THE VALUE OF *GARCINIA KOLA* (BITTER KOLA) AS FEED INGREDIENT AND ANTI-MICROBIAL AGENT FOR LAYERS AND RABBITS

BY

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CERTIFICATION

We certify that this study titled "**The value of bitter kola (*Garcinia kola*) as feed ingredient and anti-microbial agent for layers and rabbits"** was carried out by Esiegwu, Arthur Chidozie (Reg. No: 20085636428) in the Department of Animal Science and Technology, Postgraduate School, Federal University of Technology, Owerri-Nigeria.

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DEDICATION

This work is dedicated to our Lord Jesus Christ in the most Blessed Sacrament; to Him be all Glory and Honour forever, Amen.

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ABSTRACT

Two experiments were conducted to determine the value of bitter kola as feed ingredient and antimicrobial agent for laying hens and growing rabbits.
Vitamin C and mineral analysis of the bitter kola showed that it contained 0.028 mg/g magnesium, 0.025mg/g calcium, 0.161mg/g potassium, 0.057mg/g phosphorus, 0.282mg/g sodium, 0.400mg/g chlorine, 0.073mg/g sulphur, 0.02mg/g Fe, 0.003mg/g zinc, 0.05 mg/g cupper, 0.012mg/g manganese and 11.43mg/100g vitamin C. In the first experiment, four layer diets were formulated to contain bitter kola seed meal at dietary levels of 0%, 2.5%, 5.0% and 7.5%, designated as T_0, $T_{2.5}$, $T_{5.0}$, and $T_{7.5}$, respectively. Four groups of 30 Hyline brown laying hens were randomly assigned to the four treatment diets in a completely randomized design and fed for 84 days. $T_{2.5}$ had significantly ($P<0.05$) low hen-day egg production but there were no significant differences in egg weight and feed conversion ratio ($P>0.05$). There were also no significant differences in feed intake ($P>0.05$) but feed intake tended to increase with increase in dietary *Garcinia kola* seed meal. The layers on $T_{7.5}$ gained significantly ($P<0.05$) more body weight. Egg shell weight and percent shell weight, shell thickness, egg shape index, egg shell index and Haugh unit as well as egg length, egg width, yolk width, yolk length, albumen height, albumen length and albumen width were not affected by the treatments ($P>0.05$). Bitter kola tended to increase albumen weight and to decrease the yolk weight. $T_{2.5}$ had significantly ($P<0.05$) higher percent albumen whereas the control (T_0) was significantly higher ($P<0.05$) than the bitter kola groups in percent yolk weight. There were no significant effects ($P>0.05$) in most of the haematological parameters (PCV, HB, WBC and RBC). There were no traces of eosinophils, basophils and monocytes, and no significant differences in MCV, MCH and MCHC among the treatments ($P>0.05$). No significant ($P>0.05$) differences occurred in serum total protein, percent albumn, percent globulin, glucose and cholesterol. There were also no treatment effects ($P>0.05$) on the serum electrolytes (Na, K and Hco_3). Serum calcium was significantly ($P<0.05$) high at 7.5% dietary level compared to the control. Bitter kola diets did not have any effect ($P>0.05$) on liver, gizzard, abdominal fat and intestinal weights. Bitter kola tended to decrease the weight of the kidney and the heart. Dressed weights were not affected by the treatments ($P>0.05$). Bitter kola inhibited the growth of *salmonella spp, Ascaris lumbricoides* and oocyst of *Isospora belli at 5.0%* and 7.5% dietary levels. There were histological alterations of the kidney, liver and gizzard. The main cellular changes include distortion of general tissue architecture, tissue stroma proliferation, oedema of tissue, hyperthrophy and necrosis. In the second experiment, bitter kola seed meal was used in formulating four rabbit grower mash at dietary levels of 0%, 2.5%, 5.0% and 7.5%, designated as T_0, $T_{2.5}$, $T_{5.0}$, and $T_{7.5}$, respectively. Each diet was fed to 9 grower rabbits for 84 days. The *Garcinia kola* groups consumed significantly ($P<0.05$) more feed than the control. The average body weight change and average daily body weight gain of the groups on bitter kola diets decreased significantly ($P<0.05$) compared to the control. Feed conversion ratio of the control was significantly ($P<0.05$) lower than the bitter kola groups. There were no significant treatment effects ($P>0.05$) in most of the haematological parameters (PCV, HB, WBC, RBC). MCV, MCHC and MCH were also not affected by treatments ($P>0.05$). There were no traces of basophils, eosinophils and monocytes. The lymphocytes and neutrophils of the control were only significantly ($P<0.05$) higher than those of $T_{7.5}$. The platelets increased with increase in dietary levels of bitter kola. Biochemical indices (serum proteins, serum albumin, serum globulin and serum cholesterol) were not affected by the treatments ($P>0.05$). The glucose level of the bitter kola groups were drastically lowered. The electrolytes (potassium, sodium and chlorine) as well as calcium levels were not affected by the treatments ($P>0.05$). Dressed weight, liver and heart weights were not affected by the treatments ($P>0.05$). The weights of the kidney decreased with increase in bitter kola inclusion. Similarly abdominal fat decreased with increase in dietary bitter kola. At 7.5% dietary level, the weight of skin was significantly reduced ($P<0.05$). Bitter kola was unable to eliminate cysts of *Isospora bellies* in rabbits, but inhibited the growth of *Salmonella* and *Streptococcus spp* at 5.0% and 7.5% inclusion levels. *Escherichia coli* and *Staphylococus aureus* were not affected. Histological alterations of the liver and kidney were observed at 5.0% and 7.5% dietary levels. The cellular changes observed were moderate oedema with tissue stroma proliferation, glomeruli atrophy with marked cellularity within the tuft.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

There is no doubt that the cost of providing feed and drugs in livestock and poultry industry in Nigeria are steadily increasing. These costs are incident on the final consumers arising from the high cost of producing meat, eggs and milk. The high cost of feeds and drugs has greatly hampered the growth of the industry. Most of the conventional feedstuffs such as maize, soya bean meal, fish meal, meat meal etc possess nutritional qualities as proteins, energy etc without a supporting medicinal value. As such, drugs such as antibiotics and vaccines have to be procured at extra cost.

South-eastern agro–ecological zone of Nigeria is highly endowed with plants with nutritional and medicinal potentials. It has become necessary to investigate such plants for possibilities of incorporating their leaves or seeds in livestock and poultry feeds to serve as feed ingredients and/or prophylactic agents. Earlier studies at our station have shown that leaves from *Alchornia cordifolia* (Udedibie and Opara, 1998) and *Azadirchta indica* (Esonu *et al,* 2005; Obikaonu, 2009) could be of value in poultry diets. *Garcinia kola, Heckel*, commonly called bitter cola, is an indigenous medicinal tree belonging to the family *guttiferae*. It is a dicotyledonous plant found in the rain forests of Central and West Africa and grows as a medium-sized tree up to 12m high. It is grown on homesteads in southern Nigeria. The tree is well branched, evergreen, has regular fruiting cycle and bears fruits every year (Iwu, 1993).

Garcinia kola is highly valued because of its medicinal value (Hertog *et al,* 1993). The seeds are chewed as an aphrodisiac or used to cure cough, dysentery or chest cold in herbal medicine (Irvine, 1961). The seeds are also served to visitors as a sign of love and warm reception among the Igbos of Eastern Nigeria. It enjoys a folk reputation in Africa as a poison antidote (Kabangu *et al.,* 1987). The seed is also believed to act as snake repellant when placed round the compound (Nair, 1990; Daily Champion, 2004). It could serve as raw materials for pharmaceutical industries (Iwu, 1989).

Phytochemical studies have shown that the seed's constituents include biflavoids, xanthones and benzophenones. The anti-microbial properties of the seed are attributed to the benzophenones and flavonones. The seed has also shown bronchodilator effect (Orie and Ekon, 1993), anti-inflamation, anti-microbial, anti-bacterial and anti-viral properties (Ebana *et al.,* 1991; Akoachere *et al.,* 2002). It is generally assumed that the active constituent contributing to these protective effect of bitter kola on animals are the phytochemicals, minerals and vitamins (Okwu and Ekeke, 2003). The phytochemicals include alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins and lignans.

According to Okwu (2005), bitter kola contains bioactive constituents comprising saponins 11.48mg/100g, tannins 0.26mg/100g, alkaloid 0.36mg/100g. These phytochemical values tended to agree with the recent values reported by Esiegwu and Udedibie (2009) with cyanogenic glycoside as 0.54mg/100g, tannins 0.34mg/100g, saponin 10.06mg/100g and alkaloids 4.93mg/100g. Alkaloid was quite high relative to the value reported by Okwu (2005).

Some histological alterations in the liver, kidney and duodenum of rats fed diets containing 10% bitter kola extract have been reported (Braid and Grill, 1990). Oluwole and Obatomi (1992) also observed an increase in both basal and histamine-mediated gastric acid secretion of rats fed bitter kola.

Preliminary studies at our station (Esiegwu and Udedibie, 2009), showed that 2.5% dietary bitter kola induced heavier body weight and superior feed conversion ratio in broilers. Broilers placed on bitter kola diet tended to develop significantly heavier livers than the control. There were no significant differences among the groups in most of the haematological indices but *Garcinia kola* at 5.0% and 7.5% dietary levels inhibited the growth of *Salmonella Spp*, but had no effects on *Escherichia coli.*

1.2 **Statement of the Problem**

The high cost of conventional feed and drug in the livestock industry has greatly limited the growth of the industry. This has increased the cost of production and cost of livestock and poultry products by final consumers.
There is need to investigate alternative feed stuffs with both nutritional and medicinal properties with the aim of incorporating their seeds or leaves in livestock and poultry feeds to serve as feed ingredient and/or prophylactic agents.
Given the above basic problem, this study was necessitated by the need to investigate the value of bitter kola as feed ingredient and anti-microbial agent and to what extent it could be incorporated in the diets of layers and rabbits.

1.3 **Objectives of the Study**

The objectives of this study therefore were to further investigate bitter kola with a view to;

1. Determine its vitamin and mineral content ;
2. Determine the effects of graded levels of bitter kola seed meal on the performance of monogastric farm animals, using laying hens and growing rabbits as the experimental animals;
3. Determine the effects of dietary bitter kola on their intestinal microbial load;
4. Determine the egg quality indices as affected by the seed meal;
5. Determine the effects of the seed meal on haematological and serum biochemical indices of the two species of farm animals and
6. Determine the effects of dietary bitter kola on internal organs (liver, heart, and kidney) of the species.

