EFFECTS OF AQUEOUS EXTRACTS OF GRAINS OF SELIM (*Xylopia aethiopica)* AS AN ADDITIVES ON THE PRODUCTION CHARACTERISTICS OF LAYING CHICKENS

BY

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CERTIFICATION

This is to certify that this work was carried out by AKPOMIEMIE OGHENEVWEGBA in the Department of Animal Science, Delta State University, Asaba Campus, Nigeria.

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Prof. I.A. Amata (Supervisor) Date

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Prof. I. A. Amata (Head of Department) Date

DEDICATION

This work is dedicated to God Almighty who in His infinite mercy granted me life, grace, strength, wisdom and resources to start and end this race well. To God be all the Glory, Honour and Adoration.

DECLARATION

I hereby declare that, except for references to other people's work which have been duly sited, this work is solely of the author's own research conducted in the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, Delta State University, Asaba Campus. This Field Report has neither in a whole nor in part been submitted for a degree either in this university or elsewhere.

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Akpomiemie Oghenevwegba Date

Student

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ABSTRACT

The effects of aqueous extracts of grains of selim *Xylopia aethiopica* dried fruits on the production characteristics of laying chickens was investigated. One hundred and fifty Shikka Brown layers of 45 weeks old were randomly allotted into 5 treatments groups in a completely randomized design. Each treatment was replicated thrice with 10 birds per replicate and 30 birds per treatment group. *Xylopia aethiopica* was added to the drinking water at 0g, 0.20g, 0.35g, 0.50g and 0.65g per litre for 3days in T1 (control) T2,T3,T4, and T5 respectively. The birds were fed the same diet. Feed and water were served *ad-libitum*, with diet containing 17% crude protein and 2600Kcal/kg metabolizable energy and the experiment lasted for 8weeks. This was done in order to evaluate the effect of *Xylopia aethiopica* on the performance characteristics (feed intake, body weight, egg production, gut microbial population, internal and external egg quality characteristics, serological and haematological parameters, and the economic prospects of grains of selim as additive in layer production). Data collected were subjected to analysis of variance (ANOVA) and separation of means was made using Duncan's Multiple Range Test. The results of the experiment revealed that feed conversion ratio, albumen weight and length, hen-day production, and number of eggs laid, egg width, egg shell surface area, yolk diameter, yolk index, yolk weight expressed as percentage of egg weight (YEW), microbial activity and economic prospects were significantly ($P<0.05$) influenced by the use of *Xylopia aethiopica* in drinking water while body weight gain, feed intake, egg weight and length, egg volume, shell weight, shell thickness, egg shape index, shell weight expressed as percentage of egg weight (SEW), yolk weight and height, yolk colour, albumen height, haugh unit, albumen weight expressed as percentage of egg weight (AEW) and yolk cholesterol were not significantly ($p>0.05$) affected. Results revealed that layers given 0.65g/l of *Xylopia aethiopica* had the highest feed intake, total egg production, egg shape index and SEW ($P>0.05$) while yolk index, albumen length, YEW, white blood cell counts, lymphocytes and albumin were significantly ($P<0.05$) affected compared to those in control. Economic analysis of cost of total feed consumed/bird, cost/kg feed, feed cost/kg egg, cost differential/kg egg, and relative cost benefit/kg egg depicted significant differences in all the treatment groups. It was therefore concluded that grain of selim can be used in place of synthetic antibiotics as it has no detrimental effects on the health condition of the birds and since it can also support their optimum performance with more return on investment.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Xylopia aethiopica is a slim, tall, evergreen, aromatic tree which can grow up to about 20m high and 60-75cm in diameter and is found in forest zone especially along the rivers in arid areas in tropical Africa. It is of the family *Annonaceae* and order *Magnoliales (GRIN, 1985)*. The fruits are like small, twisted bean-pods, aromatic, quite pungent (scented when fresh), smooth grey bark, and slightly bitter. They are dark brown, cylindrical, 2.5 to 5cm long and 4 to 6mm thick; the contours of the seeds are visible from outside. Each pod contains 5 to 8 kidney-shaped seed grains of approximately 5mm length (Burhill, 1985). It is called kimbàà in Hausa, ùdà in Igbo èèru in Yoruba and urhierię in Urhobo. The dried fruits of *Xylopia aethiopica* when used as spice has both nutritional and medicinal value. A study investigated the composition of the essential oils from the leaves, stems, roots, barks, fresh and dried fruits of the plant and reported their antioxidant properties (Karioti *et al*., 2004). Essential oils or their constituents are odoriferous substances from plants and are extensively used as medicinal products, in the food industry as flavors and in the cosmetic industry as fragrances (Evans, 2003). Adewoyin *et al*., (2006) reported the mosquito repellent effects of the fruit essential oil of *Xylopia aethiopica*. Studies on *Xylopia aethiopica* by other researchers (Karioti *et al*., 2004) have reported its antimicrobial activity on intestinal diseases and rheumatism while Sumathykutty *et al*. (1999), Saganuwana (2009) and Ogbole *et al*. (2010) reported on the essential oils of the leaf, stem and root, barks, and fresh fruits of the plant. The ban on the use of synthetic antibiotic and the increased awareness of their residual effects on the health of consumers necessitated the need for natural and safe feed additives such as *Xylopia aethiopica* to achieve better production results of farm animals (Frankie *et al*., *2009*). The use of plant materials traditionally used as food spices, condiments and or as medicine would be more beneficial to animal and even human health than the use of synthetic antibiotics. In many tropical countries, these medicinal plants and spices are abundant and easily accessible. It has been estimated that in Nigeria over 40% of known plants serve as food whereas about 30% serve as spices and medicinal plants (Nwobegu, 2002). However, all these reports are associated with the dried fruits, with no information on the oils from the other morphological parts of the plant. Herbs, spices and plant extracts can be valuable alternatives for the health and nutrition of chickens (Manan *et al*., 2012).

***Fig 1: Xylopia aethiopica* Dried Fruits (Grains of Selim)**

1.2 Statement of the problem

The use of synthetic antibiotics in poultry production and reports on their residual effects on consumers of poultry products have led to numerous medical disorders and have generated interest in producers trying to do without antibiotics and other drugs (Frankic *et al*., 2009); because the use of synthetic drugs in animal production may increase antibiotic resistant bacteria, the accumulation of antibiotic residues in animal products and the potential to transfer resistant strains from animals to humans via the food chain (Stanacev *et al*., 2011). Therefore, there is need to adopt a more nutrition-based health strategy in future animal production and this has generated interest in producers trying to do without antibiotics and other drugs (Asekun and Adeniyi, 2004). This is important because consumers are now paying much more attention to quality and safety of poultry products they eat (Boakye *et al*., 1977). Also, the need for reduction in the prices of animal products, necessitate the use of plant supplements, which can encourage higher egg production rate and improve the quality of the animal products (Alu, 2010).

1.3 The Main Objective of the Study

The general objective of this study was to investigate the effect of using dried fruits of *Xylopia aethiopica* (Grains of selim) as additive on the production characteristics of laying birds.

1.4 Specific Objectives

The specific objectives of the study were to:

1. evaluate the effect of the grains of selim on the growth performance, gut microbial population and egg production of laying chickens;
2. evaluate the effect of the grains of selim *(Xylopia aethiopica)* on egg quality characteristics;
3. determine the effect of the grains of selim *(Xylopia aethiopica)* on the serological and heamatological parameters of laying chickens and to
4. determine the economic prospects of the grains of selim *(Xylopia aethiopica)* as an additive in layer production.

1.5 Justification of the study

The increasing cost of antibiotics and other synthetic drugs in addition to the residual effects of their use has led to numerous medical disorders in human. This led to the restriction and ban imposed in 2006 by the European Union on the use of antibiotics and one of the possible alternatives to replace antibiotics and other synthetic drugs in poultry production is by the use of phytogenic feed additives-PFAs that can serve as good alternatives to the use of synthetic drugs and antibiotics (Windisch *et al*., 2008). This necessitated the need for researchers to go into the use of natural herbs and spices that could serve as cheap and good alternatives to the use of synthetic drugs and antibiotics. The significance of using dried fruits of *Xylopia aethiopica*, as an additive in promoting growth has been reported in broiler production (Isikwenu *et al*., 2014). Therefore there is also a need to know the effect of grains of selim in the egg production and performance in laying birds in order to find out whether its use would enhance egg production as an additive.

In this study, the use of dried fruits of *Xylopia aethiopica* served as an additive just like antibiotics to ensure good health and performance.

The beneficial effects of use of dried fruits of *Xylopia aethiopica* as additive in laying birds would possibly include reduced cost of egg production and elimination of residual effects caused by synthetic antibiotics and its attendant health implications on human who consume the poultry products.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutrition

Herbs, spices and other plant extracts (phytogenic feed additives) are now the means of a new class of additives in poultry feed in place of synthetic antibiotics. The need for improvement of feed efficiency accelerated the introduction of feed additives which became widely used in animal feed for many decades. The objective delineated by scientists, is to increase production (eggs and meat) while maintaining animals in good health. (Alloui *et al*., 2014). Their uses are still limited in relation to their mode of action and aspects of application. However, complications may be encountered due to various changes in botanical origins of the plants and their extracts, transformations and compositions of plants and their extracts. But most of the investigations have studied the interactions of various active compounds and their physiological impacts and effects on production performance (Figueiredo *et al*., 2008).

Herbs, spices and plant extracts improve the feed intake when incorporated in poultry diets to replace synthetic antibiotics products in order to stimulate or promote the effective use of feed nutrients which may subsequently result in more rapid body weight gain, higher production rates and improved feed efficiency (Windisch *et al*., 2008). This also was reported by (Alloui *et al*., 2014) that "herbs and plant extracts can be incorporated in poultry feed as growth promoters such as probiotics and prebiotics". In the past decades, scientists have synthesized a large number of interesting chemical substances from medicinal plants, such as *Xylopia aethiopica* which have been of great help in the practice of replacing synthetic antibiotics with herbs and spices due to the fact that the fruit extract has been shown to be active as antimicrobial agent against gram positive and gram negative bacteria (Irvine, 1961; Burhill, 1985; Ghana Herbal Pharmacopoeia, 1992; Mshana *et al*., 2000). These researchers attested to the fact that almost every morphological part of the plant (*Xylopia aethiopica)* is used in traditional medicine for managing various ailments including skin infections, *staphylococcus aureus, streptococcus spp E. coli,* candidiasis, dyspepsia, cough and fever.

