EGG CHARACTERISTICS AND LAYING PERFORMANCE OF HENS FED GRIT-BASED DIETS FROM TWO CASSAVA (*Manihot esculenta* CRANTZ) VARIETIES SUPPLEMENTED WITH ENZYMES

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CERTIFICATION

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DEDICATION

This thesis is dedicated to *Dreams*. They do come true.

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I express my heart-felt gratitude to my late supervisor, Prof. O. O. Tewe. I had the rare privilege of being supervised by this great scholar throughout my university education right from undergraduate days. During this space of time, my perception of him grew from reverential fear to admiration. I will forever be indebted to him for the values he instilled in me, especially that of hard work. It was while working with him from my undergraduate days that I acquired the tenacity required of a livestock farmer. On his farm, he exposed me to the rudiments of day-to-day livestock husbandry. You could not work with him if you were lazy. If you were not very brilliant, Prof. Tewe could tolerate you and assist you to stretch mentally, but you could not just survive around him if you were lazy!

More than the role of supervising my academic projects, Prof. Tewe also played the role of a father. He never gave up on me when I made mistakes or committed errors of judgement. It appeared to me at a time that he even believed in me more than I believed in myself. Sometimes, he would give me assignments that I had never handled before and leave me to make all the decisions! One of such assignments that I would never forget was when he needed to castrate some piglets on his farm and he just asked me to handle the job. My heart almost jumped out of me! Yes, he knew I was an Animal Health Technologist (having obtained National Diploma in Animal Health and Production Technology) before coming to study Animal Science at the University of Ibadan, but I had never single-handedly carried out castration before. I could not afford to fail him and therefore prepared myself well to carry out the task. To my utmost relieve, the exercise was successful. And to my utmost surprise, Prof. Tewe didn't heap accolades on me. As far as he was concerned, he was not experimenting with me as he never doubted my capacity to successfully castrate the piglets. He therefore did not expect anything less of me. Period! He brought out potentials I never thought existed in me.

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ABSTRACT

Maize which serves as the main dietary energy source for poultry production is bedevilled with vicious seasonal scarcity. Cassava grits has similar metabolisable energy as maize and could replace dietary maize for laying hens. However, there is dearth of information on the effects of grits from different cassava varieties on egg attributes and performance of hens. Thus, egg characteristics and laying performance of hens fed grit-based diets from two cassava varieties with supplemental enzymes were investigated.

Eight weeks old ISA Brown pullets (n=364), weighing 0.57±0.02 kg were randomly allotted to 13 isocaloric and isonitrogenous diets, of equal number of pullets, replicated four times. Maize was replaced with TME 419 cassava grits at 0 (T_0), 33 (T_1), 66 (T_3) and 100% (T_5) without enzyme and with supplemental carbohydrase-phytase-protease enzyme-cocktail (T_2, T_4, and T_6, respectively). Similarly, maize was replaced with TMS 01/1368 cassava grits at 33, 66 and 100% without enzyme-cocktail (T_7, T_9 and T_{11}, respectively) and with the supplemental enzyme-cocktail (T_8, T_{10}, and T_{12}, respectively), in (2×3×2)+1 augmented factorial arrangement in completely randomised design. The pullets were fed *ad libitum* till week 18, then from week 19 on corresponding layer diets till week 72. Hen-Day Egg Production-HDEP (%), Yolk Weight-YW (g) and Haugh Unit-HU, were assessed at week 22-38 (Early Laying Phase-ELP), 39-55 (Mid Laying Phase-MLP) and 56-72 (Late Laying Phase-LLP). At week 72, blood (5 mL) was sampled and analysed for Alanine Amino Transferase-ALT (IU/L), Aspartate Amino Transferase-AST (IU/L) and thiocyanate (mg/dL) using standard methods. Data were analysed using descriptive statistics, regression and ANOVA at $\alpha_{0.05}$.

The HDEP of 42.3±1.2 (T_0), 42.6±1.8 (T_1) and 42.4±1.8 (T_2) at ELP; and T_2 at MLP (79.9±2.5) and LLP (73.4±3.0) were significantly higher than in other treatments. The YW at ELP in T_0 (14.2±0.3), T_1 (14.3±0.2) and T_2 (14.3±0.3); and at MLP and LLP in T_0 (17.8±0.4; 18.9±0.4), T_1 (17.7±0.4; 18.9±0.4), T_2 (18.1±0.3; 19.1±0.5), T_7 (17.7±0.5; 19.1±0.4) and T_8 (17.8±0.4; 19.1±0.5), respectively were significantly higher than in other diets. Increased dietary inclusion levels of grits resulted in linear reduction of HU at ELP (R^2 = 0.94), MLP (R^2 = 0.99) and LLP (R^2 = 0.99). The HU of 93.3±1.4 (T_0), 93.2±1.8 (T_1), 93.0±1.7 (T_2), 92.7±1.4 (T_7), and 93.4±2.0 (T_8) at ELP; 90.6±1.2 (T_0), 90.4±1.0 (T_1), 90.1±0.9 (T_2), 90.0±2.4 (T_7), and 89.9±0.9 (T_8) at MLP were significantly higher than in other diets. Similarly, HU of 87.3±1.7 (T_0), 87.2±1.5 (T_1), 86.9±1.7 (T_2), 86.8±1.0 (T_7), and 86.6±1.2 (T_8) at LLP, were significantly higher than in other diets. The ALT of hens on different treatments were similar, indicating no dietary hepatoxicity. However, AST of hens on T_0 (65.6±2.8) was significantly lower than in other diets. Thiocyanate of 0.29±0.0 (T_0) was significantly lower than 2.07±0.1 (T_5), 2.10±0.1 (T_6), 2.13±0.1 (T_{11}) and 2.13±0.1 (T_{12}).

Replacement of maize with TME 419 grits at 33% despite enzyme supplementation, in the early laying phase, and 66% with enzyme supplementation at the mid and late laying phases, sustained hen laying performance. However, egg quality decreased with the inclusion of cassava grits, despite enzyme supplementation.

Keywords: Cassava grits, ISA Brown pullets, Supplemental enzyme, Hen-day egg production, Haugh unit

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CHAPTER 1

INTRODUCTION

Energy makes up the highest percentage of poultry feed for efficient productivity (Tewe, 2004), while poultry will feed primarily to satisfy their caloric requirement (Scott *et al.,* 1982). Over the years, corn has remained the dominant energy source in poultry feed for the intensive as well as the semi-intensive rearing systems in Nigeria. Even on a global level, empirical data exist to support the fact that between 60-70% of dietary energy in poultry feed is supplied by cereal grains (Ahiwe *et al.,* 2018).

The forecast for world population was estimated to hit 9.7 billion by the year 2050 (UN DESA, 2015). Therefore, with more competition by humans for maize as food and industrial usage, the poultry industry is likely to continue to find itself more at the receiving end of the paucity of the ingredient.

The over dependence of the Nigerian poultry industry on grains especially maize portends grave danger for the sustenance of the industry. In recent years, owing to crop failure made more intense by competition with the humans, the poultry industry has witnessed crippling scarcity of maize. This resulted into importation of the grain and prompted government to open up its grain reserves to cushion the effect.

In spite of all the interventions by both government and the livestock private sector, most poultry enterprises either had to fold up or at least down size. This was to enable them cope with the crippling effect of maize scarcity and its resultant unbearable cost. With emerging trends, the situation is expected to get worse. In the USA which has become a major source of imported maize in Nigeria, maize price increased by 71.16% within the ten-year period of September 2005 to September 2015. (USDA, 2015).

There is therefore a continuous and increasing need for alternative energy sources for the poultry industry. The Nigerian Government had long ago recognised this need with

the constitution of the Presidential Task Force on Alternative Formulations in 1989. The task force ascertained the possibilities of feed formulations from a variety of alternative ingredients (among which is cassava) as energy source. Nigeria, the foremost cassava-producing nation, stands at an advantageous position in the utilisation of the root crop in livestock feeding. The annual production of cassava in Nigeria was predicted to hit 49 million metric tons in 2008, 7% increment over 2007 production statistics (FAO, 2008). This advantage is further strengthened by the fact that cassava is the highest producing crop with regard to energy output per unit area of land and also tolerant to disease, shortage of rainfall and poor soils (Garcia and Dale, 1999; Chauynarong *et al.,* 2009).

The large tonnage of cassava production in Nigeria is however yet to reflect in its actual utilisation as a livestock feed ingredient. According to statistics, only 6% of cassava utilisation is accounted for feed in Africa and Asia compared to 47% in Latin America and the Carribean (FAO, 2000). This low share of utilisation of cassava in livestock feed especially poultry has been due in part to its dusty nature when used as chips or even pellets. Another reason is the presence of cyanogenic glycosides that liberate hydrocyanic acid.

The emergence of cassava grits which is unpeeled, unsieved, unfermented, gelatinised and granular product (Tewe, 2005) has successfully eliminated the dustiness associated with cassava. This is due to gelatinisation which is brought about by application of heat during product development. Apart from aiding to eliminate dustiness, gelatinisation also improves digestibility of the starch component of cassava as cooking reportedly increased the digestibility of raw cassava starch from 48.3% to 77.9% (Tewe, 2004).

Investigations had been carried out on the utilisation of diverse forms of the whole cassava roots in poultry feed (Stevenson and Jackson,1983; Gowdh *et al.,* 1990; Tewe, 2004). However, there is dearth of data on utilisation of cassava in poultry diets with recourse to cassava varietal types which exhibit different nutritional profile. Also, while enzymes have been used in formulations comprising diverse grains (Bedford, 1995; Cowieson *et al.,*2006), few investigations have been carried out on enzyme-supplemented poultry diets containing whole cassava products. Stevenson and Jackson (1983) observed viscosity or stickiness of the excreta in poultry fed diets comprising up to 50% cassava root meal. This necessitates investigating the use of supplemental

enzyme in cassava-based diets for poultry. Choct and Hughes (1997) implicated high level of non-starch polysaccharides in increased gut viscosity in poultry. This study was therefore aimed at investigating the effects of cassava varietal type and supplemental enzyme on the performance and egg characteristics of laying chickens fed cassava grits-based diets.

1.1 Justification of the study

Caloric gap created in the poultry industry in Nigeria almost on a seasonal basis owing to scarcity and consequent unbearable cost of maize due to disease, drought, poor storage and usage for other products requires a sustainable intervention. Cassava possesses the greatest potentials to bridge the caloric gap with its reputation as a highly productive crop with regard to energy yield per unit area of land (Ravindran and Blair, 1991) and its characteristic as a crop which could be cultivated on low quality soils (Tewe, 2004)

Through vigorous breeding programmes, new varieties of cassava have emerged for different intended purposes. Studies have shown significant differences in chemical compositions of cassava due to varietal types (Manano *et al.,* 2018). However, past investigations on the utilisation of cassava in the diet of poultry were not variety-specific. There is therefore dearth of information on the effects of cassava varietal type in the diet of poultry.

Utilisation of multiple enzymes in poultry nutrition has gained prominence in commercial feed production. However, this is mostly in grain-based diets. There is still scanty information on the effects of supplemental enzyme in whole cassava-based diets of laying chickens.

1.2 Objective of the study

1.2.1 General objective

To widen the exploitation of the caloric potentials of cassava in grits form as a replacement for maize in the diets of laying chickens.

1.2.2 Specific objective

To evaluate the effect of cassava varietal type and enzyme supplementations on performance, egg characteristics, serum biochemical and haematological indices of hens fed cassava grits-based diets at the growing and laying phases.

1.3 Hypotheses

H_{01}: There are no significant differences in the performance, egg characteristics, serum biochemical and haematological indices of hens fed enzyme-supplemented cassava grits-based diets from two varieties of cassava at the growing and laying phases.

H_{A1}: There are significant differences in the performance, egg characteristics, serum biochemical and haematological indices of hens fed enzyme-supplemented cassava grits-based diets from two varieties of cassava at the growing and laying phases.

H_{02}: Interactions of grits from the different varieties of cassava and dietary enzyme supplementation will not have any significant effects on performance, egg characteristics, serum biochemical and haematological indices at the growing and laying phases

H_{A2}: Interactions of grits from the different varieties of cassava and dietary enzyme supplementation will have significant effects on performance, egg characteristics, serum biochemical and haematological indices at the growing and laying phases

CHAPTER 2

LITERATURE REVIEW

2.1 Energy requirement of poultry

Poultry can obtain their energy requirement from simple carbohydrates, lipids and protein (Ravindran, 2013). The chosen level of dietary energy in feed formulation for poultry is often the determinant of nutrients' densities in the diet. This point of view in formulating poultry diets is predicated upon the belief that poultry feed first and foremost for their energy requirements to be met, provided the feed contains the requisite essential nutrients (Scott *et al*., 1982).

Whenever dietary energy composition is altered, there will be change in intake of feed, and specifications for other nutrients must therefore be adjusted accordingly for the required intake to be maintained. The requirement of energy for metabolic purposes is as diverse as the classes of poultry and a deficiency in the required energy will adversely affect productive performance. For the sustenance of optimum productivity, modern strains of poultry are usually fed comparatively high-energy diets. Energy level of the diet adopted in each circumstance is dependent on available energy-rich feed ingredients as well as the cost. Owing to cost considerations posed by the grains, especially, maize, the utilisation of low-energy diets for poultry feeding is a common phenomenon in many developing countries (Ravindran, 2013)

In the laying hens, energy requirement for egg production is dependent on rate of lay, egg mass and composition (Larbier and Leclercq, 1994). The cost of energy of egg production has been estimated to be in the range of 8.0-13.2 MJ/kg (1,910.8 – 3,152.82 kcal/kg) of egg mass (Sakomura, 2004).

2.1.1 Bioenergetics

In commercial poultry, dietary energy has the largest chunk of the cost consideration in feed formulation. In *ad libitum* feeding, the avian species tend to eat primarily for their energy needs to be satisfied. Connection between requirement for energy and intake is essentially fundamental in empirical poultry feed formulation as the concentration of dietary energy significantly influences the intake of all other nutrients (Lopez and Leeson, 2008). Nutrients' intake can thus be regulated through the combination of different feed ingredients to form complete feed with predetermined nutrients: energy ratios (Sibbald, 1980).

Energy is by no means a nutrient but an attribute of energy-furnishing nutrients when oxidised during metabolism (NRC, 1994). The total or gross energy, otherwise known as heat of combustion, of a feed ingredient is not automatically made completely available to the bird due to indigestible substances such as the cellulose, lignin and pentosans. The gross energy, measured with the use of bomb calorimeter at 25-30 atmospheres of oxygen, is energy generated as heat when a feed substance is totally oxidised into CO_2 and H_2O (Olomu, 1995).

The intake of a unit of feed energy (IE) leads to voiding of energy in the faeces (FE), the difference between the two (IE - FE), being apparent digestible energy (ADE). Digestible energy is the energy that is absorbed in the intestinal tract. The term "apparent" however, is employed because FE comprises indigestible residues and an endogenous or metabolic fraction (FE_m) made up of worn-out cells from the intestinal mucosa, bile and digestive fluids. The FE_m represents a body maintenance cost which is not charged against the feed (Sibbald, 1980). The metabolisable energy (ME) represents the digestible energy lost in the urine, resulting from the excretion of unrequired compounds such as uric acid and ammonia (Olomu, 1995). In most countries, the ME is used as the default method for calculating energy values from feed ingredients for poultry (Farrell, 1999). In poultry, true metabolisable energy (TME) is the gross energy of the feed consumed less the gross energy of the excreta of feed origin. Net energy (NE) is metabolisable energy less the energy which is wasted as heat increment. It is made of energy utilised for maintenance, or for maintenance and production (NRC, 1994).

2.2 Cassava

2.2.1 Origin, distribution and production

Cassava (*Manihot esculenta* Crantz) was brought by the Portuguese in 1558 from America to Africa (Rogexr, 2014). In Africa, it was established and cultivated, producing and contributing above fifty percent of global output (Guira *et al.,* 2017). Cassava is a short-lived perennial shrub cultivated in areas between the latitudes 30^0 N and 30^0 S, a region that circumscribes most of the world's poorest countries (Bokanga, 1993). Owing to its adaptation to regions known for long dry spells and uncertain rainfall, cassava has the reputation of a drought-tolerant crop (Cock, 1985). Apart from success of its cultivation in drought-prone areas, cassava is also known for its tolerance to low quality soils and diseases (Chauynarong *et al.,* 2009). Cassava is the highest-yielding cultivated plant when energy production by means of specified size of land is considered (Ravindran and Blair, 1991), yielding between 25 and 60 tonnes/ha in the tropics (Garcia and Dale, 1999).

On a global level, it was estimated that about half a billion humans are dependent on cassava as a main energy source (Montagnac *et al.,* 2009). As a result, the root crop is the world's third primary source of carbohydrate food for humans. (Fauquet and Fargette, 1990). The most important cassava-producing countries in the world are Nigeria, Brazil, and Thailand, with Thailand being the largest exporter (Garcia and Dale, 1999).

2.2.2 Chemical composition of cassava root

Cassava is a carbohydrate source with a moisture content of 60-65 percent, 20-31 percent carbohydrate, 0.2-0.6 percent ether extracts, 1-2 percent crude protein, and a low level of vitamins and minerals. The crude fiber content of the carbohydrate fraction ranges from 3.2 percent to 4.5 percent (Balagopalan *et al.,* 1988*)* which comprises principally cellulose and other non-starch polysaccharides (Rattanachomsri *et al.,* 2009). Cassava root is however high in calcium and vitamin C as well as thiamine, riboflavin and nicotinic acid. The carbohydrate fraction of cassava root is primarily made up of starch, which accounts for 64-72 percent of the total. The quantum of starch increases as the plant matures and reaches its peak between 8 and 12 months after planting, after which it declines as the fibre level increases (Tewe, 2004).

In comparison to maize, which has a starch content of 28 percent amylose and 72 percent amylopectin, cassava raw starch contains 17 percent amylose and 83 percent amylopectin. (Chauynarong *et al.,* 2009) Raw cassava starch has a digestibility of 48.3%, a value that increased to 77.9% with cooking. Cassava root also contains limited quantities of sucrose, maltose, glucose and fructose (Tewe, 2004). For poultry, the metabolisable energy of cassava root meals ranged between 2,870 and 4,270 kcal ME/kg of dry matter (Khajarern and Khajarern, 1991).

Studies have however shown that due to varieties, different sources and ages during harvest, ecological conditions and processing methods, variation exists in the major components of chemical composition of cassava product (Richardson, 2013).

2.2.3 Utilisation of cassava as livestock feed

The proportions of total cassava production which is utilised as feed for livestock in Africa is perhaps understated as goats, sheep and swine are fed roots and foliage either as fresh or processed forms on small-scale farms in cassava-producing areas (Nweke *et al.,* 2002). According to Garcia and Dale (1999), the peels are also utilised as ruminant feed in tropical Africa and chips and pellets are the typical forms of feed ingredients from the roots. The chips are dried shredded root in different dimensions, shapes and quality as determined by drying rate and introduction of impurities usually sand at processing. Cassava pellets are identically cylindric products with dimensions that range from 0.5 to 0.8 cm diameter and 1.0-2.0 cm in length (Khajarern and Khajarern, 1991).

Tewe (2005) however, produced a patented product called cassava grits which is an unpeeled, unfermented, un-sieved, and gelatinised cassava product. Although roasted like the "gari" for human consumption, it is different because it is neither fermented nor peeled. Processing of fermented cassava products like the "gari" involves peeling of the roots to eliminate the thin brown outer covering and the thicker leathery parenchymatous inner covering which together constitute between 10 and 20 percent of the weight of the roots on wet basis (Obadina *et al.,* 2006). During the process of roasting like that of "gari", the starch is gelatinised which removes the property of dustiness, a major setback associated with the utilisation of most cassava products (Garcia and Dale, 1999). According to Tewe (2004), gelatinisation also improved the

digestibility of starch. Gelatinisation is the permanent loss of the structural regions in starch granules which occurs during heating in the presence of moisture. Amount of moisture and amylose-amylopectin ratio of the starch determine the range of temperature at which the granules lose their crystalline structure. (FAO, 1998).

Studies so far conducted to assess the substitution of grains, most especially with cassava meal, have shown large variations in feeding value, nutritional encumbrances as well as performance of the birds. (Jalaludin *et al.,* 1973; Omole, 1977; Tewe, 1984). Jalaludin *et al.* (1973) and Omole (1977) reported that chronic cassava toxicity lowered both hen day egg production and quality in laying chickens. However, Tewe (1984) obtained a satisfactory performance by poultry fed cassava-based diets with the total HCN of the feed not exceeding 100 mg/kg. Tewe and Bokanga (2001) reported that a mix of cassava root and leaves in the ratio 4:1 totally replaced maize in the diet of poultry and reduced cost without a loss in weight or hen day egg production.

It was observed that cassava root meal caused depression in growth with replacement levels of 48.6% in the diet (Gowdh *et al.,* 1990). According to Stevenson and Jackson (1983), diets comprising 50% cassava root meal had no major effect on body weight. They nevertheless observed that the excreta were sticky and therefore considered the root meal to be acceptable at a replacement level of not more than 30% in the diet.

2.2.4 Antinutritional factors in cassava

Compared to maize, cassava has low protein content, which necessitates its utilisation in diets of poultry to be dependent on quality of dietary protein. Also, the cyanogenic glycosides of cassava namely linamarin and lotaustralin, on hydrolysis release hydrogen cyanide (HCN) which is highly toxic (Garcia and Dale, 1999). According to Zagrobelny *et al.* (2004), cyanogenic glycosides are phytoanticipins which are documented to be available in more than 2,500 plant species. Because of their bitter taste and the release of toxic hydrocyanic acid when plant tissues are disrupted, phytoanticipins play an important role in nature's defense of plants against herbivores. There is a high concentration of cyanogenic glycosides in cassava leaf, stem and root peel (rind) (Nambisan, 1994).

According to Cooke (1983), cassava cyanide can be found in three forms: bound glycosides, cyanohydrins, and free cyanide, and each of these forms has a different toxicity and responds differently to cyanide removal techniques.

Occurring either as liquid or gas, hydrogen cyanide possesses no colour and has a slightly perceptible smell of bitter almond. It dissolves easily in water to form hydrocyanic acid or prussic acid or formonitrile which is poisonous and therefore considered to be an antinutritional factor (ATSDTR, 1993).

The cyanogenic glycosides are hydrolyzed by the enzyme linamarase found in cassava root peel, glycosidic enzymes of intestinal microflora (Fomunyam et al., 1984), acid hydrolysis in the intestine, and glucosidases found in the liver and other tissues (Padmaja and Panikkar, 1989). Maximum limits for hydrogen cyanide in cassava food is 10 ppm (10 mg/kg dry weight) (Codex Alimentarius International, 1998). According to Tewe (2004), levels not exceeding 100 ppm were considered safe for chips and pellets imported from Indonesia and Thailand into the European Union for feeding of all classes of livestock. The chemical structures of linamarin and lotaustralin have been clearly elucidated (EFSA, 2004) and are shown in Figures 2.1 and 2.2, respectively.

According to Oke (1978), powdered starch in cassava-based diets is known to elicit ulcerogenic effects on the gastric mucosa. Furthermore, the high fibre and ash content of cassava produce adverse effects in cassava-based diets and restrict the usage of other feed materials that are equally high in these components. Conversely, with adequate nutritional manipulations, cassava can be utilised to replace maize in livestock feed with a resulting dual advantage of sparing maize for human consumption and production of less costly feed and perhaps more affordable animal products.

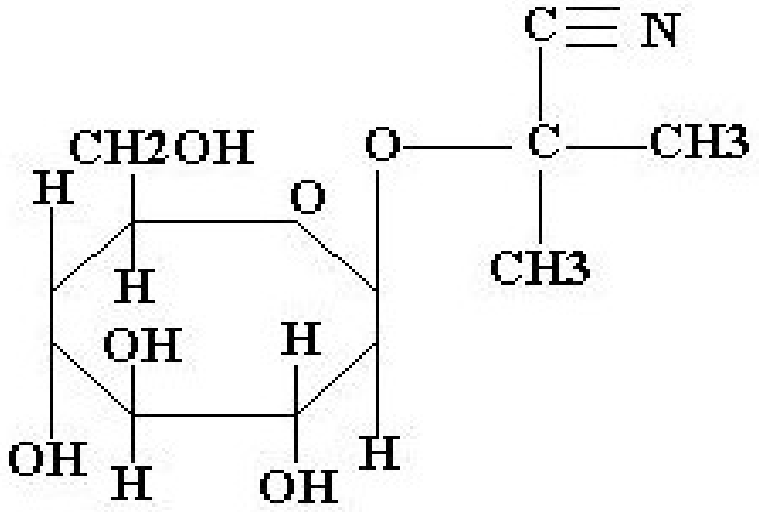

Figure 2.1: Chemical structure of linamarin

Chemical formula: $C_{10}H_{17}NO_6$

CAS Number: 554-35-8

Molecular weight: 247.247

Source: EFSA, 2004

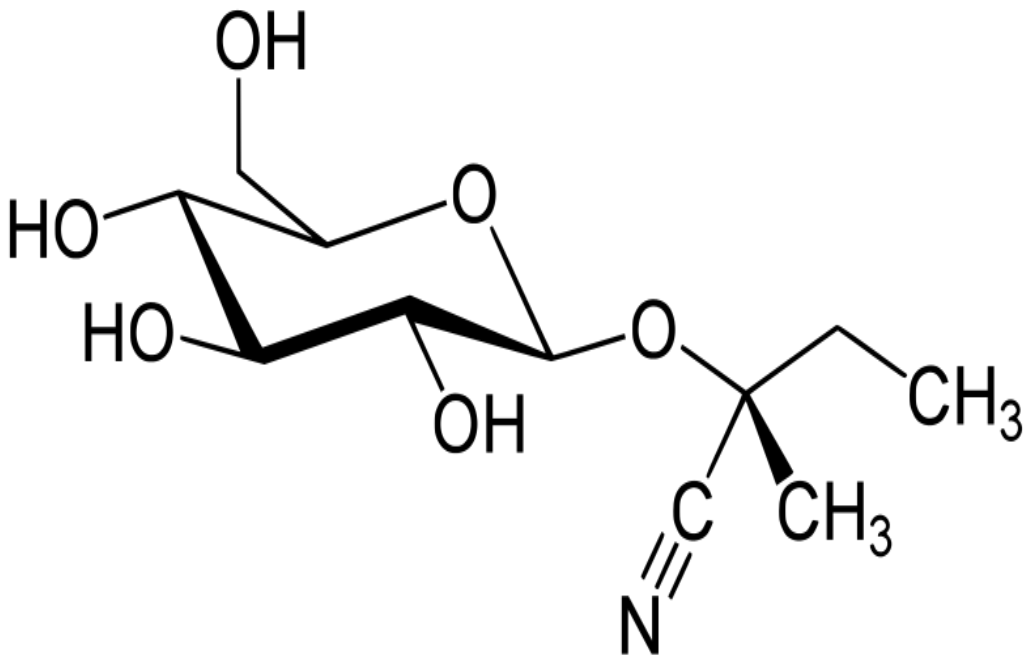

Figure 2.2: Chemical structure of lotaustralin

Chemical formula: $C_{11}H_{19}NO_6$

CAS Number: 534-67-8

Molecular weight: 261.272

Source: EFSA, 2004

2.2.5 Cyanide toxicity

Cyanogenic compounds naturally occur in many foods as component of sugars or as other compounds that occur naturally in some foods derived from plants like lima beans, almonds, soya bean, sorghum and cassava roots (ATSDR, 2006). Hydrogen cyanide and its simple salts are among very active toxicants that affect the central nervous system (CNS) resulting in immediate collapse and cessation of respiration. The earliest symptoms with smaller doses are frailty, cephalalgy, mental disorientation, sometimes nausea and vomiting. In severe toxicity, colouration of blood in the veins appears bright reddish, indicative of lack of ability of tissues to utilize oxygen (Hartung, 1982).

In studies involving rats and rabbits exposed to cyanide toxicity via the buccal cavity, the greatest accumulations of the compound were found basically in the liver and also in the blood, lung, brain and spleen. It was found that death resulted in the rats in less than 10 minutes after exposure to HCN poisoning at 1,180 ppm (Yamamoto *et al.,* 1982). According to Jantz and Uluwaduge *(*1997), consumption of foods containing low levels of cyanide for protracted periods leads to impairment of the CNS and the thyroid gland in humans. Kamalu (1995) reported that consumption of cyanide-containing food for a long period could result to loss of hearing, eye problem and impairment of ability to coordinate the muscles and cretinism. Makkar and Beckar (1998) also noted pancreatic diabetes, cobalamin inadequacy and impaired iodine absorption due to cyanide toxicity.

