enzyme combination has been put into consideration but the basic principle is that phytase enzyme reduces endogenous losses as a result of its ability to facilitate gastric and subsequent small intestinal digestion (Cowieson and Bedford, 2009a; Cowieson and Bedford, 2009b). Specific amino acids are secured more when phytase is employed, such as those involved in mucin and endogenous secretions. Application of a matrix for amino acids for a protease derived from diets which did not contain a phytase or a xylanase therefore needs to be considered with caution. Sultan *et al.* (2011) suggest that the combination of more than two enzyme classes from phytase, protease and xylanase, did not result in any further improvement in N digestibility. Each enzyme alone or in combination with one other tended to improve N digestibility, but addition of the third to a combination of the two resulted in no further improvement. Therefore, there is need to be aware of such limitations when applying feed enzymes because of the negative biological values which also may result in significant losses in performance as the enzymes fail to deliver their matrices in an additive manner.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out at the Livestock Teaching and Research Farm, Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State in the Northern Guinea Savannah zone located on longitude 11° 09° 01.78° N and 7°39°14.79°E 671m above sea level. The climate is characterized by a well-defined dry and wet seasons. The climate is relatively dry with a mean annual rainfall of 700-1400mm (Ovimaps, 2014)

3.2 Sources of Experimental Materials

Maize and the two sorghum varieties were sourced from the local market in Samaru, Zaria while other ingredients were purchased from Labar feed mill, Samaru, Zaria, Kaduna State.

3.3 Determination of Proximate Composition, Gross Energy and Anti-nutritional factors of Feed Ingredients

Proximate composition and anti-nutritional factors of the feed ingredients was determined at Biochemistry Division, National Veterinary Research Institute Vom, using the methods described by A.O.AC. (1990). The following parameters were determined: % Dry matter (DM), % Crude Protein (CP), % Crude Fiber (CF), % Ether Extract (EE) and % Nitrogen Free Extract (NFE), and the following anti- nutritional factors were determined: phytic acid, tannin and oxalate while the Gross Energy(GE) of the feed was determined at the Faculty of Chemical Engineering laboratory, Ahmadu Bello University Zaria.

3.3.1 Determination of dry matter content

A clean crucible was dried to a constant weight in a hot air oven at 100ºc, cooled in desiccators and weighed.1 gram of the finely grinded sample was accurately weighed into the previously labeled crucible and reweighed. The crucible containing the sample was kept in the oven for 24 hours, removed, cooled in desiccators and reweighed to a constant weight. The moisture content and the dry content were obtained as follows:

Weight of clean crucible = w1

Weight of crucible + sample (before heating) = w2

Weight of crucible = sample (after heating) = w3

$$\% \text{ moisture content} = \frac{w1 - w2}{W2 - w1} \times 100$$

% dry matter = 100 – moisture content

3.3.2 Determination of ash

Four porcelain crucibles were dried in an oven at 100°c for10 minutes, cooled in desiccators and weighed 2 grams of the finely grinded sample were placed in the crucibles, reweighed and transferred into a furnance set at 550ºc.The samples were left in the furnance for about 8 hours to ensure proper ashing.The crucibles containing the ash were then cooled in desecrator and then reweighed. Percent ash was calculated as:

Weight of clean crucible = w1

Weight of crucible + sample (before ashing) = w2

Weight of crucible + sample (after ashing) = w3

$$\% \text{ ash content} = \frac{w2 - w3}{w2 - w1} \times 100$$

3.3.3 Determination of ether extract

Anti bumping granules were weighed in two round bottomed flasks and 300ml of petroleum ether for the extraction poured into the flasks fitted with the soxhlet extraction unit. The extractor thimbles were weighed and 20 grams of the sample fixed into the soxhlet extraction unit with forceps and cold water circulation put on. The heating mantle switched on to allow refluxing at a steady rate. The extraction was carried out for 6 hours, the thimble removed and dried to a constant weight in an oven at 70^{o}c and reweighed. The extractable lipid content was determined as:

$$\% \text{ lipid (w/w)} = \frac{\text{weight of lipid extract}}{\text{Weight of dry sample}} \times 100$$

3.3.4 Determination of crude fiber

Two grams of finely grinded sample were put in a round bottomed flask,100ml (0.25M) sulphuric acid solution was added and the mixture boiled under reflux for 30 minutes. The hot solution was filtered under suction. The insoluble matter was washed 5 times with hot water until acid free. The residue was transferred into a flask and 100ml of hot (0,312M) NaOH solution added to the mixture and boiled again under reflux for 30 minutes and quickly filtered under suction. The insoluble residue was washed with boiling water 5 times, dried to a constant weight in an oven at 100°c, cooled in the desiccators and reweighed as C2.The weighed sample was incinerated in a muffle furnance at 550^{o}c for 2 hours, cooled in the desiccators and reweighed as C3.Calculations:

$$\text{The loss of weight on ashing} = \frac{C2 - C3}{W} \times 100$$

3.3.5 Determination of crude protein

0.2 grams of the sample was weighed into a 100ml kjedahl flask with few antibumping granules added.1 gram of the mixed catalyst (K_2SO_4 + $CuSO_4$) was added to the flask.5ml of concentrated sulphuric acid was added and 3ml of hydrogen peroxide was added to further the reaction. The flask was placed on a kjedahl digestion rack and heated slowly at first until frothing subsided then more vigorously. The heating was continued for one hour until a clear solution is obtained. At the end of the digestion the heat was turned off, the flask was cooled and the sample was transferred into a 100ml volumetric flask. The digest was transferred into Markham semi micro nitrogen still and 10ml of 40% NaOH solution was added. The samples were then steam distilled ,liberating ammonia into 100ml conical flask containing 5ml of boric acid and 3 drops of methyl red indicator changed greenish. The content of the conical flask was titrated with 0.1M HCl with the end point indicated by a change from greenish to pink colour.The volume of acid used for each titrate was noted. Percentage total nitrogen per sample was calculated using the formula:

$$\frac{V1 - V0 \times 14 \times 100 \times 100}{0.2 \times 1000 \times 10 \times 1}$$

Where :

V0 = Volume of HCl required for blank

V1 = Volume of HCl required for 10ml sample solution

M = Molarity of HCl

14 = Atomic weight of nitrogen

100 = Total volume of digest

100 = Percentage conversion

10 = Volume of distillate

1000 = to convert to liter

0.2 = Amount of sample taken in grams

3.3.6 Determination of nitrogen free extracts (NFE)

The nitrogen free extract is assumed to be the remainder of the feed after all other nutrients described above has been determined. Thus 100% minus the percentages of moisture, crude protein, crude fibre,ash and fat equals the percentage of nitrogen free extract. This is calculated as follows.

$$100 - (\%CP + \%\ Ash + \%\ Fat + \%\ CF + \%\ Moisture\ content) = NFE$$

3.3.7 Determination of Gross energy

The gross energy was determined using the oxygen bomb calorimeter (parr 64000 series). 2.0g of the sample was weighed into a combustion capsule. The combustion capsule was placed in an oxygen bomb containing 25 to 30 atmospheres of oxygen. The oxygen bomb was covered with 2000 g of water in an adiabatic calorimeter. After the bomb and calorimeter have been adjusted to the same temperature, the sample was ignited with a fuse wire. The temperature rise was measured under adiabatic conditions. From the hydrothermal equivalent of the calorimeter the temperature rise minus some small corrections for fuse wire oxidation and acid production, the caloric content of the sample was then calculated.

3.3.8 Determination of phytates

The phytate contents were determined using the method described Reddy et al, 1982. 4 grams of grinded sample were soaked in 10cm³ of 2% hydrochloric acid for 5 hours and filtered.23cm³of the filtrate was taken in a conical flask and 5cm³ of 0.3% ammonia thiocyanate solution was added .The mixture was filtrated with a standard solution of iron (iii) chloride until a brownish yellow colour persisted for 5 minutes. Three replicate determinations were performed.Phytate content was determined using the relation:

1cm³ of 0.025M FeCl3 = 6.601 mg phytate.