1.4 **Justification of the Study**

Studies on the value of bitter kola as feed ingredient and anti-microbial agent for layers and rabbits are limited and this work may serve as a reference material to scholars interested in the area. The result of this study will open a new door for scientist to research into alternative feed stuffs with nutritional and medicinal properties to serve as feed additives in the livestock industry.

It will attract research grants from government and industrialists to scientists to investigate into hidden indigenous knowledge of medicinal plants for their nutritional properties with the result of their seeds or leaves being used as feed additive.

1.5 **Scope of the Study**

The study focused on layers and rabbits. One hundred and twenty (120) layers and thirty six (36) rabbits grouped into four in a completely randomized design were given the *Garcinia kola* dietary treatments.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and distribution of bitter kola

Bitter kola, known as *Garcinia kola,* Heckel, is an indigenous medicinal plant belonging to the family *guttiferae* or *clusiaceae.* It is found in Benin, Cameroon, Democratic Republic of Congo, *Cote d'Ivoire*, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. Its natural habitat is subtropical or tropical moist lowland forests.

Bitter kola is a dicotyledonous plant found in the rain forest of Central and West Africa (Iwu, 1993). It grows in coastal rainforests in south-western and south-eastern parts of Nigeria where it is chewed as a nut and readily served to visitors as a sign of welcome. Bitter kola is enjoyed by the three major ethnic groups in Nigeria (i.e. the Yoruba's, the Igbo's and the Hausa's). Bitter kola is called *agbilu* in Igbo language and *Orogbo* in Yoruba language.

The local market for bitter kola extends beyond the southern production areas to the northern parts of the country. In Nigeria, its trade is as important as that of kola nut (*Cola nitida and C. acuminata*) in major towns and cities in southern parts of the country where the tree is endemic. Apart from being a stimulant, it has on consumption a bitter astringent and resinous taste and is used as an aphrodisiac. It is highly valued as medicinal and the fact that consumption of large quantities of it does not cause indigestion (as kola nut does) makes it a highly desired product (Dalziel, 1937). Unlike other kola nuts, bitter kola is believed to clean the digestive system, without side effect such as abdominal problems, even when a lot of nuts are eaten (Onochie and Stanfield,

1960).The root of the plant serves as a bitter chewing-sticks in west Africa while the stem bark is used as a purgative among the natives of eastern Nigeria and the latex externally applied to fresh wounds to prevent bacterial infection thereby assisting in wound healing (Uko *et al.,* 2001). It is called bitter kola because of its bitter taste or for its claimed aphrodisiac activity, respectively.

2.2 **Taxonomy of bitter kola**

Bitter kola is scientifically classified as follows:

Kingdom:	*Plantae*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Order:	*Theales*
Family:	*Clusiaceae or guttiferae*
Genus:	*Garcinia*
Species:	*G. kola*
Binomial name:	*Garcinial kola, Heckel.*

2.3 **Botany of bitter kola**

Bitter kola plant has been described as a tree of variable aspect, well branched, ever-green and grows to a height of about 12m. Towards the base of the branches are large opposite leaves (12" long by 7" broad) with short petioles, while at the extremity of the branches, the leaves are much smaller (5" by 2"). The leaves are oval, slightly dilated at the base, full green on the upper surface and greenish underneath (Heckel and Schlagdenhauffen, 1884).

The tree produces reddish yellowish or orange colored fruit. Each fruit contains two to four yellow seeds and a sour tasting pulp. The seeds when consumed have a bitter astringent taste (Ladipo, 1995).

The fruit is a berry, the size of an apple with a rugos epiderm covered entirely with rough hairs. The fruit is reddish-yellow and each fruit contains two to four brown seeds embedded in orange-coloured pulp. The fruit is about 6.25cm in diameter (Keay, 1989; Ladipo, 1995).

2.4 **Agronomic characteristics of bitter kola**

Bitter kola (*G. kola*) is endemic in the humid lowland rain-forest vegetation of the West and Central Africa sub regions. It is usually found in the coastal areas and low land plains up to 300 m above sea level with an average of 2500 mm of rainfall per annum. The major places where the plant would be found growing in the wild are forest reserve and free areas of the rain-forest or it is planted or conserved in on-farm oil-palm, cocoyam plantations. These growing regions have low altitude with annual temperature ranging from 32.15^{o}c to 21.4^{o}c and a relative humidity of 76.34% (Ntamag, 1997).

As a tropical fruit tree species, it is characterized by slow rate of growth (Ladipo, 1995). Cultivation of the plant is limited because of poor germination and the length of time it takes (about 10 - 15 years) to reach reproductive phase. In Nigeria, the demand for bitter kola is high but the production is limited due to problem of seed dormancy; untreated seeds are difficult to germinate. Farmers believe that germination of bitter kola takes about six to twelve months and that only a few seeds germinate. There is also the problem of setting up nurseries.

However, Anegheh *et al.* (2006) developed a pre-nursery treatment to break dormancy and enhance germination.

His work revealed that seed cutting (nicking) was very effective in enhancing germination of bitter kola. The seed is first raised in the nursery and then transplanted to the field. Fruiting commences in July and ends in October. Harvest continues as ripe fruits fall and are collected for the extraction of seeds.

Ladipo (1995) reported that a mature fruit tree of 10 to 15 years produces 85 to 1717 fruits with 208 to 6,112 seeds. Taking the mean of these values at 834 fruits and 2,627 nuts per tree he projected a fruit production of 26 tonnes per ha per annum with 278 trees per hectare at 6m X 6m spacing. The fruit when ripe, the green pericarp turns reddish yellow color and the fruit falls. The fruits are gathered, broken and stored in an open cool place to allow for fermentation of the pericarp and pulpy mesocarp. The fruits are threshed to release the seeds which are washed to remove the sticky mucilaginous material that sheaths it. The seeds or nuts that are not sold fresh are air dried and stored in baskets lined with jute bags. According to Ofor *et al.* (2010) storage of bitter kola in polyethene bags were favored in terms of shelf life and palatability.

2.5 **Cultivation of bitter kola**

According to *Ingenieurs Sans Engineers Frontieres without Boarders Cameroon* (2009). *Garcinia kola* is cultivated either by seeds or by cutting.

2.5.1 **Cultivation by seeds**

Prepare a suitable seed bed measuring 3m x 4m ($12m^2$) on a flat ground. The seed bed inside a shade house to protect the little plants from direct radiation of the sun and strong rains. The shade house is built from local materials like bamboo, stakes cut in forest or palm tree branches. Seeds

that germinated easily are matured ripe fruits that fall to the ground and the seeds removed. Pre-nursery germination was first done by this technique. Cutting two or three banana or plantain tree. Make a large hole on the level of its base that will allow it to destroy the central bud (meristem) of the cut plant. Insert seeds in the trunks of banana tree.

Attach firmly the two ends of each trunk. Put trunks hermetically closed under the hanger. After three months, detach the wires to recover the seeds of which will have already germinated. The germinated seeds are carefully sowed inside polyethylene bags with $^2/_3$ of it filled with a mixture of black soil and sand. After sowing in the bag, the bag is completely filled with soil. The pots of young seedlings are then laid out in the seed bed.

The seedling inside the bag is maintained by watering it every two days, weeding it, applying 3g of N.P.K 20:10:10 fertilizer every 3 months and fighting against fungal and insect attack using the appropriate fungicides and insecticides.

After 12 months, the seedlings can be planted in the field. Planting is done at the beginning of the rainy season with a standard spacing of 10m x 10m. Gently remove the seedling from the bag and put into the hole surrounded by its mound and fill it with soil. Continue with all agronomic practices. The production of fruit will approximately begin after 7 years. Note that hypogeal germination was found in seeds of bitter kola.

2.5.2 **Cultivation by cutting**

Cut the bitter kola cuttings from very tender branches, stems with young healthy leaves and vertical branches that are looking upwards. Cut

cuttings very early in the morning and preferably just after rain to avoid drying. This is put inside a wet plastic bag. This bag is tightly closed and put in the shade. The collected cuttings are put directly in the propagator. Cutting should be about 12cm. it is then germinated. The young seedlings are then carefully inserted into a polyethylene bags filled with mixture of black soil and sand up to $^{2}/_{3}$ level. After inserting the young seedling, the polyethylene bag is filled with soil to the brim. Sprinkle lightly. The pots of seedling are transferred to the seed bed and finally to the field as in propagation by seeds.

Bitter kola produces fruits between July and October.

2.6 **Some species of bitter kola**

About 400 species are found in tropical regions especially in Asia and in South Africa (Mabberly, 1987). Some of these species are:

i ***Garcinia cambogia***

The gum-resin of this species is known as *Cambodian gamboge.* Mahendran and Shyamala (2001) noted that the rind of the fruits is an astringent and may be used in Indian traditional medicine for the treatment of ulcers and haemorrhoids.

ii. ***Garcinia gaudichaudii***

Perry and Metzger (1980) in surveying the medicinal plants of east and south Asia recommended that on the Malay Peninsula, the juice from the roots is rubbed on cuts.

iii. ***Garcinia hanburyi***

The gum resin derived from this species is known as Siam *gamboge or cambodia gamboge*. It has an acrid taste and when powdered is strongly sternutatory (Wren 1975).

iv. ***Garcinia huillensis***

According to Watt and Breyer-Brandwijk (1962), a decoction of the bark is used by the Bemba as a lotion for septic and venereal sores.

v. ***Garcinia indica***

Kokum oil or kokum butter, also known as Goa butter derived from the seed of this species may be used externally to heal ulcers, fissures of the lips, chapped skin, etc.(Nadkarni, 1976).

vi. ***Garcinia kola*** (Bitter kola)

Ainslie (undated) noted that in Nigeria the powdered bark is applied to malignant tumours. The sap is used for parasitic skin diseases, and the latex or gum is applied externally to fresh wounds.

vii. ***Garcinia latissima***

Perry and Metzger (1980) in a survey of the medicinal plants of east and south East Asia, recorded that in Indonesia, the gum which flows from the wounded back is used on leg wounds.

viii. ***Garcinia mangostana***

According to Corner (1952), the rind of the edible fruit of this species is used for astringent purposes while Perry and Metzger (1980) noted that on the Malay peninsula, a decoction of the leaves with unripe banana and benzoin is applied externally for circumcision wounds.

ix. ***Garcinia morella***

According to Nadkarni (1976), the gum resin derived from this species known as Ceylon, Indian or Malabar gamboge is prepared as a paste for application to sprains, bruises, swollen hands and feet. Similarly, Perry and Metzger (1980) recorded that in China, the gum resin is used either alone as a powder or as an ingredient

in preparations for the treatment of wounds, cancerous sores and indolent ulcers. Duke-Elder and Macfaul (1972) also noted that Gamboge is irritant to the eyes of rabbits.

x. ***Garcinia oliveri***

Perry and Metzger (1980) recorded that the bark of this species with that of *Garcinia vilersiana* crushed with a little alcohol makes a poultice to apply to sprains and morbid wounds.

xi. ***Garcinia polyantha.***

In West Africa, the yellow resinous sap makes a dressing for wounds (Dalziel 1937).