In populations with long-term consumption of cassava-based food products leading to iodine deficiency, cyanide liberated from residual linamarin has been linked with goitre, though HCN can readily be eliminated in the course of processing cassava (Taga *et al*., 2008). Thiocyanate (metabolite of cyanide) has an effect on the thyroid gland which is dose-dependent and moderated by homeostatic processes which strictly direct the synthesis of thyroid hormones to facilitate a sustained systemic supply to satisfy physiologic requirements (NRC, 2005). In a situation where thiocyanate interferes with iodide uptake significantly to lower the secretion rates of thyroid hormones, circulating levels of these hormones drop. Homeostatic mechanisms moderated principally via the hypothalamo-pituitary-thyroid feedback axis are quickly

triggered to modulate thyroid hormone synthesis (NRC, 2005). As the level of these hormones in the blood decreases, the pituitary gland is stimulated by the hypothalamus through the release of thyrotropin-releasing hormone, to produce thyroid stimulating hormone (TSH). The thyroid gland is stimulated by the TSH to raise the rate of its secretion thyroid hormones. Increased TSH levels elevate thyroid secretion and prompt histological alterations for example, increased thyroid cells size and number, manifested as enlarged thyroid gland (goiter). (Manzano *et al*., 2007).

2.2.6 Detoxification of cassava

There are various methods of processing cassava to mitigate cyanide toxicity and improve on stability and shelf-life. According to Cardoso *et al.* (2005), methods of processing that involve crushing and grating efficiently detoxify cassava thoroughly as the cells are fractured, facilitating a direct contact between linamarase and the linamarin.

Techniques employed in the processing of cassava roots include peeling, drying, roasting, steaming, boiling, and fermenting. In the tropics, drying is the most popular technique of reducing cyanide and because sun-drying presents a longer duration of exposure of the cyanogenic glycosides to the enzyme linamarase, it is more efficient in cyanide detoxification than oven drying. Cyanide can almost be entirely eliminated when crushing the roots which increases the contact surface precedes sun drying. (Garcia and Dale, 1999). In Africa, two connected techniques that are frequently employed to produce cassava food products are soaking and fermentation. In West Africa, the production of "gari", which is a common staple food, involves grating of the peeled tubers, dewatering, fermentation and roasting of the fermented product. These stages of processing could bring about between 80 and 95% detoxification in the product (Padmaja, 1995).

During *in vivo* detoxification, the ingestion of sub lethal doses of cyanide stimulates the defense mechanism of the body to initiate detoxification to a less toxic product. According to Balagopalan *et al.* (1988), the principal pathway for the detoxification of cyanide is controlled in the liver by the endogenous enzyme rhodanese (thiosulfate: cyanide sulfurtransferase; E.C.2.8.1.1) which catalyses the conversion of cyanide to

thiocyanate by utilising sulphur from a donor. Conversion occurring via 3-mercaptopyruvate sulfur transferase accounts for 60–80% of a cyanide dose. There are however other minor pathways for cyanide metabolism which include incorporation into a 1-carbon metabolic pool or conversion to 2-aminothiazoline-4-carboxylic acid via reaction with cystine (ATSDR, 2006). According to Wood and Cooley, 1956, this accounted for about 15% of an injected dose of cyanide in rats. Furthermore, minute quantities are either converted to CO_2 or expelled unaltered as HCN in exhaled air (US EPA, 2010). Sorbo (1953) established that rhodanese contained an active disulfide group which takes part in the reaction. Auriga and Koj (1975) observed that rhodanese is largely spread in the body with the top concentrations found in the liver and kidney. The capacity of animals to handle cyanide is therefore contingent upon concentrations of rhodanese in the liver, which varies from one species to the other. Aminlari and Gilanpour (1991) found out that of the domestic animals studied, sheep had the highest concentration of rhodanese activity in the liver followed by the liver of camel which had one-third of the activity of that of the sheep, while the dog liver was found to have the lowest rhodanese activity, only 4% of the activity of sheep liver.

According to Westley (1981), the detoxification process by the rhodanese enzyme utilises sulphur from methionine which Adegbola (1977) observed increased the requirement for the total sulphur amino acid. However, in birds, if the requirement for methionine is met, other sources of sulphur could be utilised for the detoxification process (Oke, 1978). Thiocyanate is largely spread throughout body fluids including saliva, in which it can easily be detected. In normal health, a dynamic balance between cyanide and thiocyanate is maintained. A protein-deficient diet, especially one which is low in sulphur-containing amino-acids may impede the detoxification capacity and thus make someone more susceptible to the toxic effect of cyanide (Oke 1969).
The pathways for the metabolism of cyanide are presented in Figure 2.3.

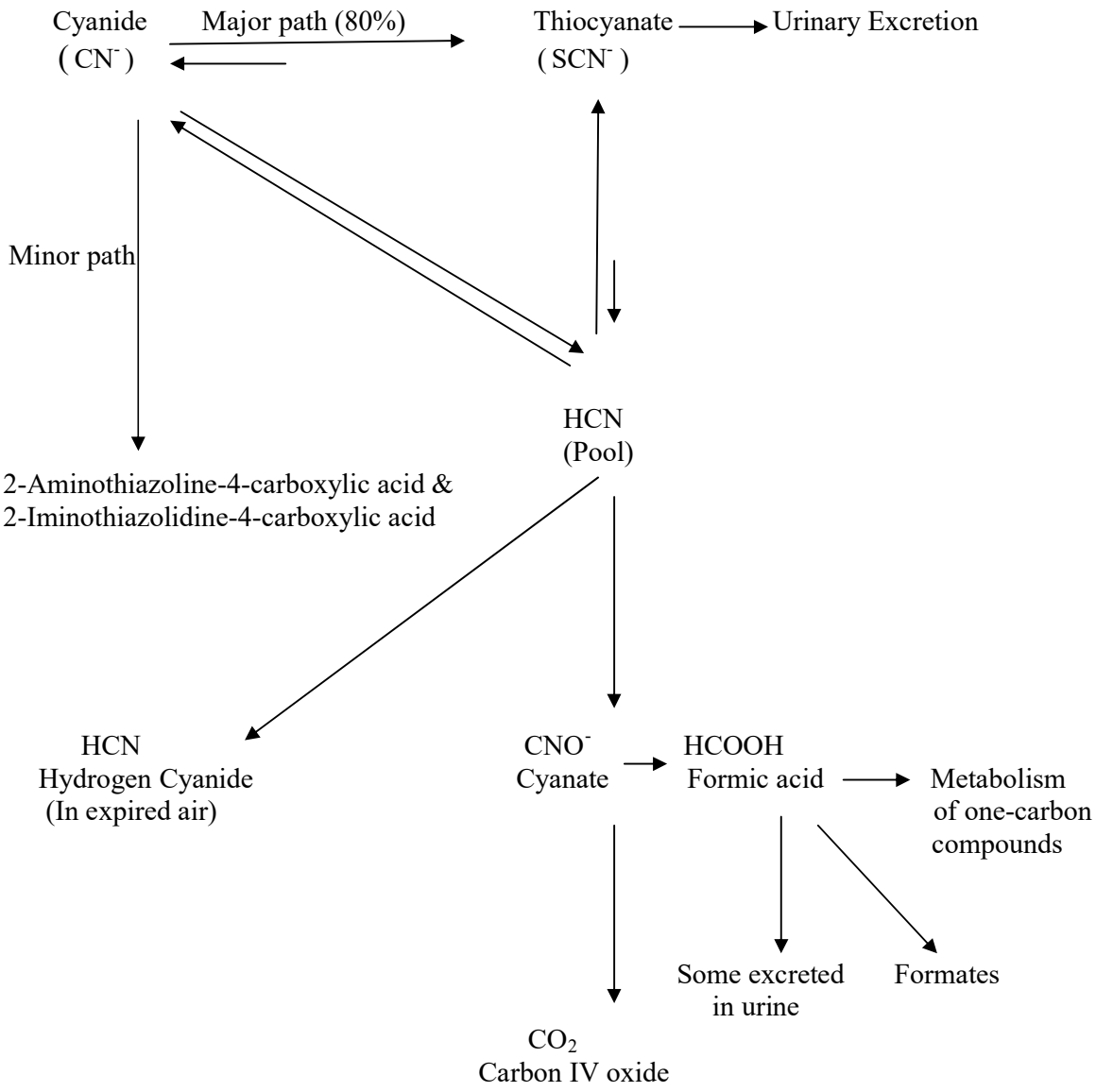

Fig 2.3: Cyanide Primary Metabolic Pathways
Source: Adapted from Ansell and Lewis (1970)

2.3 Enzymes in poultry feed

According to Khattak *et al.* (2006), enzymes are biological catalysts which aid digestion of feed nutrients. They comprise amino acids with vitamins and minerals and they catalyse digestive biochemical reactions without undergoing any change themselves. Enzymes are functional proteins that stimulate or speed up the rate of specific chemical reactions (Ferket, 1993). Ravindran (2013) stated that the deployment of exogenous enzymes in poultry nutrition is turning to a standard to circumvent the challenge of antinutritional factors and enhance digestion of feed ingredients, nutrient utilisation and bird performance. Ravindran (2013) further explained that the ability to manufacture cheap commercial feed enzymes resulted from biotechnological improvements most notably fermentation and microbiological technologies and molecular biology. These improvements have led to a rapid growth of feed enzyme market, largely in response to increasing cost of feed raw materials.

The first reported use of an enzyme product in poultry known as Protozyme dates to 1920's (Ewing, 1963). Reports of pioneering research in the 50's and 60's in the US demonstrated the importance of including enzymes in barley-based diets (Jensen *et al.,* 1957; Willingham *et al.,* 1961). This was later followed by studies conducted on deployment of phytases to enhance the availability of phosphorus from feed materials of plant sources (Nelson *et al.,*1968).

According to Ravindra (2013), it was not until much later that non-starch polysaccharidases and phytases became available in commercial quantities and the chemistry of enzymes and their manufacture specific for individual substrates also was understood. Studies have shown that combined application of different enzymes activities or enzyme admixtures, instead of pure single enzymes, results in additive effects on utilisation of nutrients and productivity of animals (Cowieson and Adeola, 2005).

2.3.1 Modes of action of enzymes

To function as catalysts, enzymes are needed in minute amounts and they catalyse or facilitate the rate of reactions without themselves being destroyed in the reactions. The best expression of the vast activity of enzyme is by a constant, $k_{cat.}$ Differently, this is known as the rate, number or frequency of turnover, which indicates the number of

molecules of substrate that can be transformed by one molecule of enzyme per time to product (Robinson, 2015).

The earlier notion of rigid structure of enzymes known as “lock and key” whereby substrate molecules fit (keys) perfectly into the active sites (locks) of enzymes molecules was extended by Koshland (1958). This was done through techniques such as X-ray crystallography to present the “induced-fit model” of substrate and enzyme binding, in which the enzyme molecule exhibits slight alterations in its shape to accommodate the binding of the substrate. Enzymes are substrate-specific; therefore, it is imperative that they are selected contingent on substrate types in the feed raw materials employed in diet formulation. This is to achieve the maximum benefit from the use of exogenous enzymes (Ravindran, 2013). According to Bedford (2018), the three principal modes of action suggested for non-starch polysaccharidases are namely; reduction of viscosity, destruction of cell wall and generation of prebiotics.

2.3.1.1 Reduction of viscosity

Diets high in non-starch polysaccharides are known to reduce gut performance as gut viscosity increases, holding increased amount of water which leads to watery and sticky droppings (Choct and Hughes, 1997). Researches indicated β 1-3, 1-4 glucanases noticeably lowered intestinal viscosity in chickens fed barley, also lowered faecal moisture and enhanced digestibility of nutrients and growth (Classen, 1996). Morgan *et al.,* (1995) discovered that supplemental enzyme in wheat-based diets significantly lowered foregut digesta viscosity in birds through hydrolysis into smaller compounds of the xylan backbone by xylanase.

2.3.1.2 Destruction of cell wall

Cell walls that encapsulate protein and starch in the endosperm of most grains utilised in livestock feed comprises non-starch polysaccharides which the fed animal is incapable of digesting. It therefore follows that supplementation with enzymes capable of breaking the cell walls should enhance diet digestibility by exposing previously encapsulated protein and starch to the activities of endogenous proteases and amylases (Bedford, 2018).

2.3.1.3 Generation of prebiotics

It is unambiguous that supplementation with non-starch polysaccharidases enhances the microflora of the ileum and caecum (Gonzalez-Ortis *et al.,* 2016) and enhances fermentation in the caecum (Masey-O'Neill *et al.,* 2014). Morgan *et al.* (1995) postulated that with provision of fermentable oligosaccharides and low molecular weight polysaccharides or prebiotics, xylanase affected the poultry GIT. The prebiotics provide source of energy to saccharolytic bacteria in the caeca, a situation which offers dual benefits to the birds. The ventriculus grinds more effectively, and digestion is improved as energy is recovered from the diets (in terms of volatile fatty acids), and also as a consequence of enterohormone responses to high butyrate levels (Furness et al. 2013; Bedford, 2018).

2.4 Formation and composition of the chicken egg

In adult hens, *ovum from the ovary is received by* the oviduct *which* offers the environment for development and possible fertilisation of the egg. The oviduct of poultry species is a highly complex and dynamic organ where ovulated yolk from the ovary is developed into egg. As opposed to what obtains in the mammalian species, hens possess just a set of functional reproductive organs, the left ovary and the oviduct as the right ones stop to develop and shrink at chick stage (Sah and Mishra, 2018). The infundibulum, a funnel-like structure engulfs the yolk for 15 minutes and here the chalazae and perivitelline membrane are added. In breeder hens, fertilisation also occurs in the infundibulum (Sauveur and De Reviers, 1988). The egg formation process progresses to the magnum for about three hours where the albumen (egg white) is secreted and layered around. From there, the process moves to the isthmus where for more than one hour, the outer and inner shell membranes are added as are some water and mineral salts (electrolytes), after which the process moves to the shell gland (uterus) for a period of about 21 hours. Here, water is also added and eggshell formation takes place with calcium carbonate as the main eggshell material (Nys *et al*., 2011).

The chicken egg consists of about 59% egg white and 31% yolk, with the shell constituting about 10% of the total weight of the egg. The chicken egg is a good source of proteins (12.3%) and an equivalent quantity of lipids (11.6%) with water constituting about 74.4% of the entire contents of the egg. All vitamins, with the

exception of vitamin C, in addition to numerous minerals and trace elements are available in the egg. The egg is a poor source of energy (148 kcal per 100 g) but a source of good quality proteins that meets the nutritional needs of humans owing to its high contents of lysine and sulphur amino acids. The egg is also a good source of easily digested fats, cholesterol, unsaturated fatty acids, choline and cephalin-rich phospholipids, phosphorus and sulphur (Nys, *et al.,* 2011).

In weight, the yolk equals about 30% of the total egg mass and consists of more than 50% dry matter. A mass of 100 g of product supplies 16 g of proteins and more than twice of this amount makes up the lipids. All egg lipids are found in the egg yolk where they are bound to proteins to form lipoproteins (Nys *et al*., 2011). About 35% of fresh egg yolk is made of lipids while triglycerides (65%) are the main lipids in the egg but phospholipids (31%) and cholesterol (4%) are also present at lower levels (Nys *et al.,* 1999).

Synthesis of the egg lipoproteins occurs in the liver after which they are mobilised to the ovary as vitellogenin and very low-density lipoproteins (VLDL). These precursors are transferred by endocytosis following their binding to oocyte-specific receptors, without any modification. For this reason, it is practically impossible to change the overall yolk lipid composition by modifying its content in the diets of hens. Nevertheless, fatty acids profile in egg yolk strongly depends on the diet. Although hens are classically fed wheat, corn and soya, eggs have low levels of saturated fatty acids (about one third). Compared with other lipids of animal origin, egg lipids contain large levels of unsaturated fatty acids (Nys, *et al.,* 2011).

Furthermore, scientific evidence abounds which suggest that the egg contains other bio-active compounds which may function in the therapeutic and prophylactic treatment of contagious and chronic diseases. Bioavailability of compounds possessing immunomodulatory characteristics and properties that militate against microbes, cancer, oxidants or hypertension have been described in eggs (Lee and Paik, 2019). The albumen contains biologically active proteins namely lysozime, ovomucoid (ovomucin), ovoinhibitor and cystatin whose activities prolong the storability of table eggs (Rakonjac *et al.,* 2014). Some of these protective substances have been isolated and produced industrially as avidin and lysozymes. Furthermore, eggs are an excellent source of a polyunsaturated phosphatidylcholine known as lecithin, a structural as well

as a functional part of every biological membrane. It acts in the rate-limiting step of activation of membrane enzymes such as superoxide dismutase. The inefficient activation of these antioxidant enzymes has been suggested to result in increased destruction of membranes by free radicals (Rock *et al.,* 1996). Lecithin in addition, is known to improve bile secretion, averting stagnation in the bladder and as a result, lowering lithogenicity (Herron and Fernandez, 2004)

2.5 Egg quality

According to Oluyemi and Roberts (2000), one of the economically important indices of performance in commercial layers apart from egg production is egg quality. Egg quality constitutes attributes which determine acceptance by consumers and it is generally described by both external and internal characteristics. The external characteristics include the size, shape, width, length, shape index, shell thickness and colour, while the internal characteristics include weight, colour, and index of the yolk; height and weight of the albumen; Haugh unit, meat and blood spot percentage (Stadelman,1977).

Poor quality of eggs does not only result in poor consumer preference but also economic losses. For instance, it is estimated that roughly 7-8% of the aggregate sum of eggs is lost to breakages during transfer and transportation of eggs from the farm to consumption thus, resulting to striking economic setbacks for the farmers, dealers and consumers (Hamilton, 1982).

Each of the specific strains of commercial chickens appears to have a range of egg characteristics specific to the strain. It was reported by Tumova *et al.* (2007) that genotype significantly influenced yolk and albumen quality, yolk index as well as egg shape index.

2.5.1 External egg characteristics

A number of factors significantly impacts on egg external characteristics. According to FAO (2003), egg size and quality factor considerations are not the same from place to place. For instance, egg weight classifications used in Africa are large for eggs weighing 65g and above, medium for eggs weighing 55-65g, while those weighing 45-55g are graded as small. In the US, the grading system is as follows: Jumbo (70g and above), Extra-large (65-70g), Large (56-65g), Medium (49-56g), Small (42-49g) and

Peewee (35-42g). There is a direct relationship between the weights of albumen, yolk and shell on one hand, and weight of the egg on the other hand (Pandey *et al.,*1986). Strain factor is also known to significantly influence egg weight (Tixier-Boichard *et al*., 2006). Age influences yolk percentage, egg white and shell deposited in the egg of laying hens. Egg weight improves as the birds grow older and a peak reached at completion of the laying phase (Scott and Silversides, 2000). The improvement in egg weight is however greatly influenced by genetic, nutrition and other environmental inputs (Silversides and Budgell, 2004).

Shell quality determinants include factors such as texture, strength, cleanliness, shape, soundness, porosity and colour (Natalie, 2009). According to Roberts (2004), eggs are candled using light or passed through an electronic crack detector to detect cracks in commercial operations. The eggshell is such an important external characteristic as the shells are covered with a cuticula which offers protection against microorganism penetration, a quality that is lost when the egg is washed. For this reason, washed eggs in the USA are always oiled to offer an alternative protective layer while in Europe, regulations do not permit washing for A-grade table eggs. Shell integrity can also be determined automatically with the use of sound detection sensors as a fully intact egg has a different resonance compared with an egg even with a hair crack (van Niekerk, 2014).

Egg colour protects the egg against deleterious radiation from the sun (Lahti, 2008), reinforces structure of eggshell (Gosler *et al*., 2005) and protects maturing embryos from thermal deterioration (King'ori, 2011). Colour of the shell could be assessed through optical contrast using a sequence of graded standards or by shell reflectivity to observe the amount of incident light which the shell surface reflects under controlled environment (Roberts, 2004).

Shell quality could be assessed directly or indirectly by different methods, some of which may require breaking of the egg (Hamilton, 1982). Direct methods of assessing the shell quality include puncture force or quasi-static compression and impact fracture force which measures the shell breaking strength. Indirect means include specific gravity, shell thickness and shell weight (Hammerle, 1969). According to Oluyemi and Roberts (2000), average egg shell thickness of the domestic fowl is 0.34mm. It

tends to be thinner in the tropics than in the temperate regions. The strength of the eggshell is not only assessed by the weight of the shell but also the quality of the make-up or fine structure of the shell. Examination of the fine structure of the shell under the electron microscope has provided explanation for relatively poor shell breaking strength in circumstances where shell weight and thickness as well as the percentage shell are excellent (Nys *et al.,* 1999).

Shell strength has a direct relationship with shell integrity and is determined by variables namely, age, nutrition and genetics of the hens. Brown eggs for instance have stronger shells than white eggs, thinner shells and more cracks are observed in older hens while calcium availability (which is a key component of the shell) is an important nutritional factor (van Niekerk, 2014). However, attention must be paid to dietary calcium to phosphorus ratio as high levels of phosphorus could impede calcium absorption, causing poor quality shell (Boorman and Gunaratne, 2001). Any disease that adversely affects the normal physiological status of the bird may also result in reduced egg quality observed through defective eggs and poor eggshells (Roberts, 2004). In addition, high ambient temperature could also result to poor eggshell owing to impaired feed intake by the laying hens which impedes the availability of blood calcium essential for formation of eggshell. Studies have however shown that when half of dietary calcium is provided in coarse particulate form in hot seasons, eggshell quality can be improved in heat-stressed hens (Nys, 1995; Nys 1999). Heat stress could also result in the reduction of carbonic anhydrase activity which leads to the formation of bicarbonate that contributes the carbonate to eggshell (Balnave *et al.,* 1989). Studies have however revealed that the eggshell quality can be improved in heat-stressed hens when they are provided with cool drinking water (Glatz, 1993).

Other stress factors such as handling of birds during relocation have also been known to negatively impact on eggshell quality. There could be a delay in the timing of oviposition in which case the laying hens retain their eggs. This could lead to increased occurrence of white-banded eggs (which occurs when the egg is held past the usual oviposition period) and slab-sided eggs (which occurs when the egg goes into the shell gland while the preceeding one is still there) (Hughes *et al*., 1986; Raynard and Savory, 1999). Administration of adrenaline injections on laying hens have been

known to simulate many of the negative results of stress on eggshell quality (Hughes *et al*., 1986).

2.5.2 Internal egg characteristics

According to Roberts (2004), the internal constitution of the chicken egg comprises the albumen and the yolk. A good quality egg should be devoid of impurities like meat and blood spots, blemishes which certain commercial sorting system will detect. Isikwenu *et al*. (1999) opined that Haugh unit and yolk index best determined internal quality of eggs while Kul and Seker (2004) highlighted internal traits like Haugh unit, albumen weight, height and ratio as well as yolk diameter, height, weight, index and ratio.

The egg yolk has two quality components to it namely the yolk colour and the strength of the perivitelline membrane surrounding the yolk. In a situation where the perivitelline membrane is weak, such as when the egg is aged, the yolk readily disintegrates (Kirunda and McKee, 2000). Texture, colour, odour as well as firmness are determinants of yolk quality (Jacob *et al*., 2000). While pigments that are of natural or synthetic sources may be included in the diet to achieve the desired egg yolk colour, countries have different preferred egg yolk colouration. For instance, in Australia, the widely acceptable colour of egg yolk on the Roche scale is about 11, while in other places, a lighter-coloured or darker appearance on the Roche scale is preferred. Regulations in some other countries like Sweden disallows the use of synthetic pigment (Roberts, 2004).

Albumen height is measured at an interval of 1cm from the yolk border, which many times with conversion is expressed as Haugh unit. According to Haugh (1937), this is used to assess the quality of the albumen (Roberts, 2004). Factors that influence albumen height are not clearly known (Williams, 1992), even though the chemical and functional attributes of the constituents of the albumen have been understood. The content and characteristics of ovomucoid seem the primary determinants of albumen height, but chemical alterations that trigger albumen height reduction during storage are less explicit (Silversides and Budgell, 2004). Decrease in albumen height has been ascribed differently to proteolysis of ovomucoid, cleavage of disulfide bonds, interactions with lysozyme and changes in the interaction between α and β ovomucins with no clear favorite (Stevens,1996).

Albumen quality is influenced by factors including genetics, bird's age and egg storage condition such as relative humidity, CO_2 and temperature (Jones, 2006). For instance, as the eggs age during storage, there is interchange of CO_2 and O_2 with evaporation of moisture through the shell. The situation increases the air chamber with a resultant effect of reduction in the albumen height. This condition is aggravated during high ambient temperature and/or low relative humidity. In the US, the condition is usually ameliorated by oiling the eggshell to prevent the exchange of CO_2 and O_2 and moisture and thus reduce ageing of the eggs (Van Niekerk, 2014). It was observed that albumen quality deteriorates as the laying hens age (Van Den Brand *et al*., 2004). According to USDA (2000), high level of ammonia build-up in the pen due to poor ventilation could also lead to poor albumen quality.

Haugh Unit, according to Van Niekerk (2014) is applied in legislation to determine the freshness of eggs which should meet a specified level before eggs are qualified to be sold. According to USDA (2000), the Haugh unit is the most accepted and widely adopted method of determining the albumen quality. The average range of Haugh unit for most eggs is 75-80 HU with 60 as a minimum value (Chukwuka *et al*., 2011). However, because the Haugh unit is determined by intrinsic factors such as the age and strain of the birds, and by the external factor of storage, its reliability was queried (Silversides, 1994).

2.5.3 Egg lipid profile

An old fear usually associated with consumption of egg is the belief that it has high level of cholesterol (Halliwell *et al.,* 1995). Egg was therefore identified by diet-heart advocates as food to avoid, although egg has the best and the least expensive, high quality protein of high biological value with excellent distribution of minerals and vitamins except vitamin C (Connor, 2000). The public concern about egg consumption was predicated on the supposition that high blood cholesterol levels and cardiovascular disease were associated with high level of cholesterol consumption.

Subsequent researches have suggested that, in contrast to saturated fatty acids and trans fatty acids, cholesterol generally in human diet and particularly in egg has scant influence on blood cholesterol and on cardiovascular disease (Eilat-Adar *et al.,* 2013). Cholesterol is the foremost sterol synthesised by animals. It is a steroid metabolite,

waxy-like in nature, which is present in all cell membranes and transported in blood plasma. Because it is an integral part of cell membranes and is transformed to hormones, cholesterol is essential for good health at a reasonable level (Naviglio *et al.,* 2012). There have been attempts to reduce the cholesterol in egg and while the total lipid of eggs cannot be altered, the quality can be enhanced by altering the fatty acid composition through the use of polyunsaturated fatty acid (PUFA)-rich dietary oils in the hen's diet (Milinsk *et al.,* 2003). Unsaturated fatty acids are known to support the production of plasma high density lipoproteins (HDL) which offer protection against atherosclerosis through the mechanism of transportation of cholesterol from the tissues to the liver where cholesterol is converted to bile acids and excreted in the biliary system (Grundy, 1989).

Other factors have also been documented to influence the egg lipid profile. Ogunwole *et al.* (2015) studied the lipid composition of eggs from laying hens on diets with five distinct proprietary vitamin-mineral premixes, reared under the battery cage and open-sided deep litter systems as influenced by days of storage. They concluded that the interactions of dietary vitamin-mineral premix, rearing system and duration of storage significantly influenced the lipid composition of eggs.

2.6 Haematological and serum biochemical indices

Blood plays a key function in carrying nutrients, metabolic wastes as well as gases within the body (Zhou *et al.,* 1999). Profiling of blood is an important tool employed in determining the status of the animal with respect to health, metabolic diseases, nutritional imbalances and welfare (Menon *et al.,* 2013). The blood acts as a pathological indicator of the status of the exposed animals to toxicants and other conditions. Its assay provides possibility of clinically investigating the occurrence of metabolites and other constituents in the system of the animals and evaluating the responses of the animals to various physiological conditions (Etim *et al.,* 2014).