3.3.9 Determination of tannin

The tannin contents were determined using Folin Denis Reagent as described by Makkar *et al*. (1993). In that method, a standard calibration curve was prepared and the Absorbance (A) against concentration of tannins at specific wave length was estimated as follows: Suitable aliquots of the tannin-containing extract (initially: 0.05, 0.2 and 0.5 mL) were pipetted in test tubes, the volume was made up to 1.00 mL with distilled water, then 2.5 mL of sodium carbonate reagent were added. Then the tubes were shaken and the absorbance was recorded at 725 nm after 40 min. The amount of total phenols was calculated as tannic acid equivalent from the standard curve.

3.3.10 Determination of oxalate

The oxalate content of the samples was determined using titration method. 2 g the sample was placed in a 250 ml volumetric flask suspended in 190 ml distilled water. 10ml 6MHCl solution was added to the sample and the suspension digested at 100ºC for 1h. The sample was then cooled and made up to 250 ml mark of the flask. The sample was filtered and duplicate portion of 125 ml of the filtrate were measured into beaker and four drops of methyl red indicator was added, followed by the addition of concentrated NH4OH solution (drop wise) until the solution changed from pink to yellow colour. Each portion was then heated to 90ºC, cooled and filtered to remove the precipitate containing ferrous ion. The filtrate was again heated to 90ºC and 10 ml of 5% CaCl2 solution was added to the sample with stirring consistently. After cooling, the sample was left overnight. The solution was then centrifuged at 2500 rpm for 5 min. The supernatant was decanted and the precipitates completely dissolved in 10 ml 20% H2SO4.

The total filtrate resulting from digestion of 2 g of the sample was made up to 200 mL. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized KMnO4 solution to a pink colour which persisted for 30 sec. The oxalate contents of the sample was then calculated (Munro and Bassir, 1969).

3.3.11 Determination of mineral content

The mineral content was determined using the method described by A.OA.C (2005) in the Biochemistry Division National Veterinary Research Institute Vom. 2.0g of sample was mixed with 20ml of nitric-per choric acid (5:1). The mixture was allow to stand overnight and then heated to 60°C on a hot plate for approximately 3 hours after which the clear solution was heated to dryness and reconstituted with de-ionized water. Calcium was determined using atomic absorption spectrophotometer and the phosphorus content was determined using phosphornely-bdonavan data method (A.O.A.C, 2005)

3.4 Experiment 1: Effect of Ronozyme® Phytase on the utilization of two varieties of sorghum (*sorghum bicolor*) by broiler chickens

3.4.1 Experimental Design and management of birds

Three hundred and seventy-five broiler chicks were allotted to five dietary treatments with 3 replicates and each having 25 birds in a completely randomized design (CRD). The birds were housed in deep litter pens with feed and water provided *ad libitum*. Heat and light were provided throughout the brooding period using kerosene stoves and electric bulbs. Routine vaccines (Gumboro and Newcastle) were also administered according to guidelines of the area.

3.4.2 Experimental diets

Five diets were formulated as shown in Tables 3.1 and 3.2 for starter and finisher diets, respectively. Diet 1 served as the control and was the maize based diet while diet 2 and 3 were Jandawa and Kaura based diets respectively. Diet 4 and 5 constituted the Jandawa and Kaura based diets with phytase respectively.

3.4.3 Performance Study

Initial and final weights of the birds were taken at the beginning and at the end of the starter and finisher phases. Feed intake and body weight changes were monitored on weekly basis. Feed/gain ratio and cost per Kg gain were computed from the feed intake and weight gain data for both phases. Mortality was recorded as it occurred.

3.4.4 Carcass Evaluation

Three birds were selected from each treatment at the end of the 8 weeks feeding trial, kept off-feed for 5 hours, weighed and slaughtered for carcass evaluation. Carcass weight, prime cuts (breast, drumstick, thigh and back) and organ weights (liver, lungs, kidney, spleen, gizzard empty and intestinal weights) were recorded. The prime cuts and organ weights were expressed as a percentage of dressed weight and live weight, respectively.

Table 3.1: Composition of Experimental Diets for Starter Broiler Chicks (0-4 Weeks)

Feed Ingredients (%)	Maize	Jandawa	Kaura	Jandawa+ Phytase	Kaura + phytase
	T1	T2	T3	T4	T5
Maize	55.80	0.00	0.00	0.00	0.00
Sorghum	0.00	55.80	55.80	55.80	55.80
Groundnut cake	18.00	18.00	18.00	18.00	18.00
Soya bean meal	22.00	22.00	22.00	22.00	22.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25
Vit/M Premix	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.20	0.20	0.20
Phytase	0.00	0.00	0.00	+0.02	+0.02
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
ME (Kcal/Kg DM)	2906	2832	2832	2832	2832
Crude protein (%)	23.30	24.14	24.14	24.14	24.14
Crude fibre (%)	4.32	4.18	4.18	4.18	4.18
Ether extract (%)	3.65	2.65	2.65	2.65	2.65
Calcium (%)	1.28	1.29	1.29	1.29	1.29
Phosphorus (%)	0.87	0.87	0.87	0.87	0.87
Lysine (%)	1.30	1.36	1.36	1.36	1.36
Methionine (%)	0.50	0.52	0.52	0.52	0.52
Cost/kg of diet(₦/kg)	80.02	91.18	80.0	91.68	80.52

Biomix broiler starter premix included per Kg diet: Vit. A, 1,000 I.U; Vit. D, 2000 IU; Vit. E, 5.0; Vit. K, 2mg; Vit. B1, 1.8 mg; Vit. B2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 0.5mg; Vit. B6 0.30 mg; Vit. B12, 0.15mg; Folic acid, 0.75mg; Biotin, 0.6mg; chlorine chloride, 300mg; Iodine, 1mg; iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg. ME= Metabolizable Energy.

Table 3.2: Composition of Experimental Diet for Finisher Broiler Chickens (5-8 Weeks)

Feed Ingredients (%)	Maize	Jandawa	Kaura	Jandawa+ phytase	Kaura+ Phytase
	T1	T2	T3	T4	T5
Maize	54.00	0.00	0.00	0.00	0.00
Sorghum	0.00	54.00	54.00	54.00	54.00
Groundnut cake	14.00	14.00	14.00	14.00	14.00
Soya bean meal	16.00	16.00	16.00	16.00	16.00
Maize offal	12.00	12.00	12.00	12.00	12.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30
Vit/Min Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Phytase	0.00	0.00	0.00	+0.02	+0.02
TOTAL	100	100	100	100	100
Calculated analysis					
ME (Kcal/Kg DM)	2908	2838	2838	2838	2838
Crude protein (%)	20.07	20.88	20.88	20.88	20.88
Crude fibre (%)	4.79	4.77	4.77	4.77	4.77
Ether extract (%)	3.23	2.26	2.26	2.26	2.26
Calcium (%)	1.24	1.26	1.26	1.26	1.26
Phosphorus (%)	0.88	0.90	0.90	0.90	0.90
Lysine (%)	1.07	1.12	1.12	1.12	1.12
Methionine (%)	0.49	0.51	0.51	0.51	0.51
Cost/kg of diet (N/kg)	74.05	84.85	74.05	85.35	74.55

Biomix broiler finisher premix included per Kg diet: Vit. A, 10,000 I.U; Vit. D3, 2000 IU; Vit. E, 23mg; Vit. K, 2mg; Vit. B1, 1.80 mg; Vit. B2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 0.5mg; Vit. B6 0.30 mg; Vit. B12, 0.15mg; Folic acid, 0.75mg; Biotin, 0.6mg; chlorine chloride, 300mg; Iodine, 1mg; iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg. ME= Metabolizable Energy

3.4.5 Bone Mineral Analysis

Three birds were selected based on the group mean weight for the bone mineral analysis. The tibia bones of the selected birds were removed carefully from the thigh and the fresh bones oven dried at 100°C to obtain a constant weight. The dry bones were ashed at 550°C for 6 hours to obtain the ash content of the bone. The bone ash was analysed for calcium content using Atomic Absorption Spectrophotometry (AAS) and the phosphorus content using flame photometry.