2.2 Use of Herbs in Organic Egg and Meat Production in Poultry

Organic egg production is gaining more importance with access to forage material such as herbs in the hen yard or supplemented with roughage in the form of silages and vegetables in

addition to the basal diet recently (The Council of the Europian Union, 2007). Hammershoj and Steenfeldt (2012) studied the effect of feeding kale (*Brassica oleracea* ssp. *acephala*), thyme (*Thymus vulgaris*) and basil (*Ocimum basilicum*) as a forage material on various egg quality parameters and egg production. They reported no significant difference in forage intake and laying rate between treatment groups but kale treatment significantly increased egg weight and higher egg shell strength. Several studies have emphasized on the importance of forage material (whole wheat, *Phacelia tanacetifolia*, *Fagopyrum esculentum* and *Linum usitatissimum*) on egg production, calcium supplements to laying hens, carotenoids in egg yolk, various egg quality parameters, conversion of oil rich forage material into specific fatty acids to the egg yolk and supply of vitamins, essential amino acids and minerals (Horsted *et al*., 2006; Hammershoj and Steenfeldt, 2009; Wood and Fearon, 2009; Hammershoj *et al*., 2010). Furthermore, different aromatic herbs, vegetables and forage material can directly transfer flavors to the egg (Tserveni-Gousi, 2001) and as a result of altered microflora composition of the intestine due to change in forage material, thereby causing new flavors to the egg (Richter and Fehlhaber 2002).

Incorporation of turmeric (*Curcuma longa*) root powder and mannan oligosaccharides in broiler ration as a feed supplement results in decrease in the fat percentage up to 1% (Al-Sultan, 2003) and 1.2% (Samarasinghe *et al*., 2003) levels over body weights. Emadi and Kermanshahi (2006) reported that supplementation of turmeric rhizome powder (0.75%) in broiler rations leads to improved carcass quality, lean meat and significant decrease in abdominal fat pad up to 57% level and heart weights to live body weight. Moreover, turmeric powder supplementation in broiler feed causes higher dressing percent up to 57% level, increased the liver weight, spleen weight and whole giblets weight (Kurkure *et al.,* 2002; Al-Sultan, 2003; Durrani *et al*., 2006). Shyama Tulsi (*Ocimum sanctum*) leaf preparation as a broiler feed supplementation for its growth promoter activity causes no significant change in the weights of the bursa, liver and spleen (Gupta and Charan, 2007). Singh *et al*. (2007) reported that supplementation of the amla and turmeric combined powder at the rate of 5g kg^{-1} in broiler feed results in enhanced dressing percentage and decreased mortality in broiler chicks. Mehala and Moorthy (2008) demonstrated that combination of *Curcuma longa* (Turmeric) and *Aloe vera* at different concentrations showed no significant change in the abdominal fat and breast muscle weights. A combination of tulsi, amla and turmeric at the rate of 0.5% has not shown significant difference in ready to cook yield percentage and giblet weights (Reddy, 2010).

2.3 Herbs and Spices and Plants Extracts as Appetite and Digestion Stimulant in Animal Nutrition

Herbs and plant extracts used in animal feed (phytogenics feed additives) are compounds of plant origin incorporated into animal feed to enhance livestock productivity through the improvement of digestibility, nutrient absorption and elimination of pathogens residents in the animal gut (Kamel, 2001; Athanasiadou *et al*., 2007) . Varieties of plant compounds are used as phytogenic feed additives (PFAs) and the most common and frequently used are herbs and spices (garlic) and other plant parts or their extracts such as the grains, leaves, oil etc. The active substances in these products can vary greatly depending on what part of the plant is used or composition, processing, the harvest season and geographical origin (Windisch *et al*., 2008). Some PFAs were sometimes seen as having a role to improve the taste and feed palatability which implies an improvement in poultry production performance. The number of studies that tested the effect of plant extracts on palatability is very limited in this specie (Alloui *et at*., 2014). In general, an increase in feed intake in chickens is much more due to additives such as organic acids, probiotics and prebiotics (Catala - Gregori *et al*., 2007). Thus, the assumption that herbs, spices and their extracts improve the feed palatability does not seem to be justified in general (Windisch *et al*., 2008). Moreover, it is believed that the phytogenic compounds can improve the digestive enzyme activity and nutrient absorption. Jamroz *et al*. (2006) demonstrated in their study that the phytogenic feed additives have a stimulatory effect on intestinal mucus in chickens and this effect is assumed to influence the adhesion of pathogens and in consequence help to stabilize the microbial equilibrium in the chicken gut.

Digestive stimulation by phytogenic additives is achieved through stimulation of saliva secretion, liver, pancreas and intestine enzymes activities, intestine function and morphohistology and metabolism (Peric *et al*., 2010). The active components of herbs and plant extracts may improve digestion and stimulate the immune function in poultry (Ghazalah and Ali, 2008).

2.4 Use of Herbs and Spices and their Extracts in Animal Nutrition

The use of synthetic antibiotics in poultry feed as a growth promoter is beneficial in improvement of production parameters and diseases prevention. However this large utilization has led to the increasing resistance of pathogens to antibiotics and the accumulation of antibiotic

residues in animal products and in the environment. This situation requires the world to restrict using antibiotics growth promoters in animal feed (Nisha, 2008). Plants and derivatives of plants play a key role in both human and animal health and have long been known to possess biological activity (Abass, 2012).

Plants extracts from a wide variety of herbs, spices and derivatives, have already been used since the antiquity. They were appreciated for their specific aroma, flavor and various medicinal properties. Studies on these compounds have shown some positive effects on antimicrobial, antioxidant and regulator of the gut flora in poultry production recently. This indicates that plant extracts can be considered as growth promoters; however evaluation procedures of their therapeutic and beneficial effects, their toxicity and interactions with prescription drugs have to be improved (Athanasiadou *et al.,* 2007).Several herbs and spices and their products or extracts have been found to improve layers performance and resulted in growth promoting effects (Evans, 2015).

2.5 Herbs and Spices and their Extracts as Antioxidants for Poultry

Herbal products are easily available, low cost, abundance and incorporated in poultry feeds to enhance the body weight gain and to increase the feed efficiency. Allison *et al*. (2013) reported that herbal extracts enhances the performance in poultry and increases the feed: gain and weight gain ratio by significantly decreasing the bacterial and oocsyt count. Issa and Abo, (2012) reported in their studies that feeding garlic powder to broilers enhances the performance, improves digestibility, digestive organs, crude protein (CP), dry matter (DM) and ether extract (EE) digestibility.

Nowadays, there has been an increase in demand for natural antioxidants in food due to its health benefits against oxidative stress and several diseases. Plant derived antioxidants are gaining more demand in poultry nutrition because their meat has high content of polyunsaturated fatty acids and susceptible to lipid oxidation (Christaki, 2012). Many plants have been identified as excellent poultry antioxidants; important among which are rosemary (*Rosmarinus officinalis*), Olive leaves (*Olea europea* L.) garden thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*), sage (*Salvia officinalis*), oregano (*Origanum vulgare*) and so forth (Madsen and Bertelsen, 1995; Botsoglou *et al*., 2002, 2005, 2013; Rahal *et al*., 2014). Among these, rosemary extracts are some of the most studied natural antioxidants in poultry products and these studies

have demonstrated the ability of rosemary products to act as natural antioxidants in various poultry products (Rojas and Brewer, 2007; Karre *et al*., 2013). Apart from these, fruits like plum, grape seed extract, cranberry, pomegranate, bearberry, pine bark extract etc. provide good alternatives to synthetic antioxidants due to the high phenolic compound in them (Karre *et al*., 2013). Spices like cinnamon, cloves, marjoram, wild marjoram, caraway, peppermint, nutmeg etc., have been shown to have antioxidant properties as they contain the compounds such as polyphenolics, lignans, flavonoids and terpenoids (Botsoglou *et al*., 2013). Among the herbal plants, tulsi (*Ocimum sanctum*) and Ashwagandha (*Withania somnifera*) have been proven as an excellent adaptogen and antistress agent and has proven to reverse the Cadmium-induced oxidative stress in chicken (Bharavi *et al*., 2010). Studies showed that active ingredients of plants have strong antioxidant effects including neutralization of superoxide, hydrogen peroxide and nitric oxide either by scavenging radicals or by increasing the production of catalase, superoxide dismutase (SOD) and glutathtion peroxidase (Yarru *et al*., 2007). Ginger contains some important metabolites and alkaloids like gingerol, shogaol, gingerdione, shogaols and other phenolic compounds which have antioxidant properties (Zhao *et al*., 2011). In thyme, important alkaloids isolated include carvacrol, thymol, caffeic acid, p-cymene-2, 3-diol and biphenylic (Bolukbasi *et al*., 2006).

2.6 Antimicrobial Activity of Herbs and Spices and other plant extracts in Animal Nutrition

Herbs and plant extracts (PFAs) are well known for their antimicrobial effects in vitro against important pathogens but also against fungi (Tatsadjieu *et al*., 2003). Most studies show a greater sensitivity of Gram +bacteria compared to Gram -bacteria (Burkhill, 1985; AJTCAM, 2008).

Phytogenics are a group of natural growth promoters (NGPs) or non-antibiotic growth promoters used as feed additives, derived from herbs, spices or other plants. Aromatic herbs and spices are added in feed or water, because they contain a large number of substances with antimicrobial, antiviral and antioxidant activities (Bolukbaşi *et al*., 2013).

Consequently, phytogenic feed additives relieve the host animals from immune defense stress during critical situations and increase the intestinal availability of essential nutrients for absorption, thereby helping animals to grow better within the framework of their genetic potential (Hashemi and Davoodi, 2010).

2.7 Antimicrobial Activity of the Essential Oil of *Xylopia aethiopica*

Several reports on the antimicrobial activity of the essential oil of *Xylopia aethiopica* have been made in the literature. For example, the essential oil as well as the crude extracts (both alcoholic and aqueous) of the plant have been shown to have antimicrobial property against a wide range of Gram positive and Gram negative bacteria, and *Candida albicans* (Boakye-Yiadom *et al*.,1977;Thomas, 1989;Tatsadjieu *et al*., 2003; Asekun and Adeniyi, 2004; Okigbo *et al*., 2005).

Xylopia aethiopica is a medicinal plant of great repute in West Africa which produces a variety of complex chemical compounds. The fresh and dried fruits, leaf, stem bark and root bark essential oils showed various degrees of activity against the Gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, the Gram negative bacteria *Pseudomonas aeruginosa* and the yeast-like fungus *Candida albicans*, using the cup plate method. However, none of the oils showed activity against *Escherichia coli* (AJTCAM, 2008). Many of these oils have been shown to exert broad spectrum antimicrobial activity (Schelz *et al*., 2006; Hammer *et al*., 1999).

Generally, the essential oils derived from plants have provided enough evidences to suggest as a tool in defending bacterial diseases in poultry (Dorman and Deans, 2000; Rota *et al*., 2004; Gopi *et al*., 2014).

2.8 Health Benefits of *Xylopia aethiopica* and Phytogenic Feed Additives (PFAs)

The potential benefits of using phytogenics in poultry nutrition are: increased feed intake, stimulation of digestion, increased growth performance, reduced incidence of disease, improved reproductive parameters, improved feed efficiency, increased profitability and reducing poultry house emissions. (Yitbarek *et al*., 2015).