According to Merck (2012), haematology is the study of numbers and morphology of cellular elements of blood namely red cells (erythrocytes), white cells (leucocytes) and platelets (thrombocytes); and utilisation of these parameters to diagnose and monitor health conditions. Apart from genotypic, age and sex factors; nutrition, environment and hormonal factors potentially lead to variations in blood parameters (Chineke *et al.,*

2006). According to Daramola *et al.* (2005), laboratory values could serve as criteria for comparison in conditions of deficient nutrition, physiology and health status of the animals.

Haemoglobin functions in transporting oxygen and carbon IV oxide in the blood. Normal value for healthy chickens as reported by Maxwell *et al*. (1990) was 10.6g/dL while value range of 7.88-9.18g/dL was reported by Oke *et al*. (2017) for laying hens under different rearing systems at peak production phase. According to Lindsay (1977), haemoglobin content of the blood falls gradually in animals with low levels of protein intake, parasitic infestations or liver damage. Red blood cells also known as erythrocytes function as carriers of haemoglobin. It therefore follows that a depressed erythrocytes count signifies a lowering of amount of oxygen that will be made available to tissues (Isaac *et al*., 2013). White blood cell and its differentials principally function in fighting infections by phagocytocis against invasion by pathogens and are also involved in immune response through the production or transportation and distribution of antibodies. Animals that present with depressed leucocyte counts therefore stand the risk of disease infection (Soetan *et al*., 2013). At peak production phase of laying hens kept in contrasting rearing systems, Oke *et al*. (2017) reported values ranging from 1.28-2.03 $\times 10^{12}$ /L for red blood cell count and 13.23-20.70 $\times 10^{9}$ / L for white blood cell count. A high level of lymphocyte counts known as lymphocytosis (lymphocytopenia being the reverse), usually is an indication of viral infection. Heterophils are the most abundant WBCs in most birds and they have lysozyme and proteins required for bactericidal activities. Monocytes are tissue macrophages, which are known as the mononuclear phagocyte system (MPS). They perform an important responsibility in the destruction of intra cellular organisms (fungi, protozoa and viruses) and transformed cells (Deldar, 1994) while eosinophils play a fundamental role of detoxification (Coles, 1986).

Alanine aminotransferase (ALT) and apartate aminotransferase (AST) are serum enzymes which values are used to estimate liver functions. They are very active in the liver and their activities could be detected in the blood where they are useful in monitoring blood serum of animals exposed to dangerous chemicals. The AST functions in catalysing reversible transfer of an α-amino group between aspartate and glutamate. It is therefore a vitally important enzyme in metabolism of amino acid.

Serum AST is used to measure hepatic function in liver disease and toxicity. It is liberated into the blood when liver cells are damaged hence the increase in blood in acute viral and toxic hepatitis, shock and progressive post necrotic cirrhosis. The ALT functions in the catalysis of the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate. Serum ALT and AST levels, and their proportions are regularly appraised clinically as biomarkers for liver health. Lohr (1975), associated an increased activity of ALT with hepatocellular damage in chickens

The ingestion of cyanide stimulates the defense mechanism of the body to detoxify cyanide to thiocyanate which according to Oke (1969) is widely distributed in the body fluids. Thus, serum thiocyanate is useful in determining dietary cyanide.

CHAPTER 3

MATERIALS AND METHODS

Experiment 1

Determination of percentage of starch content of cassava varieties TME 419 and TMS 01/1368 and starch gelatinisation of the cassava grits with sun-drying and roasting methods

3.1 Site of experiment

Preparation of the test feed material (cassava grits of varieties TME 419 and TMS 01/1368) was carried out at the Cassava Processing Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, located within the tropical rain forest zone of Nigeria at latitude 7.49º N and longitude 3.89 º E.

3.2 Determination of percentage starch content of cassava varieties TME 419 and TMS 01/1368 tubers

The starch content (fresh weight basis) of cassava tubers was assayed using the spectrophotometer method as outlined by Radley (1976). Fresh cassava tubers of the same maturity were ground, out of which 2.5g was taken and dissolved in 50mL of cold water and left to stand for 60 minutes. Thereafter, 20 mL of HCl and 150 ml of distilled water were introduced into the sample and refluxed for 120 minutes in a round bottom flask. This was cooled and neutralised with 0.5 N NaOH. With anthrone reagent, the resulting content was used for glucose determination.

To calibrate the glucose standard concentration, a sequence of solution was prepared containing 0 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm in that order. 5 mL of anthrone reagent was introduced into each of the standards and test sample and boiled in water bath for 20 minutes for occurrence of colour development. The test tubes were

cooled and absorbance was read against blank containing only 1 mL of distilled water and 5 mL of anthrone reagent at 620 nm.

Calculation of starch content (%) = Absorbance/Volume × SR × (50 ÷ 2.5) × 100 …….. Equation 1

Where SR = slope reciprocal

Volume = volume of sample used

3.3 Statistical analysis

Data from the assay were subjected to student T-test.

3.4 Production of cassava grits

Cassava tubers of varieties TME 419 and TMS 01/1368 were harvested at eleven months old at the cassava plots of IITA, Ibadan and processed into grits at the Cassava Processing Unit of the Institute. The flow chart of cassava grits production is presented in Figure 3.1.

3.5 Proximate analysis of cassava grits

Proximate composition of cassava grits was assayed according to AOAC (2000). Sampled materials were analysed in three replications. The parameters determined were crude protein, ash, ether extract, crude fibre, dry matter and nitrogen-free extracts.

3.6 Fibre fraction analysis of cassava grits

Determination of fibre fractions was carried out in triplicates using Van Soest method (AOAC, 2000). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), cellulose and hemicellulose were determined.

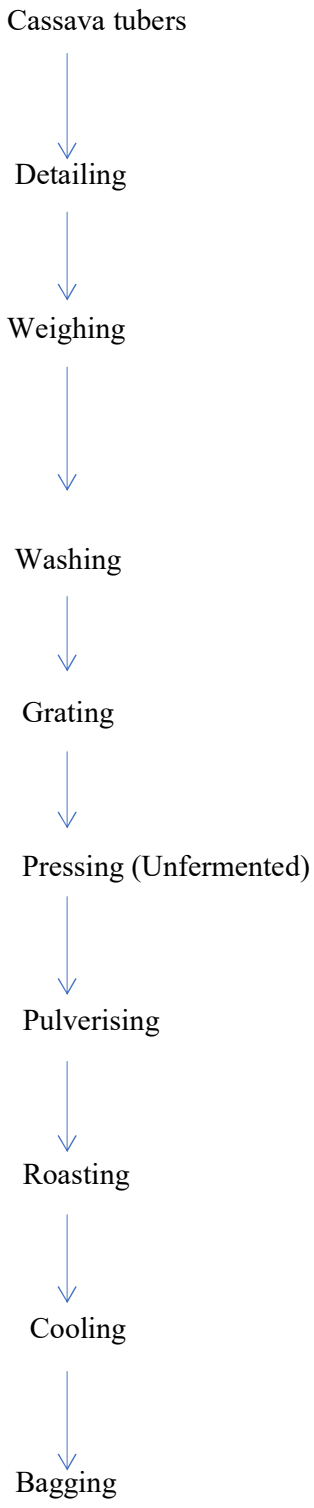

Fig 3.1: Flow Chart of Production of Cassava Grits

Source: Tewe, 2005

3.7 Determination of percentage of starch gelatinisation of sun-dried and roasted TME 419 and TMS 01/1368 grits

Percentage of starch gelatinisation of cassava grits was determined spectrophotometrically as described by Wootton and Munk (1971). Briefly, 2 g of cassava grits was macerated with 100mL distilled water in a Warring blender for 10 minutes. The suspension was centrifuged at 500rpm, then duplicate aliquots of 1mL each were diluted to 10mL with water after which 0.1 mL iodine solution was introduced. The samples' absorbance was read against a reagent blank at 600 nm. A second suspension of 2g cassava grits was made in 95 ml distilled water, to which 5 ml of a 10M aqueous solution of KOH was applied while shaking the mixture gently, it was allowed to stand for 5 minutes. The alkaline suspension was centrifuged, and 1 mL of duplicate aliquots were treated with 1 mL of 0.5M HCl, diluted with water to 10mL after which 0.1mL of iodine solution was introduced, and their absorbances were measured as stated previously.

Percentage of starch gelatinisation was then determined using the following formula:

$$\frac{A_1}{A_2} \times 100 \quad \text{……. Equation 2}$$

Where

A_1 = Absorbance of the iodine complex prepared from the aqueous suspension before alkaline solubilisation.

A_2 = Absorbance of the iodine complex prepared from the aqueous suspension after alkaline solubilisation.

3.8 Statistical analysis

Data generated from the assay were subjected to student T-test.

Experiment 2

Determination of true metabolisable energy of TME 419 and TMS 01/1368 grits

3.9 Site of experiment

The experiment was conducted at the University of Ibadan Teaching and Research Farm, Poultry Unit, situated in the tropical rain forest zone of Nigeria within latitude 7.44º N and longitude 3.89º E, with a mean altitude of 277 meters above sea level.

3.10 Management of experimental animals

ISA Brown Cockerels (n=24) aged 24 weeks of similar weights with standard husbandry records were obtained from a farm with excellent husbandry practices and randomly assigned to two dietary treatments of TME 419 and TMS 01/1368 grits. In adaptation of the Sibbald (1976) method, the grits were fed as sole feeds with a third group on fasted treatment for True Metabolisable Energy (TME) assay. Each of the birds was housed individually in a metabolic cage. There were eight replicates per treatment. The duration of the experiment was forty-eight hours. The birds were starved of feed for 24 hours to clear the gastrointestinal tract of digesta from previous feed, after which birds on dietary treatments were fed moistened 50g of grits and the excreta collection trays put in place. The birds on fasted treatment continued starvation while all the birds were allowed rights to clean drinking water. Collection trays for the excreta were withdrawn precisely 24 hours following feeding. Quantitative collection of the excreta was done, freeze dried and kept for determination of gross energy with the bomb calorimeter.

3.11 Determination of true metabolisable energy (TME) of cassava grits

TME of cassava grits TME 419 and TMS 01/1368 was determined using the following formula:

$$\text{TME (Kcal/kg)} = \frac{GE_f \times F_i - (Y_f - Y_e)}{F_i} \quad \text{…….. Equation 3}$$

Where,

GE_f = Gross energy of the feedstuff

Y_f = Gross energy discharged as excreta of fed birds

Y_e = Gross energy discharged as excreta of fasted birds

F_i = Weight of feedstuff fed (g)

3.12 Statistical analysis

Data from the assay were subjected to student T-test.

Experiment 3

Performance, haematology and serum biochemical indices of growing pullets fed enzyme-supplemented cassava grits-based diets.

3.13 Site of experiment

The experiment was conducted at the Teaching and Research Farm's Poultry Unit, University of Ibadan, Nigeria.

3.14 Management of experimental animals

ISA Brown pullets (n=364) aged eight weeks with standard records were acquired from a farm with excellent husbandry practices. The birds were assigned randomly to 13 dietary treatments replicated in quadruplicates of seven birds per replicate. The growing pullets were freely given rights to feed and water *ad libitum* while routine management including the necessary vaccination and administration of antihelminthic drugs as well as other drugs were observed.

The birds were penned in a conventional open sided deep litter house, split into 52 cubicles, each having a dimension of 1.16 m^2 floor space, with 7 birds per cubicle. The whole pen was thoroughly cleaned and sanitised with quaternary ammonium compound four weeks prior to the beginning of the experiment. The pen was then screened, fumigated and rested for a fortnight prior to receiving the birds. The assay period lasted 10 weeks.

3.15 Experimental design

The experimental design was (2×3×2)+1 augmented factorial arrangement in a completely randomised design. Maize in a standard diet was replaced by cassava grits of varieties TME 419 and TMS 01/1368, at three replacement levels of 33%, 66% and 100%, with or without enzyme supplementation.

3.16 Layout of experimental diets

A basal diet with 35% maize was formulated as control and 12 other isocaloric and isonitrogenous diets were formulated with varied levels of cassava grits inclusion and enzyme cocktail supplementations as follows:

T_0 was the basal standard maize diet; T_1 and T_2 were TME 419 cassava grits replacement of maize at 33% with 0 mg/kg and 35 mg/kg multiple enzyme supplementation, respectively; T_3 and T_4 were TME 419 cassava grits replacement of maize at 66% with 0 mg/kg and 35 mg/kg enzyme supplementation, respectively; T_5 and T_6 were TME 419 cassava grits replacement of maize at 100% with 0 mg/kg and 35 mg/kg enzyme supplementation, respectively; T_7 and T_8 TMS 01/1368 cassava grits replacement of maize at 33% with 0 mg/kg and 35 mg/kg enzyme supplementation, respectively; T_9 and T_{10} TMS 01/1368 cassava grits replacement of maize at 66% with 0 mg/kg and 35 mg/kg enzyme supplementation, respectively; T_{11} and T_{12} TM3 1368 cassava grits replacement of maize at 100% with 0 mg/kg and 35 mg/kg enzyme supplementation, respectively. The layout of the experimental diets, gross composition of the basal diets and composition and activity of the enzyme cocktail are shown in Tables 3.1, 3.2 and 3.3, respectively.

3.16.1 Performance parameters

Average daily feed intake was recorded by deducting the weight of the daily feed leftover from the weight of total feed served.

The birds were weighed weekly and accurate records of rate of gain in body weight was calculated weekly by the dissimilarity between the value obtained the previous week and that of the current one.

Feed conversion ratio (FCR) was calculated using the following formula:

$$\frac{\text{Total feed intake}}{\text{Weight gain}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 4}$$

Table 3.1: Layout of experimental diets to growing pullets

	TME 419		TMS 01/1368	
	Enzyme		Enzyme	
Replacement level (%)	0mg/kg	35mg/kg	0mg/kg	35mg/kg
33	T_1	T_2	T_7	T_8
66	T_3	T_4	T_9	T_{10}
100	T_5	T_6	T_{11}	T_{12}

T1: Diet had 33% replacement of maize with TME 419 grits without enzyme supplementation ; T2: Diet had 33% replacement with TME 419 grits and 35mg/kg enzyme supplementation; T3: Diet had 66% replacement with TME 419 grits without enzyme supplementation; T4 : Diet had 66% replacement with TME 419 grits and 35mg/kg enzyme supplementation; T5: Diet had 100% replacement with TME 419 grits without enzyme supplementation; T6: Diet had 100% replacement with TME 419 grits and 35mg/kg enzyme supplementation; T7: Diet had 33% replacement with TMS 01/1368 grits without enzyme supplementation; T8: Diet had 33% replacement with TMS 01/ 1368 grits and 35mg/kg enzyme supplementation; T9: Diet had 66% replacement with TMS 01/ 1368 grits without enzyme supplementation; T10: Diet had 66% replacement with TMS 01/ 1368 grits and 35mg/kg enzyme supplementation; T11: Diet had 100% replacement with TMS 01/ 1368 grits without enzyme supplementation; T12: Diet had 100% replacement with TMS 01/ 1368 grits and 35mg/kg enzyme supplementation.

Table 3.2: Gross composition of basal diets fed to growing pullets

Ingredients	Basal Diets (g/100g)		
	Maize	TME 419	TMS 01/1368
Maize	35.00	-	-
Cassava grits	-	35.00	35.00
Wheat offal	28.00	17.00	16.50
Palm kernel meal	24.00	21.90	21.90
Soya bean meal	8.70	22.00	22.00
Fish meal (72%)	1.00	1.00	1.00
Soya oil	-	-	0.5
Table salt	0.25	0.25	0.25
Di-calcium phosphate	2.00	1.70	1.70
Limestone	0.80	0.86	0.86
DL Methionine	-	0.04	0.04
*Vitamin-mineral premix	0.25	0.25	0.25
TOTAL	100.00	100.00	100.00
Calculated Nutrients			
Metabolisable Energy (kcal/kg)	2420.39	2406.00	2402.50
Crude Protein (%)	15.39	15.35	15.45
Crude Fibre (%)	11.72	12.04	12.80
Methionine (%)	0.32	0.31	0.31
Lysine (%)	0.79	0.82	0.82
Calcium (%)	1.11	1.11	1.11
Available Phosphorus (%)	0.45	0.45	0.45

*Vitamin-mineral premix - Vitamin A–10,000IU, Vitamin D_3–1800IU, Vitamin E–40mg, Vitamin K–1.43 mg, Vitamin B1–0.7mg, Vitamin B2–4mg, Vitamin B6–2.5mg, Vitamin B12–0.2mg, Niacin–10mg, Panthothenic–10,000mg, Folic acid –0.25mg, Biotin–100mg, Choline Chloride–300mg, Manganese–80mg, Zinc–60mg, Iron–40mg, Copper– 80mg, Iodine–0.8mg, Selenium–0.2mg, Cobalt–0.3mg, Antioxidant–100

Table 3.3: Enzyme cocktail composition and activity

Enzyme	Minimum activity (u/kg)
Cellulase	6,000,000
Xylanase	10,000,000
Beta-glucanase	700,000
Phytase	400,000
Alpha-amylase	700,000
Pectinase	70,000
Protease	700,000

Enzyme (Natuzyme) was manufactured by Bioproton PTY Ltd, Australia.

3.17 Data Collection

3.17.1 Performance parameters

Average daily feed intake was recorded by deducting the weight of the daily feed leftover from the weight of total feed served.

The birds were weighed weekly and accurate records of rate of gain in body weight was calculated weekly by the dissimilarity between the value obtained the previous week and that of the current one.

Feed conversion ratio (FCR) was calculated using the following formula:

$$\frac{\text{Total feed intake}}{\text{Weight gain}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 4}$$

3.17.2 Blood parameters

Blood (5 mL) was sampled at the end of the assay period from the birds through the jugular vein using syringe and needle. The blood samples were collected into heparinized bottles for haematological parameters while non-heparinized bottles were used to collect blood for serum biochemical parameters.

Packed cell volume (PCV) was detrmined using the micro-haematocrit method as described by Dacie and Lewis (1984), red blood cells (RBC) counts were done using the improved Neubauer haemotocytometer (Dacie and Lewis, 1984). Haemoglobin concentration (Hb) and leukocytes counts (WBC) were determined using the method described by Jain (1986) while leukocytes differential counts namely lymphocytes, heterophils, monocytes, eosinophils, and basophils were determined from blood smears prepared using the May-Grunwald-Giemsa stains with methyl alcohol fixation.

The red cell indices namely mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular heamoglobin concentration (MCHC) were calculated using the following formulae:

$$\text{MCV} = \frac{\text{Haematocrit (\%) x } 10}{\text{RBC } (\times 10^{12}/\text{ L})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\text{Equation 5}$$

$$MCH = \frac{Hb\ (g/dL) \times 10}{RBC\ (\times 10^{12}/\ L)} \quad \text{Equation 6}$$

$$MCHC = \frac{Hb\ (g/dL) \times 10}{Haematocrit\ (\%)} \quad \text{Equation 7}$$

Specimens of blood for serum biochemical analyses were allowed to coagulate and the serum was instantly isolated using the centrifuge set at 3500 rpm for a period of 600s. Serum biochemical parameters assessed were glucose (Cooper and McDaniel, 1970), triglycerides (Trinder's enzymic method), total cholesterol (Gowenlock *et al*., 1988), total protein (Reinhold, 1953), albumin (Peters *et al*.,1982), alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Rej and Hodder, 1983), and thiocyanate (Bowler, 1944). Samples were read in triplicate using spectrophotometry (UV/VIS, INESA Model 752N) at parameter-specific wavelength.

3.17.3 Apparent nutrient retention

By week 18, two pullets from each replicate were picked and arranged in a metabolic cage system and fed corresponding treatment diets for six days with feed and water given early in the morning between 7-8 am. The initial three days were regarded as period of adaptation while records of intake of feed and voided excreta were taken during the last three days. Excreta collected on a daily basis from the trays beneath the metabolic cage were weighed, dried, bulked and kept in the refrigerator. Feed and excreta were oven dried and analysed for proximate composition according to AOAC (2000).

Apparent nutrient retention was calculated as follows:

$$\frac{\text{Nutrient intake} - \text{Nutrient in excreta}}{\text{Nutrient intake}} \times 100 \quad \text{Equation 8}$$

3.18 Statistical analysis

Data were subjected to ANOVA of the General Linear Model of SAS (2002) package. Means were separated using Duncan Multiple Range Test at $\alpha_{0.05}$.

Experiment 4

Performance, haematology and serum biochemistry of laying hens fed enzyme-supplemented cassava grits-based diets.

3.19 Site of experiment

The experiment was conducted at the Teaching and Research Farm's Poultry Unit, University of Ibadan, Nigeria.

3.20 Management of experimental animals

ISA Brown point of lay pullets (n=312) aged 19 weeks from corresponding treatment groups from Experiment 3 were used for this trial. The pullets were reared in a conventional 3-tier battery cage system housed in a standard poultry house. Each of the cells had a total dimension of 40 x 45 x 50 cm^3 and floor space of 450cm^2 per bird. The pullets were weighed at the commencement of the assay period and at the conclusion of each of the phases of laying period. The birds were served both feed and water *ad libitum* while routine management including the necessary vaccination and administration of antihelminthic drugs as well as other drugs were observed. The duration of the experiment was 54 weeks (August 2016 to July, 2017) comprising of the early (22-38 weeks of age), mid (39-55 weeks of age) and late laying phases (56--72 weeks of age). The duration of each of the three phases of this experiment was eighteen weeks.

3.21 Experimental design

The experiment was designed as a (2×3×2)+1 augmented factorial arrangement in a completely randomised design. Similar to Experiment 3, maize in a standard diet was replaced by cassava grits of varieties TME 419 and TMS 01/1368 at three replacement levels of 33%, 66% and 100% with or without multiple enzyme supplementation.

3.22 Layout of experimental diets

Layout of the experimental diets of Experiment 4 was the same as those of Experiment 3 as shown in Table 3.4 and the gross composition of the basal diets of experiment is shown in Table 3.5.

Table 3.4: Layout of experimental diets fed to laying chickens

	TME 419		TMS 01/1368	
	Enzyme		Enzyme	
Replacement levels (%)	0mg/kg	35mg/kg	0mg/kg	35mg/kg
33	T_1	T_2	T_7	T_8
66	T_3	T_4	T_9	T_{10}
100	T_5	T_6	T_{11}	T_{12}

T1: Diet had 33% replacement of maize with TME 419 grits without enzyme supplementation ; T2: Diet had 33% replacement with TME 419 grits and 35mg/kg enzyme supplementation; T3: Diet had 66% replacement with TME 419 grits without enzyme supplementation; T4 : Diet had 66% replacement with TME 419 grits and 35mg/kg enzyme supplementation; T5: Diet had 100% replacement with TME 419 grits without enzyme supplementation; T6: Diet had 100% replacement with TME 419 grits and 35mg/kg enzyme supplementation; T7: Diet had 33% replacement with TMS 01/1368 grits without enzyme supplementation; T8: Diet had 33% replacement with TMS 01/ 1368 grits and 35mg/kg enzyme supplementation; T9: Diet had 66% replacement with TMS 01/ 1368 grits without enzyme supplementation; T10: Diet had 66% replacement with TMS 01/ 1368 grits and 35mg/kg enzyme supplementation; T11: Diet had 100% replacement with TMS 01/ 1368 grits without enzyme supplementation; T12: Diet had 100% replacement with TMS 01/ 1368 grits and 35mg/kg enzyme supplementation.

Table 3.5: Gross composition of basal diets fed to laying chickens

Ingredients	Basal Diets (g/100g)		
	Maize	TME 419	TMS 01/1368
Maize	55.50	-	-
Cassava grits	-	55.50	55.50
Wheat offal	9.20	-	-
Soya bean meal	21.00	23.50	23.50
Soya oil	-	1.75	2.50
Fish meal (72%)	2.00	8.00	7.50
Table salt	0.30	0.25	0.25
Di-calcium phosphate	1.60	1.25	1.25
Limestone	9.82	9.22	8.97
*Vitamin-mineral premix	0.25	0.25	0.25
Lysine HCl	0.06	-	-
DL methionine	0.17	0.18	0.18
Toxin binder	0.10	0.10	0.10
TOTAL	100.00	100.00	100.00
Calculated Nutrients			
Metabolisable energy (kcal/kg)	2600.44	2601.00	2594.20
Crude protein (%)	17.08	17.13	17.05
Crude fibre (%)	4.25	4.15	5.88
Methionine (%)	0.46	0.47	0.47
Lysine (%)	1.01	1.05	1.05
Calcium (%)	4.00	3.98	3.98
Available Phosphorus (%)	0.45	0.45	0.45

*Vitamin mineral premix - Vitamin A–10,000IU, Vitamin D3–2000IU, Vitamin E–40mg, Vitamin K–1.43 mg, Vitamin B1–0.7mg, Vitamin B2–4mg, Vitamin B6–2.5mg, Vitamin B12–0.2mg, Niacin–10mg, Panthothenic–10,000mg, Folic acid –0.25mg, Biotin–100mg, Choline Chloride–300mg, Manganese–80mg, Zinc–60mg, Iron–40mg, Copper– 80mg, Iodine–0.8mg, Selenium–0.2mg, Cobalt–0.3mg, Antioxidant–100mg

3.23 Data collection

3.23.1 Performance parameters

Weekly intake of feed was recorded as the disparity between weight of leftover feed and weight of total feed served. Weight gain (kg) was calculated as the difference between the final weight and initial weight of the birds taken at the commencement of the experiment. Egg weight was determined with a laboratory digital scale of 0.01g accuracy.

The number and weight of eggs produced per replicate for each treatment were recorded daily while Hen-day egg percentage was calculated as follows:

$$\text{Hen Day Egg Production \%} = \frac{\text{Total number of eggs produced per week}}{\text{Total number of hen-day per week}} \times 100$$

…….. Equation 9

Feed conversion ratio per egg mass (FCR/EM) was calculated as follows:

$$\text{FCR} = \frac{\text{Feed consumed}}{\text{Average Egg Mass}}$$ ……Equation 10

where,

average egg mass (g/hen/day) = % Hen-day egg production x Average egg weight (g).

3.23.2 External and internal egg characteristics

Internal and external egg characteristics were assessed at the three phases of laying. Two freshly laid eggs per replicate which amounted to eight eggs per treatment and a total of 104 eggs were sampled. Egg weight (g) was measured using a digital scale of 0.1g accuracy. Egg lengths along the longitudinal axis (mm) and egg maximum width or diameter (mm) were measured using digital Vernier caliper.

Egg shape index was calculated as:

$$\frac{\text{Average width of egg}}{\text{Average length of egg}} \times 100$$ ……. Equation 11

The eggs were broken individually on a flat plate and the yolk carefully separated from the albumen for internal egg quality indices measurement. Yolk weight was obtained by placing the yolk on a petri-dish of known weight and measuring with electronic digital scale. With the use of the micrometer gauge, the thick albumen height was assessed at 1cm away from the fringe of the yolk. Egg shell thickness was assessed at three respective locations (at the small, middle and broad ends) using a micrometer screw gauge and the average taken. The shell weight was measured using a digital scale after drying at room temperature (Scott and Silverside, 2000).

Egg shell ratio (%) was calculated as:

$$\frac{\text{Average weight of shell}}{\text{Average weight of egg}} \times 100 \quad \text{..... Equation 12}$$

Haugh Unit was determined from the relationship between albumen height and egg weight as described by Haugh (1937) from the equation;

$$HU = 100 \log_{10}(h - 1.7W^{0.37} + 7.6) \quad \text{..... Equation 13}$$

Where,

HU = Haugh Unit;

h = height of the albumen in millimeters and

W= egg weight in grams

The same procedures as the early laying phase were adopted for internal and external egg characteristics at the mid and late phases of lay with egg sampling done at 44th, 48th, and 52nd weeks of age for the mid laying phase and 63rd, 67th and 71st weeks of age for the late laying phase.

3.23.3 Yolk lipid profile

This was determined at the late laying phase of the experiment. For lipid profiling, 5mL of the mixed egg was collected with the use of syringe into bottles containing ethylene diamine tetra-acetic acid as anticoagulant. After centrifuging the samples at 1800 r/m, their analyses were done with the Hitachi 902 Auto Analyzer for total cholesterol, triglycerides, HDL and LDL. Values for VLDL were obtained by dividing the values for triglyceride by a factor of 5 in the specific egg yolk samples.