Other species of *Garcinia* found in Nigeria as well as generally across the humid low land plains of West Africa extending from Sierra Leone to Zaire according to Vivian and Faure (1996) and Angola (Keay,1989) include

i. *Garcinia livingstonei*
ii. *Garcinia gnetoides*
iii. *Garcinia standtii*
iv. *Garcinia smeath emanii*
v. *Garcinia ovalivolia*
vi. *Garcinia brevipediellata*
vii. *Garcinia manni*

2.7 Nutrient composition of bitter kola

Bitter kola contains nutrients such as proteins, carbohydrates, fibre, minerals, fat and oils. Ibekwe *et al.* (2007) reported that bitter kola seed

has poor nutrient composition but highly valued in trado-medicine due to its useful active phytochemical composition in table 2.1

Contrary to the nutrient composition of *Garcinia kola* in table 2.1, Esiegwu and Udedibie (2009) reported the nutrient composition of bitter kola as shown in table 2.2

Odebunmi *et al.* (2009) reported the moisture content of *Garcinia kola* seeds to be 60.48 ± 0.06%, dry matter of 39.52 ± 0.06%, crude fat of 4.51 ± 0.56%, crude protein of 2.48 ± 0.10%, ash content of 0.79 ± 0.005%, crude fibre of 5.23 ± 0.16% and total carbohydrates (+ fibre) of 35.64%. These values are different from what had previously been reported for bitter kola. Eleyinmi *et al.* (2006) reported a protein content of 3.95%, lipid of 4.33%, ash of 1.14% and a crude fibre content of 11.4% in the seed.

In another vein, Asaolu (2003) reported that the chemical composition of the fresh seeds of bitter kola (wet weight) contained high moisture content of 75.50% and dry weight of 24.50 while the ash content was found to be 5.90%, crude fat was 14.50%. Carbohydrate was 10.85%, crude fat was 14.50% and crude protein was found to be very low (4.25%).

Dosunmi and Johnson (1995) in comparing the nutritive value of the fresh fruit from Nigeria showed that crude protein was higher in the mesocarp (7.8%) than in the pericarp (3.9%) while the pericap was richer

in crude fibre (13.9% - 16.5%). The mesocarp was also richer in crude lipid (6.9% - 8.7%).

Unsaturated fatty acids (linoleic acid, 40.5%, oleic acid, 30.8%) are the main components of the lipids (4.5%) found in the seeds of this species (Essien *et al.*, 1995; Omode *et al.*, 1995).

Table 2.1: **Nutrient composition of *Garcinia kola* (% of dry matter)**

Components	**Amount %**
Moisture	14.60
Crude protein	0.58
Crude fibre	0.10
Ether extract	3.00
Ash	5.00
Nitrogen-free extract	91.32

Adapted from Ibekwe *et al.* (2007)

Table 2.2: **Nutrient composition of *Garcinia kola* (% of dry matter)**

Composition	**Amount%**
Dry matter %	7.30
Crude protein	2.64
Crude fibre	20.51
Ether extract	9.47
Ash	1.07
Nitrogen free extract	57.54

Adapted from Esiegwu and Udedibie (2009).

2.8. **Phytochemical, vitamin and mineral composition of *Garcinia kola***

Chemical analysis of *Garcinia kola* in Nigeria as reported by Okwu (2005) and Esiegwu and Udedibie (2009) showed that bitter kola contained a wide range of phytochemicals, vitamins and minerals as shown in tables 2.3, 2.4 and 2.5.

Esiegwu and Udedibie (2009) reported the phytochemical values as shown in table 3b.

According to Odebunmi *et al.* (2009), *Garcinia kola* has 722.10 mg/100g of potassium (K), 67.07 $\pm$ 0.12mg/kgDM of calcium (Ca), 114.83 $\pm$ 3.47mg/kgDM of magnesium (Mg), 6.10 $\pm$ 0.43 mg/KgDM of iron (Fe), 2.30 $\pm$ 0.08 mg/kgDM of zinc (Zn), and 188.57 $\pm$ 0.37mg/kgDM of phosphorus (P).

2.9. **The role of phytochemicals in the body**

The role of phytochemicals in enhancing body cell immunity against diseases in the body cannot be overemphasized.

It is believed that the active constituents contributing to the protective effect of *Garcinia kola* on animals is attributed to the presence of phytochemicals, vitamins and minerals (Okwu and Ekeke, 2003). Phytochemicals exhibit a wide range of biological activities as a result of their anti-oxidant properties.

Several types of polyphenols (phenolic acid, hydrolysable tannins and flavonoids) show anti-carcinogenic and mutagenic effects (Uruquiaga and

Table 2.3a: **Phytochemical constituents of *Garcinia kola* seeds (dry weight basis)**

Constituents	**Amount (mg/100g)**
Phenols	0.11 ± 0.20
Alkaloids	0.36 ± 0.10
Tannins	0.26 ± 0.20
Flavonoids	1.98 ± 0.20

Adapted from Okwu (2005).

Table 2.3b: **Phytochemical constituents of *Garcinia kola* seeds (dry weight basis)**

Constituents	**Amount (mg/100g)**
Cyanogenic glycosides	0.54
Tannins	0.34
Saponins	10.06
Alkaloids	4.93

Adapted from Esiegwu and Udedibie (2009).

Table 2.4: **Vitamin composition of *Garcinia kola* seeds (dry weight basis)**

Vitamins	**Amount (mg/100g)**
Thiamin (vit. B1)	0.5 ± 40.30
Riboflavin (vit. B2)	0.22 ± 0.01
Niacin (nicotinic acid)	1.60 ± 0.01
Ascorbic acid (Vit. C)	23.10 ± 0.02

Adapted from Okwu (2005).

Table 2.5: **Mineral composition of *Garcinia kola* seeds (dry weight basis)**

Mineral	Amount (mg/100g)
Macro elements	
Magnesium	0.42 ± 0.30
Calcium	0.80 ± 0.40
Potassium	2.50 ± 0.10
Phosphorus	0.33 ± 0.10
Sodium	0.72 ± 0.10
Micro elements	
Iron	17.75 ± 0.30
Zinc	2.30 ± 0.01
Copper	0.78 ± 0.20
Manganese	2.01 ± 0.50
Chromium	0.00
Cobalt	0.55 ± 0.20
Cadmium	0.29 ± 0.10

Adapted from Okwu (2005).

Leighton, 2000). It has been reported that polyphenols, particularly flavonoids inhibit the initiation, promotion and progression of tumors (Uruguiaga and Leighton, 2000; Okwu, 2004).

Flavonoids are special plant metabolites present in terrestial vascular plants chemically defined as substances composed of a carbon C6-C3-C6 skeleton with one or more hydroxyl groups and other substituents. A high proportion of flavoniods occur naturally as water soluble glycosides. Flavonoids are super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protect against all stages of carcinogenesis (Salah *et al.,* 1995; Okwu, 2004). Considerable quantities of flavonoids are consumed daily in our vegetable diets and some are particularly beneficial giving protection against cardiovascular and certain types of cancer (Dewick, 1998). Plant flavonoids are recently attracting serious attention as important dietary cancer chemoprotective agents (Hertog *et al.,* 1993; Okwu and Okwu, 2004). The biological functions of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, hepatoxins, virus and tumors (Farquar, 1996; Okwu 2004).

The presence of phenolic compounds in the seeds of bitter kola shows that it might be an antimicrobial agent. This is because phenols and plenolic compounds have been extensively used in disinfection (Okwu, 2001). Protonated phenol is used as a cleaning agent (Uruquiaga and Leighton, 2000). Extracts from the seeds of *Garcinia kola* have excellent bactericidal properties (Okwu, 2004). The presence of phenols also shows that the seeds of *Garcinia kola* could be anti-inflammatory, anti clotting, anti-oxidant, immune enhancers and hormone modulators. Phenols have been responsible for blocking specific enzymes that cause inflammation. No doubt the seeds of *Garcinia kola* have pharmacological

uses in treating coughs, throat infections, bronchitis, hepatitis (inflammation of the liver) and liver disorders (Farombi *et al.*, 2005).

Garcinia kola has high saponin content (Okwu, 2005). Saponins are glycosides containing a polycyclic aglycone moiety of either C27 steroid or C30 triterpenoid (collectively termed sapogenins attached to a carbohydrate). Saponins have the property of forming foams in aqueous solution, possessing hemolytic activity, cholesterol binding properties and bitterness (Sodipo and Akiniyi, 2000; Okwu, 2004).

Cheeke and Shull (1985) reported retardation of growth rate primarily due to a reduction in feed intake in non-ruminant animals (poultry, rats, rabbits and swine) when fed alfalfa saponins. Reduction in feed intake may occur due to unpalatability because saponins have a pronounced irritating effect on the membrane of the mouth and throat. Retardation in growth has also been noted when *Sesbania sesban* leaf meal (saponin 7.1g/kg) was incorporated in chick diet (Shqueir *et al.,* 1989).

Saponins are known to be bitter and reduce the palatability of livestock feeds and increase excretion of cholesterol concentration.

It also contains alkaloids and tannins. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stray, 1998).