The dried fruits of *X. aethiopica* (Grains of Selim) and other plant extracts are used as a spice and as herbal drugs for both human and animals. It remains an important item of local trade throughout Africa as a spice, and flavoring for food and for medicine. Every food substance consumed by animal has either a therapeutic, nutritional or toxic effect on the body, and these food substances when obtained in their crude form can be of immense help in the curing of some diseases. Plants as well have been used for therapeutic purposes and their uses are as old as the history of man. Studies have demonstrated the antioxidant and antimicrobial activity of PFAs in vitro but in vivo these results are limited. Moreover, other effects such as anti-inflammatory,

anti-fungal, anti-infectious and anti-toxigenic have been confirmed by many researches (Arczewska-Wlosek and Swiatkiewicz, 2012; Khan, 2014).

2.9 Utilization of *Xylopia aethiopica* in Poultry Nutriton

Yegani *et al*. (2006) reported that grains of selim has antimicrobial and anthelminthic activities and promotes growth in broiler chickens and that herbal product could serve as an environmental friendly alternative to the antibiotic growth promoters. Several herbal products and their extracts have been found to improve broiler performance and resulted in growth-promoting effects (Yegani *et al*., 2006, Isikwenu, 2014). Some herbs, spices and their extracts stimulate feed intake and endogenous secretions or possess antimicrobial, coccidiostatic or anthelminthic activities (Isikwenu, 2014, Hossain, 2015). The performance characteristics of the finisher broilers given different concentrations of grains of selim in drinking water were to do generally better than broilers given antibiotics in terms of final body weight, total weight gain, daily weight gain and feed conversion ratio (Isikwenu and Udomah, 2015).

Research has proven that broilers given different concentrations of grains of selim in drinking water indicate the fact that grains of selim have growth promoting potentials as antibiotics growth promoters. However there was significant decrease in abdominal fats broilers given grains of selim. This indicates that it has hypocholesteromic activities which may give a better lean meat in broilers (Isikwenu and Udomah, 2015).

Table 2.1 Phytochemical Compositions of *Xylopia aethiopica* Dried fruits

Parameters (%)		**Phytochemical Test Results**
Dry matter	87.95	Flavonoid +++
Ash	5.84	Tannin ++++
Crude fibre	10.51	Alkaloid ++
Crude protein	2.73	Steroid ++
Ether extract	9.90	Saponin +
Nitrogen free extract	58,79	Carbohydrate +

N B: + = slightly present, ++= moderately present, +++= present, ++++= strongly present
Source: (Isikwenu, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out in the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, Delta State University, Asaba Campus. The farm is located on longitude 6^0 49^0E of the Greenwich Meridian and latitude 6^0 14^0N of the Equator with annual rainfall of 1800mm to 3500mm between the period of April to October. The maximum day temperature ranges from 25.8^0C to 32.3^0C between the period of November and March and a relative humidity of 65% (Ministry of Aviation, Department of Meteorological Service Asaba, 2014).

3.2 Experimental Birds

One hundred and fifty shikka brown laying birds of 45weeks of age were used for this experiment. The birds were obtained from the Commercial section of the University Farm and were weighed individually on arrival and on a weekly interval thereafter, which lasted for the period of 8weeks (February 09^{th} - 05^{th} April 2016).

3.3 Management of the Experimental Birds

The birds were managed in deep litter system and the poultry house was partitioned into 15 pens measuring 1.8m x 1.5m each. Dwarf wooden walls and half wire mesh were used for the demarcation of the pens to permit good ventilation and prevent other predators from entering the pens. Feeding was carried out twice in a day between the early hours of 6.30am and 7.30am and 4.00pm and 5.00pm. Feed and water was provided *ad-libitum* and feed intake was determined by the difference between the quantity of feed provided the previous day and the quantity of feed left the next day. All the sanitary measures were properly enforced and accurate record keeping was carried out during the period of the study.

3.4 The Test Ingredient

The test ingredient is *Xylopia aethiopica*. The dried fruits of *X. aethiopica* were purchased from Ogbegologo Market in Asaba, Delta State, Nigeria. Extract was obtained by using water as the extracting solvent. The dried fruits of *Xylopia aethiopica* were removed from their strands,

washed and dried in the sun after which they were pulverized with mortar and pestle, and ground with a grinding machine. A sample of the test ingredient was taken to the Animal Science Research Laboratory for analysis. The paste was soaked in 1000ml of water for 72 hours, after which it was filtered out using a filter paper. The solid filtrate was dried and weighed and the value obtained was subtracted from the original weight. The balance gave the quantity of dried fruits of *Xylopia aethiopica* in the solution. The test ingredient was given via the drinking water for 3 consecutive days on the 3rd and 8th weeks of the experiment at various levels (0g/L, 0.20g/L, 0.35g/L, 0.50g/L, & 0.65g/L for T_1,T_2,T_3,T_4 and T_5) respectively.

3.5 Experimental Diet

The birds were fed with formulated feed containing 17% Crude Protein and 2600kcal/kg Metabolic Energy according to the feeding standard of Nutrient Requirement for Poultry during the period of the experiment (NRC, 1994). The experimental diet was further analyzed using the available techniques in Research Laboratory of the Department of Animal Science, Delta State University Asaba Campus for their proximate compositions.

3.5.1 Composition of the Experimental Diet (kg/100kg)

Ingredients (%)	
Maize	57.99
Soya Bean Meal	16.91
Fish Meal	3.00
Bone Meal	3.00
Wheat Offal	11.00
Oyster Shell	7.50
Common Salt	2.00
Methionine	2.00
Lysine	2.00
Total	100
Percentage Crude Protein	17.01
Metabolic Energy (Kcal/Kg)	2624.06

3.6 Weight Gain, Feed Intake and Feed Conversion Ratio

Live weight gain for each replicate was obtained by subtracting the weight gain of week 1 from that of week 2 divided by the number of birds in that pen while the weight gain was obtained by subtracting the final weight gain from the initial weight gain of the birds. Average weight gain per bird was obtained by dividing the average weight of the birds by the total number of birds in the replicate. Average Weight Gain (kg) = $\frac{\text{Final Weight – Initial Weight}}{\text{No. of birds in the replicate}}$

Feed intake was determined by subtracting the weight of the leftover feed from the initial weight of the feed, while average feed intake was obtained by dividing feed intake by the number of birds in the replicate. Feed conversion ratio was determined by dividing the kg of feed consumed (weekly feed intake) by egg weight (weekly egg production).

3.6.1 Feed Cost

The cost of the feed and the test ingredients were calculated based on the market price as at that time the experiment took place, which was used as to calculate the cost per kilogram (kg) feed consumed. The cost of total feed consumed per bird was calculated as,

Cost of total feed consumed/bird (₦) = Total feed consumed/kg/bird x Cost/kg feed consumed

Feed cost/ kg egg (₦) = $\frac{\text{Total feed consumed/kg/bird x Cost/kg feed consumed}}{\text{Total egg weight/bird/kg}}$

Cost differential/kg egg (₦) = Feed cost/kg egg – Feed cost/kg egg of control

Relative cost benefit/kg egg (%) = $\frac{\text{Feed cost/kg egg control x 100}}{\text{Cost/kg of experimental treatment}}$

3.7 Proximate Analysis

Proximate analysis of the test ingredient was carried out using the procedure by AOAC (2012).

Determination of Percentage Moisture and Total Ash: Accurate weight of 2.13g of the sample was weighed out into a known weight of crucible. This was then placed in a thermostat air oven set at 105^0C for 24hours to dry carefully. It was then cooled in desiccators for about 15-30minutes and weighed. Percentage of moisture was determined as, weight of crucible and sample before drying minus weight of crucible and sample after drying divided by the weight of the sample before drying multiplied by 100.

Percentage Total Ash: 2.0g of the sample was weighed into a known weight of crucible which was heated using an electric cooker. It was then transferred to a thermostatic muffle furnace set at 600^0C for 30minutes or until the ash turns grey powder. Thereafter the crucible was allowed to cool for about 30minutes or more in a desiccator and weighed.

$$\%\text{Total Ash} = \frac{\text{Weight of crucible + Ash - Weight of crucible}}{\text{Weight of sample}} \times 100$$

Crude Fibre: 2.0g of the sample was placed in a conical flask and was diluted with sulphoric acid and then sodium hydroxide solution which was heated on the heating mountain fibre extractor for 30-40 minutes. Thereafter the content was filtered using a filter paper. The filter paper was placed in a crucible, placed in an oven set at 105^0C for drying for about 12hours. It was cooled and weighed.

$$\%\text{Crude Fibre} = \frac{\text{Weight of filter paper + Fibre - Weight of filter paper}}{\text{Weight of sample}} \times 100$$

Crude Protein: 2.0g of the sample was weighed into 300ml of kjaldahl flask. 7.5g of anhydrous sulphate was added. 1.0g of copper sulphate, a trace of solenuim powder; 25ml of concentrated sulphoric acid and anti bombing beads were also added to the flask and mixed thoroughly. The digestion was done for about 45minutes and a bright greenish blue was observed. The digested solution was allowed to stay overnight before dilution with distilled water to avoid explosion and was transferred to a graduated flask of 250ml. Markam's kjaldahl apparatus was used to digest nitrogen and titration was carried out to obtain the titre values and averages.

$$\%\text{Nitrogen content} = \frac{\text{Average titre x Normality of acid x 1.4}}{\text{Weight of sample}}$$

Ether Extract: 2.0g of the sample was placed into a fat extraction thimble which was placed in a soxhlet extractor. The extractor was fixed into a soxhlet flask of a known weight, the soxhlet flask was filled with ¾ of petroleum ether (BP 40-60^0C) was placed on the heating mountain at 70^0C for 7hours. The process continued until all fat were extracted and the petroleum ether drained off. Main while, the cold water flow in the condenser was running through rapidly. The flask was removed and dried at 105^0C for 12hours to dry the trace of either ether or water from the flask and then cooled in a desiccator for 30minutes and weighed.

$$\%\text{Ether extract} = \frac{\text{Weight of flask + Fat – Weight of flask}}{\text{Weight of sample}} \times 100$$

Nitrogen free extract =100- (%Moisture + %Ash + %Crude Protein + %Crude Fibre + %Ether Extract)

3.8 Microbial Study/Examination

Faecal samples were collected very early in the morning a day after the administration of the test ingredient from the floor of the pen in each replicate. The samples were then taken to the research laboratory for analysis in order to identify the microbial population and presence of parasites. This analysis involved the wet preparation of the faecal sample, macroscopic and microscopic examination, preparation of the culture media and microbial population count.

3.8.1 The wet preparation of the faecal sample

1gram of the faecal sample was added to 0.1g of sodium chloride dissolved in a sterilized test tube containing 10ml of distilled water (normal saline). A drop was placed on a microscopic slide using a pasture pipette and covered with a cover slide; which was examined under the microscope for identification of parasites using the x4, x10, and x40 objectives.