3.23.4 Blood parameters

Very close to the conclusion of the late phase of Experiment 4, blood (5mL) was sampled from the jugular veins of the birds and analysed for haematological and serum biochemical parameters as described in Experiment 3.

3.23.5 Apparent nutrient retention

At the conclusion of the late laying phase, four birds per treatment were selected and arranged in a metabolic cage system and fed corresponding treatment diets for six days for apparent nutrient retention assay as described in Experiment 3.

3.24 Statistical analysis

Data were subjected to ANOVA of the General Linear Model of SAS (2002) package. Means were separated using Duncan Multiple Range Test at $\alpha_{0.05}$.

CHAPTER 4

RESULTS

4.1 Experiment 1

Percentage of starch content of cassava varieties TME 419 and TMS 01/1368, percentage starch gelatinisation of the roasted and sun-dried cassava grits and chemical composition of the roasted cassava grits

Percentage starch content of whole tuber of cassava varieties TME 419 and TMS 01/1368 are shown in Figure 4.1. The TME 419 possessed a significantly higher ($p<0.05$) starch content of 23.4% than TMS 01/1368 with 19.4%. The percentage starch gelatinisation of roasted and sun-dried TME 419 and TMS 01/1368 grits are shown in Figures 4.2 and 4.3, respectively. Roasted TME 419 grits possessed a significantly higher ($p<0.05$) starch gelatinisation of 27.13% than the sun-dried grits with 3.22%. Similarly, roasted TMS 01/1368 grits had a significantly higher ($p<0.05$) starch gelatinisation of 25.26% than the sun-dried grits with 3.10%. The chemical composition of the roasted TME 419 and TMS 01/1368 grits is shown in Table 4.1

4.2 Experiment 2

True metabolisable energy of TME 419 and TMS 01/1368 cassava grits

Results of true metabolisable energy of the two varieties of cassava grits are shown in Figure 4.4. The TME 419 elicited a significantly higher ($p<0.05$) true metabolisable energy of 2.95 g/kcal than TMS 01/1368 with 2.86 g/kcal.

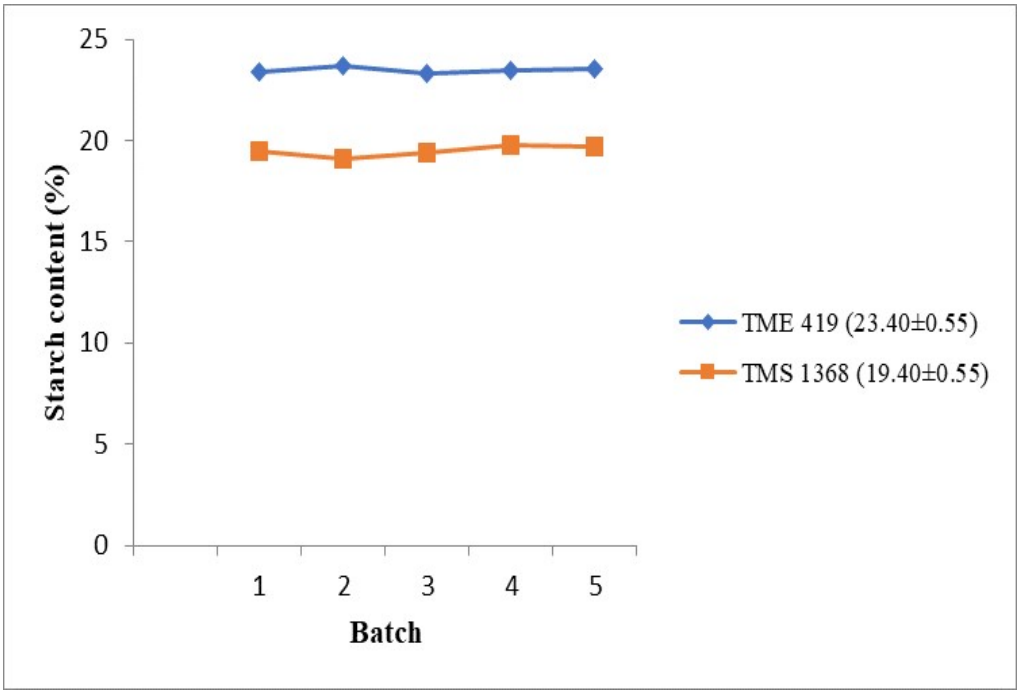

Fig 4.1: Starch content of cassava tuber varieties TME 419 and TMS 01/1368 (wet basis)

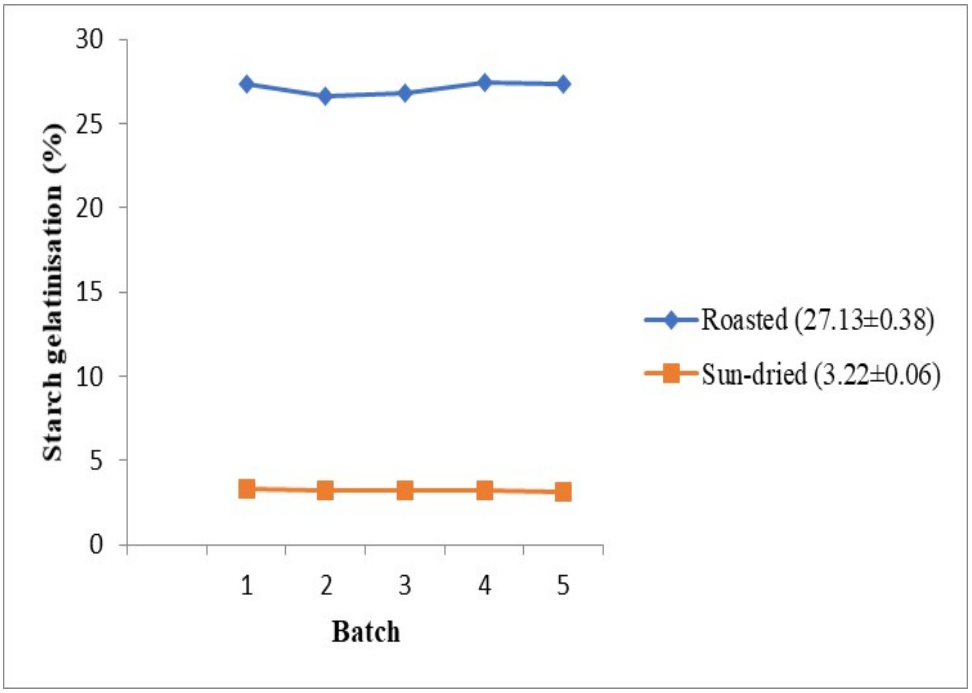

Fig 4.2: Percentage starch gelatinisation of TME 419 grits

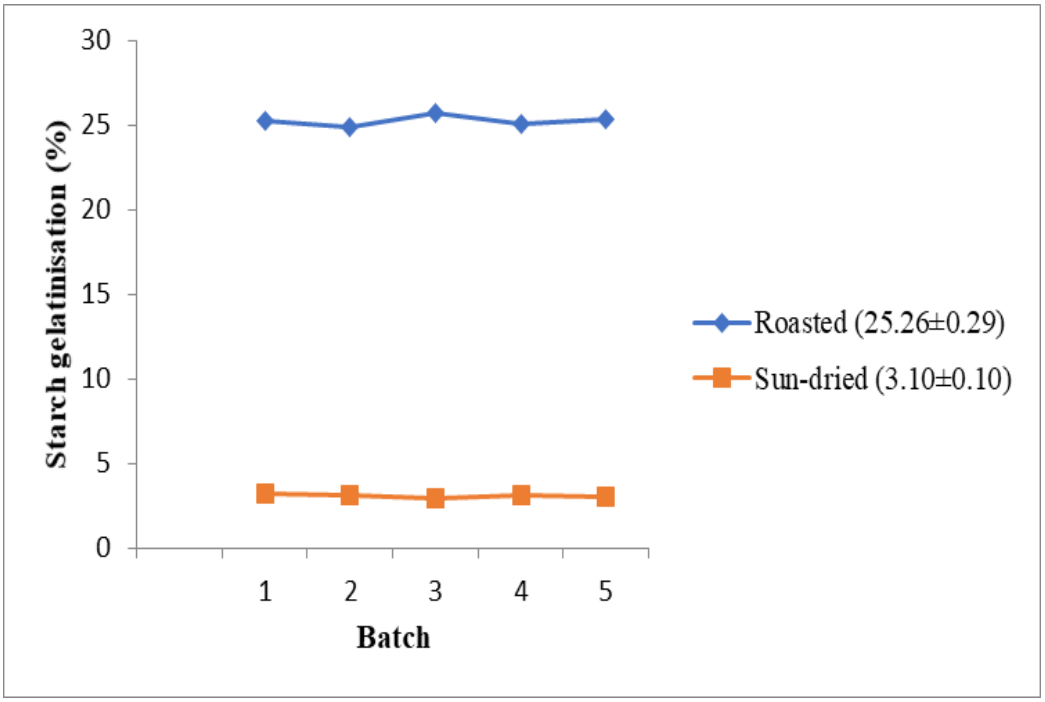

Fig 4.3: Percentage starch gelatinisation of TMS 01/1368 grits

Table 4.1 Chemical composition of cassava grits (roasted)

Composition (%)	TME 419	TMS 01/1368
Dry matter	88.00	85.80
Crude protein	2.71	3.94
Ether extract	1.50	1.80
Crude fibre	6.50	8.90
Ash	1.25	1.21
Nitrogen free extract	76.04	69.95
Phosphorus	0.06	0.05
Calcium	0.19	0.18
Hydrocyanic acid (mg/100g)	5.62	4.86

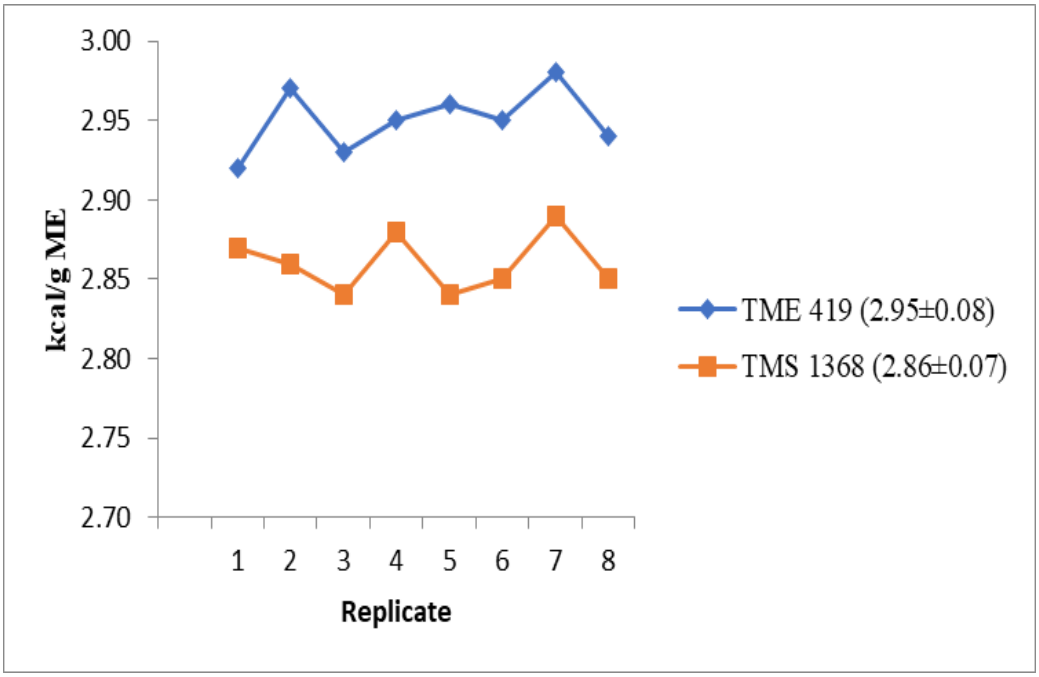

Fig 4.4: True metabolisable energy of TME 419 and TMS 01/1368 grits

4.3 Experiment 3

Performance, haematology and serum biochemistry of growing pullets fed enzyme-supplemented cassava grits-based diets.

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on the performance of growing pullets are presented in Table 4.2. The TME 419 grits-based diets elicited significantly higher ($p<0.05$) average daily gain (g/day) of 12.19 than in those fed TMS 01/1368 grits-based diets (11.13). Also, average daily gain decreased linearly with increasing level of cassava grits irrespective of variety. Growing pullets on 33% replacement levels diets had higher average daily gain (12.74 g/day) than those on 66% (11.67 g/day) and 100% (10.57 g/day) replacement levels; Pullets on diets with enzyme supplementation (35 mg/kg) had significantly higher ($p<0.05$) average daily gain (12.31g/day) than those on diets without supplemental enzyme (11.02 g/day).

Average daily feed intake (g/bird) differed significantly ($p<0.05$) between growing pullets fed TMS 419 grits-based diets (72.80) and those on TMS 01/1368 grits-based diets (75.42). Average daily feed intake also differed significantly ($p<0.05$) with the levels of replacement of maize with cassava grits in standard diet. Growing pullets on diets with 33% replacement levels had significantly lower ($p<0.05$) average daily feed intake of 72.27 g/bird than those on 66% with 74.65g/day and 100% replacement levels which had 75.44 g/day. Pullets on diets with enzyme supplementation had a significantly lower ($p<0.05$) average daily feed intake of 73.01 g/bird than 75.10 g/bird for those on diets without enzyme supplementation.

The same trend was observed in feed conversion ratio with significant differences ($p<0.05$) recorded between pullets on TME 419 grits-based diets (6.25) and those on TMS 01/1368 grits-based diets (6.69). Similarly, feed conversion ratio significantly increased with increasing levels of cassava grits in the diets from 33% (5.75), to 66% (6.54) and 100% (7.14); while pullet on diets with enzyme supplementation had significantly lower ($p<0.05$) feed conversion ratio (6.19) than pullets on diets without enzyme supplementation (6.76).

Age at first lay was not significantly affected ($p>0.05$) by the treatments and ranged from 144.94 days in enzyme supplemented diets to 146.11 days in diets without enzyme

supplementation. Although, egg weight at first lay (24.57g) was higher in pullets on TME 419 grits-based diets, there were no significant differences ($p>0.05$) among the treatments.

The cost per kg weight gain was significantly higher ($p<0.05$) in growing pullets on TMS 1368 grits-based diets (₦671.10) than in pullets on TME 419 grits-based diets (₦616.40). Similarly, cost per kg weight gain increased signifcantly as the level of replacement of maize with cassava grits in standard diet increased from 33% (₦ 575.10), to 66% (₦ 650.80) and 100% (₦ 705.40). Pullets on diets with supplemental enzyme had lower ($p < 0,05$) cost per kg weight gain ((₦ 635.80)) than those on diets without enzyme supplementation (₦ 651.70).

Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of growing pullets are outlined in Table 4.3.
Significant variations ($p<0.05$) existed in the average daily gain (g/day). Pullets on T_2 had higher average daily gain of 13.96 g/day than 12.48 in those on maize – based control diets T_0 and those on other treatments. The lowest weight gain of 9.22 g/day was obtained in pullets on T_{11}. Average daily feed intake (g/bird) was affected significantly ($p<0.05$) by the dietary treatments with the lowest intake in pullets on T_2 (69.38) and the highest intake was recorded in birds on T_{11} (77.19).
The feed conversion ratio (FCR) increased significantly ($p<0.05$) from T_2 (5.29), T_8 (5.54) and T_0 (5.59) to T_1 (5.87), T_4 (6.08), T_7 (6.29), T_{10} (6.44), T_6 (6.53), T_3 (6.74), T_9 (6.91), T_5 (7.01) T_{12} (7.27) and T_{11} (7.75). Age at first lay was not affected ($p>0.05$) by the dietary treatments, although, first egg was laid by pullets on T_4 at 141.33 days and later in those on T_5 and T_{10} at 147.67 days. Egg weight at first lay (g) was also not affected significantly ($p>0.05$) by the treatments with weight of eggs ranging from 24.07g in pullets on T_8 to 24.85 in maize standard diets (T_0). The cost per kg weight gain was significantly affected ($p<0.05$) by the treatments with the least cost in pullets on T_2 (₦ 559.41) and highest cost in those on T_{11} (₦ 725.54).

Table 4.2: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of growing pullets

	ADG (g/day)	ADFI (g/bird)	FCR	AFL (days)	EWFL (g)	Cost/kg Wt gain (₦)
Grits varieties						
TME 419	12.19[a]	72.80[b]	6.25[b]	145.00	24.57	616.40[b]
TMS 01/1368	11.13[b]	75.42[a]	6.69[a]	146.06	24.22	671.10[a]
Replacement levels (%)						
33.00	12.74[a]	72.27[b]	5.75[c]	145.92	24.43	575.10[c]
66.00	11.67[b]	74.65[a]	6.54[b]	145.42	23.39	650.80[b]
100.00	10.57[c]	75.44[a]	7.14[a]	145.25	24.35	705.40[a]
Enzyme supplementation (mg/Kg)						
0	11.02[b]	75.10[a]	6.76[a]	146.11	24.44	651.70[a]
35	12.31[a]	73.01[b]	6.19[b]	144.94	24.35	635.80[b]
SEM	1.02	2.05	0.41	16.64	2.84	10.40

[abc] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
ADG.: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio; AFL: Age at first lay; EWFL.:Egg weight at first lay; Wt: weight

Table 4.3: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of growing pullets

Grits varieties	RL (%)	Enzyme (mg/kg)	ADG (g/day)	ADFI (g/bird)	FCR	AFL (days)	EWFL (g)	Cost/kg Wt gain (₦)
Control	0	0	12.48^{b}	71.36^{c}	5.59^{de}	144.33	24.85	632.68^{d}
TME 419	33	0	12.40^{b}	71.38^{c}	5.87^{d}	145.00	24.65	583.02^{f}
	33	35	13.96^{a}	69.38^{d}	5.29^{e}	146.00	24.29	559.41^{i}
	66	0	11.44^{c}	74.89^{b}	6.74^{b}	144.33	24.42	596.33^{e}
	66	35	12.80^{b}	72.04^{c}	6.08^{cd}	141.33	24.84	576.07^{g}
	100	0	10.88^{d}	75.18^{b}	7.01^{b}	147.67	24.52	693.17^{c}
	100	35	11.67^{c}	72.58^{c}	6.53^{bc}	143.67	24.67	685.40^{c}
TMS 01/1368	33	0	11.59^{c}	74.99^{b}	6.29^{c}	147.33	24.72	593.31^{ef}
	33	35	12.93^{b}	73.03^{c}	5.54^{de}	144.33	24.07	564.67^{h}
	66	0	10.69^{d}	76.97^{a}	6.91^{b}	146.73	24.19	718.87^{ab}
	66	35	11.86^{c}	74.55^{b}	6.44^{bc}	147.67	24.08	711.91^{b}
	100	0	9.22^{e}	77.19^{a}	7.75^{a}	143.00	24.12	725.54^{a}
	100	35	10.51^{d}	75.53^{b}	7.27^{ab}	144.67	24.12	717.32^{ab}
SEM			1.02	2.05	0.41	16.64	2.84	10.40

^{abcdefghi} Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement level; ADG: Average daily gain, ADFI: Average daily feed intake FCR: Feed conversion ratio; AFE: Age at first lay; EWFL: Egg weight at first lay; Wt: weight.

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on apparent nutrient retention of growing pullets are presented in Table 4.4.

Cassava varietal type did not significantly affect ($p>0.05$) any of the nutrients' retention indices in growing pullets. Replacement levels of maize in standard diet with cassava grits did not affect significantly ($p>0.05$) retention of dry matter (DM), ether extract (EE), and crude fibre (CF) However, replacement levels significantly lowered ($P<0.05$) nitrogen retention (NR) in growing pullets as grits inclusion in the diets increased. Increasing the level of replacement of maize with grits in the diets from 33 to 100% resulted in linear decreases in the NR from 67.03 to 58.37%. Enzyme supplementation had no effect on DM, EE and CF retentions ($p>0.05$). However, enzyme supplementation significantly increased ($p<0.05$) NR with values of 63.55% in growing pullets on diets with supplemental enzyme compared to 61.39% in those on diets without enzymes.

In Table 4.5, the effects of interaction of cassava grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on apparent nutrient retention of growing pullets are presented. The DM retention was not affected by the treatments ($p>0.05$). Also, no significant differences ($p>0.05$) existed in the retention values of EE and CF by the pullets. However, significantly different ($p<0.05$) values existed in the NR of pullets with T_0, T_1, T_2, and T_7 having similar values of 68.51, 66.82, 68.16 and 67.65%, respectively, and more significantly higher than those on other treatments.

Main effects of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on haematology of growing pullets are shown in Table 4.6. The RBC, Hb, PCV, and the cellular indices namely MCV MCH, and MCHC were not significantly affected ($p>0.05$) by the cassava grits varietal difference, replacement levels of maize and enzyme supplementation. The WBC and the leukocyte differential counts namely lymphocyte, monocyte, eosinophil and basophil were also not different significantly ($p>0.05$).

Table 4.4: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on apparent nutrient retention of growing pullets

	Dry Matter (%)	Nitrogen Retention (%)	Ether Extract (%)	Crude Fibre (%)
Grits varieties				
TME 419	60.78	63.03	80.72	41.21
TMS 01/1368	61.29	61.97	81.20	40.86
Replacement levels (%)				
33.00	61.61	67.03[a]	80.80	41.74
66.00	62.16	62.10[b]	80.50	41.03
100.00	59.36	58.37[c]	81.62	40.35
Enzyme supplementation (mg/Kg)				
0	61.10	61.39[b]	81.01	41.00
35	60.97	63.55[a]	80.88	40.65
SEM	3.17	2.15	4.29	3.31

[abc] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)

Table 4.5: Effects of interaction of grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on apparent nutrient retention of growing pullets

Grits varieties	RL (%)	Enzyme (mg/Kg)	Dry Matter (%)	Nitrogen Retention (%)	Ether Extract (%)	Crude Fibre (%)
Control	0	0	62.17	68.51[a]	80.08	42.44
TME 419	33	0	61.32	66.82[ab]	80.39	41.02
	33	35	61.62	68.16[a]	80.76	42.38
	66	0	62.07	61.19[c]	80.65	41.22
	66	35	62.18	64.21[b]	79.97	40.90
	100	0	59.16	57.98[de]	80.79	41.41
	100	35	58.36	59.74[d]	81.70	40.27
TMS 1368	33	0	61.28	65.42[b]	81.04	41.42
	33	35	62.17	67.65[a]	80.95	42.12
	66	0	62.37	60.48[c]	81.06	41.82
	66	35	61.97	62.49[c]	80.27	40.06
	100	0	60.32	56.75[e]	82.32	40.28
	100	35	59.62	58.96[d]	81.59	39.31
SEM			3.17	2.15	4.29	3.31

[abcdefg] Means with identical superscripts within the same column of a group are not different significantly($p>0.05$)
RL: Replacement levels; SEM : Standard error of the mean.

Table 4.6: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on haematology of growing pullets

	RBC ($\times 10^{12}$/ L)	H_b (g/ dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC ($x10^9$/L)	Lymph (%)	Het (%)	Mon (%)	Eosi (%)	Baso (%)
Grits varieties												
TME 419	1.94	7.24	22.25	114.65	37.10	32.50	15.84	66.89	26.26	1.64	0.47	0.17
TMS 01/1368	1.96	7.43	21.20	108.11	38.05	35.05	15.79	64.60	25.54	1.57	0.37	0.20
Replacement levels (%)												
33.00	1.93	7.33	23.50	121.55	38.01	31.23	15.83	66.00	26.12	1.63	0.40	0.20
66.00	1.93	7.32	22.60	116.90	38.12	32.33	16.08	64.40	25.40	1.55	0.35	0.18
100.00	1.97	7.37	23.15	117.75	37.24	31.85	15.75	63.25	25.20	1.51	0.35	0.17
Enzyme supplementation (mg/Kg)												
0	1.95	7.34	22.20	113.62	36.95	33.04	15.76	63.47	25.12	1.52	0.30	0.17
35	1.95	7.33	23.05	117.95	37.22	31.76	14.80	64.92	25.69	1.58	0.40	0.18
SEM	0.24	0.48	2.35	10.70	3.05	4.65	1.99	4.95	2.89	0.05	0.09	0.01

RBC: Red blood cell, H_b: Haemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration: WBC: White blood cell; Lymph: Lymphocytes; Het: Heterophils; Mon: Monocytes; Eosi: Eosinophils; Baso: Basophils: SEM: Standard Error of Mean

Outlined in Table 4.7 are the main effects of cassava variety type, replacement levels of maize in standard diet and enzyme supplementation on serum biochemical indices of growing pullets. Cassava variety types did not significantly ($p>0.05$) affect glucose (GLUC), cholesterol (CHOL), triglycerides (TG), alanine aminotransferase (ALT), aspartate amino transferase (AST), total protein (TP), albumin (ALB) and thiocyanate (THIOC). Replacement levels of cassava grits did not significantly ($p>0.05$) affect GLUC, CHOL, TG ALT, AST, TP and ALB. However, birds on 100% and 66% replacement levels had significantly higher ($p<0.05$) THIOC (2.12 and 2.14 mg/dL respectively) compared to 33% replacement levels (1.98 mg/dL). Enzyme supplementation did not significantly ($p>0.05$) affect GLUC, CHOL, TG, ALT, AST, TP, ALB and THIOC.

The effects of interaction of cassava variety type, replacement levels of maize in standard diet and enzyme supplementation on serum biochemical indices of laying birds at grower phase are shown in Table 4.8. Blood glucose was not affected significantly ($p>0.05$) and ranged from 121.02 (T_5) to 122.88 (T_{12}). The CHOL observed was not significantly affected ($p>0.05$) and ranged from on 43.11 in T_{10} to 47.55 in T_8. Lowest value observed for TG was 229.5 in T_0 and highest value of 247.00 observed in T_7 but they were not significantly different ($p>0.05$). The ALT values observed were not significantly different ($p>0.05$) and ranged from 5.23 in T_8 to 6.08 in T_3. The AST values observed in growing pullets were similarly not affected significantly ($p>0.05$) by the interaction effects and ranged from 48.97 in T_9 to 51.55 in T_4. The values for TP did not elicit any significant difference ($p>0.05$). However, values ranged from 4.43 in T_4 to 4.65 in T_1. The interaction effects did not significantly affect ($p>0.05$) ALB values among the treatments. THIOC recorded in T_3 (2.16), T_5 (2.22), T_6 (2.22), T_9 (2.27), T_{11} (2.25), T_{12} (2.23) indicated statistical equivalence and were significantly higher ($p<0.05$) than the other treatments. The least value however was observed in standard maize-based control diet T_0 (0.32)

Table 4.7: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on serum biochemical indices of growing pullets

	GLUC (mg/dL)	CHOL (mg/dL)	TG (mg/dL)	ALT (IU/L)	AST (IU/L)	TP (g/dL)	ALB (g/dL)	THIOC (mg/dL)
Grits varieties								
TME 419	125.38	47.15	239.43	5.87	51.36	4.54	1.81	2.08
TMS 01/1368	125.92	45.57	240.95	5.68	50.25	4.55	1.80	2.12
Replacement levels (%)								
33.00	125.40	46.33	239.22	5.50	51.66	4.57	1.80	1.98[b]
66.00	126.35	46.34	242.74	5.77	50.69	4.50	1.84	2.12[a]
100.00	125.19	46.39	238.64	6.02	50.10	4.56	1.78	2.14[a]
Enzyme supplementation (mg/Kg)								
0	124.79	46.19	241.98	5.74	51.08	4.56	1.80	2.17
35	126.50	46.52	238.30	5.80	50.54	4.53	1.81	2.13
SEM	6.12	4.24	21.74	0.96	4.06	0.08	0.19	0.06

[ab] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
GLUC: Glucose; CHOL: Cholesterol; TG: Triglycerides; ALT: Alanine animotransferase; AST: Aspartate aminotransferase; TP:Total protein; ALB: Albumin; THIOC: Thiocyanate; SEM: Standard Error of the Mean

Table 4.8: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on serum biochemical indices of growing pullets

Grits Varieties	RL (%)	Enzyme (mg/Kg)	GLUC (mg/dL)	CHOL (mg/dL)	TG (mg/dL)	ALT (IU/L)	AST (IU/L)	TP (g/dL)	ALB (g/dL)	THIOC (mg/dL)
Control	0	0	121.53	45.84	229.50	5.30	49.40	4.58	1.83	0.32^{e}
TME 419	33	0	121.93	47.07	237.52	5.44	52.67	4.65	1.86	2.08^{b}
	33	35	121.94	47.46	235.50	5.99	52.87	4.49	1.80	1.85^{d}
	66	0	122.29	47.39	244.17	6.08	51.53	4.54	1.81	2.16^{ab}
	66	35	122.55	47.32	240.03	5.61	51.55	4.43	1.82	2.10^{b}
	100	0	121.02	46.56	243.50	6.07	50.47	4.56	1.77	2.22^{a}
	100	35	122.54	47.08	234.67	6.01	49.10	4.58	1.79	2.19^{a}
TMS 1368	33	0	121.44	43.26	247.00	5.38	51.84	4.55	1.76	2.04^{b}
	33	35	121.30	47.55	235.87	5.23	49.21	4.57	1.79	1.95^{c}
	66	0	121.78	47.46	243.85	5.36	48.97	4.51	1.85	2.27^{a}
	66	35	121.79	43.11	242.50	6.02	50.72	4.53	1.86	1.97^{c}
	100	0	121.33	45.34	234.83	6.06	51.05	4.55	1.77	2.25^{a}
	100	35	122.88	46.59	241.17	5.98	49.72	4.56	1.71	2.23^{a}
SEM			6.12	4.24	21.74	0.96		0.08	0.19	0.06

^{abcde} Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL:Replacement levels; ES: Enzyme supplementation; GLUC: Glucose; CHOL: Cholesterol; TG: Triglycerides; ALT: Alanine animotransferase; AST: Aspartate aminotransferase; TP:Total protein; ALB: Albumin; THIOC: Thiocyanate; SEM: Standard Error of Mean.