3.4.6 Digestibility Study

A digestibility trial was carried out at the end of the experiment. Three (3) birds were selected from each treatment. The birds were kept in individual cages and allowed an adjustment period of five days before total faecal collection began which also lasted for five days. Each bird was offered a known amount of experimental diet. Faecal sample was collected, weighed and oven-dried at 65 °C for 24 hours. The dried faecal samples were assayed for their nutrient contents using the methods described by AOAC (1990). Nutrient digestibility was determined for crude protein, ether extract, crude fibre, ash and nitrogen-free extract using the formula:

$$\% \text{ Digestibility of Nutrient} = \frac{Amount\ of\ Nutrient\ intake - Amount\ of\ nutrient\ output}{Amount\ \ 0f\ Nutrient\ intake} \times 100$$

3.4.7 Determination of Serum Calcium and Phosphorus

3.4.7.1 *Calcium*

Procedure: 2.0 mls sample of clear blood serum was transferred by means of a pipette to a 250 mls Erlenmeyer flask. The sample was diluted to 50 mls with calcium-free distilled water. 0.4mls of 9 N KaOH solution and 1 drop of ammonium purpurate indicator was added. The

sample was rapidly titrated with constant swirling to the same purple end-point reached in a titrated blank (Elliott, 1952)

3.4.7.2 *Phosphorus*

Procedure: Three mls of a suitable dilute standard or sample was measured into a calibrated Klett tube. 0.5 mls of 1: 2 nitric acid was added and mixed by thorough shaking. Then 0.5 mls of 0.25 per cent ammonium vanadate solution was added and mixed by shaking. Finally 0.5 mls of 5 per cent ammonium molybdate, made to a volume of 5 mls with water, and mix by inversion. After the mixture has stood for 5 minutes, it was read in the calorimeter with the No. 42 (blue) filter. A blank was prepared with 3 ml. of 7.5 per cent trichloroacetic acid and the reagents added in the same amounts and ordered as the standard. In every case the calculations were based upon the reading after subtraction of the blank reading (Klett, 1952).

3.5 Experiment 2: Response of broiler chickens fed Kaura based diets supplemented with enzymes

3.5.1 Experimental Design and management of birds

Four hundred broiler chickens were allotted to five dietary treatments with 3 replicates and each having 25 birds in a completely randomized design (CRD). The birds were housed in deep litter pens and managed with all necessary routine management practices with feed and water provided *ad libitum*.

3.5.2 Experimental diets

Five diets were formulated as shown in Tables 3.3 and 3.4 for starter and finisher diets respectively. Diet 1 served as the control and was the maize based diet while diets 2 and 3

were Kaura based diet with phytase and protease respectively. Diet 4 and 5 constituted the Kaura based diet with G2 G and protease and G2 G in combination respectively

3.5.3 Performance Study

Initial and final weights of the birds were taken at the beginning and at the end of the starter and finisher phases. Feed intake and body weight changes were monitored on weekly basis. Feed/gain ratio and cost per Kg gain were computed from the feed intake and weight gain data for both phases. Mortality was recorded as it occurred.

3.5.4 Carcass evaluation

Three birds were selected from each treatment at the end of the 8 weeks feeding trial, kept off-feed for 5 hours, weighed and slaughtered for carcass evaluation. Carcass weight, prime cuts (breast, drumstick, thigh and back) and organ weights (liver, lungs, kidney, spleen, gizzard empty and intestinal weights) were recorded. The prime cuts and organ weights were expressed as a percentage of dressed weight and live weight, respectively.

Table 3.3: Composition Broiler Starter Diet

Feed Ingredients (%)	Maize	Kaura+ Phytase	Kaura+ Protease	Kaura+ G2G	Kaura+ Protease + G2G
	T1	T2	T3	T4	T5
Maize	53.30	0.00	0.00	0.00	0.00
Sorghum	0.00	55.80	55.80	55.80	55.80
Groundnut cake	18.00	19.00	19.00	19.00	19.00
Soya bean meal	19.50	16.00	16.00	16.00	16.00
Maize offal	5.00	5.00	5.00	5.00	5.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Common salt	0.25	0.25	0.25	0.25	0.25
Vit/M Premix	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.20	0.20	0.20
Phytase	0.00	+0.02	0.00	0.00	0.00
Protease	0.00	0.00	+0.02	0.00	+0.02
G2G	0.00	0.00	0.00	+0.02	+0.02
TOTAL	100	100	100	100	100
Calculated analysis					
ME (Kcal/Kg DM)	2889	2838	2838	2838	2838
Crude protein (%)	23.11	23.09	23.09	23.09	23.09
Crude fibre (%)	4.63	4.36	4.36	4.36	4.36
Ether extract (%)	3.53	2.47	2.47	2.47	2.47
Calcium (%)	1.33	1.32	1.32	1.32	1.32
Phosphorus (%)	0.86	0.88	0.88	0.88	0.88
Lysine (%)	1.23	1.24	1.24	1.24	1.24
Methionine (%)	0.50	0.51	0.51	0.51	0.51
Cost/kg of diet (N/kg)	77.89	77.60	78.30	78.10	79.30

Biomix broiler starter premix included per Kg diet: Vit A, 1,000 I.U; Vit D, 2000 IU; Vit E, 5.0; Vit K, 2mg; Vit B1, 1.8 mg; Vit B2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 0.5mg; Vitamin B6 0.30 mg; Vit B12, 0.15mg; Folic acid, 0.75mg; Biotin, 0.6mg; chlorine chloride, 300mg; Iodine, 1mg; iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg. ME= Metabolizable Energy.

Table 3.4: Composition of Experimental Diet for Finisher Broiler

Feed Ingredients (%)	Maize	Kaura+ Phytase	Kaura+ Protease	Kaura+ G2G	Kaura+ Protease+ G2G
	T1	T2	T3	T4	T5
Maize	54.50	0.00	0.00	0.00	0.00
Sorghum	0.00	57.00	57.00	57.00	57.00
Groundnut cake	16.45	14.45	14.45	14.45	14.45
Soya bean meal	15.00	14.50	14.50	14.50	14.50
Maize offal	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Vit/M Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Phytase	0.00	+ 0.02	0.00	0.00	0.00
Protease	0.00	0.00	+0.02	0.00	+0.02
G2 G	0.00	0.00	0.00	+0.02	+0.02
TOTAL	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
ME (Kcal/Kg DM)	2910	2856	2856	2856	2856
Crude protein (%)	20.12	20.06	20.06	20.06	20.06
Crude fibre (%)	4.83	4.57	4.57	4.57	4.57
Ether extract (%)	3.42	2.34	2.34	2.34	2.34
Calcium (%)	1.26	1.27	1.27	1.27	1.27
Phosphorus (%)	0.87	0.89	0.89	0.89	0.89
Lysine (%)	1.71	1.07	1.07	1.07	1.07
Methionine (%)	0.49	0.50	0.50	0.50	0.50
Cost/kg of diet (N/kg)	78.26	76.76	77.46	77.74	78.92

Biomix broiler finisher premix included per Kg diet: Vit. A, 10,000 I.U; Vit. D3, 2000 IU; Vit. E, 23mg; Vit. K, 2mg; Vit. B1, 1.80 mg; Vit. B2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 0.5mg; Vit. B6 0.30 mg; Vit. B12, 0.15mg; Folic acid, 0.75mg; Biotin, 0.6mg; chlorine chloride, 300mg; Iodine, 1mg; iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg. ME= Metabolizable Energy

3.5.5 Digestibility

A digestibility trial was carried out at the end of the experiment. Three (3) birds were selected from each treatment. The birds were kept in individual cages and allowed an adjustment period of five days before total faecal collection began which lasted for five days. Each bird was offered a known amount of experimental diet. Faecal sample was collected, weighed and oven-dried at 65 °C for 24 hours. The dried faecal samples were assayed for their nutrient contents using the methods described by AOAC (1990). Nutrient digestibility was determined for crude protein, ether extract, crude fibre, ash and nitrogen-free extract using the formula:

$$\% \text{ Digestibility of Nutrient} = \frac{Amount\ of\ Nutrient\ intake - Amount\ of\ nutrient\ output}{Amount\ \ 0f\ Nutrient\ intake} \times 100$$

3.5.6 Haematological Evaluation

At the end of the finisher phase, 2 mls blood samples were taken from three birds per treatment using 5 mls syringe and was put into bottles with ethylenediamine tetra acetic acid (EDTA) and taken to the laboratory for haematological study. The samples were taken to the haematological laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, for analysis of packed cell volume (PCV), Haemoglobin (Hb) count and total proteins (TP) and differential cell counts such as Neutrophils (%), Lymphocytes (%), Monocytes (%) and Basophils (%).