3.8.2 Microbial population count (serial dilution of the samples)

This involved the serial dilution of the sample ranging from 10^{-1}- 10^{-5}. 1 gram of the sample was added to 0.1g of sodium chloride dissolved in 10ml of distilled water (normal saline) in a sterilized test tube and mixed thoroughly (ie the 1^{st} test tube-10^{-1}) while the other test tubes contained 9ml of normal saline each. 1ml was drawn from the 1^{st} (10^{-1}) into the 2^{nd} (10^{-2}) and then mixed thoroughly. Again 1ml was added to the 3^{rd} test tube (10^{-3}). The dilution was made up to the 5^{th} test tube (10^{-5}). 1ml was drawn from the 5^{th} test tube (10^{-5} dilution) for inoculation into the Petri dishes and was cultured using the pour plating method. This was done for each of the replicates.

3.8.3 Preparation of the media

MacConkey Agar and Nutrient Agar were used in the preparation of the bacteria media while Sabouraud Dextrose Agar was used in preparing the fungi media according to the manufacturers specifications.

MacConkey Agar: 12.9g of this Agar was weighed on an analytical balance and was dissolved in 250ml of distilled water. It was gently heated to dissolve completely for 5minutes and was then sterilized by autoclaving at 121^0C for 15minutes. It was cooled at a room temperature before dispensing into the sterilized Petri dishes.

Sabouraud Dextrose Agar: 16.3g of the Agar was weighed and then dissolved in 250ml of distilled water; heated gently to dissolve completely for 5minutes. It was autoclaved at 121^0C for 15minutes, cooled at a room temperature before pouring into the Petri dishes.

Nutrient Agar: 10g of the Agar was weighed on analytical balance and dissolved in 300ml of distilled water. It was heated for 5minutes to completely dissolve and was then sterilized by autoclaving at 121^0C for 15 minutes.

Peptone Agar: 3.8g of this Agar was dissolved in 250ml of distilled water. It was heated to dissolve completely and transferred into sterilized test tubes. It was autoclaved at 121^0C for 15minutes.

When cooled, about 16ml of the media was poured on the Petri dishes containing 1ml of the dilution. The inoculated dishes were allowed to solidify and later transferred to the incubator for incubation at 37^0C for 24hours. After 24hours of incubation, colony growths were counted. The average colony count for each treatment was divided by 0.1 and multiplied by the dilution factor to obtain the total count per ml. The value obtained was further multiplied by 10 to give the total count of bacteria colony per gram of the sample.

3.8.4 Isolation of bacteria (Gram staining technique)

This was done by making a thin smear of the bacteria isolate by placing a drop of distilled water at the glass slide. An inoculating wire loop was sterilized by flaming and allowed to cool a bit; it was used to pick the isolate from the pure culture prepared. The isolate was rubbed on the slide by making a smear and was allowed to air dry. The smear was then flooded with crystal violet stain for 60seconds and then rinsed with distilled water. The smear was flooded with iodine solution and left for 60 seconds and then rinsed with distilled water; after which it was stained with acetone (acid alcohol) and left for 60seconds before rinsing with distilled water. Again the slide was finally stained with safranin for 60seconds and rinsed with distilled water. The smear was allowed to dry and was observed under microscope using oil immersion objective

(x100). The gram negative bacteria stained pink while the gram positive bacteria stained purple according to Gram's description of staining procedures in routine bacteriology (1884).

Biochemical characterization of the isolate: The following biochemical tests were carried out

Indole production test: This test was carried out by inoculating an isolate into peptone water and incubated at 37^0C for 48hours with 3ml of kovac's reagent added into the isolate that has been inoculated into the peptone water. A rose pink colour shows a positive result (presence of *E. coli).*

Catalyst test: This test was used to differentiate those bacteria that produce the enzyme catalyst from non catalyses producing bacteria. The test was done by adding few drops of hydrogen peroxide into the suspension of organism on a microscopic slide which resulted to the production of effervescence of the mixture. The reaction that showed effervescence shows a positive (presence of *staphylococcus spp*) by releasing oxygen from hydrogen peroxide by the bacteria that produced enzyme catalyst while a non effervescence showed a negative. The equation for the reaction is showed below,

$$2H_2O_2 \xrightarrow{\text{Staph spp.}} 2H_2O + O_2$$

Motility Test: This test was carried out by hanging drop technique. It was done by placing a drop or 2 drops of normal saline on a cover slide and the inoculum was introduced with aid of a sterilized wire loop into the cover slide. The edge of the cover slide was greased with Vaseline, picked with a cavity slide through the Vaseline edge and the cover slide was turned on inverted position. It was then placed under the microscope for observation of the movement of the organism which showed upward movement (Gram negative) or no movement at all (Gram positive).

3.9 Determination of the Egg Quality Parameters

Egg were collected and recorded on daily basis, replicate by replicate and the following parameters were calculated

$$\text{Total egg produced/bird} = \frac{\text{Total no. of egg produced/replicate during the period of the experiment}}{\text{Total number of birds in that replicate}}$$

$$\text{Total egg produced/bird/week} = \frac{\text{Total eggs produced/bird}}{\text{Weeks of the experiment}}$$

Total egg weight/bird (kg) = $\frac{\text{Average egg weight x Total eggs produced/bird}}{1000}$

Eggs were collected from each treatment and were randomly selected at the end of the experiment for egg quality parameters analysis. The eggs were properly cleaned and weighed with sensitive analytical balance. The eggs length and width was determined with Vernier Calliper, while the egg volume was determined by using a measuring cylinder. The egg shape index was calculated by dividing egg width by egg length. Shell thickness was determined with Micrometer Screw Guage, while shell samples from the broad, middle and narrow portions of the eggs was measured for shell thickness and the mean was calculated as,

Egg shell surface area (ESSA) = Egg Length x Egg Width (cm^2).

Shell weight expressed as % of egg weight (SEW) = $\frac{\text{Egg shell weight}}{\text{Egg weight}} \times \frac{100}{1}$

The yolk height and diameter was measured using Vernier Calliper after breaking the egg and separation of yolk from the albumen while the yolk colour was determined by using the yolk colour fan. The yolk index was calculated by dividing yolk height by yolk width. Haugh unit was determined according to Stadelman and Cotterill (1986).

Haugh Unit (HU) = $100 \times \log (h - 1.7w^{0.37} + 7.6)$

Where, h = Observed height of the albumen (mm)

w = Weight of the egg (g)

Yolk weight expressed as percentage of egg weight (YEW) (%) = $\frac{\text{Yolk weight}}{\text{Egg weight}} \times \frac{100}{1}$

Albumen weight expressed as a percentage of egg weight (AEW) (%) = $\frac{\text{Albumen weight}}{\text{Egg weight}} \times \frac{100}{1}$

3.10 Hen-day egg production and Hen-Housed egg production

Hen-day egg production was determined by diving number of eggs produced per day by the total number of birds in the replicate multiplied by 100 (expressed in percentage) and hen-housed egg production was obtained by the total number of eggs laid during the period of the experiment by the total number of birds housed as at when the experiment began (expressed in numbers).

3.10.1 Percentage Mortality

The percentage of the mortality was obtained by dividing the total numbers of birds that died in each replicate by initial number of birds in each of the replicates.

3.10.2 Egg Mass Production

This was obtained by multiplying the total egg produced per replicate by the average weight of the eggs in the replicate.

Egg Mass Production = Total Egg Produced x Average weight.

3.11 Serological Analysis

Blood samples were collected from each replicates through the subclavicular wing vein into the plain bottles containing no EDTA (ethylene diamine tetra-acetic acid) at the end of the experiment. The blood was kept at a room temperature for 45minutes in order to clot and was later centrifuged to obtain the serum which was then used for serological analysis such as Total Protein, Glucose, Aspertate aminotransferase (AST), Alanine aminotrasferase (ALT), Albumin, Creatinine, Triglyceride and Cholesterol. Globulin concentration was calculated as the difference between Total Protein and Albumin concentration. Using the instruction of the Randox laboratories limited United Kingdom and Teco diagnostics U.S.A analytical kits and formulae, serological parameters were manually calculated**.** Total blood serum protein (basophils, eosinophils, lymphocytes, monocytes, and neutrophils) were obtained by dividing each differential count by the total number of differential count and multiply by 100 (expressed in percentage)

Yolk cholesterol concentration was determined by Zlatkis colourimetric method as described by Varley (1969).

3.12 Haematological Parameters

Blood samples were collected into EDTA bottles for analysis such as Packed Cell Volume (PCV), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Heamoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were all determined as follows;

Mean Corpuscular Volume (MCV) = $\frac{\text{PCV x 10}}{\text{RBC}}$

Mean Corpuscular Heamoglobin (MCH) = $\frac{\text{Hb x 10}}{\text{RBC}}$

Mean Corpuscular Haemoglobin Concentration (MCHC) = $\frac{\text{Hb x 100}}{\text{PCV}}$

PCV was determined by using the Winthrobes Micro-haematocrit technique. Haemoglobin was obtained by mixing the blood sample with Drabkin,s solution in a ratio of 1:250 at a wavelength of 625nm. The absorbance reading was obtained by using a digital photo colorimeter.

3.13 Chemical Analysis

The phytochemical and proximate compositions of the test ingredient (*Xylopia aethiopica*) were determined using the techniques of the Association of Official Analytical Chemists (AOAC, 2012).

3.14 Statistical Analysis

Data collected were calculated, tabulated and analyzed; and their errors were calculated as standard error of means (SEM)

The data collected were subjected to statistical analyses using SPSS (version 20) and a one-way Analysis of variance (ANOVA). Duncan's Multiple Test (1955) was used to separate and show means that differed significantly using SPSS (version 20) at 5% level.

3.15 Experimental Design/Model

The birds were randomly assigned to five treatment groups (T1 - T5) of 30 birds each in a Completely Randomized Design (CRD). Each treatment was further divided into 3 replicates of 10 birds each, and was placed in a pen in line with the design of the experiment.

The design model for this experiment was;

$X_{ij} = U + T_i + E_{ij}$

Where,

X_{ij} = Observation made on an animal X in the experiment involving the jth individual receiving ith treatment level.

U = Population mean common to all observations/observation common to every individual measured or sampled.

T_i = Effect of treatment where i is the level of that treatment/Effect due to treatment at level i (i = 1, 2, 3, 4, & 5).

E_{ij} = Random error involved in the experimentation/experimental error

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Proximate and Phytochemical Compositions of *Xylopia aethiopica* Dried fruits

The results of the proximate composition of *Xylopia aethiopica* are presented in Table 4.1. Results show that; dry matter 88.05%, moisture 11.95%, ash 5.92%, crude fibre 11.03%, crude protein 3.20%, ether extract 9.50%, and nitrogen free extract 58.58%. The presence of carbohydrate, saponin, steroid, alkaloid, flavonoid and tannin were also observed.

4.2 Proximate Composition of the Diet

Results of the proximate composition of the layers diet in Table 4.2 indicated that dry matter content contains 90.61%, moisture content 9.39%, Ash 6.00%, crude fibre 6.00%, crude protein 17.05%, ether extract 5.00%, and the nitrogen free extract 56.56%.