Tannin has astringent properties, quickens the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). The presence of tannins in *Garcinia kola* explains their use in treating wounds, various ulcers, hemorhoids, cold and burns in herbal medicine (Igboko, 1983; Maduinyi, 1983). The high tannin content may have also contributed to the bitterness in *Garcinia kola*.

Tannins are water soluble phenolic compounds of plants with a molecular weight of 7500 and with the ability to precipitate gelatin and

other proteins from aqueous solution. Tannin forms complexes with proteins. These complexes can either be reversible or irreversible. If the tannin is present in excess, all the protein available is precipitated. However, when the protein is present in excess, the complexes remain soluble (Haslam, 1989). The main anti-nutritional effects of tannins are reduction in voluntary feed intake, diminished digestibilities of nutrients, adverse effects upon rumen metabolism and toxicity. The role of tannin in reducing voluntary feed intake is not clearly understood. However, high levels of tannin may depress feed intake by slowing down digestion of dry matter (DM) in the rumen, react with the outer cellular layer of the gut, and this diminishes the permeability of the gut wall (Mitjavila *et al*., 1977) or cause depression in feed intake due to unpalatability. Tannin in plant tissue may precipitate salivary proteins, causing an unpalatable astringent taste in the mouth. Condensed tannin (CT) in *leucaena leucocephala* caused poor nitrogen retention and low apparent metabolizable energy in poultry (D' Mello and Acamovic, 1989). In rabbit and rat, condensed tannin from the browse plant Robin's pseudoacacia caused reduced feed intake, reduced growth and coprophagy (Raharjo *et al.,* 1990) and reduced protein digestibility (Horigone *et al.,* 1988) respectively. Free tannins can form complexes with dietary proteins (Vaithiyanathan and kumar, 1993) as well as endogenous proteins including enzymes. The proteins bound with tannins are most unlikely to undergo normal metabolism. Further tannin–enzyme interaction would inhibit the enzyme activity. Indeed tannins inhibit a broad spectrum of enzymes in *in-vitro* assays (Horigone *et al.,* 1988; kumar, 1992a). Salawu *et al.* (1999) reported that small amount of condensed tannins (20 - 40g/kg dm) can exert beneficial effect on protein metabolism in ruminant by slowing rapid microbial degradation of dietary protein and increasing protein out flow from the rumen thus increasing

absorption of amino acids in the small intestine of the animal. The tannin content of cassava leaf when consumed by ruminant could serve as a coat for protein thus enhancing by-pass protein and effective feed utilization.

Salawu *et al.* (1999) also reported that feeding tannin to sheep reduced the risk of bloat and parasite. However, feeding cassava leaf meal up to 20% in poultry has been reported to decrease weight gain and feed efficiency. The decrease in weight gain might not only be connected to HCN but high consumption of tannin.

2.10 **The role of vitamins and minerals in the body**

Garcinia kola contains vitamins and minerals that are required for the normal functioning of the body metabolic and physiological processes. Vitamins, though present in trace amounts, are very essential for the body metabolism. *Garcinia kola* contains niacin and thiamin, and niacin is necessary for the prevention of the disease, pellagra, while a deficiency of thiamin results in a disease condition known as beri beri.
A deficiency of riboflavin, one of the constituents of bitter kola, produces symptoms such as inflammation of the tongue, lesion of the eyes and lips, congestion of the conjunctiva blood vessels and desquamation of the skin.

Garcinia kola contains ascorbic acid and lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body. Therefore, the clinical manifestation of scurvy (haemorrhage from mucous membrane of the mouth and gastrointestinal tract) anaemia, pains in the joints and defects in skeletal calcification can be related to the association of ascorbic acid and normal connective tissue metabolism (Hunt et al., 1980, Okwu, 2004). Ascorbic acid also plays a

role in the transformation of cholesterol into bile acid in the liver. It is required for the hydroxylation of proline to hydroxyl proline and of lysine to hydroxylysine. The hydroxylated forms of these amino acids are found in collagen, and so accounts for the role of the vitamins in maintaining normal connective tissue. The reducing property is vital in some physiological process such as in the absorption of dietary iron. Iron is absorbed in the ferrous state (Fe^{2+}) rather than the ferric (Fe^{3+}) form and the reducing ability of ascorbate accounts for the enhanced absorption of the metal in the presence of the vitamin (Hunt et al., 1980, Okwu, 2004). Ascorbic acid has an ability to act as an antioxidant, to prevent or at least to minimize the formation of carcinogenic substances from dietary materials. Nitroso which are known to be carcinogenic can be formed from the reaction of nitrites with certain amino compounds *in vivo*. The nitrite is formed from the oxidation of nitrate, which are commonly incorporated into foodstuffs used in human nutrition. In this sequence ascorbate can therefore prevent the oxidation of nitrate. The presence of ascorbic acid in *Garcinia kola* makes it possible to initialize the seed of the plant in herbal medicine for the treatment of common cold and prostrate cancer. It also accounts for the use of *Garcinia kola* plant for normal wound healing (Okwu, 2004).

Garcinia kola contains minerals which are required for maintaining body osmotic balance and regulation of body metabolism. Calcium and phosphorous are required for skeleton and bone formation.

The zinc present in Garcinia kola may suggest the valuable role of the plant in the management of diabetes, which results from insulin malfunctioning. Zinc is essential for the production of insulin, a hormone and carbonic anhydrase, an enzyme in the body. Iron is a component of haemoglobin. It helps oxygen transport. The low sodium content of

Garcinia kola may be an advantage due to the direct relationship of sodium intake with hypertension in human. (Dahl, 1972).

11 The role of chemical nutrients in the body

The body requires chemical nutrients such as carbohydrates, proteins and lipids for the normal functioning of the body. *Garcinia kola* contains a lot of carbohydrates which could serve as source of energy for the body. It also contains proteins, though in small quantity which could help in building up and repair of body tissues. Lipid content of *Garcinia kola* could equally provide enough energy for body activities. All the properties or qualities of *Garcinia kola* go to show it as a food substance (Okwu, 2005).

2.12 Uses of *Garcinia kola* seed

Garcinia kola is a tropical flowing plant found in western and central Africa and it produces brown nut-like seeds. It has been used in African culture for centuries for medicinal purposes. *Garcinia kola* contains dimeric flavonoid, which is believed to have many healing benefits.

i. Cold remedy: *Garcinia kola* is often used to treat the symptoms of colds. It is suggested in particular for coughs and sneezing.
ii. Impotence: *Garcinia kola* is sometimes believed to cure impotence.
iii. Knee osteoarthritis: *Garcinia kola* has been successfully used to treat patients suffering from knee osteoarthritis, according to a study published in the journal of orthopaedic surgery. It reduced pain and swelling and improved movement.
iv. Immunity: *Garcinia kola* is known for its anti-inflammatory and anti-oxidant properties. It is used to prevent infections and viruses, especially of the immune system.
v. Hop substitute: *Garcinia kola* is used as a substitute for hops in brewing lager beer. It is especially useful in preventing beer spoilage.

2.13 **Other values of *Garcinia kola* plant**

Garcinia kola plant is a plant with every part useful ranging from the root of the plant to its seed. The root of the plant serves as a bitter chewing stick in West Africa. The stem of *Garcinia kola* serves as a chewing stick for many people in southern Nigeria (Olabanji *et al.,* 1996; Uko *et al.*, 2001; Okwu and Ekeke, 2003). The products of three *Garcinia kola* species are widely used in Ghana and 70% of its use is as chewing sticks. These are brought in urban markets as an alternative to tooth paste and brush (Adu-Tutu *et al.,* 1999).

The raw stem bark of *Garcinia kola* serves as a purgative, the powdered bark is applied to malignant tumours, the sap is used for curing parasitic skin diseases and the latex or gum is used internally against gonorrhea and applied externally on fresh wounds to prevent bacteria infection. *Garcinia kola* is cultivated throughout West Africa for its edible fruit and seeds which are used as a rejuvenating agent for masticatory purposes and as a general antidote (Ibiblio, 1983). *Garcinia kola* is chewed in Igbo land and is presented to visitors as a sign of peace and welcome. It is also used to entertain guest during ceremonies and festivities. It is popular among Nigerians for nervous alertness and for induction of insomnia when chewed.

Traditionally, the nuts of *Garcinia kola* are chewed as a masticatory substance to stimulate the flow of saliva (Leakey, 2001). The kernels of the nuts are widely traded and eaten as a stimulant (Omode *et al.,* 1995; Atawodi *et al.*, 1995; Leakey, 2001). *Garcinia kola* is believed to clean the digestive system, without side effects such as abdominal problems, even when a lot of it is eaten (Onochie and Stanfield, 1960). In traditional medicine, *Garcinia kola* is dried, ground and mixed with honey to make a

traditional cough mixture. Currently, the ground *Garcinia kola* is mixed with water and given to new born babies to relieve pains.

Experimentations using *Garcinia kola* kernels as hop substitutes in several indigenous alcoholic drinks as well as a flavour enhancer in the beverage industry also exist (FDA, 1999). Ofor *et al.* (2004) identified several ethno-botanical uses to which the indigenes of Imo state in South–eastern Nigeria put the *Garcinia kola* seeds. These include as an antidote to snake bites, poison and overdose, for cough, vomiting and as a snake repellent. The seeds which serve as a bitter stimulant also serve as a snake repellent when they are placed round the compound (Nair, 1990; Daily Champion, 2004). The seed is used in the treatment of diarrhoea (Braid, 1991), bronchitis and throat infections (Orie and Ekon, 1993; Adesina *et al.,* 1995), liver disorders (Iwu *et al.,* 1990) and enjoys a folk reputation in Africa as a poison antidote (Kabangu *et al.,* 1987). According to Farombi *et al.* (2005), the seeds of *Garcinia kola* have pharmacological uses in treating coughs, throat infections, bronchitis, hepatitis and liver disorders.

The by-products of *Garcinia kola* plants are also useful to mankind. The wood makes excellent fuel source. Its dense rounded crown makes it an ideal tree for shade around homestead. The branches are used as chewing stick because of its taste and anti-bacterial activities of its extract (Taiwo *et al.,* 1999).