3.5.7 Statistical Analysis

Data obtained in the experiment were statistically analysed using the General Linear Model Procedure of SAS and Significant difference between treatments means were separated by Duncan's Multiple Range Test (SAS, 2002). The model used for this design was:

$Y_{ij} = \mu + t_i + e_{ij}$

Where Y_{ij} = Individual observation.

μ = Overall mean.

t_i = Effect of treatment diet.

e_{ij} = Experimental error.

CHAPTER FOUR

4.0 RESULTS AND DISCUSIONS

4.1 Proximate composition and anti-nutrients of two varieties of sorghum

The proximate compositions and anti-nutrients of Kaura and Jandawa varieties of sorghum are presented in Table 4.1. The result of proximate composition showed that Kaura and Jandawa had crude protein of 8.53% and 9.27%, nitrogen free extract 83.37% and 84.28%, ether extract 2.08% and 1.52%, respectively. The values for crude protein recorded for the two varieties were within the range of 6 to 10% as reported by Gualtieri and Rapaccini (1990) and Rooney and Serna-Saldivar (2000) though lower compared to values of Etuk *et al*. (2012) who reported a value of 10.48%. This variation may be due to the differences in varieties of the sorghum used, soil nutrients and storage conditions. Nitrogen free extract values obtained in this study was in line with Etuk *et al*., (2012) and Subramanian and Metta (2000) who reported values of 76.60%, each. This was an indication that sorghum is a good source of carbohydrate for poultry and other livestock. Ether extract was lower than the 3.7% value reported by Etuk and Ukaejiofo (2007).

The values obtained for the two varieties for anti-nutrients showed that Kaura had a value of 1.07mg/100g for tannin, 2.96mg/100g for phytic acid and 60mg/100g for oxalate compared to Jandawa which had 0.84mg/100g, 2.21mg/100g, 145mg/100g for tannin, phytic acid and oxalate, respectively.

Table 4.1: Nutrients and Anti-nutrients of sorghum varieties

Nutrients (%)						
Sorghum Varieties	**Dry matter**	**Crude protein**	**Crude fibre**	**Ether extract**	**NFE**	**Ash**
Kaura	91.35	8.53	4.71	2.08	83.37	1.31
Jandawa	87.16	9.27	3.47	1.52	84.28	1.46

Anti-nutrients (mg/100g)			
	Phytic acid	**Tannin**	**Oxalate**
Kaura	2.96	1.07	60
Jandawa	2.21	0.84	145
Maize	1.92	0.51	76

4.2 Experiment 1: Effect of Ronozyme® Phytase on the utilization of two varieties of sorghum (S*orghum bicolor*) by broiler chickens

4.2.1 Performance of broiler chicks fed two varieties of sorghum with and without enzyme inclusion (0-4 weeks)

The performance of broiler birds fed Jandawa and Kaura varieties of sorghum with and without phytase inclusion is presented in Table 4.2. There were significant ($P<0.05$) differences in the final body weight, weight gain and feed intake.

Addition of phytase to diets had a significant effect on feed intake. Jandawa based diet and Kaura based diet with phytase enzyme (T4 and T5) improved feed intake in broiler chicks. Phytase enzyme may have decreased passage rate of feed stuffs in digestive tract, degraded the cell wall of ingredients, increased availability of nutrients and thus, improve the feed intake. This result agrees with Viverous *et al.*(2002) and Aksakal and Bilal (2002) who reported that adding phytase to broiler chick diets increased feed intake.

Broiler chicks on the maize and Jandawa diets (T1 and T2) had significantly lower feed consumption than all the other treatments which were at par. This could be as a result of the high oxalates present in the red sorghum variety. Oxalates have been reported to form complexes with mineral particularly calcium thereby making them unavailable to the body, cause irritation of the gut and resulting in low feed intake and inhibit protein and energy utilisation in broilers (Agwunobi *et al.,* 2002; Okereke, 2012).

Birds fed Kaura with phytase had significantly ($P<0.05$) higher final body weight and weight gain. The phytase may have increased the availability of nutrients. This result is supported by the findings of Dilger *et al*., (2004) and Khan *et al*., (2013) who reported that phytase addition in broiler feed greatly enhanced the growth rate and weight gain of chicks.

Table 4.2: **Performance of broiler chicks fed two varieties of sorghum with and without enzyme inclusion (0-4 weeks)**

Parameters	Maize	Jandawa	Kaura	Jandawa+ phytase	Kaura+ phytase	SEM
	T1	T2	T3	T4	T5	
Initial wt (g/bird)	43.53	43.40	43.53	43.40	43.30	0.16
Final wt (g/bird)	722.10^{b}	755.50^{b}	755.60^{b}	755.53^{b}	820.57^{a}	21.38
Weight gain(g/bird)	678.57 ^{b}	712.10 ^{b}	712.07 ^{b}	712.13 ^{b}	777.27^{a}	21.46
Feed intake (g/bird)	1347.63 ^{b}	1313.23 ^{b}	1412.40^{a}	1369.97^{a}	1440.87^{a}	42.63
Feed/gain ratio	1.98	1.84	1.99	1.92	1.85	0.05
Feed cost/kg gain (₦)	158.44 ^{b}	167.67^{c}	159.01 ^{b}	176.33^{d}	148.96^{a}	4.27

a,b,c,d Means with different superscripts on the same row are significantly different (P<0.05)
SEM: Standard error of means

Feed /gain ratio was not significantly ($P > 0.05$) different across treatments. The Kaura based diet with phytase increased feed consumption but with a significant increase in weight gain. Therefore, birds efficiently utilized Kaura diets. This supports the findings of Aksakal and Bilal (2002) who reported that addition of phytase to broiler chicken diet increased feed intake but there was no significant effect on FCR. Also, Viverous *et al*. (2002) reported that due to increasing feed intake simultaneously with body weight, effect of phytase supplementation on FCR of broiler chicks was not significant.

Feed cost per kilogram weight gain showed that Kaura based diet with phytase had the least cost of production (₦148.96) compared to other treatments with ₦158.44, ₦167.67, ₦159.01 and ₦176.33 for T1, T2, T3 and T4 respectively. The use of phytase with Kaura significantly reduced the cost of producing broiler chickens than the traditional maize based diet at the starter phase.

4.2.2 Performance of finisher broiler fed two varieties of sorghum with or without enzyme inclusion (5-8 weeks)

The performance of broiler finishers fed Jandawa and Kaura varieties of sorghum with or without enzyme inclusion is as presented in Table 4.3. There was no significant ($P>0.05$) difference in the final body weight, feed intake, weight gain, and feed to gain ratio across all the dietary treatments. However, some of the values of sorghum based diet for final body weight were higher compared to maize based diets. This report is in line with Blaha *et al.* (1984) who observed that broiler chicken given diet with sorghum had higher weight gains than those given diets with maize. However, feed/gain ratio was not significantly ($P<0.05$) different across treatment diet. Therefore, sorghum could also be used successfully as the only cereal components of diet for broilers

The feed cost required to produce one kilogram of meat was lowest and similar for Kaura with enzyme and maize diets (T1 and T5). This was followed by Kaura and Jandawa without enzyme, and then Jandawa with enzyme which had the highest cost of production probably because the red sorghum is not largely produced here in the north but in the western part of the country unlike the Kaura variety which is largely produced in the northern part of the country.

These results indicated that phytase can enhance the nutritive value of sorghum-based broiler diets for best performance and for economic benefits.