4.3 Effect of *Xylopia aethiopica* as an additive at Graded Levels on the Performance Characteristics of Laying Chickens in Drinking Water

The results of the statistical analysis on the effect of *Xylopia aethiopica* given to laying birds at varying levels via drinking water are shown in Table 4.3.

The result showed that the mean initial body weight and the mean final body weight of the birds were not significantly different ($P>0.05$). In the mean initial body weight, birds on treatment 1 and 2 had the highest values and birds on treatment 5 had the lowest value ranging from 1.59-1.63kg/bird. However, the mean final weight of treatment 4 had the highest value (1.60kg/bird) while treatment 1 had the lowest value (1.46kg/bird), though the values were statistically the same.

There was no significant difference ($P>0.05$) in the mean total feed intake however the feed conversion ratio was significantly different ($P<0.05$). Birds in treatment 3 and 5 were significantly higher ($P<0.05$) in feed conversion ratio than birds in treatment 2 but birds in treatment 1 and 4 had similar values.

Results of the mean total egg produced indicated no significant different ($P>0.05$), birds in treatment 5 had the highest value of 398.33 and treatment 3 had the lowest value of 357.67. However there was a significant difference ($P<0.05$) in the mean of the total egg produced per

bird and the mean total egg produced/bird/week. Birds in treatment 2 were significantly different ($P<0.05$) from birds in treatment 3 but were similar to birds in treatment 1, 4, and 5.
Mean average weight of eggs was not significantly different ($P>0.05$) but value fell within the range of 53.91-58.91g.

There were significant differences ($P<0.05$) in the mean values of the total egg weight per bird and the hen day egg production. Birds in treatment 2 were significantly different ($P<0.05$) from those birds in treatment 3 but were similar to birds in treatment 1, 4, and 5. Mean egg mass production values were found between the ranges of 20267.24-22700.72 but no significant difference was observed. There were also no significant differences ($P>0.05$) between the means of the hen house egg production and the mortality of the birds among the treatment groups.

Table 4.1 Proximate and Phytochemical Compositions of *Xylopia aethiopica* Dried fruits

Parameters	Percentage (%)
Dry matter	88.05
Moisture	11.95
Ash	5.92
Crude fibre	11.03
Crude protein	3.02
Ether extract	9.50
Nitrogen free extract	58.58
Carbohydrate	+ve
Saponin	+ve
Steroid s	+ve
Alkaloid	+ve
Flavonoid	+ve
Tannin	+ve

+ve = Present

Table 4.2 Proximate Composition of the Diet

Parameters	Percentage (%)
Dry matter	90.61
Moisture	9.39
Ash	6.00
Crude fibre	6.00
Crude protein	17.05
Ether extract	5.00
Nitrogen free extract	56.56

Table 4.3 Effect of *Xylopia aethiopica* as an additive at Graded Levels on the Performance Characteristics of Laying Chickens in Drinking Water

Parameters	T1 (Control)	T2 0.20g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
Initial Body Weight (kg)	1.63	1.63	1.61	1.60	1.59	0.01
Final Body Weight (kg)	1.46	1.50	1.52	1.60	1.52	0.03
Total Feed Intake/Bird (kg)	5.08	5.18	5.44	5.84	5.84	0.17
Feed Intake/Bird/Day (g)	90.65	92.50	97.12	104.23	104.46	3.01
Feed Conversion ratio	2.24^{ab}	1.96^{b}	2.58^{a}	2.32^{ab}	2.64^{a}	0.09
Total Egg Produced	368.67	388.68	357.67	368.33	398.33	11.61
Total Egg Produced/Bird	41.15^{ab}	46.09^{a}	37.04^{b}	42.68^{ab}	41.19^{ab}	1.25
Total Egg Produced/Bird/Wk	5.15^{ab}	5.76^{a}	4.63^{b}	5.34^{ab}	5.15^{ab}	0.16
Average Egg Weight (g)	55.36	58.49	56.74	58.91	53.91	0.77
Total Egg Weight/Bird (kg)	2.28^{ab}	2.70^{a}	2.10^{b}	2.52^{a}	2.22^{ab}	0.08
Hen Day Egg production (%)	73.49^{ab}	82.30^{a}	66.14^{b}	76.22^{ab}	73.54^{ab}	2.24
Hen House Egg Production (%)	65.83	69.41	63.87	65.77	71.13	2.07
Egg Mass Production	20580.66	22700.72	20267.24	21710.64	21352.46	681.58
Mortality (%)	1.00	1.33	0.33	1.33	0.33	0.34

a-b Means within the row with different superscripts are different at $P<0.05$. SEM: Standard error of the mean

4.4 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the External Egg Quality Characteristics of Laying Chickens in Drinking Water

External egg quality characteristics of laying birds given *Xylopia aethiopica* in drinking water are presented on Table 4.4. The result shows that the egg weight were all similar (P>0.05) in all treatments, with values ranging from 53.36 to 58.91g.

The egg length, egg volume, egg shell, egg shell thickness, egg shape index, and shell weight expressed as percentage of egg weight were similar (P>0.05) in all the treatment groups.

On mean egg width, treatments 2, 3,4 and 5 given 0.2g/l, 0.35g/l, 0.5g/l and 0.65g/l respectively of the test ingredient were similar (P>0.05) but treatments 2,4 and 5 were significantly difference (P<0.05) from birds in treatment 1.

The result of the mean egg shell surface area shows that birds in treatment 2 and 4 were significantly different (P<0.05) from birds in treatment 1 and 5 but similar (P>0.05) to the birds in treatment 3 while birds in treatment 3 were similar (P>0.05) to birds in treatment 1 and 5.

Table 4.4 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the External Egg Quality Characteristics of Laying Chickens in Drinking Water

Parameters	T1 (Control)	T2 0.2.g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
Egg Weight (g)	53.36	58.49	56.74	58.91	53.91	0.77
Egg Length (cm)	5.30	5.47	5.37	5.53	5.30	0.04
Egg width (cm)	3.97^{b}	4.13^{a}	4.03^{ab}	4.10^{a}	4.13^{a}	0.02
Egg Volume (ml)	53.00	55.67	55.33	57.00	54.00	0.76
Egg Shell Wt (g)	6.62	6.68	6.62	7.06	6.90	0.18
EST (mm)	0.31	0.30	0.31	0.30	0.31	0.00
Egg Shape Index	0.75	0.76	0.75	0.74	0.78	0.01
SEW (%)	11.96	11.41	11.67	11.98	12.78	0.29
ESSA (cm^2)	21.02^{b}	22.60^{a}	21.56^{ab}	22.69^{a}	21.90^{b}	0.23

a-b Means within the row with different superscripts are different at $P < 0.05$. SEM: Standard error of the mean, Wt: Weight, EST: Egg shell thickness, SEW: Shell Weight Expressed as percentage of Egg Weight, ESSA: Egg Shell Surface Area

4.5 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the Internal Egg Quality Characteristics of Laying Chickens in Drinking Water

Results on the internal egg quality of laying birds given *Xylopia aethiopica* at graded levels are presented on Table 4.5. Yolk weight was found within the range of 14.76g-16.44g, 1.50cm-1.87cm for yolk height, 7.00-8.00 for yolk color and 0.47-0.63cm for albumen height. There were no significant differences ($P>0.05$) between the means of the yolk weight, yolk height, yolk color and albumen height among the treatment groups. The yolk diameter for treatments 1, 2, 3, and 4 were significantly different ($P<0.05$) from birds in treatment 5. The mean yolk index of birds in treatment 5 were significantly different ($P<0.05$) from birds in treatments 2 and 3 but similar ($P>0.05$) to birds in treatments 1 and 4.

Treatment 4 was significantly higher ($P<0.05$) in albumen weight than birds in treatment 5 but was similar ($P>0.05$) to birds in treatment 1, 2, and 3 while the birds in treatments 1, 2, and 3 were similar ($P>0.05$) to birds in treatment 5.

The mean albumen length of birds in treatment 5 were significantly different ($P<0.05$) from birds in treatment 2 but similar ($P>0.05$) to birds in treatments 1, 3, and 4. Birds in treatments 1, 2, 3, and 4 were similar ($P>0.05$) in albumen length.

Haugh unit ranges from 66.85-80.16 and the means between treatments row were not statistically different ($P>0.05$). The mean yolk weight expressed as percentage of egg weight (YEW) of birds given the highest level of *Xylopia aethiopica* (treatment 5, 0.65g/l) were significantly different ($P<0.05$) from treatment 1 (control) and birds given 0.2g/l (treatment 2), 0.35g/l (treatment 3), and 0.50g/l (treatment 4). Statistically, there was no significant difference ($P>0.05$) between the means of the albumen weight expressed as percentage of egg weight (AEW). The mean yolk index of birds in treatment 5 were significantly different ($P<0.05$) from birds in treatments 2 and 3 but similar ($P>0.05$) to birds in treatments 1 and 4.

Table 4.5 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the Internal Egg Quality Characteristics of Laying Chickens in Drinking Water

Parameters	T1 (Control)	T2 0.02g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
Yolk Weight (g)	14.76	15.23	15.45	15.66	16.44	0.25
Yolk Height (cm)	1.77	1.50	1.63	1.73	1.87	0.06
Yolk Diameter (cm)	3.97^{a}	3.83^{a}	3.97^{a}	3.77^{a}	3.50^{b}	0.05
Yolk Index	0.45^{ab}	0.39^{b}	0.41^{b}	0.46^{ab}	0.53^{a}	0.02
Yolk Colour	7.00	7.67	8.00	7.67	8.00	0.25
Albumen Weight (g)	31.50^{ab}	32.56^{ab}	32.58^{ab}	33.69^{a}	29.23^{b}	0.61
Albumen Height (cm)	0.63	0.60	0.47	0.50	0.47	0.03
Albumen length (cm)	7.17^{ab}	6.27^{b}	6.67^{ab}	7.37^{ab}	7.63^{a}	0.19
Haugh Unit	80.16	76.20	66.85	68.48	67.43	2.60
YEW (%)	26.70^{b}	26.05^{b}	27.20^{b}	26.58^{b}	30.60^{a}	0.57
AEW (%)	56.81	55.65	57.44	57.21	54.20	0.56

a-b Means within the row with different superscripts are different at $P < 0.05$. SEM: Standard error of the mean, YEW: Yolk Weight Expressed as percentage of Egg Weight, AEW: Albumen Weight Expressed as percentage of Egg Weight.