2.14 Effect of Garcinia kola on organ characteristics

Uko *et al.* (2001) reported that water extract from *Garcinia kola* fed to rats did not significantly influence the organ of the control and experimental rats; however, there was a dose-related decrease in size of livers, lungs and hearts of rats fed the plant extract. The organs (testes, kidney, liver, heart, lungs and brain) did not show any microscopic alterations in any of the treatment groups. It has also been reported by Braid (1989) that male rats fed diets containing 10% (w/w) dry powdered seeds of *Garcinia kola* for six weeks caused marked inhibition of gastro-intestinal motility, protected against castor oil induced diarrhoea, prolonged pentobarbital sleeping time, caused marked retardation of growth but did not affect organ weights compared to pair fed controls. However, the report of Braid and Grill (1990) revealed some histological alterations in the liver, kidney and duodenum of rats fed diets containing 10% (w/w) dry powdered bitter kola for six weeks. The main cellular changes included vacuolation of duodenal villous epithelial cells, numerous intracytoplasmic vacuoles in hepatocytes and mild hydropic degeneration in cells of the renal proximal tubular epithelium. Contrary to the report of Braid (1989) and Uko *et al.* (2001), Esiegwu and Udedibie (2009) reported significantly heavier livers in *Garcinia kola* groups than the control group for broiler chicks placed on similar treatments for 8 weeks at 0%, 2.5%, 5.0%, and 7.5% dietary levels.

2.15 Effect of bitter kola on haematological and serum biochemical indices.

Uko *et al.* (2001) reported that water extract from *Garcinia kola* administered to rats did not cause any significant differences between blood samples from the control and experimental rats for HB, PVC, RBC and erythrocyte indices. However, there was a general inverse

relationship between the erythrocyte values (HB, PVC and RBC) and increased doses of the plant extract. There was also a significant proliferation of total leucocytes count in blood samples from the experimental rats which depended on the doses of the extract. The work also showed that bitter kola extract decreased total plasma proteins, albumin concentrations but slightly increased total and conjugated bilirubin levels.

Similarly, Adedeji *et al.* (2005) reported the effect of different dietary inclusion levels of bitter kola on blood profiles of rats. The rats were placed on diets containing 0% (w/w), 5% (w/w), 10% (w/w) and 20% (w/w) levels of *Garcinia kola* for six weeks. It was observed that there was no significant difference between rats fed control diet and those in various dietary groups for all the blood parameters checked with the exception of the lymphocyte count which had a significant difference in all dietary groups less than the control. According to Esiegwu and Udedibie (2009), there were no significant differences among the groups fed *Garcinia kola* in most of the haematological indices; however, the control group had ($p<0.05$) more RBC than the *Garcinia kola* groups but significantly lower WBC.

2.16 **Effect of *Garcinia kola* on reproduction**

Garcinia kola seeds contain biflavonoid (kolaviron) capable of having anti-inflammatory properties (Braid, 1993) and is a natural anti-oxidant (Olatunde *et al.,* 2002; Terashima *et al.,* 2002). The importance of the anti-inflammatory property of *Garcinia kola* is necessary because ovulation, an important process in female reproductive function, is believed to be an inflammatory process (Epsey, 1980; Epsey, 1994). Ovulation is brought about by a luteinising hormone (LH) surge. This surge of LH causes the follicle to rupture and hence ovulation. According

to Gaytan *et al.* (2002), ovulation can be blocked experimentally by high doses of anti-inflammatory drugs administered before the LH surge because once the level starts to rise, it may not be stopped by any drug. Akpantah *et al.* (2005) reported that *Garcinia kola* seed extract administered to rats at 200 mg/kg body weight altered oestrous cycle in rats, partly inhibited ovulation as evidenced by the reduced no of ova in the oviduct compared to the control, with a significant decrease in the weight of foetuses from the treated rats. Also 7% of the foetuses from pregnant rats which received treatment for the first five days of gestation had malformed left upper limb or morphological anormalies. It was also reported by Uko *et al.* (2001) that rats fed *Garcinia kola* seed extract exhibited increased libido (sexual instinct) for the male rats justifying the use of *Garcinia kola* by natives as an aphrodisiac, but did not improve pregnancy rates in female rats as a measure of the male fertility index. The anti-inflammatory property of *Garcinia kola* seed may be responsible for the observed effect in blocking ovulation when administered to rats before the surge of luteinsing hormone (Freeman, 1988). The anti-inflammatory property of flavonoids is believed to result from inhibition of cyclo-oxygenase enzyme (Liang *et al.*, 1999). Akpantah *et al.* (2005) suggest that *Garcinia kola* seed may block ovulation by inhibiting cyclo-oxygenase activity and prostaglandin synthesis. Some flavonoids suppress the formation of cyclo-oxygenase – 2, thus playing an important role in the prevention of cancer and inflammation. This property is under trial in chemo-prevention potentials against human cancers as many types of cancer cells use cyclo-oxygenase – 2 to propagate (Liang *et al.,* 1999). Cyclo-oxygenase-2 (COX-2) deficient mice suffer from defect in reproductive functions such as ovulation and fertilization (Lim *et al.,* 1997).

2.17 **Anti-microbial activities of *Garcinia kola***

Garcinia kola stem has been shown to contain a complex mixture of phenolic compounds such as biflavonoids, xanthones and benzophenone (Iwu and Igboko, 1982) which have anti-microbial activity as kolanone (Hussain *et al.,* 1982), kola flavonone and garcinia flavonone (Iwu, 1993). Phytochemical studies have shown that the seeds constituents include biflavonoids, xanthones and benzophenones. The seeds of *Garcinia kola* are known to have a general antidote effect in traditional medicine in Africa. The seeds are believed to possess aphrodisiac properties and are used for the treatment of catarrh and abdominal colicky pain. In addition, their use is believed to improve singing voice and relieve cough (Irvin, 1961).

Extracts from the bark, stem and seed of *Garcinia kola* have been reported to inhibit the growth of *plasmodium falciparum* by well over 60% *in vitro* at a concentration of 6mg/ml (Tona *et al.,* 1999).

The antimicrobial activities of aqueous extract of three Nigerian medicinal plants, *Vernonia amygdalina* (bitter leaf, BL), *Garcinia kola* (Bitter kola, BC) and *Gongronema latifolium* (Utazi, UT) and their blends were evaluated against several test organisms, *staphylococcus aureus, Bacillus subtilis*, *Escherichia coli* and *streptococcus salivanus*. The study revealed that media containing a crude extract of UT showed no zone of growth inhibition against *Escherichia coli* and *Streptococcus salivanus* while BC had no effect on *Escherichia coli* at all. UT: BL: BC and BL: BC blends were the most active blends while BL was the most active single plant extract (Oshodi *et al.,* 2004).

A study by Akoachere *et al.* (2002) to investigate the anti-bacterial activity of bitter kola *(Garcinia kola)* and ginger *(Zingiber officianale)* on four respiratory tract pathogens, viz *Staphylococcus aureus,*

Streptococcus pyogens, Streptococcus pneumoniae and Haemophilus influenza revealed that the extract from ginger and *Garcinia kola* exhibited anti-bacterial activity against the pathogens. A study by Elekwa (2003) on the effect of aqueous extracts of *Garcinia kola* seeds on membrane stability of human erythrocytes showed the possible use of the extract for the management of sickle cell crisis. The seed of *Garcinia kola* has shown anti-inflammatory, anti-bacterial, anti-microbial, and anti-viral properties (Ebana *et al.,* 1991; Akoachere *et al.,* 2002). According to Esiegwu and Udedibie (2009), *Garcinia kola* seed meal fed to broiler chicks at 5.0% and 7.5% dietary levels suppressed the growth of *Salmonella species* in the birds but had no effect on *Escherichia coli.* In laboratory tests, bitter kola was found to halt the deadly disease caused by Ebola virus in its tracks. Compounds from the plant have also proved effective against some strains of flu, a contagious respiratory disease also called influenza (Iwu, 1993). The seed of *Garcinia kola* has also shown a mild bronchodilator effect (Orie and Ekon, 1993). A study to investigate the anti-ulcerogenic and gastric acid lowering effects of *Garcinia kola* seeds in male albino rats containing 25%, 50% and 75% by weight of bitter kola showed a dose-dependent inhibition of gastric acid secretions and indomethacin-induced ulceration (Ibironke *et al.,* 1996). According to Aniche and Uwakwe (1990), the chemical, brewing and anti-microbial properties of *Garcinia kola* were compared with traditional hops; hops had higher concentration of organic acid than *Garcinia kola*. Laboratory brewing trials with *Garcinia kola* and hops gave beers with similar chemical properties; organolepticallly, *Garcinia kola* beer was as acceptable to tasters as hopped beer except that it had improved bitterness and finally *Garcinia kola* and hop extracts exerted similar anti-microbial effects on two beer spoilage micro-organisms *(Lactobacillus delbruckii and Candida vini).*

2.18 Effect of kolaviron (a *Garcinia kola* extract) on animals

The pharmacodynamic mechanism of *Garcinia kola* is anchored on kolaviron (Farombi *et al.,* 2000; Farombi, 2004; Adaramoye *et al.,* 2005). Kolaviron is a yellow solid when extracted and is a mixture of Garcinia biflavonones GBI, GB2 and kola flavanone (KF). Kolaviron, a type of biflavonoids obtained from *Garcinia kola* seeds produced significant hypoglycemic effects when administered intraperitoneally to normal and alloxan diabetic rabbits at a dose of 100mg/kg. The fasting blood sugar in normoglycaemic rabbits was reduced from 115mg/100ml to 65mg/100ml after 4 hours and in alloxan diabetic rabbits, the blood sugar was lowered from 506mg/ml to 285mg/ml after 12 hours (Iwu *et al.,* 1990).

Kolaviron, a *Garcinia kola* extract has been shown to modulate the hepatoxicity of carbon tetrachloride, galactosamine, aminata toxin, paracetamol thioacetamide and 2-acetyl-aminofluorene in various experimental models. The hepatoprotective effect of *Garcinia kola* seed extract was investigated in rats treated with high doses of paracetamol. The extracts when administered at 100mg/kg three times daily for 5 consecutive days reduced paracetamol – (800, 1000, 1200mg/kg), induced lethality from 50, 90, and 100% to 0, 20 and 40%, respectively (Akintowa and Essien, 1990).