Table 4.3: Performance of finisher broiler fed two varieties of sorghum with or without enzyme inclusion (5-8 weeks)

Parameters	Maize	Jandawa	Kaura	Jandawa+ phytase	Kaura+ phytase	SEM
	T1	T2	T3	T4	T5	
Initial wt. (g/bird)	722.10	755.50	755.60	755.53	820.57	21.38
Final wt. (g/bird)	2272.22	2339.78	2257.86	2282.52	2395.24	83.86
Wt gain (g/bird)	1550.12	1584.28	1502.33	1526.99	1574.67	70.17
Feed intake (g/bird)	3902.50	4040.00	3883.20	4036.10	3999.50	143.58
Feed/gain ratio	2.52	2.53	2.59	2.64	2.54	0.40
Feed cost/kg gain	186.60[a]	216.65[c]	191.54[b]	225.61[d]	189.36[a]	3.19

a,b,c,d Means with different superscripts on thesame row are significantly different (P<0.05)
SEM: Standard error of means

4.2.3 Performance of broiler chickens fed two varieties of sorghum with phytase inclusion (0-8 weeks)

The result of the performance of broiler chickens fed two varieties of sorghum with phytase inclusion is shown on Table 4.4. There was no significant (P>0.05) difference across dietary treatments on final body weight, body weight gain and feed conversion ratio for both the starter and finisher phase. However, final body weight and weight gain were numerically higher in birds fed treatment 5. This agreed with Fakorede (1997) who observed increase in feed intake on diets that were supplemented with phytase. In most cases, the exogenous enzymes are known to improve feed intake and utilization of high fibre diets and diets rich in non-starch polysaccharide (Kyle, 2010).

In the overall performance of birds, feed cost per kilogram weight gain was lowest and best for birds fed Kaura with enzyme (₦169.60) compared to other treatments while Jandawa had significantly (P<0.05) higher feed cost per kilogram weight gain (₦200.97).

The trend in weekly weight gain and weekly feed consumption of broiler chickens fed different diets are shown in appendix 1 and 2, respectively. In the trend for weekly weight gain, there was persistent increase of weight gain of broiler birds from week 1(one) to week 6 (six) but from week 7 (seven), the trend showed obvious decrease in the weight gain of the birds for all treatments. The weekly feed intake increased as the birds grew older. This is indicative that at week 6 (six) optimum performance was reached and continuous keeping /raising of the birds will only incur more expenses for feed consumption with little body weight gain for the birds.

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4.4: Performance of broiler chickens fed two varieties of sorghum with phytase inclusion (0-8 weeks)

Parameters	Maize	Jandawa	Kaura	Jandawa+ phytase	Kaura+ phytase	SEM
	T1	T2	T3	T4	T5	
Initial wt(g/bird)	43.53	43.40	43.53	43.40	43.30	0.16
Final wt(g/bird)	2272.20	2339.80	2257.90	2282.50	2395.2	83.86
Weight gain (g/bird)	2228.67	2296.40	2214.37	2239.10	2351.90	83.89
Feed intake (g/bird)	5270.0	5353.3	5295.7	5406.0	5438.4	170.31
Feed/gain ratio	2.37	2.33	2.39	2.41	2.31	0.03
Feed cost/kg gain (₦)	172.52^{b}	192.16^{c}	175.28^{b}	200.97^{d}	169.60^{a}	2.56

a,b,c,d Means with different superscripts on thesame row are significantly different (P<0.05)
SEM: Standard error of means

4.2.4: Nutrient digestibility and mineral profile of broilers fed Kaura and Jandawa with and without phytase inclusion

The results of protein, ether extract, crude fibre, ash, Nitrogen free extract and dry matter digestibility by broiler chickens fed two varieties of sorghum with and without enzyme supplementation are presented in Table 4.5. Birds fed diets with phytase (Treatments 4 and 5) had significant ($P < 0.05$) higher digestibility in terms of ash and Calcium than the control diet and diets without enzyme (treatments 1, 2 and 3). Phytase inclusion in the Jandawa based diet had a positive influence on gastrointestinal tract digestive enzymes thus causing the observed increased digestibility. These results were in agreement with previous findings on broiler nutrient digestibilility reported by Rutherfurd, *et al*.(2004); Onyango.(2005) and Mondal, *et al*.(2007) who worked on the effect of phytase on broiler diets.

4.5: Nutrient digestibility and mineral profile of broilers fed two varieties of sorghum with and without phytase inclusion

Parameters	Maize	Jandawa	Kaura	Jandawa+ phytase	Kaura+ phytase	SEM
	T1	T2	T3	T4	T5	
Nutrients						
Protein (%)	78.71[b]	78.96[b]	77.44[b]	83.64[a]	78.71[b]	2.10
Ether extract (%)	84.33[a]	76.47[a]	70.84[b]	76.75[a]	67.94[b]	6.24
Crude fibre (%)	52.36[a]	49.24[b]	47.35[b]	59.79[a]	48.54[b]	4.80
Ash (%)	54.47[b]	59.87[a]	57.15[b]	67.65[a]	60.06[a]	4.47
N.F.E (%)	72.77[a]	71.93[a]	71.18[b]	77.69[a]	72.39[a]	2.88
Dry matter (%)	74.35[b]	73.50[b]	72.59[b]	78.88[a]	73.49[b]	2.73
Minerals						
Calcium (%)	52.00[b]	58.33[b]	71.33[ab]	80.33[a]	82.00[a]	6.41
Phosphorus (%)	44.33	45.00	49.67	42.33	47.67	7.99

a,b, Means with different superscripts on the same row are significantly different ($P<0.05$)
SEM: Standard error of means

4.2.5 Bone and serum mineral contents of broilers fed two varieties of sorghum with and without phytase inclusion

The result of mineral analysis of the tibia bones of broiler chickens fed Kaura and Jandawa with and without enzyme inclusion is in Table 4.6. There were significant ($P<0.05$) differences across the dietary treatments. Diets with phytase enzyme inclusion had higher tibia calcium and phosphorus content. Phytase inclusion increased tibia calcium of birds fed Jandawa based diet with phytase (up to 6%) and tibia phosphorus (up to 3%) and Kaura based diet with phytase enzyme (up to 7%) and tibia phosphorus (up to 3%). This is a good indication of increased availability of phosphorus from phytate mineral complex by the action of phytase as also earlier reported by Sabestian, *et al*. (1997) and Mondal, *et al*. (2007).

The result of blood serum calcium and phosphorus of broiler chickens fed Kaura and Jandawa based diets with and without enzyme inclusion is also in Table 4.6. It showed that here was no significant ($P > 0.05$) difference across treatment diets. However, the blood serum of birds fed with Jandawa based diet and Kaura based diet with phytase inclusion had numerically higher values of calcium (1.84 and 1.88mmol/l) and phosphorus (2.61 and 2.50mmol/l) and for birds fed diets without phytase enzyme inclusion had lower values of calcium (1.78 and 1.76mmol/l) and phosphorus (2.02 and 2.14mmol/l) respectively.

Table 4.6: Bone and blood serum calcium and phosphorus of broilers fed two varieties of sorghum based diets with or without phytase inclusion

Parameters	Maize	Jandawa	Kaura	Jandawa+ phytase	Kaura+ Phytase	SEM
	T1	T2	T3	T4	T5	
Bone						
Calcium (%)	29.11[a]	23.39[b]	24.69[ab]	29.13[a]	31.33[a]	3.42
Phosphorus (%)	13.27[a]	10.52[b]	11.11[ab]	13.11[a]	14.10[a]	1.55
Serum						
Calcium (mmol/l)	1.81	1.78	1.76	1.84	1.88	0.09
Phosphorus (mmol/l)	2.02	2.14	2.27	2.61	2.50	0.24

a,b, Means with different superscripts on the same row are significantly different ($P < 0.05$)
SEM: Standard error of means

4.2.6 Carcass characteristics and organ weight of broiler chickens fed two varieties of sorghum with and without phytase inclusion

The carcass characteristics and organ weight of broiler chickens as presented in Table 4.7 showed that there was significant ($P<0.05$) difference in the dressed weight of the birds. Birds fed with two varieties of sorghum with and without phytase inclusion had a higher dressed weight compared to the control diet (maize based diet). This result showed that sorghum can adequately replace maize without having any deleterious effect on broiler chickens.