4.4 Experiment 4

Performance, haematology and serum biochemistry of laying hens fed enzyme-supplemented cassava grits-based diets.

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on the performance of laying hens at early laying phase are shown in Table 4.9. It was noted that cassava variety type affected ($p<0.05$) performance of laying birds at the early phase of lay. The live weight (g/bird) observed in birds on TME 419 grits-based diets (1598.23) was higher significantly ($p<0.05$) compared to TMS 01/1368 grits-based diets (1548.77). Higher average daily feed intake (g/bird) was observed in birds on TMS 01/1368 grits-based diets (96.58) compared to TME 419 grits-based diets (95.28). The hen day egg production (%) observed on TME 419 grits-based diets (39.41) was higher ($p<0.05$) compared to TMS 01/1368 grits-based diets (38.08) at early laying phase. It was noted that birds on TME 419 grits-based diets had higher ($p<0.05$) egg weight (45.54) compared to TMS 01/1368 (43.95). Higher feed conversion ratio ($p<0.05$) was noted in birds on TMS 01/1368 grits-based diets (7.51) compared to TME 419 (6.83). Replacement levels of maize with cassava grits in standard diet affected ($p<0.05$) performance of laying birds at early phase of lay. It was observed that birds on 33% replacement levels with cassava grits had higher ($p<0.05$) live weight (1676.45) in contrast with the remaining treatments. However, the lowest ($p<0.05$) live weight was observed in laying hens on 100% replacement levels (1482.50). Average daily feed intake observed in 33% replacement levels (93.58) was lower ($p<0.05$) in contrast with other dietary treatments. The highest average daily feed intake ($p<0.05$) was observed in 100% replacement levels (98.11). Hen day egg production (HDEP) values in laying hens on 33% replacement levels (41.74) was higher ($p<0.05$) in contrast with other dietary treatments. Nevertheless, the lowest ($p<0.05$) hen day production was noted in laying hens on 100% replacement levels (36.08). Higher egg weight ($p<0.05$) was noticed in laying hens on 33% replacement levels (49.63) in contrast with other treatments. The lowest ($p<0.05$) egg weight at early laying phase was observed in 100% replacement levels (41.6). Also, higher FCR ($p<0.05$) was noticed in 100% replacement levels (8.66) compared to the remaining treatments. However, lowest ($p<0.05$) FCR was

observed in laying hens on 33% replacement levels (5.63). Supplemental enzyme significantly ($p<0.05$) increased live weight of birds (1583.03) compared to birds on diets without supplemental enzyme (1563.97). Average daily feed intake observed in birds on enzyme supplemented diet (95.10) was lower ($p<0.05$) in contrast with laying hens on diets devoid of supplemental enzyme (96.36). However, hen day production was higher significantly ($p<0.05$) in laying hens on diets with enzyme supplementation (39.10) compared to laying hens on diets devoid of supplemental enzyme (38.39) at early laying phase. There was no significant difference ($p>0.05$) between the egg weight observed in birds on diets with enzyme supplementation (44.93) and birds on diets without enzyme supplementation (44.61). Significantly higher ($p<0.05$) feed conversion ratio was observed in birds on diets without enzyme supplementation (7.31) compared to those on diets with supplemental enzyme (7.04) at early laying phase.

The relationship between replacement levels of maize with cassava grits in standard diet and HDEP in laying hens at early laying phase is shown in Figure 4.5. The regression curve shows that an optimum level of 35% replacement of maize in a standard diet with cassava grits supported an HDEP of 41.7%. The R^2 value of 0.85 indicated that the HDEP of laying hens at early laying phase was up to 85% dependent on cassava grits replacement levels. The regression equation is shown below:

$Y = -0.000x^2 – 0.018x + 42.33, \quad (R^2 = 0.85)$ .....Equation 1

The effects of interaction of cassava variety type, replacement levels of maize in standard diet and supplemental enzyme on the performance of laying hens at early phase of lay are outlined in Table 4.10. Significantly higher ($p<0.05$) live weight was observed in birds on T_2 (1705.11) compared to other treatments at early laying phase. The average daily feed intake observed in T_5 (98.01), T_6 (98.50), T_9 (97.62), T_{10} (97.60), T_{11} (97.95), T_{12} (98.00) were similar ($p>0.05$) and significantly ($p<0.05$) higher compared to other treatments at early laying phase. Birds on T_2 exhibited least ($p<0.05$) intake of feed (91.88). Significantly higher ($p<0.05$) hen day egg production was observed in T_0 (42.27), T_1 (42.58) and T_3 (42.38) compared to other treatments. Birds on T_{11} (35.48) and T_{12} (35.53) had the lowest ($p<0.05$) hen day production at early laying phase. Egg weight values observed in T_0 (50.08), T_1 (50.88), T_2 (50.55),

T_7 (48.82) and T_9 (49.25) were similar and significantly higher ($p<0.05$) compared to other treatments. The FCR observed in T_2 (5.16), was significantly ($p<0.05$) lower compared to other dietary treatments at early laying phase. However, birds on T_5 (8.66), T_6 (8.51), T_{11} (8.76) and T_{12} (8.77) had the highest ($p<0.05$) FCR at early laying phase.

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and supplementation with enzyme on external egg characteristics of laying hens at early laying phase are outlined in Table 4.11. Egg length observed in eggs of laying birds on TME 419 grits-based diets (46.36) was higher significantly ($p<0.05$) than in those on TMS 01/1368 grits-based diets (45.14) at early laying phase. The same trend was noted for egg width which was higher ($p<0.05$) in eggs of laying birds on TME 419 grits-based diets (35.90) compared to those on TMS 01/1368 grits-based diets (34.86). However, shape index observed in eggs from laying birds on TME 419 grits-based diets (77.44) was not different ($p>0.05$) from those on TMS 01/1368 grits-based diets (77.23). Egg shell weight of birds on TME 419 grits-based diets (4.98) was not different significantly ($p>0.05$) in contrast with TMS 01/1368 grits-based diets (5.01). It was observed that cassava variety type did not significantly ($p>0.05$) affect shell thickness (0.37mm) of eggs. Shell ratio was not influenced ($p>0.05$) by cassava variety type and ranged from 9.17 in eggs of birds on TME 419 grits-based diets to 9.18 in birds on TMS 01/1368 grits-based diets.

Cassava grits replacement levels did not change ($p>0.05$) external egg characteristics at early laying phase except egg length and width. It was observed that egg length in laying hens on 33% cassava grits replacement levels (50.60) was higher significantly ($p<0.05$) in contrast with the remaining treatments. The lowest ($p<0.05$) egg length was observed in 100% cassava grits replacement (42.12). Egg width observed in 33% cassava grits replacement (39.1) was increased significantly ($p<0.05$) in contrast with the other replacement levels. However, the lowest ($p<0.05$) egg width was observed in 100% cassava grits replacement (32.61). Supplemental enzyme did not significantly influence ($p>0.05$) external egg characteristics measured.

Table 4.9: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of hens at early laying phase

	LW (g/bird)	ADFI (g/ bird)	HDEP (%)	EW (g)	FCR
G2rits varieties					
TME 419	1598.23[a]	95.28[b]	39.41[a]	45.54[a]	6.83[b]
TMS 01/1368	1548.77[b]	96.58[a]	38.08[b]	43.95[b]	7.51[a]
Replacement levels (%)					
33.00	1676.45[a]	93.58[c]	41.48[a]	49.63[a]	5.63[c]
66.00	1561.58[b]	96.10[b]	39.07[b]	43.27[b]	7.23[b]
100.00	1482.50[c]	98.11[a]	36.08[c]	41.43[c]	8.66[a]
Enzyme supplementation (mg/Kg)					
0	1563.97[b]	96.36[a]	38.39[b]	44.61	7.31[a]
35	1583.03[a]	95.10[b]	39.10[a]	44.93	7.02[b]
SEM	17.09	1.01	0.70	1.45	0.25

[abc] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
LW.: Live Weight; ADFI: Average daily feed intake; HDEP: Hen day egg production; EW: Egg weight; FCR: Feed Conversion Ratio

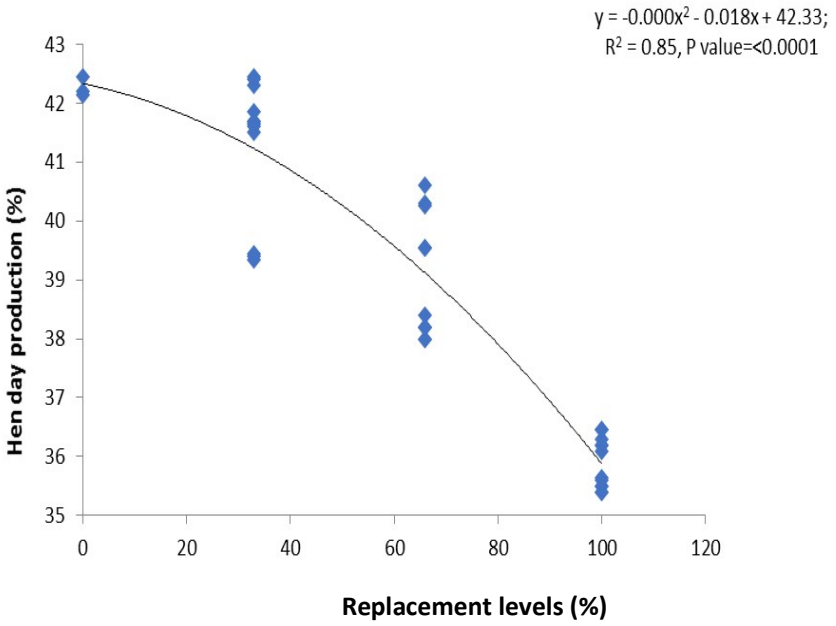

Figure 4.5: Relationship between replacement levels of maize with cassava grits in standard diet and hen day egg production at early laying phase.

Table 4.10: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of hens at early laying phase

Grits varieties	Replacement Level (%)	Enzyme (mg/Kg)	LW (g/bird)	ADFI (g/bird)	HDEP (%)	EW (g)	FCR
Control	0	0	1666.49^{c}	94.00^{b}	42.27^{a}	50.08^{a}	5.61^{d}
TME 419	33	0	1687.62^{b}	94.13^{b}	42.58^{a}	50.88^{a}	5.43^{d}
	33	35	1705.11^{a}	91.88^{d}	42.38^{a}	50.55^{a}	5.16^{e}
	66	0	1563.21^{f}	95.05^{b}	39.55^{d}	44.57^{b}	6.72^{c}
	66	35	1595.19^{e}	94.14^{b}	40.38^{c}	44.33^{b}	6.53^{c}
	100	0	1515.33^{h}	98.01^{a}	36.18^{f}	41.05^{c}	8.66^{a}
	100	35	1522.92^{h}	98.50^{a}	36.38^{f}	41.88^{c}	8.51^{a}
TMS 01/1368	33	0	1635.98^{d}	95.32^{b}	39.40^{d}	48.82^{a}	6.43^{c}
	33	35	1677.00^{bc}	93.01^{c}	41.75^{b}	49.25^{a}	5.51^{d}
	66	0	1539.63^{g}	97.62^{a}	38.07^{e}	42.02^{c}	7.84^{b}
	66	35	1548.27^{g}	97.60^{a}	38.27^{e}	42.17^{c}	7.81^{b}
	100	0	1442.05^{i}	97.95^{a}	35.48^{g}	41.43^{c}	8.76^{a}
	100	35	1449.69^{i}	98.00^{a}	35.53^{g}	41.37^{c}	8.71^{a}
SEM			17.09	1.01	0.70	1.45	0.25

^{abcdefghi} Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)LW.: Live Weight; ADFI: Average daily feed intake; HDEP: Hen day egg production; EW: Egg weight; FCR: Feed Conversion Ratio

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The effects of interaction of grits variety type, replacement levels of maize in standard diet and enzyme supplementation on egg external characteristics at early laying phase are presented in Table 4.12. Egg length observed in T_0 (51.44), T_1 (51.41) and T_2 (51.88) was significantly higher ($p<0.05$) compared to other treatments at early laying phase. The lowest ($p<0.05$) egg length was noted in T_5 (41.75), T_6 (42.29), T_{11} (42.19) and T_{12} (42.23). Significantly higher ($p<0.05$) egg width was observed in T_0 (39.69), T_1 (39.86), T_2 (40.16) compared to other treatments at early laying phase. Shell weight, egg shape index, shell thickness and shell ratio were not affected ($p>0.05$) by interaction of cassava variety type, grits replacement levels and enzyme supplementation at early laying phase.

The main effects of grits variety type, replacement levels of maize in standard diet and enzyme supplementation on egg internal characteristics at early laying phase are depicted in Table 4.13. Yolk weight observed in eggs of birds on TME 419 grits-based diets (12.83) was higher ($p<0.05$) compared to TMS 01/1368 (12.36). Albumen height, Haugh unit and yolk ratio were not influenced ($p>0.05$) with cassava variety type. It was observed that 33% cassava grits replacement levels resulted in higher ($p<0.05$) albumen height (8.44) compared to other replacement levels with 100% replacement resulting in the lowest albumen height (7.27). Significantly elevated ($p<0.05$) Haugh unit was noted in 33% replacement (93.09) compared to other replacement levels. Egg yolk weight of birds on 33% replacement (13.99) was higher ($p<0.05$) in contrast with other treatments. However, yolk ratio was not significantly affected ($p>0.05$) by cassava grits replacement levels in birds at early laying phase. Supplemental enzyme did not influence ($p>0.05$) albumen height, Haugh unit, yolk weight and yolk ratio.

Table 4.11: Main effects of cassava grits varieties, replacement levels of maize in standard diet and supplemental enzyme on external egg characteristics of hens at early laying phase

	EL (mm)	EWi (mm)	SI (%)	SW (g)	ST (mm)	SR (%)
Grits varieties						
TME 419	46.36[a]	35.90[a]	77.44	4.98	0.37	9.17
TMS 01/1368	45.14[b]	34.86[b]	77.23	5.01	0.37	9.18
Replacement levels (%)						
33.00	50.60[a]	39.14[a]	77.25	4.98	0.37	9.33
66.00	44.46[b]	34.39[b]	77.34	5.05	0.36	9.13
100.00	42.12[c]	32.61[c]	77.42	4.93	0.36	9.06
Enzyme supplementation (mg/Kg)						
0	45.68	35.36	77.41	4.95	0.36	9.12
35	45.82	35.40	77.27	5.02	0.37	9.23
SEM	1.17	1.00	3.12	0.45	0.01	1.45

[abc]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
EL:Egg length; EWi: Egg width; SI: Shape Index; SW: Shell weight; ST: Shell thickness; SR: Shell ratio

Table 4.12: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on external egg characteristics of hens at early laying phase

Grits Varieties	RL (%)	Enzyme (mg/kg)	EL (mm)	EWi (mm)	SI (%)	SW (g)	ST (mm)	SR (%)
Control	0	0	51.44[a]	39.69[a]	77.15	5.07	0.37	9.12
TME 419	33	0	51.41[a]	39.86[a]	77.44	4.97	0.37	9.19
	33	35	51.88[a]	40.16[a]	77.53	5.15	0.36	9.33
	66	0	45.58[c]	35.32[c]	77.50	4.77	0.36	9.16
	66	35	45.24[c]	34.97[c]	77.29	5.18	0.37	9.15
	100	0	41.75[e]	32.33[e]	77.44	4.98	0.37	9.09
	100	35	42.29[e]	32.78[e]	77.44	4.86	0.37	9.08
TMS 01/1368	33	0	49.16[b]	38.02[b]	77.34	4.86	0.37	9.22
	33	35	50.22[b]	38.51[b]	76.69	4.95	0.37	9.56
	66	0	43.55[g]	33.70[d]	77.39	5.15	0.37	9.03
	66	35	43.49[d]	33.56[d]	77.15	5.12	0.37	9.17
	100	0	42.19[e]	32.62[e]	77.32	4.97	0.37	8.99
	100	35	42.23[e]	32.72[e]	77.47	4.93	0.37	9.08
SEM			1.17	1.00	3.12	0.45	0.01	1.45

[abcde] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement levels; EL: Egg length; EWi: Egg width; SI: Shape Index; SW:Shell weight; ST: Shell thickness; SR: Shell ratio

Table 4.13: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on internal egg characteristics of hens at early laying phase

	Albumen Height (mm)	Haugh Unit	Yolk Weight (g)	Yolk Ratio
Grits varieties				
TME 419	7.94	91.65	12.83[a]	25.57
TMS 01/1368	7.79	91.43	12.36[b]	25.31
Replacement levels (%)				
33.00	8.44[a]	93.09[a]	13.99[a]	25.55
66.00	7.90[b]	92.07[b]	12.29[b]	25.54
100.00	7.27[c]	89.49[c]	11.50[c]	25.24
Enzyme supplementation (mg/kg)				
0	7.85	91.49	12.56	25.39
35	7.88	91.59	12.63	25.49
SEM	0.51	0.70	0.40	2.12

[abc]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)

Relationship between the maize replacement levels with cassava grits in the standard diet and Haugh unit at early laying phase is shown in Figure 4.6. The relationship was negative, the Haugh Unit related linearly to the replacement levels of maize with cassava grits in standard diets. The relationship is further explained by the regression equation 2 below:

$Y = -0.053x + 95.14$ ($R^2 = 0.936$) ..... Equation 2

The R^2 value of 0.936 depicted that the Haugh Unit of eggs in laying hens at early laying phase was up to 93.6% dependent on cassava grits replacement levels. The relationship was significant ($p<0.05$)

Effects of interaction of cassava grits varietal type, replacement levels in standard diet and supplemental enzyme on internal characteristics of eggs of laying hens at early laying phase are shown in Table 4.14. Similar albumen heights were observed in the eggs of pullets from control T_0 (8.52), T_1 (8.53), T_7 (8.34), and T_8 (8.47) and were higher ($p<0.05$) in contrast with the remaining treatments at the early laying phase. Higher ($p<0.05$) Haugh unit was obtained in hens on control T_0 (93.33) T_1 (93.26), T_2 (93.03), T_7 (92.73), T_8 (93.36) compared to eggs from other treatments at the early laying phase. Yolk weight of eggs from pullets on control T_0 (14.17) T_0 (14.28), and T_2 (14.34) were identical ($p>0.05$) but higher significantly ($p<0.05$) in contrast with eggs from other treatments at the early laying phase. Yolk ratio of eggs from pullets in all the treatments were similar ($p>0.05$) at the early laying phase.

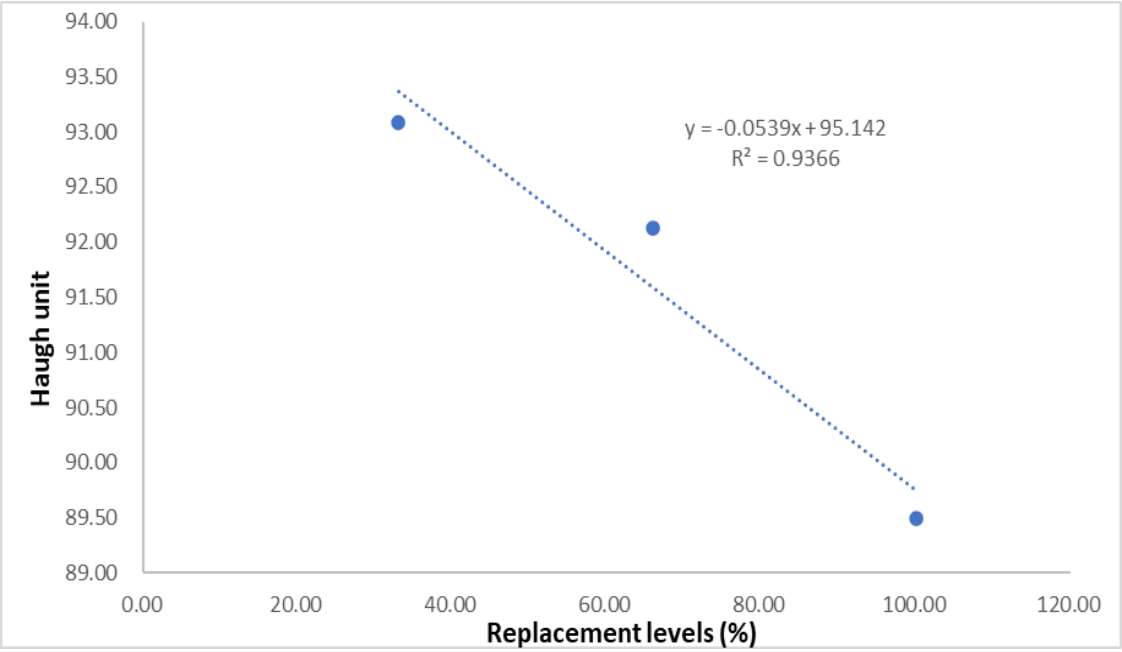

Figure 4.6: Relationship between replacement levels of maize in standard diet with cassava grits and Haugh unit at the early laying phase.

Table 4.14: Effects of Interaction of cassava grits varieties, replacement levels of maize in the standard diet and supplemental enzyme on internal egg characteristics of hens at the early laying phase

Grits Varieties	RL (%)	Enzyme (mg/Kg)	Albumen height (g)	Haugh unit	Yolk weight (g)	Yolk ratio
Control	0	0	8.52[a]	93.33[a]	14.17[a]	25.49
TME 419	33	0	8.53[a]	93.24[a]	14.28[a]	25.51
	33	35	8.46[a]	93.03[a]	14.34[a]	25.86
	66	0	8.02[b]	92.31[b]	12.69[c]	25.71
	66	35	8.01[b]	92.29[b]	12.63[c]	25.81
	100	0	7.25[c]	89.51[c]	11.42[f]	25.38
	100	35	7.30[c]	89.55[c]	11.58[e]	25.38
TMS 01/1368	33	0	8.34[a]	92.73[a]	13.57[b]	25.49
	33	35	8.47[a]	93.36[a]	13.75[b]	25.37
	66	0	7.79[b]	91.75[b]	11.83[de]	25.11
	66	35	7.69[b]	91.90[b]	12.02[d]	25.49
	100	0	7.27[c]	89.39[c]	11.57[e]	25.21
	100	35	7.25[c]	89.45[c]	11.43[f]	25.21
SEM			0.51	0.70	0.40	2.12

[abcdef] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement levels; ES: Enzyme supplementation; SEM: Standard error of the mean

Main effects of cassava grits variety type, replacement levels of maize with grits in standard diet and supplemental enzyme on the performance of laying hens at the mid laying phase are presented in Table 4.15**.** Laying pullets on TME 419 grits-based diets had higher ($p<0.05$) live weight of 1748.15g compared to 1687.18 for those on TMS 01/1368. However, pullets on TMS 01/1368 grits-based diets elicited significantly higher ($p<0.05$) average daily feed intake of 118.37g/bird compared to 117.24g for those on TME 419. Hen day egg production obtained for pullets on TMS 01/1368 grits-based diets (72.57) was lower significantly ($p<0.05$) than 73.64 obtained for pullets on TME 419. Weight of eggs obtained for pullets on TME 419 grits-based diets (56.00) was remarkably higher ($p<0.05$) in contrast with those from TMS 01/1368 (54.94). Significantly higher ($p<0.05$) FCR was obtained in pullets on TMS 01/1368 grits-based diets (3.05) than from TME 419 (2.88) at the mid-laying phase. Varying maize replacement levels with cassava grits changed remarkably ($p<0.05$) the performance of laying pullets at the mid laying phase. Significantly higher ($p<0.05$) live weight was obtained for pullets on 33% maize replacement with grits (1828.40) than in pullets on other dietary treatments. The lowest ($p<0.05$) live weight of 1613.85g was obtained for pullets on 100% maize replacement with grits. Pullets on 100% maize replacement with cassava grits (119.88) had remarkably higher ($p<0.05$) average daily feed intake in contrast with to those on 66% (118.11) and 33% (115.42) maize replacement with grits at the mid laying phase. Significantly increased ($p<0.05$) hen day egg production was noted in laying hens on 33% maize replacement with cassava grits (77.71) compared to other dietary treatments at the mid laying phase. Significantly higher ($p<0.05$) egg weight of 60.86g was observed in laying hens on 33% cassava grits replacement compared to laying hens on cassava grits replacements at 66% and 100% with egg weight of 55.14 and 50.42, respectively, at mid laying phase. However, the FCR observed in laying hens on 100% cassava grits replacement (3.59) was significantly higher ($p<0.05$) compared to other dietary treatments. The lowest ($p<0.05$) FCR was observed in birds on 33% cassava grits replacement (2.44) at mid laying phase. It was observed that live body weight was significantly increased ($p<0.05$) in laying hens on diets with enzyme supplementation (1734.11) compared to the laying hens on diets without supplemental enzyme (1701.21) at the mid laying phase. Average daily feed intake of laying hens on enzyme supplemented diets (117.03) decreased remarkably

($p<0.05$) in contrast with those on diets devoid of supplemental enzyme (118.48). Significantly increased ($p<0.05$) hen day production was noted in laying hens on enzyme-supplemented diets (73.71) in contrast with those on diets devoid of supplemental enzyme (72.33). It was observed that egg weight of birds on enzyme supplemented diets (55.57g) was not different ($p>0.05$) from those on diets without supplemental enzyme (55.38). Significantly increased ($p<0.05$) FCR was noticed in laying hens on diets without enzyme supplementation (3.00) in contrast with those on enzyme-supplemented diets (2.93) at mid laying phase.

The relationship between replacement levels of maize with cassava grits in standard diet and HDEP in laying hens at mid laying phase is shown in Figure 4.7. From the regression curve, it could be depicted that an optimum level of 40% replacement of maize in a standard diet with cassava grits supported an HDEP of 78.5%. The R^2 value of 0.68 indicated that the HDEP of laying hens at mid laying phase was up to 68% dependent on cassava grits replacement levels. The regression equation is shown below:

$Y = -0.002x^2 + 0.153x + 75.53, \quad (R^2 = 0.68)$ .....Equation 3

The effects of interaction of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on performance of laying hens at the mid laying phase are presented in Table 4.16. Significantly higher ($p<0.05$) live weight was observed in birds on T_2 (1877.28) compared to other treatments at mid laying phase. The lowest ($p<0.05$) live weight was observed in T_{11} (1560.17) at mid laying phase. The average daily feed intake observed in T_5 (120.07), T_6 (119.88), T_9 (119.13), T_{11} (119.75) and T_{12} (119.87), increased significantly ($p<0.05$) in contrast with the other treatments at mid laying phase. Birds on T_2 (114.07) exhibited the lowest ($p<0.05$) average daily feed intake. Higher ($p<0.05$) hen day egg production was observed in T_2 (79.90) compared to other treatments. Egg weights observed in T_0 (60.83), T_1 (60.83), T_2 61.28), T_7 (60.44), and T_8 (60.90), were similar and significantly higher ($p<0.05$) compared to other treatments at mid laying phase. The FCR observed in T_2 (2.35), decreased remarkably ($p<0.05$) in contrast with the remaining dietary treatments at mid laying phase. However, birds on T_{11} (3.66) and T_{12} (3.68) had the highest ($p<0.05$) FCR at mid laying phase.