In addition, the anti-hepatotoxic properties of *Garcinia kola* have been evaluated using four experimental toxins, namely carbon tetrachloride, galactosamine, alpha amanitin and phalloidin. Kolaviron, a fraction of the defatted ethanol extract and two billabongs of *Garcinia kola* seed (GB1 and GB2), significantly modified the action of this hepatitis. At 100mg/kg orally, the test substances reduced thiopental-induced sleep in CC14 poison rats and protected microsomal enzymes against phalloidin toxin

(Iwu *et al.,* 1987). Kolaviron from *Garcinia kola* seeds reduced lethal poisoning of mice by phalloidin. The biflavanones GB1, GB2 and kolaflavanone were isolated as the active constituents (Iwu, 1985).

Kolaviron, an extract of *Garcinia kola* has been shown to prevent lipid peroxidation products and protect biomembranes against oxidative damage by acting as *in vivo* anti-oxidant in animal studies (Farombi *et al.,* 2000). *Garcinia kola* inhibited *in vitro* lipid peroxidation of rat liver homogenate in a dose-dependent manner (Adegoke *et al.,* 1998). Possible anti-atherogenic effects of kolaviron (a *Garcinia kola* seed extract) in hypercholesterolaemic rats were investigated and it was revealed that kolaviron reduced plasma cholesterol levels and the relative weight of the heart in cholesterol fed animals (Adaramoye *et al.,* 2005).

Garcinia kola (*G. kola*) clinically appeared to have a significant analgesic/anti-inflammatory effects in knee osteoarthritis patients. According to Olayinka *et al.* (2008), *Garcinia kola* is effective in improving locomotors function and significant pain reduction in patients with knee osteoarthritis.

2.19 Safety of *Garcinia kola*

Garcinia kola is safe taken with or without other foods. Taking it an hour before or after meals may help to increase the absorption of the key ingredients.

Food does not affect the metabolism of *Garcinia kola* and may buffer the effects of mild indigestion (Iwu, 1986).
Kolaviron does not appear to have a pronounced effect on drug metabolizing enzymes (Farombi *et al.,* 2000) and no known interaction with orthodox medications (Okoli, 1991).

2.20 The feed value of *Garcinia kola* for farm animals

Although *Garcinia kola* is being chewed or eaten by many Nigerians and in most parts of Africa as an aphrodisiac, for the medicinal value or for pleasure. Not much work has been done to determine its feed value for animals in terms of feed intake, growth rate and feed conversion ratio. However, Uko *et al.* (2001) reported that water extract from *Garcinia kola* administered to growing winstar rats in three doses of 0, 10, 20 mg/100g body weight of rats daily to respective group of 15 rats for a period of 70 days showed depressive effect on appetite and water intake with resultant poor feed utilization efficiency and weight gain of rats in a dose-dependent manner.

Contrary to the report of Uko *et al.* (2001), Esiegwu and Udedibie (2009) reported that *Garcinia kola* seed meal in broiler diets at 0%, 2.5%, 5.0% and 7.5% levels fed to groups of 30 broiler chicks for 8 weeks recorded no significant differences in feed intake among the groups ($P>0.5$) but the group on 2.5% b *Garcinia kola* diet had significantly ($P<0.5$) heavier body weight and superior feed conversion ratio than the other groups. *Garcinia kola* has being reported to improve digestibility when chewed in small pieces before any meal (Kafaru, 1998). Improved digestibility could enhance intake and consequent gain in weight.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The study was carried out in the Poultry Unit of the Teaching and Research Farm of the School of Agriculture and Agricultural Technology and Animal Science Laboratory of the Federal University of Technology, Owerri, Imo State, Nigeria. The serum biochemical/haematological, microbial and histopathological aspects of the study were done at the Federal Medical Centre, Owerri. The mineral analysis was done at the plant physiology laboratory, University of Port Harcourt.

3.2 Sources, processing and analysis of bitter kola (*Garcinia* kola)

The bitter kola seeds used in the study were bought from Omuma market in Oru East Local Government Area of Imo State, Nigeria. The bitter kola seeds were cut into small pieces with kitchen knife, spread evenly on a mat and sun-dried for 7 days. It was then milled into a fine powdery bitter kola seed meal (BKSM). Samples of the meal were subjected to mineral and vitamin analysis according to AOAC (1990).

Procedure for acid digestion of organic materials for metal determination

Mineral analyses were determined according to the method of Shahidi *et al*. (1999).
2.0g of the bitter kola sample was weighed into a kjeldahl flask; 25ml of concentrated nitric acid (HNO_3) was added (for pre-oxidation) and the sample pre-digested by heating gently at 200 - 250^{o}c for 30 minutes. When the brown fumes produced initially had ceased, the beaker was set down from the heating mantle and allowed to cool. About 10ml of

concentrated nitric acid (HNO3) was thereafter added and digestion was continued for more 60 minutes. Digestion was stopped when a clear digestion was obtained. Initially, thick white fumes formed at first, subsided as digestion progressed and finally left colourless residue behind. The beaker was set down from the heating mantle and allowed to cool very well.

Distilled water was added to make up solution to 50ml. Finally, the solution was filtered through whatmen no.42, 150mm diameter filter paper into a 50ml volumetric flask.

The resulting solution was analysed for heavy metals using the atomic absorption spectrophotometer (AAS).

Atomic absorption spectrophotometry is based on the principle that metallic elements in the ground state will absorb light of the same wavelength which they emit when given excited element is passed through a flame containing ground state atoms of that element, the intensity of the transmitted radiation will decrease in proportion to the amount of ground state elements in the flame.

The metals (minerals) analysed included calcium (424.7nm), magnesium (285.2nm), sodium (589nm), potassium (766.5nm), iron (248.3nm), zinc (213.9nm), copper (324.7nm), manganese (279.5nm), phosphorus (660nm), nitrogen(N_2), sulphur(S) and chloride(Cl). Vitamin C was also analyzed.

3.3 Feeding trials

Feeding trials were carried out to determine the value of *Garcinia kola* (bitter kola) as feed ingredient and anti-microbial agent for:

i. Laying hens and
ii. Rabbits.

Plate 1: Bitter kola tree

Plate 2: Bitter kola fruits

Plate 3: Bitter kola seeds

3.3.1 **Trial with laying hens**

3.3.1.1 **Experimental diets**

The *Garcinia kola* seed meal was used to formulate four layers mash (17% cp) at inclusion levels of 0%, 2.5%, 5.0% and 7.5%, respectively. The ingredient compositions of the diets are shown in table 3.1.

3.3.1.2 **Experimental birds and design**

One hundred and twenty, 36 weeks old laying hens of Hyline breed were used for the experiment. The birds were randomly divided into four groups of 30 layers each and each group randomly assigned to one of the four treatment diets in a completely randomized design (CRD). Each group was further subdivided into three replicates of 10 layers each and each replicate housed in a deep litter compartment measuring 2m x 1.5m. Water was provided *ad libitum*. The birds were weighed at the beginning of the experiment to obtain their initial body weights and at the end of the feeding trial to determine their body weight changes. The trial lasted for 12 weeks.

3.3.1.3 **Data collections**

Data were collected on the initial and final body weight, daily feed intake, hen-day egg production, egg weights, internal and external egg characteristics. Feed intake was determined by weighing the feed offered and the left-over the following day. The difference between the two values was taken as feed taken. Feed conversion ratio was determined by dividing average feed intake by average daily egg weight. A crate of eggs

Table 3.1: **Ingredient and calculated chemical composition of the experimental layers diets**

	Dietary levels of *Garcinia kola* seed meal			
Ingredients (%)	0	2.5	5.0	7.5
Maize	48.00	45.50	43.00	40.50
Bitter kola	0.00	2.50	5.00	7.50
Soya bean meal	15.00	15.00	15.00	15.00
Fishmeal	2.00	2.00	2.00	2.00
Blood meal	3.00	3.00	3.00	3.00
Wheat offal	14.00	14.00	14.00	14.00
Palm kernel cake	7.00	7.00	7.00	7.00
Bone meal	10.00	10.00	10.00	10.00
Salt	0.25	0.25	0.25	0.25
*Tm/Vit. premix	0.25	0.25	0.25	0.25
L-lysine	0.25	0.25	0.25	0.25
L-Methionine	0.25	0.25	0.25	0.25
Calculated Chemical Composition (% of dm)				
CP	17.95	17.80	17.64	17.48
CF	4.52	4.96	5.41	5.85
EE	3.85	3.97	4.10	4.24
Ash	4.47	3.23	3.22	3.22
NFE	69.21	70.04	69.63	69.21
Ca	3.75	3.75	3.75	3.75
P	2.10	2.17	2.16	2.16
Lysine	1.18	1.18	1.17	1.16
Methionine	0.57	0.57	0.56	0.56
ME (mcal/kg)	2.52	2.51	2.50	2.48

*Provided the following per kg of feed: vitamin A, 10,000iu; vitamin D_3, 2000 iu; vitamin B1, 0.75mg; nicotinic acid, 25mg; calcium pantothenate, 12.50mg; vitamin B_{12}, 2.5mg; vitamin k_3, 2.5mg; vitamin E, 2.5mg; cobalt, 0.40mg; biotin, 0.50mg; folic acid, 10.0mg; choline chloride, 25mg; copper, 8.00mg; manganese, 64mg; iron, 32mg; zinc, 40mg; iodine, 0.8mg; flavomycine, 100mg; spiromycine, 5mg; DL-methionine, 50mg; selenium, 0.16mg; L-lysine, 120mg.

from each replicate was weighed weekly to obtain average egg weights. Eggs were collected daily at 8.30 am and 4.00 pm.

Egg Quality Indices: egg shell thickness, yolk width, albumen index and Haugh unit (HU)) were determined in the middle and end of the experiment.

Egg weight – Three eggs were collected weekly from each replicate, labeled and taken to the laboratory and weighed to the nearest 0.01g using sensitive top loading electric balance.

Egg width – This was measured using vernier caliper – the result obtained from the measurement of egg circumference and egg length were used to compute the egg shape index.

Egg length – The length of each egg was measured from the pointed end to the blunt end with the aid of vernier sliding caliper.

Egg shape index – This was done according to Anyanwale *et al.* (2006), using the formula:

$$\text{Egg Shape Index} = \frac{\text{Egg width}}{\text{Egg length}}$$

Egg shell index (1) – This was calculated according to Sauver (1988) and Iposu *et al.* (1994), using the formula:

$$\text{Egg shell index (1)} = 100\ SW / S$$

Where:

SW = shell weight (g)

S = surface (cm^2)

S was calculated from egg weight

(EW).

$$S = K.\ EW^{2/3}$$

Where K has a value of 4.67 for egg weight less than 60g, 4.68 for egg weight between 60g and 70g and 4.69 for egg weight greater than 70g respectively.