The dressing % was significantly ($P < 0.05$) influenced by dietary treatments with treatments 3 and 5 having higher values (71.28 and 71.57). This result could be as a result of the variety of the sorghum used (Kaura variety) though it also showed that diets with enzyme inclusion performed better than diets without enzyme. This result showed that phytase inclusion in poultry diet could increase dressing percentage significantly ($P<0.05$). This supports Viverous *et al*. (2002) who observed that the use of phytase enzyme improved dressing percentage of broiler birds. The improvement in dressing percent could be as a result of phytase increasing the release of amino acids from phytate complex.

Significant differences ($P<0.05$) were also observed in values of breast (prime cut) and kidney and gizzard (organ weights) but with no particular trend. Dietary treatments had no significant ($P > 0.05$) effect on the drumstick, thigh, back, liver, lungs, spleen and intestinal weight.

Table 4.7: Carcass characteristics and organ weight of broiler chickens fed two varieties of sorghum with and without phytase inclusion

Parameters	Maize	Jandawa	Kaura	Jandawa+ phytase	Kaura+ phytase	
	T1	T2	T3	T4	T5	SEM
Live wt (g/bird)	2126.00	2216.70	2150.00	2166.70	2300.00	109.76
Dressed weight(g/bird)	1303.30^{c}	1416.70^{bc}	1533.30^{b}	1466.70^{b}	1650.00^{a}	113.92
Dressing %	61.23^{b}	64.00^{b}	71.28^{a}	67.54^{ab}	71.57^{a}	3.34
Cut parts expressed as percentage of dressed weight (%)						
Breast	28.70^{a}	31.45^{a}	27.41^{a}	25.17^{b}	26.67^{ab}	2.41
Drumstick	16.29	15.88	14.25	16.67	13.27	0.73
Thigh	19.01	17.52	17.17	17.02	18.06	1.19
Back	23.23	23.81	20.87	21.55	21.18	1.30
Organ weight expressed as percentage of live weight (%)						
Liver	2.11	2.52	2.38	2.01	2.52	0.25
Lungs	0.45	0.58	0.77	0.60	0.61	0.18
Kidney	0.50^{b}	0.56^{ba}	0.64^{a}	0.43^{c}	0.61^{ba}	0.05
Spleen	0.06	0.06	0.05	0.06	0.07	0.01
Gizzard	2.17^{b}	2.64^{a}	2.04^{c}	2.34^{a}	2.18^{b}	0.20
Intestine weight	3.18	3.62	3.95	3.02	3.68	0.36

a,b,c, Means with different superscripts on the same row are significantly different ($P<0.05$)
SEM: Standard error of means

4.3 Experiment 2: Response of broiler chickens fed Kaura based diets with enzymes inclusion

4.3.1: Performance of Broiler Chicks fed Kaura Based diets with Enzyme Inclusion (0-4 weeks)

The performance of broiler chicks fed different enzymes in Kaura based diet is shown in Table 4.8. The result showed that birds on the Kaura based diet with combination of protease and G2 G performed significantly ($P < 0.05$) better compared to birds fed Kaura based diet with protease alone but similar to the G2 G supplemented diet. This could be due to the synergy of the two enzymes releasing more protein and energy for tissue synthesis. Birds fed Kaura with phytase inclusion showed the least performance which may be explained by earlier reports by researchers that phytic acid is not the limiting factor, but that tannin is the major issue that limits sorghum utilisation (Etuk *et al.,* 2012). Consequently, diets supplemented with protease and G2 G performed better by releasing the protein and energy bound by tannin.

Feed conversion was not significantly ($P > 0.05$) different for all treatments. Birds fed Kaura diets compared favourably with maize diets in converting feed nutrients to muscle.

The cost of feed per Kg weight gain was not significantly ($P > 0.05$) different for all the treatments. The addition of enzymes to Kaura diets neither increased feed cost nor cost of producing broiler meat significantly.

Table 4.8: **Performance of Broiler Chicks fed Kaura Based diets with Enzyme Inclusion (0-4 weeks)**

Parameters	Maize	Kaura+ phytase	Kaura+ Protease	Kaura+ G2G	Kaura+ Protease+ G2G	
	T1	T2	T3	T4	T5	SEM
Initial wt (g/bird)	87.88	87.42	87.88	87.88	88.33	0.68
Final wt (g/bird)	1166.7^{a}	948.0^{c}	1065.3^{b}	1118.7^{a}	1135.3^{a}	21.23
Wt gain (g/bird)	1078.8^{a}	891.4^{c}	977.5^{b}	1030.8^{ba}	1047.0^{a}	18.00
Feed intake (g/bird)	1900.5^{b}	1874.3^{b}	1990.0^{ab}	2038.8^{a}	2100.9^{a}	39.45
Feed/gain ratio	1.96	2.10	2.04	1.98	2.01	0.14
Feedcost/Kg gain(₦/Kg)	152.66	162.96	159.73	154.64	159.39	8.41

a,b,c, Means with different superscripts on the same row are significantly different ($P < 0.05$)
SEM: Standard error of means

4.3.2 Performance of Finisher Broiler Fed Kaura based diets and enzyme inclusion (5-8 weeks)

The performance of broiler finisher chickens fed different enzyme inclusion in Kaura based diet is shown in Table 4.9. Kaura diet supplemented with protease, G2 G and the combinations had significantly ($P<0.05$) higher final body weight than the phytase supplemented diet. This is in line with the report of Khan *et al*. (2006) who reported that enzyme supplementation improved productive performance of birds fed sunflower-corn based diets.

There were no significant ($P > 0.05$) differences in feed /gain ratio and feed cost per kilogram gain across all dietary treatments. Similar to the observation at the starter phase, birds fed Kaura diets compared favourably with maize diets in converting feed nutrients to muscle and the addition of enzymes to Kaura diets did not increase cost of producing broiler meat significantly.

Table 4.9: **Performance of Finisher Broiler Fed Kaura based diets and enzyme inclusion (5-8 weeks)**

Parameters	Maize	Kaura+ Phytase	Kaura+ Protease	Jandawa+ G2G	Kaura+ Protease+ G2G	SEM
	T1	T2	T3	T4	T5	
Initial wt (g/bird)	936.7	858.0	865.3	918.7	935.3	41.23
Final wt (g/bird)	2486.3[a]	2284.9[b]	2405.5[a]	2473.8[a]	2454.5[a]	62.97
Body wt gain (g/bird)	1549.7	1436.9	1540.2	1555.1	1519.2	61.55
Feed intake (g/bird)	3605.3	3508.8	3743.1	3689.2	3685.7	84.21
Feed/gain ratio (g/bird)	2.33	2.44	2.44	2.31	2.44	0.11
Feed cost/Kg gain (₦/Kg)	182.34	187.29	189.00	179.58	192.11	11.49

a,b, Means with different superscripts on the same row are significantly different ($P<0.05$)
SEM: Standard error of means

4.3.3 Nutrient Digestibility of Finisher Broiler Fed Kaura based diets with enzyme inclusion

The result of nutrient digestibility trial is shown on Table 4.10. There was no significant ($P>0.05$) difference in the coefficient digestibility of dry matter, gross energy, crude protein, crude fibre, nitrogen free extract and ash of broiler chickens fed Kaura and different enzyme inclusion in all the dietary treatments. However, the experimental diets significantly affected ($P<0.05$) the digestibility of ether extract. Ether extract was significantly lowest for the enzyme combination diet and highest for the maize based diet though, this could be as a result of the higher energy content of the maize compared to that of sorghum.

Numerically, the values of coefficient of digestibility of dry matter, crude protein, crude fibre, ash and nitrogen free extract were higher for all the treatments except for Treatment 2 which is the Kaura based diet with phytase. This could be as a result of the presence of enzymes of protease and G2 G releasing much of the available nutrients bound by the presence of tannin in Treatments 3, 4 and 5.

Roxazyme G2G and protease enzyme reduces gut viscosity and nutrient entrapment (mostly starch and protein) and led to better digestion in broilers (Bedford, 2000). Thus, the improved digestibility of nutrients observed in diets with protease and G2 G enzymes could be due to disruption of plant cell wall fraction by xylanase and β-glucanase in Roxazyme G2G and protease. This disruption liberates locked protein and starch for easier digestion, thus improving feed digestibility and the broiler chicken performance. This was supported by the reports of Khan *et al. (*2006) who reported that enzyme supplementation improved productive performance of birds fed sunflower-corn based diets.