Table 4.15: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of hens at mid laying phase

	LW (g/bird)	ADFI (g/ bird)	HDEP (%)	EW (g)	FCR
Grits varieties					
TME 419	1748.15[a]	117.24[b]	73.64[a]	56.00[a]	2.88[b]
TMS 01/1368	1687.18[b]	118.37[a]	72.57[b]	54.94[b]	3.05[a]
Replacement levels (%)					
33.00	1828.40[a]	115.42[c]	77.71[a]	60.86[a]	2.44[c]
66.00	1710.74[b]	118.11[b]	75.43[b]	55.14[b]	2.86[b]
100.00	1613.85[c]	119.88[a]	67.22[c]	50.42[c]	3.59[a]
Enzyme supplementation (mg/Kg)					
0	1701.21[b]	118.48[a]	72.33[b]	55.38	3.00[a]
35	1734.11[a]	117.03[b]	73.71[a]	55.57	2.93[b]
SEM	12.49	1.02	1.01	1.00	0.06

[abc]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
LW.: Live Weight; ADFI: Average daily feed intake; HDEP: Hen day egg production; EW: Egg weight; FCR: Feed Conversion Ratio

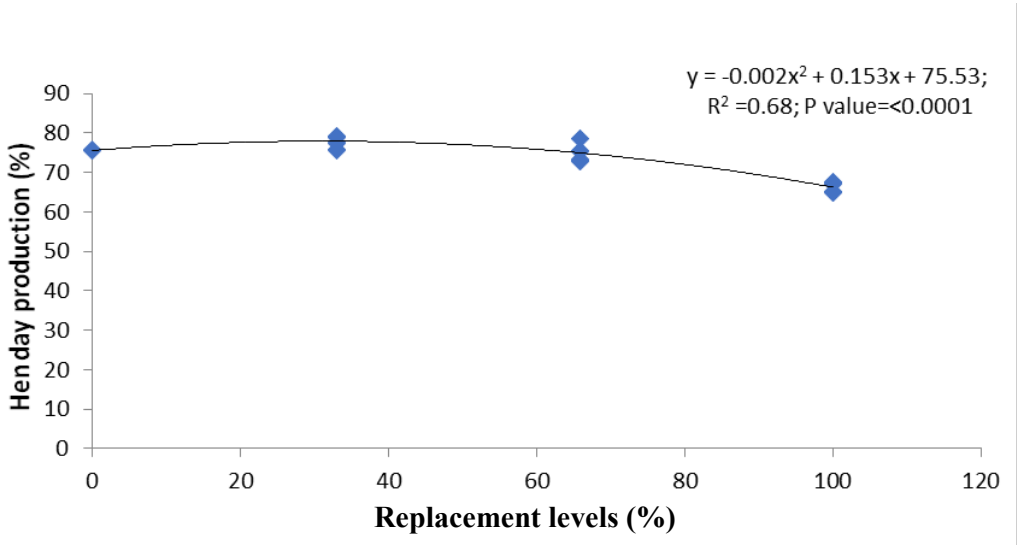

Figure 4.7: Relationship between replacement levels of maize with cassava grits in standard diet and hen day egg production at the mid laying phase.

Table 4.16: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of hens at mid laying phase

Grits varieties	RL (%)	Enzyme (mg/kg)	LW (g/bird)	ADFI (g/bird)	HDEP (%)	EW (g)	FCR
Control	0	0	1827.72^{b}	115.23^{c}	75.48^{b}	60.83^{a}	2.59^{f}
TME 419	33	0	1833.56^{b}	115.85^{c}	75.93^{b}	60.83^{a}	2.46^{g}
	33	35	1877.28^{a}	114.07^{d}	79.90^{a}	61.28^{a}	2.35^{h}
	66	0	1712.33^{e}	117.78^{b}	74.32^{c}	56.35^{b}	2.77^{d}
	66	35	1754.78^{d}	116.97^{b}	76.43^{b}	56.17^{b}	2.65^{e}
	100	0	1646.17^{h}	120.07^{a}	67.68^{d}	50.56^{d}	3.54^{b}
	100	35	1644.78^{h}	119.88^{a}	67.76^{d}	50.78^{d}	3.50^{b}
TMS 01/1368	33	0	1779.78^{c}	116.45^{bc}	76.40^{b}	60.44^{a}	2.55^{f}
	33	35	1828.00^{b}	115.28^{c}	76.31^{b}	60.90^{a}	2.40^{h}
	66	0	1680.28^{g}	119.13^{a}	74.37^{c}	53.89^{c}	3.03^{c}
	66	35	1695.56^{f}	118.98^{a}	74.26^{c}	54.13^{c}	2.99^{c}
	100	0	1560.17^{j}	119.75^{a}	67.00^{d}	50.18^{d}	3.66^{a}
	100	35	1584.28^{i}	119.87^{a}	67.12^{d}	50.16^{d}	3.68^{a}
SEM			12.49	1.02	1.01	1.00	0.06

^{abcdefghij}Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement levels; LW.: Live Weight; ADFI: Average daily feed intake; HDEP: Hen day egg production; EW: Egg weight; FCR: Feed Conversion Ratio

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on external egg characteristics at mid laying phase are presented in Table 4.17. Cassava variety type did not remarkably influence ($p>0.05$) external egg characteristics measured. Eggs of pullets on TME419 grits-based diets had similar ($p>0.05$) egg length (52.10) with those on TMS 01/1368 (51.16). Egg width from pullets on TME 419 grits-based diets (39.94) did not remarkably differ ($p>0.05$} from those on TMS 01/1368 (39.22). Egg shape index in birds on TME 419 grits-based diets (76.66) and that of TMS 01/1368 grits-based diets (76.67) were similar ($p>0.05$). Shell weight in eggs of laying hens on TME 419 grits-based diets (5.11) was observed to be similar to that of TMS 01/1368 (5.01). Similarities were also observed between the shell thickness in eggs of laying hens on TME 419 grits-based diets (0.38) and those on TMS 01/1368 grits-based diets (0.37). Replacement levels of maize with cassava grits had no remarkable influence ($p>0.05$) on external egg characteristics estimated at mid laying phase with the exception of egg length and egg width. The egg length in the pullets on 33% maize replacement (56.09) was remarkably higher ($p<0.05$) in contrast with the remaining treatments. Replacement levels of maize with cassava grits changed significantly ($p<0.05$) the egg width of laying hens at the mid laying phase with values of 42.96, 39.59 and 36.19 mm at 33, 66 and 100% replacement levels, respectively. However, enzyme supplementation did not remarkably influence ($p>0.05$) any of the estimated external egg characteristics.

The effects of interaction of cassava grits varietal type, replacement levels of maize in standard diet and dietary supplementation with enzyme on external egg characteristics of laying hens at the mid laying phase are outlined in Table 4.18. Egg length of laying hens on T_0 (56.09), T_1 (55.76), T_2 (56.56), T_7 (55.66) and T_8 (56.40) was higher remarkably ($p<0.05$) in contrast with egg length from other dietary treatments. Egg width in T_0 (42.89), T_1 (42.67), T_2 (43.31), T_7 (42.57) and T_8 (43.27) was remarkably higher ($p<0.05$) in contrast with the remaining treatments. The effects of interaction of cassava variety, replacement levels and enzyme supplementation on egg shape index, shell weight, thickness and ratio were not remarkable ($p>0.05$) at the mid laying phase.

Table 4.17: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on external egg characteristics of hens at the mid laying phase

	EL (mm)	EWi (mm)	SI (%)	SW (g)	ST (mm)	SR (%)
Grits varieties						
TME 419	52.10	39.94	76.66	5.23	0.38	8.88
TMS 01/1368	51.16	39.22	76.67	5.15	0.37	8.88
Replacement levels (%)						
33.00	56.09[a]	42.96[a]	76.58	5.39	0.38	9.03
66.00	51.62[b]	39.59[b]	76.69	5.06	0.38	8.91
100.00	47.18[c]	36.19[c]	76.72	5.13	0.37	8.70
Enzyme supplementation (mg/Kg)						
0	51.44	39.40	76.61	5.19	0.38	8.84
35	51.83	39.76	76.71	5.21	0.38	8.93
SEM	1.72	1.20	3.98	0.45	0.01	0.46

[abc]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
EL:Egg length; EWi: Egg width; SI: Shape Index; SW: Shell weight; ST: Shell thickness; SR: Shell ratio

Table 4.18: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and supplemental enzyme on external egg characteristics of hens at mid laying phase

Grits Varieties	RL (%)	ES (mg/Kg)	EL (mm)	EWi (mm)	SI (%)	SW (g)	ST (mm)	SR (%)
Control	0	0	56.09[a]	42.89[a]	76.48	5.48	0.38	9.04
TME 419	33	0	55.76[a]	42.67[a]	76.53	5.47	0.38	8.87
	33	35	56.56[a]	43.31[a]	76.58	5.51	0.38	9.14
	66	0	52.57[b]	40.29[b]	76.64	5.15	0.37	8.93
	66	35	52.85[b]	40.57[b]	76.77	5.11	0.38	8.79
	100	0	47.33[d]	36.26[d]	76.60	5.03	0.38	8.69
	100	35	47.56[d]	36.54[d]	76.84	5.16	0.37	8.89
TMS 01/1368	33	0	55.66[a]	42.57[a]	76.49	5.52	0.38	9.01
	33	35	56.40[a]	43.27[a]	76.72	5.07	0.38	9.09
	66	0	50.45[c]	38.68[c]	76.67	4.98	0.38	8.94
	66	35	50.61[c]	38.72[c]	76.69	5.04	0.38	9.01
	100	0	46.88[d]	35.96[d]	76.74	5.05	0.38	8.58
	100	35	46.94[d]	36.02[d]	76.68	5.29	0.38	8.63
SEM			1.72	1.20	3.98	0.45	0.01	0.46

[abcd] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement levels; ES: Enzyme supplementation; EL:Egg length; EWi: Egg width; SI: Shape Index;;SW:Shell weight;ST: Shell thickness; SR: Shell ratio

Main effects of cassava grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on internal egg characteristics of laying hens at the mid laying phase are shown in Table 4.19. Cassava grits variety type (TME 419 and TMS 01/1368) did not significantly influence (p0.05) the assessed egg internal characteristics. Replacement levels of maize with cassava grits significantly affected ($p<0.05$) the measured internal egg characteristics except for yolk ratio. Albumen height was remarkably higher ($p<0.05$) at 33% replacement (8.23) compared to the other replacement levels. The lowest albumen height ($p<0.05$) was observed in diets with 100% replacement levels (7.03). The same trend was observed in Haugh unit and yolk weight at 33% replacement levels with significantly higher ($p<0.05$) values of 90.18 and 17.82, respectively. Enzyme supplementation in diets of laying hens at the mid laying phase elicited no remarkable effect ($p>0.05$) on measured internal egg characteristics. Relationship between Haugh unit and replacement levels of maize in standard diet with cassava grits at the mid laying phase is depicted in Figure 4.8. The relationship was negative, linear and significant ($p<0.05$). The relationship is further shown in regression equation 4 below:

$Y= -0.06x + 92.12$ ($R^2 = 0.999$) ..... Equation 4

The interaction effects of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on internal egg characteristics of laying hens at mid laying phase is shown in Table 4.20. Similar ($p>0.05$) albumen heights (mm) were noted in eggs from the pullets on control T_0 (8.30), T_1 (8.29), T_2 (8.25), T_7 (8.24), and T_8 (8.22). The values were remarkably higher ($p<0.05$) in contrast with the remaining treatments at mid laying phase. Also, the Haugh units of eggs of laying hens on control T_0 (90.58) T_1 (90.47), T_2 (90.13), T_7 (90.08), T_8 (90.07) were similar but were remarkably higher ($p<0.05$) than in other treatments at mid laying phase. Yolk weights of eggs (g) from the laying hens on control T_0 (17.81), T_1 (17.74), and T_2 (18.08), T_3 (16.44), T_4 (16.34), T_7 (17.36), and T_8 (17.82) were similar ($p>0.05$) but were higher remarkably ($p<0.05$) in contrast with the remaining treatments at mid laying phase. Effect of treatment on yolk ratio in all the treatments were similar ($p>0.05$) at the mid laying phase.

Table 4.19: Main effects of cassava grits varieties, replacement levels of maize in standard diet and supplemental enzyme on internal egg characteristics of hens at the mid laying phase

	Albumen Height (mm)	Haugh Unit	Yolk Weight (g)	Yolk Ratio
Grits varieties				
TME 419	7.68	88.31	16.48	28.86
TMS 01/1368	7.56	87.85	16.12	28.59
Replacement levels (%)				
33.00	8.23[a]	90.18[a]	17.82[a]	28.77
66.00	7.60[b]	88.05[b]	16.13[b]	28.72
100.00	7.03[c]	86.02[c]	14.91[c]	28.68
Enzyme supplementation (mg/kg)				
0	7.63	87.99	16.26	28.70
35	7.61	88.17	16.35	28.75
SEM	0.40	1.10	1.40	2.23

[abc]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)

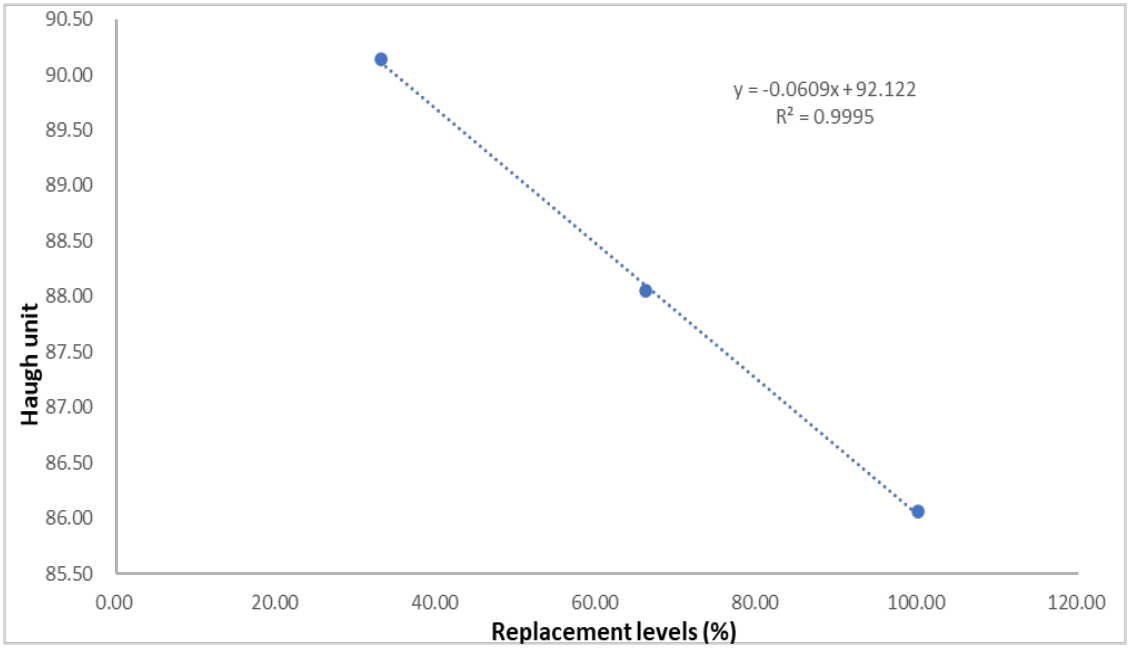

Figure 4.8: Relationship between Haugh unit and replacement levels of maize in standard diet with cassava grits at the mid laying phase.

Table 4.20: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and supplemental enzyme on internal egg characteristics of hens at the mid laying phase

Grits Varieties	RL (%)	Enzyme (mg/Kg)	Albumen height (g)	Haugh unit	Yolk weight (g)	Yolk ratio
Control	0	0	8.30[a]	90.58[a]	17.81[a]	28.79
TME 419	33	0	8.29[a]	90.44[a]	17.74[a]	28.89
	33	35	8.25[a]	90.13[a]	18.08[a]	28.93
	66	0	7.76[b]	87.77[c]	16.44[ab]	28.80
	66	35	7.69[b]	88.98[b]	16.34[ab]	28.92
	100	0	7.04[c]	86.34[d]	14.98[c]	28.54
	100	35	7.00[c]	86.56[d]	15.22[c]	29.06
TMS 01/1368	33	0	8.24[a]	90.04[a]	17.66[a]	28.64
	33	35	8.22[a]	89.94[a]	17.82[a]	28.60
	66	0	7.49[b]	87.82[c]	15.95[b]	28.80
	66	35	7.47[b]	87.62[c]	15.77[b]	28.38
	100	0	7.02[c]	85.45[d]	14.70[c]	28.54
	100	35	6.98[c]	85.71[d]	14.82[c]	28.58
SEM			0.40	1.10	1.40	2.23

[abcd] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement levels;

The main effects of cassava grits variety type, replacement levels of maize with grits in standard diet and supplemental enzyme on the performance of laying hens at late laying phase are presented in Table 4.21**.** Laying birds on TME 419 grits-based diets had remarkably higher ($p<0.05$) live weight (g) of 1769.92 than 1704.32 for TMS 01/1368. However, laying hens on TMS 01/1368 grits-based diets had remarkably higher ($p<0.05$) intake of feed (g) of 120.51 than those on TME 419 with average daily feed intake of 119.78. Laying hens on TMS 01/1368 grits-based treatments had remarkably lower ($p<0.05$) hen day production of 66.70% than those on TME 419 grits-based treatments that had 67.78%. Weight of eggs (g) from laying hens on TME 419 grits-based diets was 59.18 and was higher significantly ($p<0.05$) in contrast with laying hens on TMS 01/1368 with 57.91. The FCR with value of 3.34 was observed in laying hens on TMS 01/1368 grits-based diets but this was significantly lowered ($p<0.05$) in laying hens on TME 419 grits-based diets with FCR value of 3.09 at late laying phase. Varying replacement levels of cassava grits significantly affected ($p<0.05$) performance of laying birds at late laying phase. Laying hens on diets with 33% cassava grits replacement of maize had significantly higher ($p<0.05$) live weight of 1856.19g than laying hens on 66% and 100% replacements with live weights of 1732.19g and 1622.96, respectively. Average daily feed intake of 122.32 g/bird was observed in laying hens on diets with 100% cassava grits replacement of maize and was remarkably higher ($p<0.05$) in contrast with values observed in laying hens on 66% and 33% replacements with values of 120.48g and 117.63g, respectively, at late laying phase. Significantly higher ($p<0.05$) hen day production of 71.56 % was noted in laying hens on diets with 33% cassava grits replacement of maize than in dietary treatments with 66% and 100% replacements with values of 69.44% and 60.28%, respectively. Remarkably higher ($p<0.05$) egg weight (g) was noted in hens on 33% cassava grits replacement (62.53) than in those on 66% (57.60) and 100% (55.85) cassava grits replacement at late laying phase. However, the FCR observed in hens on 100% cassava grits replacement with maize (3.93) increased significantly ($p<0.05$) in contrast with the values in hens on diets with 66% replacement (3.11) and 33% replacement (2.63), at the late laying phase. It was observed that enzyme supplementation remarkably increased ($p<0.05$) live body weight (1754.02g) of hens on diets with enzyme supplementation compared to the hens on diets without supplemental enzyme (1720.21g). Average daily feed intake of hens on enzyme supplemented diets (119.84 g/bird) was remarkably lower ($p<0.05$) in contrast with hens on diets without

supplemental enzyme (120.45). Hen day egg production (%) was remarkably higher ($p<0.05$) in hens on diets with enzyme supplementation (66.77) than that of hens on diets without enzyme supplementation (65.41). It was however observed that egg weight (g) of hens on enzyme supplemented diets (59.09) was not remarkably different ($p>0.05$) from those on diets without supplemental enzyme (58.42). The FCR in laying hens fed on diets without enzyme supplementation (3.27) was remarkably higher ($p<0.05$) in contrast with the hens on enzyme-supplemented diets (3.18), at late laying phase.

The relationship between replacement levels of maize with cassava grits in standard diet and HDEP in laying hens at late laying phase is shown in Figure 4.9. The regression curve shows that 30% replacement level of maize in a standard diet with cassava grits supported an optimum HDEP of 71.8%. The R^2 value of 0.69 indicated that the HDEP of laying hens at late laying phase was up to 69% dependent on cassava grits replacement levels. The regression equation is shown below:

$Y = -0.002x^2 + 0.108x + 70.32$ $(R^2 = 0.69)$ .....Equation 5

The effects of interaction of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on the performance of laying hens at late laying phase are presented in Table 4.22. Live weight (g) in laying hens on T_2 (1901.33) was noted to be significantly higher ($p<0.05$) than in other treatments at the late laying phase. The lowest live weight was observed in T_{11} (1575.39) and T_{12} (1576.94) at the late laying phase. The average daily feed intake (g/bird) in T_5 (122.26), T_6 (122.16), T_{11} (122.43) and T_{12} (122.44), were similar and significantly higher ($p<0.05$) than the values in the other treatments at late laying phase. Laying hens on T_2 (115.92) had the lowest average daily feed intake ($p<0.05$). Hen day egg production (%) in T_2 (73.40) increased significantly ($p<0.05$) in contrast with the remaining treatments with $T_{5,}$ $T_{5,}$ T_{11} and T_{12} having the least hen day egg production of 60.14, 60.45, 59.78 and 60.40, respectively. Egg weights (g) of hens on $T_{0,}$ $T_{1,}$ $T_{2,}$ T_7 and T_8 were 62.21, 62.15, 62.97, 62.29, and 62.87, respectively. They were similar and remarkably higher ($p<0.05$) than egg weights of hens in the other treatments, at late laying phase. The FCR observed in T_2 (2.53) and T_8 (2.59) were similar and remarkably lower ($p<0.05$) in contrast with other dietary treatments at late laying phase. However, hens on T_{11} (4.01) and T_{12} (4.02) had the highest FCR at late laying phase.

Table 4.21: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of hens at late laying phase

	LW (g/bird)	ADFI (g/ bird)	HDEP (%)	EW (g)	FCR
Grits varieties					
TME 419	1769.92[a]	119.78[b]	67.78[a]	59.18[a]	3.09[b]
TMS 01/1368	1704.32[b]	120.51[a]	66.70[b]	57.91[b]	3.34[a]
Replacement levels (%)					
33.00	1856.19[a]	117.63[c]	71.56[a]	62.53[a]	2.63[c]
66.00	1732.19[b]	120.48[b]	69.44[b]	57.60[b]	3.11[b]
100.00	1622.96[c]	122.32[a]	60.28[c]	55.85[c]	3.93[a]
Enzyme supplementation (mg/Kg)					
0	1720.21[b]	120.45[a]	65.41[b]	58.42	3.27[a]
35	1754.02[a]	119.84[b]	66.77[a]	59.09	3.18[b]
SEM	30.19	0.50	1.01	1.20	0.07

[abc]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
LW.: Live weight; ADFI: Average daily feed intake; HDEP: Hen day egg production; EW: Egg weight; FCR: Feed Conversion Ratio

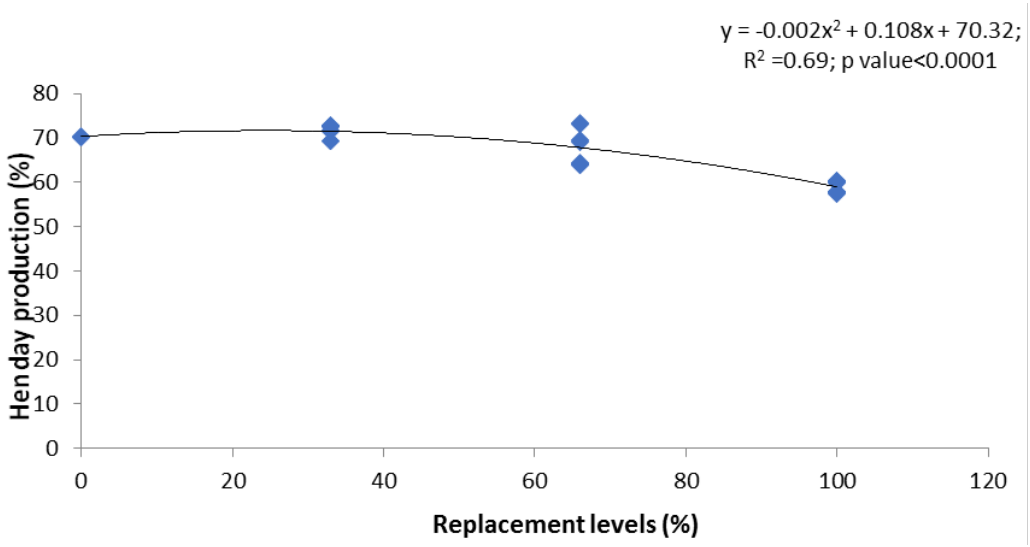

Figure 4.9: Relationship between replacement levels of maize in standard diet with cassava grits and hen day egg production at the late laying phase.

Table 4.22: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on performance of hens at late laying phase

Grits varieties	RL (%)	Enzyme (mg/kg)	LW (g/bird)	FI (g)	HDEP (%)	EW (g)	FCR
Control	0	0	1859.61^{b}	120.28^{c}	70.32^{b}	62.21^{a}	2.75^{e}
TME 419	33	0	1866.22^{b}	118.02^{d}	70.82^{b}	62.15^{a}	2.66^{f}
	33	35	1901.33^{a}	115.92^{e}	73.40^{a}	62.97^{a}	2.53^{g}
	66	0	1731.72^{d}	120.23^{c}	69.85^{c}	58.66^{b}	2.96^{d}
	66	35	1780.72^{c}	120.07^{c}	70.91^{b}	59.57^{b}	2.76^{f}
	100	0	1652.61^{f}	122.26^{a}	60.14^{d}	55.84^{c}	3.85^{b}
	100	35	1686.89^{e}	122.16^{a}	60.45^{d}	56.08^{c}	3.84^{b}
TMS 01/1368	33	0	1798.39^{c}	118.49^{d}	70.83^{b}	62.29^{a}	2.74^{e}
	33	35	1858.83^{b}	118.09^{d}	70.89^{b}	62.87^{a}	2.59^{g}
	66	0	1696.94^{e}	121.38^{b}	69.87^{c}	55.91^{c}	3.38^{c}
	66	35	1719.39^{d}	120.34^{c}	69.93^{c}	56.23^{c}	3.35^{c}
	100	0	1575.39^{g}	122.43^{a}	59.78^{d}	55.97^{c}	4.01^{a}
	100	35	1576.94^{g}	122.44^{a}	60.40^{d}	55.83^{c}	4.02^{a}
SEM			30.19	0.50	1.01	1.20	0.07

^{abcdefg}Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL:Replacement levels; LW.: Live Weight; ADFI: Average daily feed intake; HDEP: Hen day egg production; EW: Egg weight; FCR: Feed Conversion Ratio

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on external egg characteristics at late laying phase are presented in Table 4.23. Cassava variety type elicited no remarkable effect ($p>0.05$) on external egg characteristics with the exception of egg width at the late laying phase. Egg width of hens on TME 419 grits-based diets (40.09mm) significantly increased ($p<0.05$) in contrast with that of hens on TMS 01/1368 grits-based diets with a value of 39.43 at the late laying phase. Replacement levels of maize with cassava grits also elicited no remarkable influence ($p>0.05$) on eternal egg characteristics measured at late laying phase with the exception of egg length and egg width. Egg length (mm) observed in hens on 33% grits replacement (56.82) was remarkably higher ($p<0.05$) than in other treatments. Similarly, egg width (mm) in diets with 33% grits replacement levels (43.09) improved significantly ($p<0.05$) compared to diets with 66% replacement (39.77), and 100% replacement (36.42) at the late laying phase. Shell weight was significantly improved ($p<0.05$) from 5.00g in treatments without enzyme to 5.37g in treatments with enzyme supplementation. The other egg external characteristics measured were not significantly affected ($p>0.05$) by enzyme supplementation at late laying phase.