Egg specific gravity (ESG) – ESG was estimated from the weight of the egg and shell as outlined by poultry adviser (1992).

ESG = EW / [0.9680 (EW-SW) + (0.4921 SW)]

Egg shell thickness – Each egg used for internal egg quality measurement was broken on a small Petri-dish. Shell thickness was measured with a micrometer screw guage. The measurement was taken from the pointed end, the middle and round part of the egg and the mean obtained. The reading was taken to the nearest 0.01mm.

Egg shell weight – Egg shell was sun-dried for 2 days and the weight taken using sensitive top loading electric weighing balance to the nearest 0.01g. The shell weight was expressed as a percentage of the egg weight and recorded as percent shell (% shell) for individual eggs.

Yolk weight - The yolk was carefully separated from the albumen using a plastic egg separator and weighed individually with an electronic sensitive weighing balance to the nearest 0.01g.

Relative yolk weight - This was calculated in percentage by relating the yolk weight (to the nearest gramme) to the whole weight of the particular egg and multiplied by 100.

Yolk width – The yolk width was measured around the widest horizontal circumference using vernier caliper.

Albumen weight – The albumen of the broken fresh egg was carefully separated from the yolk and weighed. The albumen weight was expressed as a percentage of the egg weight and recorded as percent

albumen (% albumen) for individual egg sample (i.e. relative albumen weight).

Albumen height – This was taken as the height of the thick white of the chalazae at a point about the midway between the inner and outer circumference of thick white with tripod micrometer (P 6085 spherometer) having an accuracy of 0.01mm.

Haugh unit (HU) – Egg weight and albumen height were used to compute the Haugh unit for individual egg sample using the simplified formula of Haugh (1937) as cited by Asuquo *et al.* (1992).

$$HU = 100 \log (H + 7.5 - 1.7 W^{0.37}).$$

Where HU = Haugh unit

H = Height of thick albumen in millimeter.

W = weight of eggs in grams

Hen day production percentage (HDPP) –

The HDPP was calculated as

$$HDDP = \frac{\text{No. of eggs laid per group X 100}}{\text{Total no of birds alive}}$$

Egg breaking strength (EBS) – this was calculated using the formula suggested by Arad and Marder (1982) and presented as

EBS = 50.86 X [EW] 0.915

Where EW = egg weight (gram)

3.3.1.4 Microbial studies

Intestinal droppings of the laying hens were collected at the first week and last week of the experiment for parasitological analysis. The

droppings were cultured for the presence of *Salmonella spp*., *Escherichia coli*, *streptococcus spp.* and *Staphylococcus aureus*. Parasites isolated were cyst of *Isospora belli* and *Ascaridia lumbricoides.*

Procedure for culturing of bacteria in droppings/ feacal samples

The micro-organisms were cultured according to the method of Monica (2000). The specimen was inoculated into a tetrathionate broth and peptone water sugar. It was further inoculated at *Salmonella Shigella* agar (SSA) and deoxycholate citrate agar (DCA). Thereafter it was incubated at 37^{o}c for 24 hours. Urease test was done. The specimen was also inoculated on kingler iron agar (KIA) cultures and incubated at 37^{o}c for 24 hours and the pathogens were examined.

Procedure for analysis of droppings/ faecal samples

A smear of the sample was made on a slide with eosin. The slide was covered with a cover slip avoiding all air bubbles. X10 and X40 objective lens was used to view the specimen microscopically for parasites.

3.3.1.5 **Haematological studies**

Blood samples were collected from 4 birds per treatment at the end of the experiment from the wing veins of the birds using syringe and needle and placed in the specimen bottles with EDTA (Ethylene Diamine Tetra Acetate) for haematological studies. Blood was analyzed within three hours of collection for haemoglobin (Hb) level, white blood cells (WBC), red blood cells (RBC), packed cell volume (PCV), platelets, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), neutrophils, basophils, eusinophil, lymphocytes and monocytes using standard methods (Monica 1984). PCV, RBC, WBC and Haemoglobin

concentration were determined using wintobes microhaematocrit improved neubauer haemocytometer and cyanomethaemoglobin methods respectively. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) were computed according to Jain (1986).

(i) **Haemoglobin estimation (HB)**

The Sahli method was employed in estimating haemoglobin. This involved drawing 0.02mls of blood into 0.01N HCL to form brownish coloured acid haematinin in a Sahli pipette. The sample was placed in a sahli haemoglobin meter and diluted drop by drop with the acid until the colour matched that in the sahli haemoglobin meter and the value recorded in g/100mls (WHO, 1980).

(ii) **White blood cell (leucocyte) count**

Before counting the number of white blood cells present in the sample, a dilution was first prepared which lysed the red cells thus making the white cells readily visible. The suitable diluent used was 2% acetic (20ml/l) tinged with gent ray violent. This in addition to destroying the red cells also stained the white cell nuclei. The blood sample was diluted by washing 50ml blood taken into a displacement pipeltte into 950ml of diluent to give a final dilution of 1ml in 20. The dilute sample was mixed and loaded into the counting chamber. The white cells present in the 4 corner $1mm^2$ area and those in the central $1mm^2$ area were counted. The final white cell count for the whole blood sample was calculated using the basic formula thus:

White cell count = $N \times DF \times 10^6/A \times D$ per litre

Where N = the number of cells

DF = dilution factor

10^6 = converts to cell per litre

A = area of chamber counted

D = the dept of chamber

(iii) **Red blood cells (erythrocytes) count**

The diluent used for visual red cell count was a solution of formal citrate prepared by mixing 10ml of formalin, 40% formaldehyde with 1 litre of trisodium citrate solution, 31.3g per litre. The blood was taken into positive displacement pipette into 40ml of diluent to give a final dilution of 20:1. The diluted sample was mixed and loaded into counting chamber. When the cells had settled out of suspension, the number lying on 5 of the 0.04mm^2 areas were counted using the improved Neubauer haemocytometer (WHO, 1980).

(iv) **Packed cell volume (PCV)**

The packed cell volume was determined by centrifugation using a micro haematocrit technique. The blood was centrifuged at approximately 1200rpm in a special centrifuge (microhaematocrit centrifuge) which automatically attains the correct speed. The PCV was subsequently determined by measuring the height of the red cell column and expressing this as a ratio of the height of the blood column. A PCV reactor was then used to determine the height of both the red cell and total column simultaneously and these were converted to the final result thus;

PCV = Height of red blood cell column/Height of total blood column.

(v) **Mean cell volume (MCV)**

This is the mean volume of the red cells expressed as fento litres.

It was calculated from the PCV and red cell count thus:

MCV = PCV/total red cells

(vi) **Mean cell haemoglobin (MCH)**

This is the amount of haemoglobin present in the average red cells, expressed as picogram. It was calculated from the haemoglobin and red cell count thus:
MCH = Hb x 10/Total red cells (pg)

(vii) **Mean cell haemoglobin concentration (MCHC)**

This is the amount of haemoglobin in 100ml of packed red cells as opposed to the amount present in whole blood. It was calculated from the haemoglobin and PCV and the result expressed as grams per 100ml.
MCHC = Hb/PCVg/100ml.

3.3.1.6 **Serum biochemical analysis**

The pre-blood treatments included:
i) Collection of the blood into clean dry pyrex tubes.
ii) Separation (by centrifugation) to obtain clear (non-blood stained) serum specimen for biochemical assays.
Blood samples were collected from 4 birds per treatment at the end of the experiment from the wing veins of the birds using syringe and needle and placed in the specimen bottles without EDTA (Ethylene Diamine Tetra Acetate) for biochemical studies. Blood serum biochemical indices determined included total proteins, glucose, cholesterol, calcium, sodium, potassium, chloride and carbonate.

(i) **Total protein (Biuret method)**

Principles: - Serum protein forms a violet blue complex with copper ions in alkaline solution. A method using a sample blank is recommended in order to avoid errors due to turbidity.

Techniques: - 25ml each of biuret reagent and blank biuret reagent was pipetted into test tubes for standard and test for each of standard and sample blanks respectively.

50ml of standard (100g/l) or samples were added to each pair of test tubes. A reagent blank was set up for each batch and contains 2.5ml of biuret reagent and 50ml of water. Each of these tubes was mixed and allowed to stand at room temperature for 30minutes. The absorbance's were measured at 540nm (yellow-green filter, ilford No. 605) while setting the spectrometer to zero with blank biuret reagent. The absorbance of the sample blank was first read, followed by the reagent blank and then the tests (Reinhold, 1953).

(ii) **Serum albumin (Method: by Bromo Cresol Green BCG)**

Principle: Albumin has the ability to bind with certain dyes. When Bromo Cresol Green binds to albumin, there is shift in the dyes peak absorption wavelength.

Technique: 4.0ml of working dye solution was pipetted into test tubes. 2.0ul of sample solution was added and mixed. The absorbance was read within 30 seconds at 632nm setting the colorimeter to zero with the working dye solution (Henry, 1974).

Calculation;

Absorbance text x Concentration of standard absorbance STD = Mg urea 100ml.

Where concentration standard = 50mg/100ml.

(iii) **Serum globulin**

This was determined by difference as follows.

Serum globulin = Total protein – Serum albumin

(iv) **Serum calcium**

Calcium values were determined by complex metric procedure which measure calcium in the serum directly (Gitelman, 1967). This is based on the principle that calcium reacts with cresolphthalein complexone in 8-hydroxyquinoline to form a coloured complex (purple colour) that absorbs at 570nm (550-580nn).
The intensity of the colour is proportional to calcium concentration.