Table 4.10: Nutrient Digestibility of Finisher Broiler Fed Kaura based diets with enzyme inclusion

Parameters	Maize	Kaura+ Phytase	Kaura+ Protease	Kaura+ G2G	Kaura+ Protease+ G2G	SEM
	T1	T2	T3	T4	T5	
Dry matter (%)	80.87	77.65	82.00	79.98	80.35	1.69
Gross energy (%)	83.65	86.83	85.26	82.98	80.99	2.28
Crude Protein (%)	83.63	82.20	84.16	84.40	83.74	0.74
Ether extract (%)	91.57[a]	88.26[ab]	89.93[a]	89.69[a]	83.66[b]	1.72
Crude fibre (%)	87.41	83.71	87.19	84.38	84.77	1.69
Ash (%)	77.88	77.13	79.36	80.22	78.74	1.35
Nitrogen free extract (%)	71.48	69.52	73.83	72.10	72.56	1.32

a,b,c Means with different superscripts on the same row are significantly different ($P < 0.05$)
SEM: Standard error of means

4.3.4 Haematological indices of Finisher Broiler Fed Kaura based diets with enzyme inclusion

The haematological parameters of broiler chickens is presented in Table 4.11. The results showed that the packed cell volume (PCV), haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC) and Total protein(TP) were not significantly($P > 0.05$) different between treatments. The non-significant effect of diets on the values indicates that the animals were not anaemic and also implies that the dietary proteins were of good quality (Abu *et al*., 1998). The PCV values obtained from this study were within the normal range of 22-35% (Schalm *et al.*,1975).Total protein in all the enzyme based treatments were comparatively higher than the maize based treatment. This may be attributed to the presence of enzymes in the other diets which helped to improve digestion of nutrients in the feed.

The non significant effect ($P > 0.05$) of neutrophils, lymphocytes, monocytes and basophils shows the effectiveness of enzymes in reducing the anti-nutrients in sorghum.

The blood contains several metabolites which provide useful information on nutritional status and clinical investigation. They are also readily available and fast means of assessing clinical and nutritional health status of animals on feeding trials because ingestion of dietary components have measurable effects on blood composition (WHO.,1963, Egbunike *et. al*., 2009). Generally, the result showed that blood quality and health of birds were not compromised by feeding of Kaura one of the two popular Sorghum varieties in Nigeria.

Table 4.11: Haematological indices of Finisher Broiler Finisher Fed Kaura based diets with enzyme inclusion

Parameters	Maize	Kaura+ phytase	Kaura+ protease	Kaura+ G2G	Kaura+ Protease+ G2G	SEM
	T1	T2	T3	T4	T5	
PCV (%)	29.00	29.67	28.69	30.00	27.67	0.97
Hb (g/dl)	9.73	9.87	9.63	9.97	9.17	0.31
WBC Count ($\times 10^9$/l)	5.67	3.70	3.10	3.13	5.27	1.21
RBC($\times 10^6$/l)	4.70	4.23	4.67	5.03	4.57	0.42
Total Protein (g/dl)	3.07	3.60	3.40	3.13	3.20	0.25
Differential Cell Counts						
Neutrophils (%)	3.67	5.00	8.67	4.00	9.67	2.02
Lymphocytes (%)	95.00	94.33	90.33	94.00	88.00	2.90
Monocytes (%)	0.00	0.00	1.00	0.33	0.67	0.56
Basophils (%)	1.33	0.67	0.00	0.33	1.67	0.98

LOS: Level of significance.
SEM: Standard error of means

4.3.5 Carcass Characteristics and Organ weights of Finisher Broiler Fed Kaura based diets with enzyme inclusion

The carcass cuts and organ weights expressed as percentage of dressed weight and live weight, respectively are presented in Table 4.12. Carcass evaluation of birds showed similar trend as that of growth performance. Dressed weight showed that birds on treatments 1, 3, 4, and 5 were significantly ($P<0.05$) different from birds on dietary treatment 2. This could be as a result of the presence of enzymes of protease and G2 G releasing much of the available nutrients bound by the presence of tannin. Dressing percentage of back and organs like kidney, lungs and intestine length and weight had significant ($P< 0.05$) differences across dietary treatments but with no defined patterns. Other parameters were not significantly ($P>0.05$) different. Adejinmi. *et al* (2007) indicated that carcass quality is closely related to feed intake and level of nutrients especially protein and energy. Therefore, the differences obtained across treatments could be as a result of the differences in the final weight of the birds at the end of the experiment.

Table 4.12: Carcass Characteristics and Organ Weights of Broilers Fed Kaura Based diets with Enzymes

Parameters	Maize	Kaura+ Phytase	Kaura+ Protease	Kaura+ G2G	Kaura+ Protease+ G2G	SEM
	T1	T2	T3	T4	T5	
Live weight (g/bird)	2450.01	2326.67	2436.67	2446.67	2443.33	56.12
Dressed weight (g/bird)	1720.15^{a}	1500.47^{b}	1641.58^{a}	1695.30^{a}	1661.22^{a}	59.4
Dressing percent (%)	70.21^{a}	64.49^{b}	67.37^{ab}	69.29^{ab}	67.99^{ab}	1.49
Cut parts expressed as percentage of dressed weight (%)						
Breast	29.59	29.05	28.19	27.85	27.90	1.17
Drumstick	15.01	15.42	15.34	15.61	15.53	0.60
Thigh	16.37	17.14	16.11	17.16	17.10	0.50
Back	20.80^{b}	22.47^{a}	21.68^{a}	20.44^{b}	21.46^{a}	0.67
Organ weights expressed as percentage of live weight (%)						
Liver	2.22	2.14	2.06	2.11	1.90	0.21
Lungs	0.53^{a}	0.63^{a}	0.60^{a}	0.49^{b}	0.66^{a}	0.08
Kidney	0.46^{ba}	0.39^{b}	0.51^{ba}	0.44^{ba}	0.53^{a}	0.04
Spleen	0.06	0.08	0.07	0.08	0.09	0.02
Gizzard empty	2.30	2.72	2.56	2.28	2.65	0.18
Intestinal weight	3.76^{b}	4.45^{a}	3.62^{b}	3.56^{b}	3.92^{a}	0.28

a,b,c Means with different superscripts on the same row are significantly different ($P<0.05$)
SEM: Standard error of means

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

The inclusion of the enzymes Ronozyme® phytase, Ronozyme® protease and Roxazyme® G2 G were considered in improving broiler chicken diets based on sorghum as a replacement for maize. Two feeding trials were carried out to determine the effects of these enzymes in broiler diets using two common varieties of sorghum namely Kaura and Jandawa.

In the first experiment, the starter phase showed that chicks fed Kaura with phytase inclusion had significantly ($P<0.05$) higher final body weight and weight gain. Feed cost/Kg gain for the eight (8) weeks period showed that birds fed Kaura with phytase had significantly ($P<0.05$) lower cost of production. The dressing percentage was significantly ($P<0.05$) highest for birds fed Kaura diets (T3 and T5). Diets with phytase inclusion had significantly ($P< 0.05$) higher tibia calcium and phosphorus contents. There were no significant ($P>0.05$) differences in blood calcium and phosphorus but values were numerically higher for diets with phytase.

Results of the second experiment showed that chicks fed Kaura with protease and G2 G inclusion had significantly ($P<0.05$) highest final body weight and weight gain. Feed/gain ratio and feed cost/Kg gain were not significantly ($P>0.05$) different. Finisher broiler chickens showed significant ($P<0.05$) differences in the final body weight. Kaura diet with phytase had significantly lower final body weight and dressing percentage than all the other treatments.

Parameters measured for blood quality were not significantly ($P > 0.05$) different for all the treatments.

At the end of the experimental trials, enzyme addition improved growth performance, bone mineral contents. Health status of the birds was not compromised and feed cost of production was significantly reduced.