Effects of interaction of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on external egg characteristics of laying hens at late laying phase are outlined in Table 4.24. Remarkably higher ($p<0.05$) egg length (mm) was noted in hens on T_0 (56.75), T_1 (56.49), T_2 (57.36), T_7 (56.45) and T_8 (57.09) than in other dietary treatments. The lowest ($p<0.05$) egg length was observed in T_5 (47.82), T_6 (48.19) T_{11} (48.14) and T_{12} (47.89), at late laying phase. Egg width (mm) observed in T_0 (43.03), T_1 (42.90), T_2 (43.41), T_7 (42.73) and T_8 (43.33) were remarkably higher ($p<0.05$) than in other treatments. However, laying hens on T_5 (36.29), T_6 (36.60) T_{11} (36.53) and T_{12} (36.25) had the lowest ($p<0.05$) egg width at late laying phase. Shell weight values observed in T_2 (5.34), T_4 (5.45), T_6 (5.35), T_8 (5.36), T_{10} (5.37) and T_{12} (5.35) were higher remarkably ($p<0.05$) in contrast with other treatments, at late laying phase. The interaction effect of variety, replacement levels and enzyme supplementation were not significant ($p>0.05$) on egg shape index, shell thickness and shell ratio at late laying phase**.**

Table 4.23: Main effects of cassava grits varieties, replacement levels of maize in standard diet and supplemental enzyme on external egg characteristics of hens at late laying phase

	EL (mm)	EWi (mm)	SI (%)	SW (g)	ST (mm)	SR (%)
Grits varieties.						
TME 419	52.83	40.09[a]	75.88	5.19	0.37	8.88
TMS 01/1368	51.99	39.43[b]	75.85	5.17	0.38	8.92
Replacement levels (%)						
33.00	56.82[a]	43.09[a]	75.84	5.23	0.38	8.40
66.00	52.39[b]	39.77[b]	75.90	5.18	0.38	8.96
100.00	48.01[c]	36.42[c]	75.85	5.13	0.37	9.15
Enzyme supplementation.						
(mg/kg)	52.18	39.60	75.89	5.00[b]	0.37	8.51
0	52.64	39.92	75.83	5.37[a]	0.37	8.88
35						
SEM	2.82	1.50	2.09	0.20	0.004	0.67

[abc]Means with identical superscripts within the same column of a group are not different significantly (p>0.05)
EL:Egg length; EWi: Egg width; SI: Shape Index; SW: Shell weight; ST: Shell thickness; SR: Shell ratio

Table 4.24: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on external egg characteristics of hens at late laying phase

Grits Varieties	RL (%)	Enzyme (mg/kg)	EL (mm)	EWi (mm)	SI (%)	SW (g)	ST (mm)	SR (%)
Control	0	0	56.75[a]	43.03[a]	75.82	5.02[b]	0.38	8.99
TME 419	33	0	56.49[a]	42.90[a]	75.95	5.11[b]	0.38	8.50
	33	35	57.36[a]	43.41[a]	75.69	5.34[a]	0.38	8.53
	66	0	53.15[b]	40.30[bc]	75.83	4.91[b]	0.38	9.03
	66	35	53.48[b]	41.01[b]	75.97	5.45[a]	0.38	8.96
	100	0	47.82[c]	36.29[d]	75.88	4.98[b]	0.37	9.11
	100	35	48.19[c]	36.60[d]	75.95	5.35[a]	0.37	9.17
TMS 01/1368	33	0	56.45[a]	42.73[a]	75.82	5.09[b]	0.38	8.52
	33	35	57.09[a]	43.33[a]	75.90	5.36[a]	0.38	8.51
	66	0	51.11[b]	38.86[c]	76.02	4.99[b]	0.38	8.97
	66	35	51.33[b]	38.90[c]	75.79	5.37[a]	0.38	8.93
	100	0	48.14[c]	36.53[d]	75.88	4.87[b]	0.37	9.03
	100	35	47.89[c]	36.25[d]	75.69	5.35[a]	0.37	9.15
SEM			2.82	1.50	2.09	0.20	0.004	0.67

[abcd] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement levels; EL:Egg length; EWi: Egg width; SI: Shape Index; SW:Shell weight; ST: Shell thickness; SR: Shell ratio

Main effects of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on internal egg characteristics of laying hens at late laying phase are presented in Table 4.25. Cassava grits variety type did not significantly influence ($p>0.05$) the egg internal characteristics measured as there were similar albumen height (mm) of 7.22 and 7.10; Haugh unit of 85.21 and 84.86; yolk weight (g); and yolk ratio of 30.04 and 30.33 in eggs of hens on TME 419 grits-based and TMS 1368 grits-based diets, respectively. Replacement levels of maize with cassava grits significantly affected ($p<0.05$) the internal egg characteristics measured with the exception of yolk ratio. Albumen height (mm) was noted to be higher significantly ($p<0.05$) at 33% replacement level (7.70) than in 66% replacement (7.18) and 100% replacement level (6.60). Haugh Unit was also significantly higher ($p<0.05$) at 33% replacement level with a value of 86.90, than at 66% and 100% replacement levels with values of 85.22 and 83.02, respectively. The same trend was observed in the egg yolk weight where eggs of hens on diets with 33% replacement of maize with cassava grits had yolk weight of 19.06g which was higher significantly ($p<0.05$) in contrast with the yolk weight of hens on 66 and 100% replacement levels with values of 17.49g and 16.31g, respectively. None of the internal egg characteristics measured was however remarkably affected ($p>0.05$) by enzyme supplementation in the diets of the hens at the late laying phase.

The relationship between Haugh unit and replacement levels of maize in standard diet with cassava grits at late laying phase is shown in Figure 4.10. The relationship was negative with Haugh unit linearly related to replacement levels of maize with cassava grits in standard diet. The relationship was explained by the regression equation:

$Y= 0.058x + 88.91$ $(R^2 = 0.995)$ ..... Equation 6

The effects of interaction of cassava grits varietal type, replacement levels of maize in standard diet and supplemental enzyme on internal egg characteristics of laying hens at late laying phase are shown in Table 4.26. Similar albumen heights (mm) were observed in control T_0 (7.59), T_1 (7.72), T_2 (7.70), T_3 (7.32), T_4 (7.33) T_7 (7.74), and T_8 (7.64) and increased remarkably ($p<0.05$) in contrast with the remaining treatments at late laying phase. Haugh units observed in T_0 (87.29) T_1 (87.25), T_2 (86.71), T_7 (87.24), T_8 (87.46) were identical and were higher remarkably ($p<0.05$) in contrast with the remaining

treatments at late laying phase. Yolk weight values observed in control T_0 (18.94), T_1 (18.90), and T_2 (19.09), T_4 (18.19), T_7 (19.10), and T_8 (19.13) were equivalent and remarkably higher ($p<0.05$) than the other treatments at late laying phase. Yolk ratio observed in all the treatments did not vary ($p>0.05$) significantly at late laying phase.

Main effects of cassava grits varietal type, replacement levels in standard diet and supplemental enzyme on apparent nutrient retention of laying hens are presented in Table 4.27. Cassava varietal type elicited no remarkable effect ($p>0.05$) on nutrients' retention in laying hens. Diets based on TME 419 grits had retention values (%) of 83.83, 67.71, 82.80 and 69.81 for dry matter, nitrogen, ether extract, and crude fibre, respectively while TMS 1368 grits-based diets had similar retention values of 82.71, 67.50, 82.81 and 69.18 for dry matter, nitrogen, ether extract, and crude fibre, respectively. Replacement levels of maize in standard diet with cassava grits did not significantly affect ($p>0.05$) retention values of dry matter, ether extract, and crude fibre. Replacement levels however significantly affected ($p<0.05$) nitrogen retention (NR) in laying hens with 33, 66 and 100% maize replacement with grits having 70.30, 67.40 and 64.90% NR respectively. Enzyme supplementation elicited no significant effect ($p>0.05$) on retention values for dry matter, ether extract and crude fibre. Supplemental enzyme however remarkably improved ($p<0.05$) the NR with values of 69.26 and 65.90% in laying hens on diets with supplemental enzyme and those on diets without enzyme supplementation, respectively.

Effects of interaction of cassava grits varietal type, replacement levels in standard diet and enzyme supplementation on apparent nutrient retention in laying hens are outlined in Table 4.28. The interaction effect shows remarkable differences ($p<0.05$) only in NR (%) with T_2 (71.98) and T_8 (71.70) having similar and significantly higher values than in other treatments. The least NR (%) were observed in T_5 (63.00) and T_{11} (62.35).

Table 4.25: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on internal egg characteristics of laying hens at late laying phase

	Albumen height (mm)	Haugh unit	Yolk weight (g)	Yolk ratio
Grits varieties				
TME 419	7.22	85.21	17.73	30.04
TMS 01/1368	7.10	84.86	17.51	30.33
Replacement levels (%)				
33.00	7.70[a]	86.90[a]	19.06[a]	30.38
66.00	7.18[b]	85.22[b]	17.49[b]	30.27
100.00	6.60[c]	83.02[c]	16.31[c]	29.20
Enzyme supplementation (mg/kg)				
0	7.18	85.17	17.54	30.05
35	7.41	84.89	17.69	29.96
SEM	0.50	1.20	1.01	2.16

[abc]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)

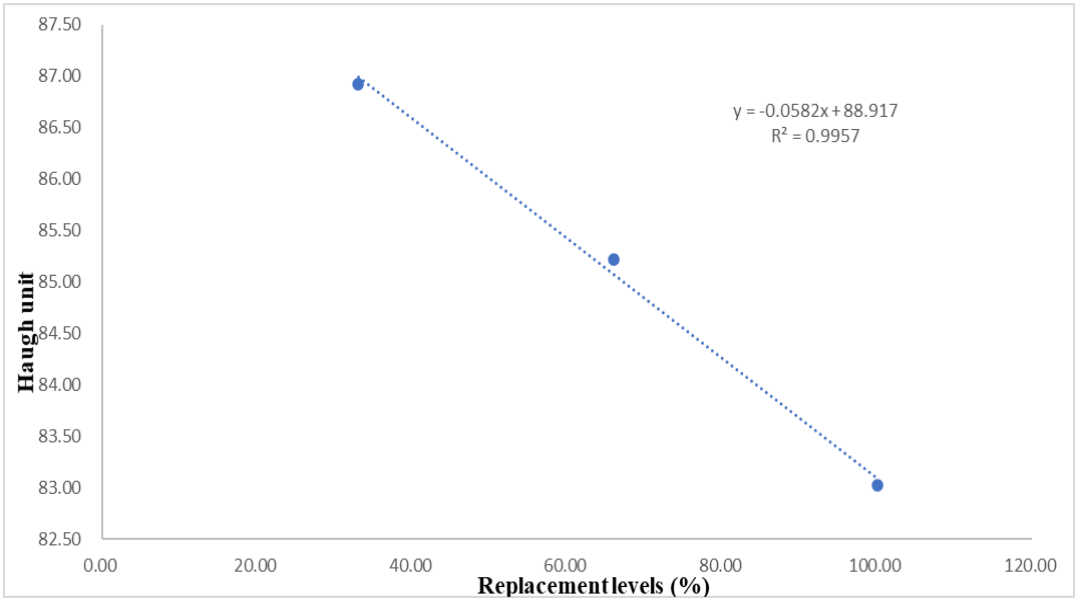

Figure 4.10: Relationship between Haugh unit and replacement levels of maize in standard diet with cassava grits at late laying phase

Table 4.26: Effects of interaction of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on internal egg characteristics of laying hens at late laying phase

Grits Varieties	RL (%)	Enzyme (mg/kg)	Albumen height (g)	Haugh Unit	Yolk weight (g)	Yolk ratio
Control	0	0	7.59[a]	87.29[a]	18.94[a]	30.29
TME 419	33	0	7.72[a]	87.24[a]	18.90[a]	30.39
	33	35	7.70[a]	86.91[a]	19.09[a]	29.97
	66	0	7.32[ab]	85.24[b]	17.76[b]	30.18
	66	35	7.33[ab]	85.42[b]	18.19[ab]	30.25
	100	0	6.69[c]	83.62[c]	16.08[c]	30.17
	100	35	6.57[c]	82.78[c]	16.33[c]	30.55
TMS 01/1368	33	0	7.74[a]	86.76[a]	19.10[a]	30.51
	33	35	7.64[a]	86.64[a]	19.13[a]	30.25
	66	0	7.03[b]	85.30[b]	16.93[bc]	30.12
	66	35	7.04[b]	84.93[b]	17.06[bc]	30.24
	100	0	6.60[c]	82.93[c]	16.45[c]	30.67
	100	35	6.56[c]	82.75[c]	16.38[c]	30.68
SEM			0.50	1.20	1.01	2.16

[abc]Means with identical superscripts within the same column of a group are not different significantly (p>0.05)
RL: Replacement levels.

Table 4.27: Main effects of grits varieties, replacement levels of maize in standard diet and supplemental enzyme on apparent nutrient retention of laying hens

	Dry Matter (%)	Nitrogen Retention (%)	Ether Extract (%)	Crude Fibre (%)
Grits varieties				
TME 419	83.83	67.71	82.80	69.81
TMS 01/1368	82.71	67.50	82.81	69.18
Replacement levels (%)				
33.00	84.82	70.30[a]	81.39	70.78
66.00	83.22	67.40[b]	83.35	69.81
100.00	81.68	64.90[c]	83.66	67.89
Enzyme supplementation (mg/kg)				
0	82.85	65.90[b]	82.75	69.38
35	83.69	69.26[a]	82.85	70.62
SEM	4.09	2.02	4.05	3.87

[abc] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)

Table 4.28: Effects of interaction of grits varieties, replacement levels of maize in standard diet and enzyme supplementation on apparent nutrient retention of laying hens

Grits varieties	Replacement Level (%)	Enzyme (mg/kg)	Dry Matter (%)	Nitrogen Retention (%)	Ether Extract (%)	Crude Fibre (%)
Control	0	0	86.37	68.60^{b}	80.24	70.94
TME 419	33	0	84.68	68.90^{b}	81.50	70.91
	33	35	86.24	71.98^{a}	81.29	72.03
	66	0	83.29	67.14^{bc}	82.92	69.96
	66	35	84.17	68.54^{b}	83.40	70.07
	100	0	81.95	63.00^{d}	83.86	67.99
	100	35	82.34	66.40^{c}	83.72	67.91
TMS 01/1368	33	0	83.86	68.52^{b}	81.36	69.57
	33	35	84.53	71.70^{a}	81.39	70.42
	66	0	82.16	67.28^{bc}	83.35	69.64
	66	35	83.28	68.70^{b}	83.64	69.58
	100	0	80.88	62.35^{d}	83.41	67.98
	100	35	81.57	66.05^{c}	83.60	67.81
SEM			4.09	2.02	4.05	3.87

^{abcd} Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on haematology of laying hens are outlined in Table 4.29. Laying hens on diets based on TME 419 grits and TMS 1368 grits did not elicit any remarkable differences ($p>0.05$) in RBC (2.08 and 2.12 x 10^{12} /L, repectively); Hb (8.45 and 8.56 g/dL, respectively); PCV (26.25 and 25.10 %, respectively)**;** MCV (125.95 and 119.04 fL, respectively); MCH (40.80 and 40.65 pg, respectively); and MCHC (32.30 and 34.18 g/L, respectively); WBC (20.24 and 19.69 x 10^{9}/L, respectively); as well as the leukocyte differential counts. In like manner, replacement levels of maize with grits in standard diets and enzyme supplementation elicited no remarkable differences ($p>0.05$) the haematological indices measured in the laying hens.

Main effects of cassava variety type, replacement levels of maize in standard diet and supplemental enzyme on the biochemical indices of the serum of laying hens are outlined in Table 4.30. Laying hens on diets based on TME 419 grits and TMS 1368 grits elicited no remarkable differences ($p>0.05$) in serum glucose (153.02 and 150.21 mg/dL, respectively); cholesterol (74.20 and 76.05 mg/dL, respectively); triglycerides (376.59 and 378.25, respectively); alanine aminotransferase (5.15 and 5.21 IU/L, respectively); aspartate aminotransferase (73.42 and 73.08 IU/L, respectively); total protein (5.26 and 5.45 g/dL, respectively); albumin (3.19 and 3.18 g/dL, respectively) and thiocyanate (2.12 and 2.15 mg/dL, respectively) . Replacement levels of maize with cassava grits in standard diets significantly affected ($p<0.05$) only the serum thiocyanate. A value of 2.35 mg/dL was obtained in 100% replacement level which increased significantly ($p<0.05$) in contrast with 66% replacement level (2.11) and 33% replacement level (1.95). Supplemental enzyme did not remarkably affect ($p>0.05$) the serum biochemical indices measured.

The effects of interaction of cassava variety type, replacement levels of maize in standard diet and supplemental enzyme on the biochemical indices of the serum of laying hens are presented in Table 4.31. Only aspartate aminotransferase (AST) and thiocyanate were significantly affected ($p<0.05$) by the interaction effects. The AST observed in control T_0 (65.59) was significantly lower ($p<0.05$) than the remaining treatments which were identical to each other ($p>0.05$). The serum thiocyanate recorded in T_0 (0.29) was also lower significantly ($p<0.05$) in contrast with the remaining diets, with T_6 (2.07), T_7 (2.10), T_{11} (2.12) and T_{12} (2.13) having the highest values.

Table 4.29: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on haematology of laying hens

	RBC ($\times 10^{12}$/ L)	H_b (g/ dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/L)	WBC ($x10^9$/L)	Lymph (%)	Het (%)	Mon (%)	Eosi (%)	Baso (%)
Grits varieties												
TME 419	2.08	8.45	26.25	125.95	40.80	32.30	20.24	67.63	20.29	2.04	0.41	0.10
TMS 01/1368	2.12	8.56	25.10	119.04	40.65	34.18	19.69	66.98	19.74	2.01	0.40	0.09
Replacement levels (%)												
33.00	2.07	8.37	25.70	124.53	40.51	32.63	19.92	67.50	19.97	2.12	0.38	0.08
66.00	2.10	8.49	24.90	118.70	40.62	34.13	20.73	68.05	20.79	2.05	0.41	0.09
100.00	2.13	8.50	26.55	125.05	39.84	32.15	19.86	67.35	19.91	2.02	0.37	0.08
Enzyme supplementation (mg/Kg)												
0	2.09	8.49	27.10	130.02	40.65	31.45	19.54	67.60	19.59	2.11	0.38	0.09
35	2.10	8.42	24.35	116.15	40.02	34.46	19.78	68.25	19.83	2.02	0.39	0.10
SEM	0.05	0.45	3.41	15.50	3.55	3.50	1.62	3.55	1.97	0.08	0.03	0.01

RBC: Red blood cell, H_b: Haemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration: WBC: White blood cell; Lymph: Lymphocytes; Het: Heterophils; Mon: Monocytes; Eosi: Eosinophils; Baso: Basophils: SEM: Standard Error of Mean

Table 4.30: Main effects of cassava grits varieties, replacement levels of maize in standard diet and enzyme supplementation on serum biochemical indices of laying hens

	GLUC (mg/dL)	CHOL (mg/dL)	TG (mg/dL)	ALT (IU/L)	AST (IU/L)	TP (g/dL)	ALB (g/dL)	THIOC (mg/dL)
Grits varieties								
TME 419	153.02	74.20	376.59	5.15	73.42	5.26	3.19	2.12
TMS 01/1368	150.21	76.05	378.25	5.21	73.08	5.45	3.18	2.15
Replacement levels (%)								
33.00	149.41	73.06	368.92	5.13	73.47	5.23	3.21	1.95[c]
66.00	151.08	75.10	378.38	5.18	72.98	5.42	3.14	2.11[b]
100.00	150.84	77.22	385.11	5.22	73.49	5.33	3.20	2.35[a]
Enzyme supplementation (mg/kg)								
0	149.09	75.32	369.75	5.15	73.55	5.29	3.17	2.33
35	152.14	74.93	385.15	5.20	72.95	5.33	3.19	2.34
SEM	15.83	7.89	35.40	0.10	2.90	0.20	0.08	0.02

[ab]Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
GLUC: Glucose; CHOL: Cholesterol; TG: Triglycerides; ALT: Alanine animotransferase; AST: Aspartate aminotransferase; TP:Total protein; ALB: Albumin; THIOC: Thiocyanate; SEM: Standard Error of the Mean

Table 4.31: Effects of interaction of grits varieties, replacement levels of maize in standard diet and enzyme supplementation on serum biochemical indices of laying hens

Grits Varieties	RL (%)	Enzyme (mg/kg)	GLUC (mg/dL)	CHOL (mg/dL)	TG (mg/dL)	ALT (IU/L)	AST (IU/L)	TP (g/dL)	ALB (g/dL)	THIOC (mg/dL)
Control	0	0	151.51	73.74	365.07	5.10	65.59^{b}	5.21	3.28	0.29^{f}
TME 419	33	0	153.90	71.19	362.91	5.01	73.59^{a}	5.23	3.25	1.94^{de}
	33	35	153.92	72.93	372.66	5.19	73.48^{a}	5.20	3.18	1.92^{e}
	66	0	152.78	74.14	358.60	5.14	74.06^{a}	5.30	3.21	2.01^{c}
	66	35	153.95	72.52	394.39	5.21	72.65^{a}	5.34	3.10	2.00^{c}
	100	0	152.92	77.34	374.34	5.15	73.42^{a}	5.31	3.15	2.07^{ab}
	100	35	153.84	76.88	396.50	5.21	73.29^{a}	5.20	3.23	2.10^{a}
TMS 01/1368	33	0	146.80	74.42	364.54	5.20	73.85^{a}	5.20	3.11	1.98^{d}
	33	35	146.30	73.55	375.41	5.13	73.05^{a}	5.26	3.29	1.97^{d}
	66	0	148.71	76.45	363.19	5.18	72.99^{a}	5.40	3.15	2.04^{b}
	66	35	149.95	77.26	397.21	5.20	72.21^{a}	5.23	3.11	2.04^{b}
	100	0	146.05	78.15	394.55	5.24	73.38^{a}	5.25	3.17	2.12^{a}
	100	35	149.88	76.23	374.52	5.29	72.99^{a}	5.17	3.25	2.13^{a}
SEM			15.83	7.89	35.40	0.10	2.90	0.20	0.08	0.02

^{abcdef}Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
RL: Replacement levels; GLUC: Glucose; CHOL: Cholesterol; TG: Triglycerides; ALT: Alanineanimotransferase; AST: Aspartate aminotransferase; TP:Total protein; ALB: Albumin; THIOC: Thiocyanate; SEM: Standard Error of Mean

The main effects of cassava grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on egg lipid profile of laying hens are outlined in Table 4.32. Significant differences did not exist ($p>0.05$) in the values for egg total cholesterol (md/dL) of hens on TME 419 grits-based diets (12.75) and those on TMS 1368-based diets (12.87). Replacement levels of maize with cassava grits in standard diets also conferred no remarkable differences ($p>0.05$) on the egg total cholesterol (mg/dL) as 33, 66 and 100% replacement levels had values of 12.73, 13.03 and 13.07, respectively. In the same vein, enzyme supplementation in the diet did not alter remarkably ($p>0.05$) total cholesterol, as eggs of hens on enzyme-supplemented diets had a value of 13.06 mg/dL while those on diets without enzyme supplementation had a value of 12.86 mg/dL. The same trend was observed for the triglycerides, high-density lipoproteins, low-density lipoproteins and very low-density lipoproteins as grits varietal type, replacement levels of maize in standard diet and enzyme supplementation did not significantly affect ($p>0.05$) lipid parameters in eggs of laying hens.

Main effects of grits varietal type, replacement levels of maize in standard diet and enzyme supplementation on cost of feed conversion ratio of laying hens are presented in Table 4.33. Laying hens on TME 419 grits-based diets had cost of FCR (₦496.97) which was remarkably lower ($p<0.05$) than cost of FCR of hens on TMS 01/1368 grits-based diets (₦516.99). Replacement levels of maize with cassava grits in standard diet also elicited remarkable differences ($p<0.05$) in the cost of FCR with values of ₦386.29, ₦457.42 and ₦677.22 at 33%, 66% and 100% replacement levels, respectively. Laying hens on diets with enzyme supplementation also had a significantly lower cost of FCR (₦501.65) than those on diets without enzyme supplementation (₦512.31).

The effects of interaction of grits varietal type, replacement levels in standard maize diet and enzyme supplementation on cost of feed conversion ratio in laying hens are outlined in Table 4.34. Significantly lower ($p<0.05$) cost of FCR (₦372.61) was observed in T_2 than in the other treatments. The highest cost of FCR was observed in T_5 (₦679.20), T_6 (₦675.75),, T_{11} (₦675.01), and T_{12} (₦678.91).

Table 4.32: Main effects of grits varieties, replacement levels of maize in standard diet and enzyme supplementation on egg lipid profile of laying hens

	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Grits varietal type					
TME 419	12.75	40.63	3.39	1.18	8.21
TMS 01/1368	12.87	41.42	3.46	1.11	8.35
Replacement levels (%)					
33.00	12.73	39.85	3.38	1.39	8.05
66.00	13.03	41.40	3.41	1.51	8.21
100.00	13.07	40.33	3.45	1.40	8.13
Enzyme supplementation (mg/kg)					
0	12.86	40.44	3.39	1.38	8.13
35	13.06	41.91	3.37	1.27	8.34
SEM	1.09	3.41	0.18	0.38	0.37

TC: Total cholesterol; TG: Triglycerides; HDL: High density lipoproteins; LD: Low density lipoprotein; VLDL: very low density lipoproteins; SEM: Standard Error of the Mean

Table 4.33: Main effects of grits varieties, replacement levels of maize in standard diet and enzyme supplementation on average cost of feed conversion ratio in laying hens

	Average cost of FCR (₦)
Grits varieties	
TME 419	496.97[b]
TMS 01/1368	516.99[a]
Replacement levels (%)	
33.00	386.29[c]
66.00	457.42[b]
100.00	677.22[a]
Enzyme supplementation (mg/kg)	
0	
35	512.31[a]
	501.65[b]
SEM	10.05

[abc] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
FCR: Feed conversion ratio

Table 4.34: Effect of interaction of grits varieties, replacement levels of maize in standard diets and enzyme supplementation on average cost of feed conversion ratio in laying hens

Grits varieties	Replacement levels (%)	Enzyme supplementation (mg/Kg)	Average cost of FCR (₦)
Control	0	0	405.10[e]
TME 419	33	0	388.95[f]
	33	35	372.61[g]
	66	0	443.84[c]
	66	35	421.47[d]
	100	0	679.20[a]
	100	35	675.75[a]
TMS 01/1368	33	0	402.91[e]
	33	35	380.71[f]
	66	0	483.96[b]
	66	35	480.43[b]
	100	0	675.01[a]
	100	35	678.91[a]
SEM			10.05

[abcdefghi] Means with identical superscripts within the same column of a group are not different significantly ($p>0.05$)
FCR: Feed conversion ratio

CHAPTER 5

DISCUSSION

5.1 Experiment 1

Percentage starch contents (wet basis) which was higher in TME 419 than in TMS 01/1368 fresh roots harvested at age 11 months conforms to the review of Ayetigbo *et al.* (2018), on the characteristics of root, flour and starch of biofortified yellow-flesh and white-flesh cassava varieties. The authors reported a higher starch content (%) ranging from 13.47 to 30.97 in white-flesh cassava variants and between 14.29 - 20.00 in yellow-flesh variants in Nigeria. This was also indicated in the result of proximate analysis of grits from the two varieties which had a crude estimate of carbohydrate fraction (nitrogen-free extract) of 76.04% and 69.95% for TME 419 and TMS 01/1368, respectively.