Table 4.6 Microscopy Examination of the Faecal Sample of Laying Chickens Given *Xylopia aethiopica* as an Additive at Graded levels in Drinking Water

Parameter	T1(control)	T2 (0.2g/L)	T3 (0.35g/L)	T4 (0.50g/L)	T5 (0.65g/L)
Faecal appearance before administration	Soft faecal sample	Normal formed faeces	Normal formed soft faeces	Normal formed faeces	Normal formed faeces
Observation before administration	*Escherichia. Coli.* Uric acid crystals.	*Ova of Trichomonas hominis,* Flat worm, uric acid crystals.	Round worm, uric acid crystals.	Round worm, uric acid crystals	Cyst of *Escherichia. Coli* uric acid crystals
Faecal appearance after the Ist administration	Soft faecal sample	Normal formed faeces with whitish particle	Normal formed faeces with whitish particle	Normal formed faeces with whitish particle	Normal formed faeces with whitish particle
Observation after the Ist administration	Flat worm, round worm, *Escherichia. Coli.*	*Ova of Trichomonas hominis,* uric acid crystals.	Uric acid crystals.	Uric acid crystals.	No ova or cyst of parasite seen
Faecal appearance after the 2nd administration	Soft formed faeces	Normal formed faeces with whitish particle	Normal formed faeces with whitish particle	Normal formed faeces with whitish particle	Normal formed faeces with whitish particle
Observation after the 2nd administration	Round worm, flat worm, *Escherichia. Coli.*	Uric acid crystals.	No ova or cyst of parasite	Uric acid crystals.	Uric acid crystals.

Table 4.7 Isolation of the Population of Bacteria and Fungi Strains of Laying Chickens Given *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water

Parameter	T1 (control)	T2 (0.2g/L)	T3 (0.35g/L)	T4 (0.50g/L)	T5 (0.65g/L)
Observation before administration	Profuse growth of microbes and fungi	Heavy growth of microbes and fungi	Profuse growth of microbes and fungi	Scanty growth of microbes	Profuse growth of microbes and fungi
Organism confirmed before the administration (Bacteria)	*Staphylococcus spp, streptococcus spp, Escherichia coli*	*Staphylococcus spp*	*Staphylococcus spp*	*Staphylococcus spp*	*Escherichia coli Staphylococcus spp, streptococcus spp,*
Organism confirmed before the administration (Fungi)	Yeast cells, *Candida albicans*	*Aspergillus niger*	*Candida albicans,* yeast cells	No growth of microbes	*Candida albicans*
Observation After the Ist administration	Profuse growth of microbes and fungi	Scanty growth of microbes	Scanty growth of microbes	No growth of microbes	Scanty growth of microbes
Organism confirmed After the Ist administration (Bacteria)	*Staphylococcus spp, Escherichia coli, streptococcus spp,*	*Staphylococcus spp, streptococcus spp,*	*Staphylococcus spp,*	*Staphylococcus spp*	*Staphylococcus spp, streptococcus spp,*
Organism confirmed After the Ist administration (Fungi)	Yeast cells, *Candida albicans*	No growth of fungi	No growth of fungi	No growth of fungi	No growth of fungi
Observation After the 2nd administration	Profuse growth of microbes and fungi	Scanty growth of microbes	Scanty growth of microbes	Scanty growth of microbes	Scanty growth of microbes
Organism confirmed After the 2nd administration (Bacteria)	Yeast cells, *Candida albicans Staphylococcus spp, streptococcus spp, E. coli*	No growth	No growth	No growth	No growth

4.8 Microbiological Results of the Faecal Samples of Laying Chickens Given *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water

The results of the microscopy of the faecal samples, population of the fungi and bacteria colony count, and the microbial activity in the faecal samples of the birds before, after the first and the second administration of treatments at varying levels are shown in Tables 4.6, 4.7, and 4.8 respectively.

Microscopy Examination of the Faecal Samples

The microscopy result (Table 4.6) showed that the faecal samples of all the treatment groups were the same except the control treatment that was a bit softer than the other treatments. Before the administration of the test ingredient, treatment 1 (control) had cyst of *Escherichia coli (E. coli)*, treatment 2 had ova of Trichomonas *hominis*, flat worm, treatment 3 and 4 had round worm while treatment 5 had *E. coli*. After the first administration, treatment 1 faecal sample was soft while all the others treatments were normal with whitish particles. *E. coli*, flat worm, and round worm were found in treatment I, Trichomonas *hominis* in treatment 2, but treatments 3, 4, and 5 had no ova or parasite. At the end of the second administration, *E. coli*, round worm, and flat worm were found in treatment 1, treatment 2 had uric acid crystals. Treatment 3, 4, and 5 contained no ova or parasite.

Isolation of the Population of Bacteria and Fungi Strains

Before the administration of the test ingredient (Table 4.7) profuse growth of microbes was observed in all the treatment groups except treatment 4 (scanty growth of microbes). Treatment 1 had *E. coli, staphylococcus spp, streptococcus spp* (bacteria) and yeast cells and *Candida albican* (fungi). Treatment 2 staphylococcus spp, and *Aspergillus niger* (fungi), treatment 3 had staphylococcus spp, *Candida albican* and yeast cells. Treatment 4 had *staphylococcus spp,* while treatment 5 had *E. coli, staphylococcus spp, streptococcus spp* and *Candida albican.* After the first administration, the growths were scanty in treatment 2, 3, 4, and 5 but profuse growth was observed in treatment 1. All the fungi organism were completely eliminated in treatment 2, 3, 4, and 5 except treatment 1 (control) which did not receive the *Xylopia aethiopica*. The bacteria colonies were still seen in treatment 2, 3, 4, and 5 but there was reduction in the total colony counted. At the end of the second administration, the results of the population of the bacteria showed a drastic reduction. Only traces of the presence of bacteria were observed.

Microbial Activity

The results (Table 4.8) showed that before the administration of the *Xylopia aethiopica*, the microbial activity of treatment 1 (control), treatments 3 and 5 were significantly higher ($P<0.05$) than birds in treatment 4 but similar ($P>0.05$) to treatment 2.

After the first administration of the test ingredient, the result revealed that treatments 2, 3, and 5 were similar ($P>0.05$) but different from birds in treatment 4. Birds in treatment 1 (control) was significantly higher ($P<0.05$) than birds in other treatment groups. There was a reduction in the microbial load. The second administration showed that treatment 1 (control) was significantly higher ($P<0.05$) in microbial presence than treatment 4 but similar ($P>0.05$) to treatments 2, 3, and 5. The microbial load was drastically reduced in all the treatment groups.

Table 4.8 Microbial Activity in Faecal Sample of Laying Chickens Given *Xylopia aethiopica* Dried Fruits as an Additive at Graded Levels in Drinking Water

Parameters	T1 (Control)	T2 0.20g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
Microbial count before administration of Xa ($10x10^5$cfu/ml)	105.39^{a}	86.00^{ab}	94.67^{a}	58.00^{b}	107.33^{a}	6.06
Percentage Reduction	20	43	55	56	47	
After the 1st Administration of Xa ($10x1\ 0^5$cfu/ml)	84.67^{a}	48.67^{ab}	42.33^{ab}	25.33^{b}	57.33^{ab}	7.30
Percentage Reduction	65	62	66	65	69	
After the 2nd Administration of Xa ($10x10^5$cfu/ml)	29.67^{a}	18.67^{ab}	14.33^{ab}	9.00^{b}	17.67^{ab}	2.53

a-b Means within the row with different superscripts are different at $P < 0.05$. SEM: Standard error of the mean. Xa: *Xylopia aethiopica*

Table 4.9 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the Haematological Characteristics of Laying Chickens in Drinking Water

Parameters	T1 (Control)	T2 0.20g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
PCV (%)	28.33^{b}	30.00^{ab}	32.00^{ab}	32.67^{a}	34.00^{a}	0.72
RBC (10^6)	50.00	43.33	46.00	45.67	47.67	1.67
WBC (10^3)	5.80^{ab}	4.73^{b}	5.70^{ab}	5.47^{ab}	6.40^{a}	0.21
Hb (g/dl)	9.90^{c}	10.20^{c}	10.90^{bc}	11.37^{b}	12.70^{a}	0.29
MCV (fl)	5.85	6.97	7.21	7.15	7.12	0.27
MCH (pg)	2.06	2.37	2.41	2.54	2.67	0.98
MCHC (g/dl)	43.95	34.06	33.37	35.61	37.60	0.70

a-b Means within the row with different superscripts are different at $P < 0.05$. SEM: Standard error of the mean PVC: Packed cell volume, RBC: Red blood cell counts, WBC: White blood cell counts, Hb: haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglonin concertration

4.9 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the Haematological Characteristics of Laying Chickens in Drinking Water

The results of the haematological characteristics of laying birds given *Xylopia aethiopica* at varying levels as additive are shown in Table 4.9. Results of the haematological parameters of the packed cell volume (PCV) showed that the PCV levels of treatments 4 and 5 were significantly higher ($P<0.05$) than treatment 1 but similar to treatments 2 and 3. The average means of the red blood cells (RBC) and their corresponding value did not significantly ($P>0.05$) differ among the treatment groups. White blood cells (WBC) increased significantly ($P<0.05$) in response to the *Xylopia aethiopica* administration. Birds of treatment 5 were significantly higher ($P<0.05$) than the birds in treatment 2 while treatments 1, 3, and 4 were similar ($P>0.05$) to treatment 5. Haemoglobin (Hb) values in treatment 5 were statistically different ($P<0.05$) from treatments 1, 2, 3, and 4. Treatments 1, 2, and 3 were similar ($P>0.05$) while treatment 4 was different ($P<0.05$) from treatments 1 and 2 but similar ($P>0.05$) to treatment 3. The average means of the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the means of the mean corpuscular haemoglobin concentration (MCHC) did not differ significantly ($P>0.05$) in all the treatment groups.

4.10 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on Differential Count of Laying Chickens in Drinking Water

The results of the effect of giving dried fruits of *Xylopia aethiopica* at varying levels on differential count are shown in Table 4.10.

The results showed that there was a significant difference ($P<0.05$) in Eosinophils counts among the treatment groups. Treatment 1 had the highest value (6.00%), treatment 4 had the least figure (1.00%) while treatments 2 and 5 were statistically the same. Lymphocytes count showed that treatments 4 and 5 were significantly higher ($P<0.05$) than treatment 1 but similar ($P>0.05$) to treatments 2 and 3. There were no significant difference ($P>0.05$) in monocytes and neotrophil in treatment groups.