5.2 Conclusion

Complete replacement of maize with Kaura and Jandawa variety of sorghum did not impair body weight, feed intake, feed conversion ratio, nutrient digestibility, carcass and organ weights and bone mineral content in the diets of broiler chicken. Phytase inclusion in sorghum based broiler diets improved growth performance and calcium content of bones.

Roxazyme® G2 G and the combination with Ronozyme Protease in Kaura based diets improved growth performance for broiler starter chicks and also resulted into comparable performance with maize based diets for broiler finishers. Haematological indices showed that blood quality and the health of chickens were not compromised with the feeding of Kaura diets supplemented with enzymes.

5.3 Recommendation

Sorghum, particularly Kaura variety with Ronoxyme® phytase, Ronozyme® protease combined with Roxazyme® G2 G inclusion should be used for effective utilization and improved growth performance by broilers chickens.

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APPENDIX 1

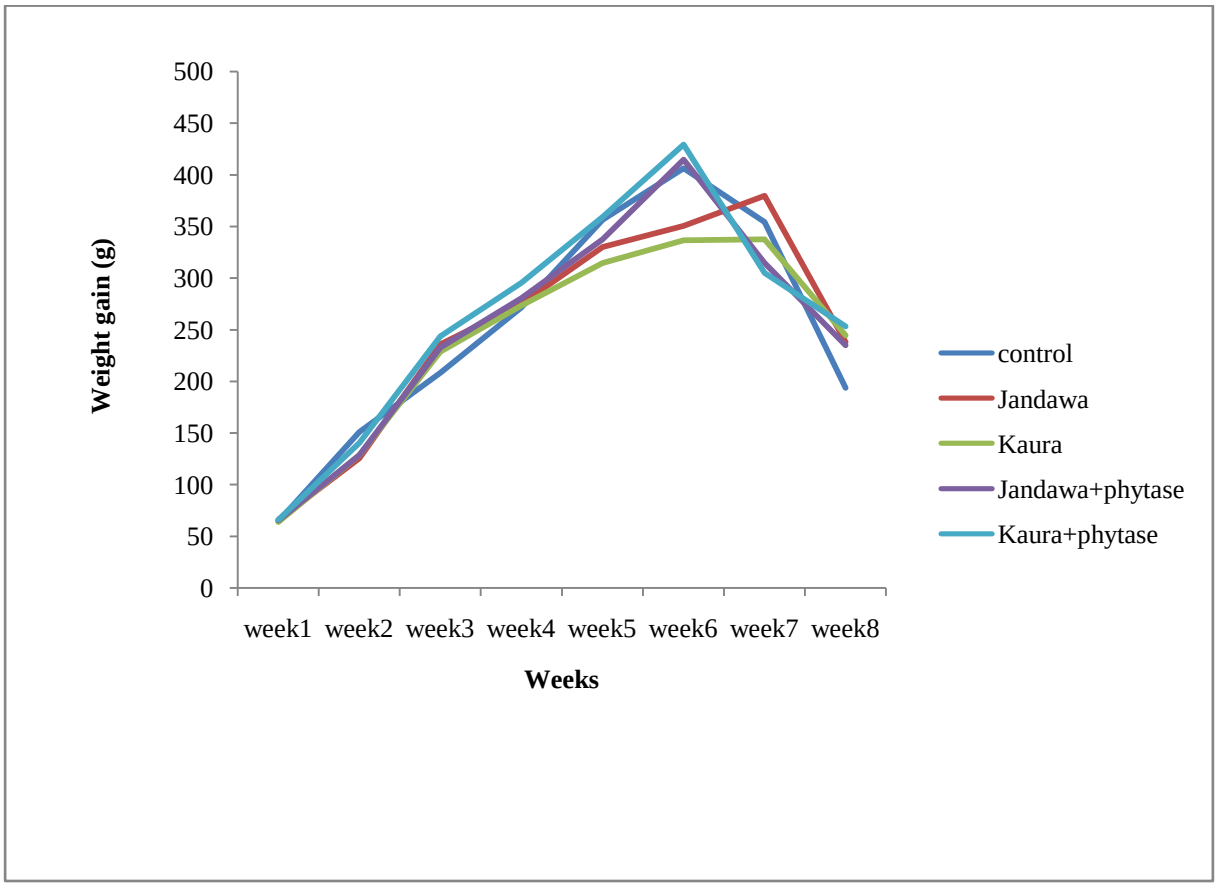


Figure 1: Weekly weight gain of broiler chickens fed Kaura and Jandawa based diets with and without phytase enzyme inclusion (0-8 weeks)

APPENDIX 2

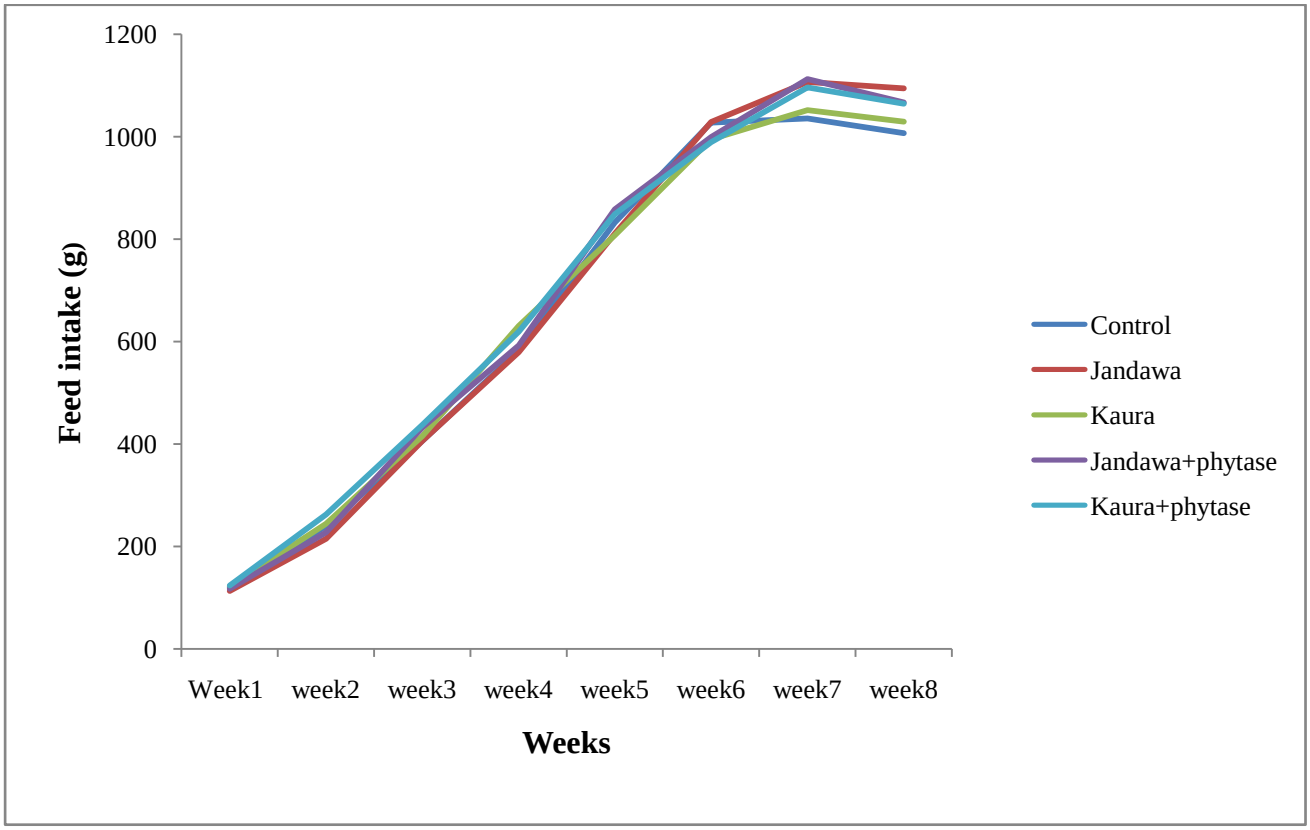


Figure 2: Feed intake of broiler chickens fed Kaura and Jandawa based diets with and without enzyme inclusion (0-8 weeks)