The effect of sun-drying and roasting methods of preparing the test cassava feed materials indicated a higher percentage of starch gelatinisation (%) of cassava grits in the roasting than in sun-drying method. This wide variation in percentage of starch gelatinisation between the two processing methods might be due to the differences in temperature-time regime of the two processing (drying) methods. Moisture content of the pressed cassava pulp was usually as low as 30%. According to Aichayawanich *et al.* (2011), at this level of moisture, gelatinisation of cassava starch begins to set in at 102.89 °C. Roasting in an open pan attains this temperature (up to 120 °C) and for 5-10 minutes brings about high level of gelatinisation which confers the gritty nature on the roasted product. This temperature was practically unachievable with sun-drying and little gelatinisation takes place as the pulp took a longer time to dry with continuous loss of the moisture required for gelatinisation. Hence, the cassava product was powdery as there was no grittiness conferred.

The higher the degree of crystallinity of native starch, the lower its digestibility (Björck *et al.,* 2000). However, according to Svihus (2014), when native starch is treated with high temperatures in the presence of moisture, the granular structure is ruptured by the process of gelatinisation, rendering it more available for the action of α-amylase and thereby improving its digestibility. Therefore, cassava grits so obtained by roasting method as prescribed (Tewe, 2005), used in the feeding trials was a gelatinised product which improved the digestibility of the starch fraction of cassava. Tewe (2004), indicated that cooking improved digestibility of raw starch from 48.3% to 77.9%, thereby enhancing the caloric value of the product. Also, owing to gelatinisation, the product therefore was gritty in nature, eliminating dustiness usually associated with cassava flour, thereby enhancing the sensory value of the product.

5. 2 Experiment 2

True metabolisable energy (kcal/g ME) value obtained in TME 419 grits was greater than in TMS 01/1368 grits. This result could be due to higher starch content and percentage of starch gelatinisation in TME 419 cassava variety**.** Several authors have reported varying values of true metabolizable energy for cassava roots. Buitrago *et al.* (2002) reported a range of 3000-3200 kcal/kg while Egena (2006), Khajarern and Khajarern (1991), and Olugbemi *et al.* (2010) reported values of 3200 kcal/kg, 3154 kcal/kg and 3279 kcal/lg respectively. The variations or lack of consistency in the reported values by the different authors apparently might be due to differences in the adopted processing methods and also the large array of cassava varieties with respective differences in nutrient and starch compositions. Manano *et al.* (2018) reported five different starch compositions for five different cultivars of cassava with a wide range of values between 66.72 to 84.42%. The variations in the true metabolizable energy values reported by authors could also be due to variations in age of harvesting of the roots for processing into feed material. According to Tewe (2004), the starch composition of cassava reaches its peak between 8 and 12 months after planting, after which starch composition begins to decline with an increase in the fibre composition.

The chemical composition of the two varieties of grits indicated variations in the nutrients profile. Although the factors of processing methods, ecological conditions, and ages at harvesting could cause variations in the chemical composition of the grits,

variety of cassava is a major factor of the observed dissimilarities in the chemical composition (Richardson, 2013). Higher nitrogen free extract of TME 419 grits is a pointer to greater soluble carbohydrate and higher caloric value of the variety compared to TMS 01/1368.

5.3 Experiment 3

Main effect of cassava grits varietal type on performance of growing pullets showed average daily gain (g/day) was remarkably higher in growing pullets fed TME 419 grits-based diets than in those fed TMS 01/1368 grits-based diets. In spite of the nutritionally balanced formulation of diets based on each of the varieties, this could be due to better utilisation of TME 419 grits-based diets than TMS 01/1368 grits-based diets. In addition, TME 419 grits had higher percentage starch gelatinisation and lower crude fibre composition which could impact positively on the performance of birds.

The result of the present experiment showing decrease in average daily gain with increasing level of replacement of maize with cassava grits in the standard diet is in agreement with the report of Gowdh *et al.* (1990). The authors reported that cassava root meal elicited growth depressing effect when cassava was included in the diets of poultry at levels up to 48%. Similarly, Weurding *et al.* (2003), found that pea-corn diets consistently elicited higher weight gain than tapioca-corn diets. According to Gomes *et al.* (2005), cassava starch is more digestible than maize starch due to the higher amylopectin content of cassava starch. This could be as a result of the more extended surface area of amylopectin with its average molecular weight of $10^5 – 10^6$, larger than amylose with average molecular weight of 10^4 (Foster, 1965). Also, the glucose molecules of amylose starch are more bound together by hydrogen bonds than in amylopectin starch with several branched chain of glucose molecules thereby making amylose less susceptible to attack by the amylase enzyme (Leach, 1965) According to Weurding *et al.* (2003), highly digestible starch could be converted to glucose quickly when the other nutrients have not yet been absorbed. Since part of the dietary energy is used by the GIT to support digestive and absorptive processes, the digestion of highly digestible starch in the upper small intestine will not provide the lower small intestine with glucose for its energy requirement. In such a case, amino acids may be oxidised for the purpose of providing the energy requirement which

could hamper the utilization of amino acids for tissue formation and growth. This could be responsible for the lower body weight in pullets on diets having higher levels of cassava grits inclusion.

Enzyme supplementation at 35 mg/kg in the diets of growing pullets resulted in higher average daily gain compared to those on diets without supplemental enzyme. This could be due to enhanced utilisation conferred by the multiple enzymes on the feed of the growing pullets formulated with the minimum industry metabolisable energy baseline of 2,400kcal/kg. The result is in tandem with claims of the proprietor of the multiple enzymes that the enzyme would compensate for about 2.5% reduction in energy or 70 kcal/kg and also 25% increase in fibre from standard diet formulation. Non-starch polysaccharidases in the feed benefit the birds in a number of ways including the production of fermentable prebiotics which supply a source of energy for the saccharolytic bacteria in the caeca which results in increased recovery of energy from the diet (Bedford 2018). Analysis of both poultry feed and digesta under the microscope gave evidence for the presence of intact cells and also supported rupture of the cell wall through the employment of enzymes that degrade endosperm cell wall when the digesta get to the proximal small intestine thus releasing the encapsulated nutrient for enzymatic activities (Bedford and Autio, 1996). This promotes digestibility of nutrients by allowing endogenous proteases and amylases quicker attack on the hitherto encased protein and starch.

Effects of interaction of cassava grits varietal type, replacement levels of maize with cassava grits in standard diets and enzyme supplementation showed T_2 (33% replacement of maize in standard diet with TME 419 grits, supplemented with enzyme) resulted in lower average daily feed intake, lower FCR and higher average daily gain than in other treatments. This result could be due to the higher energy density of the diet brought about by a combination of higher starch level in TME 419 grits and increased recovery of energy from the digesta in the gut due to the action of non-starch polysaccharidases in the feed. Poultry feed primarily to satisfy their energy requirement and according to Greger (2011), poultry could change their intake of feed within a sizeable gamut of feed energy levels to meet their daily energy needs. Albuquerque *et al.* (2003) reported a reduction in feed intake in chickens due to increased density of dietary energy and according to Leeson *et al.* (1996), the intake of energy is a fundamental influence on growth rate.

There was no significant difference in age at first lay among the treatments. This result appears to support the argument that body weight has little effect on sexual maturity of pullets. In their work with broiler breeders, Brody *et al.* (1980) indicated that prior to initiation of oviposition, the condition of age had to be satisfied irrespective of body weight. Leeson and Summers (2009) opined that the argument for the role of body weight at sexual maturity, is in fact not too important, because the pullets showed some compensatory growth prior to the time of first egg.

The cost per kg weight was affected by the dietary treatments with the least cost in T_2 containing 33% TME grits with supplemental enzyme, and highest cost in T_{11}. Although, the unit cost of cassava grit at the time of the feeding trial was 53.5% the cost of maize, this did not reflect in the cost of weight gain/kg. This was because more protein materials had to be incorporated into the diets with increasing inclusion levels of cassava grits in order to compensate for its protein deficit compared to maize. Also, FCR generally became poorer as levels of replacement of maize with cassava grits increased from 33 to 100%.

There was no effect of cassava grits varietal type on apparent nutrient retention in growing pullets. This might be due to the fact that the anti-nutritional factor in cassava (HCN) did not adversely affect nutrient uptake in the gastro intestinal tract of growing birds. Replacement levels of maize in standard diet with cassava grits did not influence retention of DM, EE, and CF, but impacted NR in growing birds. The observed lower NR with increasing cassava grits inclusion could be due to higher HCN content which resulted in higher percentage of excreted nitrogen from cyanide rather than from protein nitrogen. Okoh (1983) reported that after injecting rats with one dose of cyanide, there was detection of 89% of excreted radioactivity in the urine in 24 hours. The result therefore may not necessarily indicate only lower protein utilisation in treatments with higher level of cassava grits inclusion, but also indicative of cassava cyanide detoxification mechanism.

Addition of enzyme cocktail to the experimental diets had no effect on retention of DM, EE and CF. However, NR was influenced in growing birds on diets with supplemental enzyme compared to those on diets without supplemental enzymes. This

could be due to the synergistic effect of the enzyme cocktail used with the non-starch polysaccharidases in the cocktail breaking the cell walls that encapsulates protein in the feed ingredients, which Bedford (2018) suggested is then exposed to digestion by the action of proteases.

Cassava grits variety type, replacement levels of maize with grits in standard diet and enzyme supplementation did not remarkably affect the haematological parameters measured, namely RBC, Hb, PCV, WBC and the leukocyte differential counts. All values obtained were compared with those obtained for the maize control diet and within the normal range reported for healthy pullets of their age (Mistruka and Rawnsley, 1977; Oke *et al.,* 2017). Haematological parameters in birds are influenced by a few factors including age, sex, nutrition, health status, rearing system, and climate. In the present experiment, the normal range of WBC and leukocyte differential counts indicated that no pathological effect or immunological disorder was induced by cassava grits in the diets. Normal Hb concentration in pullets fed cassava grits-diets could reflect a low and therefore tolerable level of HCN in the diets of the pullets. High cyanide level in the diets would have been expected to cause a depression in Hb concentration as cyanide has affinity to bind to ferric ion (Fe^{3+}) in methaemoglobin in erythrocyte thereby affecting haemoglobin concentrations and interfering with cellular respiration (Frankenberg and Sorbo, 1975).

Serum biochemical indices measured were glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate amino transferase, total protein, albumin and thiocyanate. They were not altered significantly with grits varietal difference, replacement levels of maize by grits and enzyme supplementation in growing pullets. Conversely, serum thiocyanate increased as replacement levels of maize with cassava grits in the diets increased. Cyanide causes changes in glucose metabolism leading to 100% increase in conversion of glucose by pentose-phosphate pathway (Isom *et al*., 1975). The glucose values which were not different among the treatments could therefore be adduced to the low level of cyanide in the cassava grits-based diets which could not elicit any notable effect on glucose metabolism. Since serum glucose levels were not different, the triglyceride levels were also not expected to follow suit. This is because the main source of the acetyl-CoA required for the synthesis of fatty acids in non-ruminants is glucose (Chesworth *et al.,* 1998).

The non-significant values obtained for the serum total protein and albumin values could be as a result of the experimental diets which were formulated to be iso-nitrogenous. Leveille *et al.* (1961) reported that serum protein of growing chicks was significantly altered by dietary protein. Ogunwole *et al.* (2017) observed as not significantly different, the serum total protein values of broilers on isocaloric diets based on two varieties of cassava grits at the finisher phase. Similarly, Mosobalaje *et al.* (2019) observed values for serum total protein and albumin of pullet chicks on isonitrogenous cassava root products-based diets were not significantly different. The statistically insignificant values obtained for ALT and AST indicated normal liver functions in the experimental birds. According to Kaplan *et al.* (2003), damage to the liver often resulted into elevated values of the enzymes in the blood. Higher levels of serum thiocyanate with increasing inclusion of cassava grits in the diets could be attributed to higher levels cassava in the affected diets and efficient mechanism of the system in the conversion of hydrogen cyanide to the less toxic metabolite thiocyanate. A comparatively low level of serum thiocyanate at higher levels of cassava inclusion could be an indication of inefficient conversion of hydrogen cyanide to thiocyanate that could be occasioned by inadequate dietary sulphur-containing amino acid. Garcia and Dale (1999) observed that HCN are converted *in vivo* to thiocyanate by the liver enzyme rhodanase and that this process used sulphur from methionine.

5.4 Experiment 4

For the same reasons adduced for growing pullets in Experiment 3, TME 419 grits-based diets significantly increased live weight and significantly reduced average daily feed intake of hens at early, mid and late phases of laying phase compared to TMS 01/1368. Also, live weight significantly reduced, and average daily feed intake significantly increased with higher replacement levels of maize with grits from 33 to 66 and 100% in the diets of hens at the three phases of lay. Likewise, enzyme supplementation significantly increased live weight while it significantly reduced average daily feed intake at all phases of lay.

Hen day egg production was noted to be remarkably higher in birds on TME 419 grits-based diets than in TMS 01/1368 grits-based diets at the three phases of egg laying. This could be due to the presence of more soluble carbohydrate in TME 419-based

grits, though all the diets were formulated to be isocaloric and isonitrogenous. The TME 419 grits had more starch and less dietary fibre than TMS 01/1368 grits which indicated more soluble carbohydrate in TME 419 grits-based diets. As replacement level of maize with cassava grits was increasing from 33, 66 to 100%, hen day egg production was observed to show corresponding decrease at all phases of egg lay. This could be attributed to increasing content of highly digestible starch in cassava grits compared to that of maize. According to Weurding *et al.* (2003), the highly digestible starch of cassava could be converted to glucose quickly and absorbed in the upper small intestine when other nutrients have not yet been absorbed, leaving amino acids to be catabolized in the enterocytes of the lower small intestine to generate the energy demand of the digestive and absorptive processes, thereby hampering post-enteral availability of amino acids which is a key requirement for protein synthesis, growth and egg production**.** This is suggestive of the fact that protein efficiency in the chickens is higher in slowly digestible starch diets than in diets with highly digestible starch as amino acids are required in the right proportion for egg production. Enzyme supplementation significantly increased the hen day egg production. This would be due to better utilization of the feed because of the supplementation of the diet with the enzyme cocktail. The result was also in tandem with the manufacturer's claim of improved egg production and feed efficiency However, relationship between grits inclusion level and hen day egg production as described by regression equations obtained from figures 4.5, 4.7 and 4.9 shows that optimum Hen Day Egg Production of 41.7% was obtained at 35% replacement of maize with cassava grits at the early laying phase; 78.5% was obtained at 40% replacement of maize with cassava grits at the mid laying phase, while 71.8% Hen Day Egg Production was obtained at 30% replacement of maize with cassava grits at the late laying phase, respectively.

Egg weight at 33% replacement level of maize with the cassava grits compared favourably with standard diet irrespective of enzyme supplementation and cassava variety at the three phases of laying. However, at higher replacement levels, there was declined egg weight. As suggested by Ledvinka *et al.* (2012), egg weight is influenced by dietary total protein, methionine and other essential amino acids. The declining egg weight as replacement level of maize with cassava grits in the feed increased could therefore be due to diminishing available of methionine for egg weight. This is because

the critical amino acid is channeled into the detoxification of HCN to thiocyanate by the rhodanese enzyme.

The FCR of laying hens is a function of feed intake and egg mass which takes into cognizance percentage hen day egg production and egg mass. At the three phases of lay, pullets fed TME 419 grit-based diets had better FCR than those on TMS 01/1368 grit-based diets. Although, enzyme supplementation significantly improved FCR, the parameter was observed to be significantly poorer with corresponding increase in the levels of replacement of maize with cassava grits in the diets. All the cassava grits-based diets at 33% replacement level of maize with grits at all phases of laying elicited FCR that was either significantly improved or not significantly different compared to maize-based diet. This however is with the exception of TMS 01/1368 grit-based diet without enzyme supplementation which elicited poorer FCR compared to the maize-based diet. These FCR results were expected as the parameters for measuring FCR in laying hens, largely followed the same trend.

The egg shape index which is a derivative of egg width and egg length, was not affected by any of the factors of grits variety, replacement levels of maize with cassava grits and enzyme supplementation at the three phases of lay compared to the maize standard diet. This is in tandem with Tumova *et al.* (2007) who implicated genotype as a significant factor affecting egg shape index. Shell weight, thickness and ratio parameters are collectively indicative of the strength of the eggshell which is an important attribute in commercial egg production. Results obtained at all phases of laying indicated that eggshell weight, thickness and ratio were not altered significantly by the factors of cassava grits variety, replacement levels of maize in standard diet by cassava grits and enzyme supplementation. This was apart from shell weight which was improved at the late laying phase by enzyme supplementation. This could be ascribed to the effect of phytase activity (400,000 u/kg) in the enzyme cocktail which disrupted the phytate-mineral complexes binding calcium. The high phytate contents in plant feed materials could reduce calcium availability necessary for egg shell formation through the binding of calcium in phytate-mineral complexes (Li *et al.,* 2017) At the late laying phase, egg shell quality usually decreased as calcium becomes further unavailable due to a decrease in its intestinal uptake.

The albumen height and corresponding Haugh unit observed in all phases of egg laying were similar due to the effect of cassava grits variety and enzyme supplementation. However, Haugh unit decreased correspondingly with increasing replacement levels of maize with grits from 33 to 66 and 100% in the diets. The obtained values however did not fall below the average range of 75-80 Haugh unit reported for most eggs (Chukwuka *et al*., 2011). The values obtained were also noted to decrease as the age of the laying pullets increased. Williams (1992) similarly opined that the factor which mostly influenced albumen quality and by implication, the Haugh unit of freshly laid eggs is the age of the pullets. According to the author, as flock age advanced, Haugh unit scores decreased. There have been claims of poor albumen quality by farmers who have used cassava in the diet of laying hens. Cassava in ungelatinised form in the diet is powdery in nature, a situation observed by Panigrahi *et al.* (1992) to cause watery excreta. This could lead to increased exposure of laying pullets to ammonia, a circumstance which Benton and Brake (2000) suggested would increase albumen pH which consequently leads to albumen liquefaction and therefore poor albumen quality and Haugh unit.

At all phases of laying, yolk weight was observed to reduce significantly with corresponding increase in the level of replacement of maize with cassava grits in the diets of laying hens irrespective of enzyme supplementation. This trend was also observed with respect to grits variety except for the early laying phase. This could be due to the weight of the eggs which was significantly reduced with corresponding increase in the level of replacement of maize with cassava grits (See Tables 4.9, 4.15 and 4.21). According to Ledvinka *et al.* (2012) the weight of the yolk (as well as that of the albumen) is known to be highly correlated positively with the egg weight, an attribute directly influenced by dietary total protein, methionine and other essential amino acids. As the level of replacement of maize with cassava grits increased from 33 to 66 and 100%, there could be an equally diminishing methionine utilisation for egg size. This could be the case as the rhodanese enzyme catalysed the detoxification of cyanide to thiocyanide and methionine channeled into the process as the Sulphur donor. This also suggests a need to slightly increase methionine supplementation of

cassava-based diets above the levels normally included in maize-based diets to cater for cyanide detoxification.

Similar trend noticed in apparent nutrient retention of the pullets at the growing phase was largely observed at the laying phase. Apparent nutrient retention was not affected by cassava grits varietal type in laying hens. This could be due to the fact that nutrient uptake was not adversely affected by the anti-nutritional factor in cassava (HCN). Replacement levels of maize in standard diet with cassava grits had no effect on retention of DM, EE, CF, but influenced NR in the laying hens. Lower NR with increasing cassava grits inclusion could be as a result of higher HCN content which reflected in higher percentage of excreted nitrogen from cyanide rather than from protein nitrogen. Okoh (1983) reported that after a dose of administration of cyanide on rats through injection, analysis within 24 hours indicated 89% of excreted radioactivity in the urine. The result therefore may necessarily not be a true reflection of poorer protein utilisation in the treatments with higher inclusion levels of cassava grits, but indicative of the cassava cyanide detoxification mechanism.

Enzyme supplementation had no effect on retention of DM, EE and CF. However, NR in laying hens on enzyme-supplemented diets increased in contrast with that of pullets on diets without enzyme supplementation. As was the case at the growing phase, this could be due to the combined effect of the non-starch polysaccharidases in the enzyme cocktail hydrolysing the protein-encapsulating cell walls of the feed particles for exposure to digestion by proteases (Bedford, 2018).

Cassava grits variety type, replacement levels of maize in standard diet with grits, and enzyme supplementation did not influence the RBC, Hb, WBC and the leukocyte differential counts of the laying hens. All values obtained were comparable to those observed in laying hens on maize control diet and were also within the normal range for healthy birds of their age with reference to values obtained by Mistruka and Rawnsley (1977) and Oke *et al.* (2017).

Apart from age, sex, rearing systems, state of health and climatic conditions, another key determining factor of haematological parameters in chickens is nutrition or dietary content (Iheukwumere and Herbert, 2002). The observed normal ranges of WBC and

leukocyte differential counts which are the system's defense mechanisms against antigens and transformed cells, connoted that cassava grits in the diets of the laying hens did not induce morbidity or immunosuppression.

Normal red blood cells and Hb concentration observed in the laying hens fed cassava grits-based diets could indicate a low and consequently a very tolerable level of HCN in the diets. High cyanide level in the diets would have been expected to induce a depression in Hb concentration because of the affinity of cyanide to bind to ferric ion (Fe^{3+}) in methaemoglobin in erythrocyte thereby affecting haemoglobin concentrations and interfering with cellular respiration (Frankenberg and Sorbo, 1975). This result could also indicate the absence of hepatotoxicity and liver damage by the HCN content in the diets despite the prolonged feeding of the diets to the laying hens. According to Lindsay (1977), liver damage could result in depression of haemoglobin.

Similar results of serum biochemical indices observed in the growing pullets were recorded in the laying hens. The results indicated that glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate amino transferase, total protein, albumin and thiocyanate were not remarkably affected ($p>0.05$) by the grits varietal difference and enzyme supplementation. Cholesterol, triglycerides and thiocyanate were the only serum biochemical indices that were significantly affected ($p<0.05$) by replacement levels of maize with cassava grits in the diets of the laying hens.

Cyanide causes changes in glucose metabolism leading to 100% increase in conversion of glucose by pentose-phosphate pathway (Isom *et al*., 1975) resulting in depressed blood glucose. The glucose values so obtained which were not significantly different could therefore be due to the low level of cyanide in the cassava grits-based diets which could not induce any significant effect on glucose metabolism. Serum triglyceride levels that were not significantly different could be related to the serum glucose levels as the main source of the acetyl-CoA required for the synthesis of fatty acids in non-ruminants is glucose (Chesworth *et al.,* 1998). The result of serum ALT and AST which were within the normal range for the liver enzymes for most healthy laying hens further indicated there was no hepatoxicity induced by the cassava grit-based diets as the values obtained compared favourably with that of the maize-based diets.

The egg yolk lipid profile was not significantly affected by cassava grits variety, grits inclusion level and enzyme supplementation. Factors known to affect the egg yolk lipid quality in laying hens include incorporation of dietary polyunsaturated fatty acid (PUFA)-rich oils, vitamin E, vitamin-mineral premix and their interaction with rearing system (Milinsk *et al.,* 2003; Irandoust and Ahn, 2015; Ogunwole *et al.,* 2015). Barring these factors, utilisation of cassava grits in the diets of laying hens therefore would not be expected to adversely alter the quality of lipid profile of egg yolk compared to maize-based diets.

The average cost per kg weight gain, which is the cost of feed intake relative to hen-day egg production and average egg weight, was significantly influenced by cassava grits variety, grits inclusion level and enzyme supplementation. The least cost of ₦372.61 was recorded in pullets on T_2 (33% replacement of maize with TME 419 grits supplemented with enzyme). This result was in consonance with the FCR obtained for pullets at the early, mid and late laying phases in which the FCR value of T_2 was remarkably lower than the that of maize-based diet and the other treatments. (See Tables 4.10, 4.16 and 4.22). Although, cost/kg of cassava grit at the time of the feeding trial was ₦75 as against ₦140 for maize, this advantage did not reflect in the average cost of FCR for all the cassava grits-based diet treatments. This was because more expensive levels of protein materials had to be incorporated into the diets, as levels of replacement of maize with cassava grits increased in order to compensate for the protein deficiency of cassava grits. Furthermore, FCR (as occasioned by performance indices of feed consumption, hen-day hen production and egg weight) generally became poorer as inclusion level of cassava grits in the diets increased from 33 to 100%.

CHAPTER 6

SUMMARY AND CONCLUSION

The evaluation of percentage starch content of varieties TME 419 and TMS 01/1368 cassava roots and percentage starch gelatinisation of the respective grits with sun-drying and roasting methods was carried out. Results obtained showed that TME 419 root had more starch content than TMS 01/1368. Also, much higher percentage of starch gelatinisation was achieved with roasting method of producing cassava grits than sun-drying.

Evaluation of true metabolisable energy of TME 419 and TMS 01/1368 grits was carried out with mature cockerels. Results indicated TME 419 grits had higher true metabolisable energy than TMS 01/1368 grits.

Studies with growing pullets indicated that feed conversion ratio improved with 33% replacement of maize in standard diet with both TME 419 and TMS 01/1368 grits supplemented with enzyme. Cost of weight gain per kg body weight was lowered with 33 and 66% replacement with TME 419 and 33% replacement with TMS 01/1368 grits, supplemented with or without enzyme, compared to control diet. However least cost was obtained with 33% replacement with TME 419 grits with enzyme supplementation.

In the laying hens, 33% replacement of maize in standard diet with TME 419 grits with or without supplemental enzyme showed similar hen day egg production as the control diet at the early laying phase. At the mid and late laying phases, 33% replacement of maize with TME 419 grits with supplemental enzyme elicited improved hen-day egg production compared to control diet. However, at the mid and late laying phases, up to 66% replacement with TME 419 grits with supplemental enzyme showed similar hen-day egg production as the control diet. Haugh unit was similar in control diet and diets with 33% replacement of maize with TME 419 and TMS 01/1368 grits, with or

without supplemental enzyme. Increased dietary inclusion levels of grits resulted in linear reduction of Haugh unit at early laying phase (R^2 = 0.94), mid laying phase (R^2 = 0.99) and late laying phase (R^2 = 0.99). At the three laying phases, egg shell thickness of hens on control diet was equivalent to that of hens on 33, 66 and 100% replacement levels of maize in standard diet with TME 419 and TMS 01/1368 grits with or with supplemental enzyme. Laying hens on the three levels of replacement of maize in standard diet with either TME 419 or TMS 01/1368 grits, supplemented with or without enzyme also had similar serum alanine aminotransferase (ALT) as the control diet. Same trend was observed for the egg yolk lipid profile. The average cost of FCR was significantly lower in hens on 33% replacement of maize with TME 419 grits with or without supplemental enzyme and same replacement level with TMS 01/1368 with supplemental enzyme compared to control diet. The least cost of FCR was however observed in 33% replacement of maize with TME 419 grits with supplemental enzyme. Average cost per kg of cassava grits (both TME 419 and TMS 01/1368) was ₦75 which was 53.5% of cost per kg of maize within the period of the experiment.

6.1 Contributions to knowledge

The results in the study showed that:

- Varietal difference and processing method could affect the caloric potentials of cassava grits. TME 419 cassava grits had significantly higher starch content than TMS 1368, while roasting processing method elicited significantly higher starch gelatinisation than the sun-drying method.

- Performance as indicated by hen-day egg production in laying hens was sustained by replacement of maize in standard diet with roasted TME 419 grits at 33% irrespective of enzyme supplementation, in the early laying phase, and 66% with enzyme supplementation at the mid and late laying phases. However, regression analyses showed that 35% replacement could sustain an optimum hen day egg production of 41.7% at the early laying phase while 40% replacement could sustain an optimum hen day egg production of 78.5% and 30% replacement sustain 71.8% hen day egg production at the mid and late laying phases, respectively.

- Internal egg quality as depicted by the Haugh Unit elicited by hens on standard maize diet and those on 33% replacement of maize with TME 419 and TMS 1368 grits with or without supplemental enzyme, was similar. The Haugh Unit decreased as the replacement level of maize with cassava grits increased.
- There was no hepatotoxic response from the laying hens as indicated by the serum alanine aminotransferase and aspartate aminotransferase with the inclusion of TMS 419 and TMS 1368 cassava grits in the diets up to 100% replacement of maize in standard diet.

In conclusion, performance in laying hens was sustained by replacement of maize with roasted TME 419 grits at 33% despite enzyme supplementation, in the early laying phase, and 66% with enzyme supplementation at the mid and late laying phases. Egg quality however decreased with inclusion of cassava grits, irrespective of supplemental enzyme.

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