Table 4.10 Effect of *Xylopia aethiopica* as an additive at Graded Levels on Differential Count of Laying Chickens in Drinking Water

Parameters	T1 (Control)	T2 0.20g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
Eosinophils (%)	6.00^{a}	1.33^{bc}	4.67^{ab}	1.00^{ac}	1.33^{bc}	0.68
Lymphocytes (%)	38.00^{b}	38.00^{b}	38.00^{b}	45.33^{a}	45.33^{a}	1.03
Monocytes (%)	6.67	0.33	0.67	0.33	0.67	1.32
Neotrophils (%)	55.33	56.00	53.67	53.33	53.33	0.56

a-b Means within the row with different superscripts are different at $P < 0.05$ SEM: Standard error of the mean

Table 4.11 Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the Serological Parameters of Laying Chickens in Drinking Water

Parameters	T1 (Control)	T2 0.20g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
Total protein (g/dl)	5.65	5.77	5.87	6.15	6.38	0.14
Albumin (g/dl)	4.75^{b}	4.87^{b}	5.08^{ab}	5.19^{ab}	5.42^{a}	0.09
Globulin (g/dl)	0.90	0.90	0.79	0.96	0.96	0.10
Glucose (mmol/L)	8.87	8.86	8.82	8.13	9.38	0.37
Creatinine (mg/dl)	3.52	4.31	3.47	2.60	4.12	0.34
(AST)(u/l)	261.07^{a}	248.50^{ab}	220.75^{ab}	143.80^{ab}	80.80^{b}	26.92
(ALT)(u/l)	19.11^{ab}	33.88^{ab}	42.57^{a}	13.77^{b}	32.88^{ab}	4.06
Triglyceride (egg)	162.91	246.70	157.85	133.70	106.72	23.87
Triglyceride (serum)	225.63	374.24	254.58	227.92	287.49	36.65
Cholesterol (egg)(mg/dl)	230.00	245.32	253.06	226.45	249.03	5.87
Cholesterol (serum)(mg/dl)	101.62^{b}	155.92^{a}	103.79^{b}	151.43^{a}	154.22^{a}	7.78

a-b Means within the row with different superscripts are different at $P < 0.05$. SEM: Standard error of the mean, AST: Aspertate aminotransferase, ALT: Alanine aminotransferase

4.11 Effect of *Xylopia aethiopica* as an Additive on the Serological Parameters of Laying Chickens in Drinking Water

Results of the serological parameters of layer birds on the effect of dried fruits of *Xylopia aethiopica* as additive at varying levels are shown in Table 4.11

The result shows that total protein had no significant ($P>0.05$) difference among treatment groups. Albumin values were significantly different ($P<0.05$). Treatment 5 was significantly ($P<0.05$) higher than treatments 1 and 2 while treatments 3 and 4 were similar ($P>0.05$) to treatment 5. The average means of globulin, glucose and creatinine had no significant difference ($P>0.05$) in all the treatment groups. On the mean of the aspertate aminotransferase (AST) and alanine aminotransferase (ALT) there were significant differences ($P<0.05$). AST in treatment 1 (control) significantly differed ($P<0.05$) from treatment 5 but similar ($P>0.05$) to treatments 2, 3 and 4. ALT in treatment 3 was significantly higher ($P<0.05$) than treatment 4 while treatments 1, 2 and 5 were similar ($P>0.05$) to treatment 3. Triglyceride in egg and serum, and cholesterol in egg had no significant differences ($P>0.05$) among the treatment groups. Cholesterol values in serum were significantly ($P<0.05$) affected, treatments 2, 4, and 5 were significantly ($P<0.05$) higher than treatments 1 and 3.

4.12 Economic Analysis of Laying Chickens on the Effect of Using of *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water

Results of the economics Analysis of layer birds on the effect of dried fruits of *Xylopia aethiopica* as additive at varying levels are shown on Table 4.12

Result of the study revealed that birds in all the treatment groups consumed the same quantity of feed statistically, that is to say that there were no significant differences ($P>0.05$) in the total feed consumed per bird among the treatment groups. Cost per kg feed consumed and the cost of total feed consumed per birds (₦) were significantly increased ($P<0.05$). Treatment 5 was significantly higher than treatment 4, 3, 2 and 1 retrogressively due to the increase in the quantity of *Xylopia aethiopica.* In both cost per kg feed consumed and the cost of total feed consumed per birds (₦), treatment 1 was the cheapest with values of 99.67 and 506.26 while treatment 5 had the highest values respectively. Feed cost per kg egg was significantly affected ($P<0.05$). Treatment 5 had the highest value while treatments 3 and 4 were significantly different from 1 and 2. Treatment 2 was least among the treatment groups. This implies that feed cost per kg egg

in treatment 2 was the cheapest. Cost differential per kg egg weight was significant ($P<0.05$). Birds in treatment 2 had the least value since cost increases as the level of the concentration increases. Treatment 5 was significantly higher than treatment 3 and 4. The relative cost benefit was also significantly ($P<0.05$) affected. Treatment 2 had the highest value (110.52%) while treatment 5 had the lowest value (69.39%)

Table 4.12 Economic Analysis of Laying Chickens on the Effect of *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water

Parameters	T1 (Control)	T2 0.20g/L	T3 0.35g/L	T4 0.50g/L	T5 0.65g/L	SEM
Total feed consumed (kg /bird)	5.08	5.18	5.44	5.84	5.85	0.17
Cost of total feed consumed/bird (₦)	506.26^{c}	542.47^{bc}	596.90^{abc}	679.80^{ab}	710.48^{a}	26.54
Cost/kg feed (₦)	99.67^{e}	104.72^{d}	109.72^{c}	116.40^{b}	121.45^{a}	2.09
Feed cost/kg egg (₦)	222.04^{d}	200.91^{e}	284.24^{b}	269.76^{c}	320.01^{a}	11.47
Cost differential/kg egg (₦)	0.00^{d}	-21.13^{e}	62.20^{b}	47.72^{c}	97.97^{a}	9.97
Relative cost benefit/kg egg (%)	100.00^{b}	110.52^{a}	78.12^{d}	82.31^{c}	69.39^{e}	4.01

a-c Means within the row with different superscripts are different at $P < 0.05$

SEM: Standard error of the mean

DISCUSSION

Proximate and Phytochemical Compoisitions of *Xylopia aethiopica* Dried Fruits

The results of the analysis of *Xylopia aethiopica* dried fruits (Grains of selim) proved it contains the following phytochemicals: saponin, carbohydrate, flavonoid, tannin, alkaloid and steroid which have been reported to act as antioxidant, antimicrobial, and pharmacological effects against bacteria. Results of the study agree with the findings of Isikwenu and Udomah (2015) and also similar to that of Ezekwesili *et al*. (2010) but reported the traces of glycosides and the absence of alkaloid. Alkaloid has been found to be the most important bioactive constituents of natural and valuable products or supplements as reported by Edeoga *et al*. (2005) while steroid has been reported to have a lowering effect on blood cholesterol in experimental animals and humans (Law, 2000). Flavonoid prevents oxidative cell damage and Saponin has wide range of biological properties to recover homeostasis (Uzodike and Onuoha (2010). These observations may be supportive of the use *of X. aethiopica* fruits in alleviating post-natal pains in women by traditional health practitioners (Nnodim *et al*., 2011).

The proximate composition of the diet *Xylopia aethoipica* showed that the dry matter content, moisture content, crude fibre and the crude protein content reported in this study agree with the findings of Isikwenu and Udomah (2015) but there was a relative difference in the ash (5.92%) and ether extract (9.5%), when compared with his finding of 5.84% and 9.9% respectively but were similar in others parameters (Table 4.1).

Proximate Composition of the Diet

The proximate composition of layer's mash used in this study meets the recommended nutrients requirement for the experimental birds and was also in line with the nutrient requirement standards of NRC (1994) for laying birds.

Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the Performance Characteristics of Laying chickens in Drinking Water

The results of the study revealed that *Xylopia aethiopica* as additive at graded levels influenced the body weight of the laying birds (Table 4.3). The result showed that giving *Xylopia aethiopica* (dried fruits) in drinking water increased total egg production and egg mass production from 368.67 - 398.33 and 20267.24 – 22700.72 respectively, but significant

differences were not recorded among the mean values. Production might have been enhanced by the presence of some active phytochemicals in *Xylopia aethiopica* which improved the health status as well as the reproduction performance of the birds. This finding is similar to that of Akhtar *et al*. (2003) who reported a decrease in body mass with increase in egg production and egg mass by supplementation of layers ration with *Nigella sativa .L*. (Kalongi seeds). The study also revealed that the use of *Xylopia aethiopica* decreased body weight of the birds except in treatment 4 where the values of the initial weight and final weight were the same. Though significant differences were not observed in the weight values, *Xylopia aethiopica* treated diets were better than control. Since the decrease in body weight is negatively correlated with egg production, reduction in body weight of the laying birds by giving dried fruits of *Xylopia aethiopica* in drinking water as additive is considered a favourable factor in increasing egg production. The findings of this study also agree with the report of Chike and Adienbo (2011) that the active ingredients present in the dried fruits of *Xylopia aethiopica* attributed to the decrease in the body weight of the animals Woode *et al.* (2012) reported that the reduction in the body weight was as a result of the xylopic acid content of the extract. Similar finding was also reported by Eze (2012). Birds in treatment 5 (0.65g of XA) amongst the other treatment groups had the highest feed intake, total egg production and hen house production (HHP) with no significant differences in values. FCR in this present study showed that the use of *X. aethiopica* increased FCR/kg egg from 2.24-2.64.This result agrees with Obodo *et al*. (2013) who reported that the decreased in growth performance at high feed conversion ratio appeared to have affected the body weight negatively; and the degree to which this factor contributed to this effect is dose and duration dependent. FCR were ranging from 1.96-2.64 which is in harmony with the report of Isikwenu and Udomah (2015) but slightly lower. Feed intake increased as the level of *Xylopia aethiopica* increased. HDP in this study was lower (63.87-71.13%) than what was reported by Dairo and Ogunmodede (2004) (76.11 – 79.62%). However, variation was mainly due to age of the birds. The study revealed that there was improved egg mass production. Mortality rate was not significant which proves that the use of *Xylopia aethiopica* (Dried fruits) is effective by improving immunity and can be used in place of synthetic antibiotics.

Effect *of Xylopia aethiopica* as an Additive at Graded Levels on the External Egg Quality Characteristics of Laying Chickens in Drinking Water

The egg weight reported in this study (53.36-58.91g) was higher than the finding of Nworgu *et al*. (2012) but in agreement with the report of Akhtar *et al*. (2003) who reported 54.12-58.48g by supplementation of *Nigella sativa .L.* seed in layers diet and that of Odunsi *et al*. (2002) (57.80-59.90g). It was observed that birds in the test groups (treatments 2, 3, 4, and 5) had a better performance in egg weight, egg length, egg width, egg volume, shell weight, egg shell surface area than birds in the control (treatment 1) although not significant in some. This shows that the use of aqueous extract of *Xylopia aethiopica* (dried fruits) as additive has positive influence on external egg qualities. Egg shell thickness was found within the range of 0.30-0.31mm while egg shape index were within 0.74-0.78. Shell weight expressed as percentage of egg weight (SEW) is 11.41-12.78%.

Effect *of Xylopia aethiopica* as an Additive at Graded Levels on the Internal Egg Quality Characteristics of Laying Chickens in Drinking Water

The results of the study shows that yolk weight and yolk colour, though not significant, were higher in treatment groups that used *Xylopia aethiopica* than the control. Albumen height and haugh unit decreased at approximately constant rate over time, Nworgu *et al*. (2012) in his finding using basil leaf (Ocimum gratissimum) as supplement reported the same decreased in albumen and haugh unit. Haugh unit and albumen height in this study were highest in control (80.16 and 0.63cm) when compared with the birds given *Xylopia aethiopica* extract. The higher haugh unit observed in control showed a better albumen quality (Nworgu *et al*., 2012) than birds given *Xylopia aethiopica*. Albumen weights were within the range of 29.23-33.69g, while yolk weight 14.76-16.44g. This finding is similar to that of Awosanya *et al*. (1998) who reported albumen weight 29.23-36.95g, and yolk weight of 11.36-16.44g. The yolk diameter, yolk index, and albumen length, and yolk weight expressed as a percentage of egg weight (YEW) were significantly ($P<0.05$) influenced by the aqueous extract. All the parameters listed above, except yolk diameter, treatment 5 (birds given 0.65g/l) had the highest performance. Albumen weights expressed as a percentage of egg weight (AEW) were ranging from 54.20-57.44%.

Microbiological Results of the Faecal Samples of Laying Chickens Given *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water

Result of the faecal microbial population of the birds showed that the birds were greatly affected positively by the use of the aqueous extract of *Xylopia aethiopica* dried fruits. The enumeration of the faecal sample revealed that there was significant increased reduction in microbial load in response to *Xylopia aethiopica* in all the concentrations. Treatment 5 (0.65g/l) with the highest concentration had the best performance. This report is in harmony with that of Isikwenu *et al*. (2014) and Karioti *et al*. (2004) who also reported decrease in the microbial population of the gut intestinal tract of broilers as the concentration of the grain of selim increased. The aqueous fruit extract of *Xylopia aethiopica* has been shown to be active as antimicrobial agent against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria, as well as the fungus, (*Candida albicans* and *Aspergillus niger*) found in this study. This report is the same with the findings of Tatsadjieu *et al*. (2003); Asekun and Adeniyi, (2004); and Okigbo *et al*. (2005). *Candida albicans and Aspergillus niger* were the most sensitive, followed by *Staphylococcus aureus and streptococcus spp* while the least sensitive was *E coli*. Xylopic acid has also demonstrated activity against the fungus Candida albicans, Woode *et al*. (2012). This study also proved that *Xylopia aethiopica* can be used as an antihelmintic which is in line with the report of Evans (2003) and Hammer *et al*. (1999). This validates the use of the dried fruits of *Xylopia aethiopica* in place of synthetic antibiotics and in various disease conditions by traditional health practitioners.

Effect of *Xylopia aethiopica* as an Additive at Graded Levels on the Haematological Characteristics of Laying Chickens in Drinking Water

The results of the haematological indices of the layer birds given grains of selim and the response variables for the different treatments are shown on Table 4.9. The packed cell volume, white blood cells & haemoglobin were slightly increased as the aqueous extract concentration increases. This report corroborates the report of Vivian *et al*. (2015) who supplemented ginger and garlic in broiler chickens. Red blood cells, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were not significantly increased in response to the different concentration of the aqueous extract of *Xylopia aethiopica* dried fruits. This finding agrees with that of Isikwenu and Udomah (2015) who also administered

Xylopia aethiopica dried fruits as additive in broiler chickens. Red blood cells transport oxygen and carbon dioxide in the body and also manufacture haemoglobin. The higher value in red blood cells signifies a greater ability for these functions and a better state of health while WBC (Neotrophils, Lymtocytes, Monocytes, Eosiniphils) protects the body against infection, Al-Mashhadani *et al*. (2011). Results of the test for red blood cell membrane stabilization showed that the extract prevented haemolysis suggesting that it maintains the integrity of the erythrocytes and possibly acts by enhancing active transport across the membrane of the erythrocytes as opposed to osmosis or by reducing the permeability of the erythrocyte membrane to water. The result of the red blood counts of this present study corroborates the reports by Ezekwesili (2010) who also used *Xylopia aethiopica,* that various herbal remedies are endued with the potency of stabilizing erythrocyte membrane.

Effect of *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water on Differential Count

Results showed that neutrophils and monocytes were not significantly influenced by the use of *Xylopia aethiopica* in the treatment groups but lymphocytes were slightly increased as the concentration of the aqueous extract increased. This is related to the slight increase in white blood cells which helps in protecting the body against infections. This report is similar to that of Esonu *et al*., (2006) that significant differences in lymphocytes values exist between treatment groups using neem leaf.

Effect of *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water on the Serological Parameters of Laying Chickens

The result of the serum biochemistry revealed that there were no significant difference in total protein (TP), glubulin, glucose and creatinine. These findings are in agreement with those of Onu (2010) who reported that the supplementation of ginger (0.25%) in the basal diet of broiler chicks do not result in any significant difference in terms of total protein, globulin, glucose and creatinine. TP increased numerically with no significance while there was slight increase in albumin as the level of concentration increased in this study. Albumin and globulin are the major protein found in the blood and albumin concentration in the blood can fluctuate depending on the nutrition of the animals or as a result of diseases (infections); there is often a drop in case of

malnutrition and infection. Globulin protein consists of antibodies, enzymes and other types of proteins (Vivian *et al*., 2015). Albumin content in this study was significantly increased in test groups. This finding is similar to that of George *et al*. (2015) who supplemented ginger in broilers diet. The use of *Xylopia aethiopica* caused slight decrease on glucose level in test groups when compared to the control except treatment 5 which had the highest value. This is similar with the finding of Kappel *et al.* (2013) who demonstrated the beneficial effects of banana leaf (*Musa paradisiaca)* on regulation of glucose homeostasis and concluded that it has the ability to improved carbohydrate metabolism. This was attributed to the presence of active phytochemicals such as flavonoid found in *Xylopia aethiopica.* Creatinine level in this study varied numerically among test groups. Treatments 3 and 4 had the lowest values with no significant difference between the test groups and the control. This shows that the use of *Xylopia aethiopica* as additive in drinking water has no negative effect on the kidney. The reports on Aspertate amino transferase (AST) and cholesterol in serum in this study are similar with the report of Mathivanan and Edwin (2012) who fed Andrographis paniculala as an additive in broiler diet. Deshpande (2006) also reported that the dietary supplementation of tulsi leaf powder (Ocimum sanctum) commonly known as Indian ginseng causes a significant increase in serum cholesterol in layers. Triglyceride in serum and yolk triglyceride and yolk cholesterol did not show any significant differences between treatment groups compared to the control group. The non significant different in triglyceride in serum is similar to the report of Yahya *et al*., (2014) who used ginger root as an additive in broiler chickens. Alanine amino transferase (ALT) and yolk cholesterol increased with significant differences and decreases at 0.50g/l (T4). Yolk cholesterol in the present study is lower than the reports of Nworgu *et al*. (2012) (312-232 mg/d) who used basil leaf. Bitman and Wood (1990) noted that regardless of the method of expression (mg/g yolk, mg/g or (mg/ egg) the cholesterol content of eggs from domestic fowl varies among species. In chicken yolk cholesterol content can be influenced by breed/strain (Shafey, 1993) and Shafey *et al*., 2000), age of the hen (Shafey *et al*., 2001), rate of egg production (Jiang *et al*., 1991), yolk size (Harris and Wilcox, 1963) and fatty acids present in the diet (Summers *et al*., 1996).

Economic Analysis of Laying Chickens on the Effect of Using of *Xylopia aethiopica* as an Additive at Graded Levels in Drinking Water

The influence of the aqueous extract of grains of selim on the layer birds was beneficial economically in terms of production cost. There were significant differences in cost per kg feed consumed, cost of total feed consumed per bird, total feed cost per kg egg, total egg weight per bird per kg, cost differential and relative cost benefit among treatment groups. Treatment 5 was significantly increased in cost per kg feed consumed, cost of total feed per bird, total feed cost per kg egg and cost differential while in relative cost benefit treatment 2 was highly significance. Since the major challenge of the poultry farmer in improving performance of the birds to ensure a better net return while minimizing cost of production. This report is in harmony with that of Isikwenu and Udomah (2015) who reported finanacial increase in the production cost with the inclusion of *Xylopia aethiopica* in broilers. Therefore the study revealed that *Xylopia aethiopica* dried fruits (grains of selim) have proven to be one of the natural alternatives to antibiotics with a low cost of production.

CHAPTER FIVE

5.0 SUMMARY

The study investigated the effects of grains of selim (*Xylopia aethiopica* dried fruit) as additive on performance characteristics of laying birds. One hundred and fifty birds were used and the experiment lasted from February–April 2016. To achieve this, the following experiments were carried out. The proximate composition of the diet (feed and test ingredient) and the phytochemical composition of the test ingredient were investigated. The test ingredient was given at varying concentrations (0.2g/l-0.65g/l) via drinking water. Effect of the grains of selim on the performance (body weight, feed intake, feed conversion ratio, total egg production, egg weight, hen day production, hen house production, egg mass and mortality), gut microbial population, (total bacteria and fungi colony count), egg quality characteristics (external & internal), haematological & serological parameters and the economic potentials of using dried grains of selim were all investigated.

5.1 CONCLUSION

Results from this study revealed that the administration of the aqueous extract in drinking water to the laying birds at these levels did not adversely affect their performance, external and internal egg characteristics, haematology and serum chemistry. However it is beneficial to the health of the birds because the study revealed that it helps in reducing the gut microbial population as antibiotics with no residual effect. Therefore, the study has proven that it can be used in place of synthetic antibiotics since it is cost beneficial and readily obtainable in the local market which is an added advantage. Based on the finding of this study therefore, *Xylopia aethiopica* dried fruit has a great medicinal repute which produces a variety of complex chemical compounds which may possible influence body weight, feed intake, egg production and general performance. The dried fruits showed various degrees of activity against the Gram positive and Gram negative bacteria. Based on the findings, it is recommended that phytogenic feed additives such as *Xylopia aethiopica* be used to replace antibiotic feed additives in order to prevent the deposition of toxic substances in poultry meat which may invariably be harmful to man when such products are consumed.

5. 2 RECOMMENDATIONS

Based on the results of the research work on the proximate and phytochemical composition of the *Xylopia aethiopica* dried fruits and its influence in response to the various test groups as additive via drinking water on the general performance of the laying birds, the following are recommended;

- *Xylopia aethiopica* dried fruits can be used as additive in layers production via drinking water.
- From the result of the gut microbial population, the aqueous extracts of *Xylopia aethiopica* can be used as a natural alternative antibiotic and as antihelminthic to replace synthetic antibiotics with residual effect.
- Based on the performance characteristic of the birds and the economic cost benefit of the finding, poultry farmers are advised to use natural antibiotics such as *Xylopia aethiopica* dried fruits among many others in order to enhance production and reduce cost at 0.20g/litre.

5.3 CONTRIBUTIONS TO KNOWLEDGE

- The study established that *Xylopia aethiopica* aqueous extracts has antimicrobial and antihelminthic activities and can be used in place of synthetic antibiotics to improve performance in laying birds at 0.65g/litre.
- The study confirmed that utilization of *Xylopia aethiopica* by laying birds has no adverse effect on egg quality and consumers of poultry products.
- The use of *Xylopia aethiopica* established the fact that it promotes good health in laying birds.

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