Procedure: This involved the use of calcium colour reagent, calcium buffer and calcium standard. Blank, standard, control and treatments tubes were labelled appropriately and 1.0ml of working reagent (buffer and standard) was pipetted into each tube and 0.02ml (20ul) of serum sample added to the respective tubes, mixed and allowed to stand for sixty second (60s) at room temperature. Spectrophotometer was thereafter zeroed with a blank at 570nm wavelength. Absorbance of all tubes were read and recorded. The value of calcium was calculated as follows;

$$\text{Calcium (mg/dl)} = \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

(v) **Serum cholesterol**

This was determined by the ferric chloride-sulphuric acid reaction (modified Leffler method) (Elletson and Caraway, 1970). 5.0ml of isopropyl alcohol was added to 0.2mls of serum in one test-tube and 0.2ml of cholesterol standard in another test tube. The former was mixed thoroughly and centrifuged. 1.0ml of each supernatant fluid was pipetted into the bottom of a dry-glass-stopped 15ml centrifuge tube. Another 1.0ml of isopropyl alcohol was pipetted into a clean dry tube to act as blank; 3.0ml of glacial acetic acid was added to all the tubes and mixed. Subsequently, 0.3ml of iron was added and mixed. Thereafter at about

15 seconds intervals, 3.0ml of concentrated sulphuric acid was added to one tube at a time and allowed to cool. Each solution was measured for absorbance viz: standard and unknown against the reagent blank at 560nm. Cholesterol was determined as follows:

$$\text{Mg cholesterol/100ml} = \frac{\text{Unknown}}{\text{Standard}} \times 200$$

(vi) **Serum glucose**

Serum sugar was determined by the Tinder method (Tinder, 1959). The principle behind this methodology is the oxidation of B-D-Glucose by glucose oxidase to produce D-gluconic acid and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4-aminoantipyrine and phenol substitute, P-HBS, in the presence of peroxidase to yield a red quineoneiminedye. The amount of coloured complex formed is proportional to glucose concentration and can be photometrically measured.

Procedure: Blank, standard, control and treatment test tubes were labelled and 1.0ml of working reagent were pipetted into all tubes and placed in 37^0c heating bath for five (5) minutes. 0.005ml (5ul) of serum sample was added to each tube mixed and incubated at 37^0C for ten (10) minutes. Spectrophotometer was there after zeroed with blank sample and absorbance of all tubes read at 500nm. Sugar was there after calculated as follows

$$\text{Concentration of sugar (mg/dl)} = \frac{\text{Treatment}}{\text{dard}} \times \text{concentration of Standard}$$

(vii) **Serum sodium**

The colorimetric method was employed (Tietz 1976). This method involved the use of filtrate reagent, acid reagent, sodium colour reagent and sodium standard. Blank, standard, control and treatment test tubes were well labelled and 1.0ml of filtrate reagent pipetted to all the tubes. 0.05ml (50ul) of serum sample was added to all tubes and distilled water to the blank and shaken vigorously. Tubes were centrifuged at high speed (1500G) for ten minutes and the supernatant tested by adding 1.0ml of acid reagent to all tubes.

0.05ml (50ul) of supernatant were added to all tubes and mixed. 0.05ml of colour reagent was also added to all tubes and mixed. Spectrophotometer was zeroed with distilled water at 550nm. Absorbance of all tubes were read and recorded. Concentration of sodium were calculated as follows.

$$\text{Conc. of STD (mEq/L)} = \frac{\text{(Abs. of Blank - Abs of S)}}{\text{(Abs. of Blank- Abs of STD)}} \times \text{Conc. of STD (mEq/L)}$$

Where S = sample serum

Abs = Absorbance

Conc = concentration

STD = Standard

(viii) **Serum Chloride**

The procedure used to determine the chloride content of serum was based on the modified colorimetric method of skeggs and Hochestrasser (1964). This is based on the principle that chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a colour complex that absorbs light at

480 nm. The intensity of the colour produced is directly proportional to the chloride concentration.

Procedure: Chloride reagent and chloride calibrator were used. Blank, calibrator and treatment test tubes were labelled and 1.5ml chloride reagent was pipetted to each tubes. 0.01ml (10ul) of calibrator or sample serum was added to respective tubes, mixed and incubated at room temperature for five (5) minutes. Spectrophotometer was set to 480 nm and zeroed with reagent blank. Absorbance reading of all tubes were read and recorded. Chloride concentration was calculated using the following formular;

$$\text{concentration of chloride (mEq/L)} = \frac{\text{Abs. of unknown}}{\text{Abs. of calibrator}} \times \text{concentration of calibrator}$$

Where

Abs = Absorbance.

(ix) **Serum potassium**

Serum potassium was determined by the colorimetric method (Tietz, 1976). This is a direct spectrophotometric measurement of potassium in blood or plasma based on the principle that sodium tetraphenylboron is used in specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration.

Procedure: Potassium reagent and potassium standard were used. The standard, control, treatment and blank test tubes were labelled. 1.0ml of potassium reagent were pipetted to all tubes. 0.01ml (10ul) of samples were added to all tubes, mixed and left at room temperature for 3 minutes. Wavelength of spectrophotometer were set to 500 nm and then

zeroed with reagent blank. Absorbance of all tubes were read and recorded. The concentration of potassium were calculated thus;

$$\text{conc. of potassium(mEq/L)} = \frac{\text{Abs. of unknown}}{\text{Abs. of STD (mEq/L)}} \times \text{conc. of STD}$$

Where

Abs = Absorbance

STD = Standard

Conc. = Concentration.

(x) **Serum Bicarborate**

Bicarbonate concentration in Serum was measured by the spectrophotometric procedures (Natelson, 1951).

3.3.1.7 Carcass evaluation

At the end of the feeding trial, four birds were randomly selected from each treatment, weighed, slaughtered, defeathered and eviscerated. The live weights and dressed weights were recorded and the internal organ weights (liver, kidney, heart, gizzard and intestine) as well as abdominal fat were expressed as percentage of live weight.

3.3.1.8 Histological studies

The internal organs (liver, kidney, heart, and gizzard) after being weighed, were preserved in 10% formalin and taken to laboratory for histological observations. Histographs of organ tissues were also taken.

Histological procedure

Histological studies were carried out according to Baker *et al* (1989). Excised organs were fixed in 10% formol saline for 24hrs after which they were dehydrated in ascending grades of alcohol (70%, 80%, 90%, Absolute) and dealcoholized in xylene (three changes) for 30mins. The tissues were impregnated/infiltrated in molten paraffin wax and subsequently embedded

using disposable plastic embedding moulds. The embedded tissues were sectioned with a Rotary microtome and sections stained with haematoxylin and eosin (H/E) staining procedure.

Microscopy

The sections were examined under the microscope (DM 500 leica binocular microscope) and observations noted.

Photomicrography

The examined slides were photomicrographed with luca DM 500 binocular microscope with photomicrographic accessories.

3.3.1.9 **Cost Analysis**

Efficiency of production was determined as follows: -
Cost of feed (₦/kg) = a
Kg feed/kg wt. gain = b
Cost of feed/kg wt. gain = a x b

3.3.1.10 **Statistical analysis**

Data collected were subjected to Analysis of Variance (Snedecor and Cochran, 1978). Where analysis of variance indicated significant treatment effects, means were compared using Duncan's New Multiple Range Test (DNMR) as outlined by Obi (1990).

3.3.2 **Trial with rabbits**

3.3.2.1 **Experimental diets**

The bitter kola seed meal was used to formulate four rabbit growers mash at inclusion levels of 0%, 2.5%, 5.0%, and 7.5%, respectively. The ingredient composition of the diets is shown in table 7.

3.3.2.2 **Experimental rabbits and design**

Thirty-six eight-week old growing rabbits of mixed breeds were purchased from a reputable rabbit farm in Ahoada L.G.A of Rivers State.

Table 7: **Ingredient and calculated chemical composition of the experimental diets.**

	Dietary levels of bitter kola seed meal			
Ingredients (%)	0.00	2.50	5.00	7.50
Maize	47.00	44.50	42.00	39.50
Bitter kola	0.00	2.50	5.00	7.50
Soya bean meal	9.00	9.00	9.00	9.00
Fishmeal	2.00	2.00	2.00	2.00
Blood meal	1.00	1.00	1.5	1.5
Wheat offal	26.00	26.00	25.5	25.5
Palm kernel cake	11.00	11.00	11.00	11.00
Bone meal	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25
*Tm/Vit premix	0.25	0.25	0.25	0.25
L-lysine	0.25	0.25	0.25	0.25
L-methionine	0.25	0.25	0.25	0.25
Calculated Chemical Composition (% of dm)				
CP	16.31	16.15	15.99	15.84
CF	5.71	6.15	6.60	7.05
EE	4.47	4.61	4.74	4.88
Ash	3.72	3.72	3.71	3.70
NFE	69.79	69.37	68.96	68.53
Ca	1.24	1.24	1.24	1.24
P	1.12	1.11	1.10	1.09
Lysine	1.03	1.03	1.02	1.01
Methionine	0.55	0.54	0.54	0.53
ME (mcal/kg)	2.54	2.53	2.51	2.50

*To provide the following per kilogram of diet: Vit. A, 10,000 iu; Vit. D3, 2000 iu; Vit. E, 5 iu; Vit. K, 2mg; Riboflavin, 4.20mg; Vit. B_{12}, 0.01mg; Panthotenic acid, 5mg; Nicotinic acid, 20mg; Folic acid, 0.5mg; Choline, 3mg; Mg, 56mg, Fe, 20mg; Cu, 10mg; Zn, 50mg; Co, 125mg.

The rabbits were randomly divided into four groups of nine (9) rabbits each and each group randomly assigned to one of the four treatment diets in a completely randomized design (CRD). Each group was further sub-divided into three replicates of three (3) rabbits each. The rabbits were housed in 1m by 0.7m hutch. They were weighed at the beginning of the experiment to obtain their initial body weights and weekly thereafter. Feed and water were supplied *ad libitum* in separate earthen troughs.

3.3.2.3 **Data collection**

Data were collected on initial body weights and weekly body weights. These were used to calculate their weight gain and average daily weight gain. Feed intake was recorded daily. Feed intake was determined by weighing the feed offered and the left-over the following day. The difference between the two values was taken as feed consumed. Feed conversion ratio was determined by dividing average daily feed intake by average daily gain (ADG).

3.3.2.4 **Microbial studies**

Faecal samples were collected at the first week and last week of the experiment for parasitological analysis. The faecal samples were cultured for the presence of *Salmonella* spp., *Escherichia coli, Staphylococcus aureus* and *Streptococcus* species. Parasytes isolated were cyst of *Isospora belli* and *Ascaridia lumbricoides.* Procedures for culturing/analyses of micro-organism and parasites were as in layer trial above.

3.3.2.5 **Haematological and serum biochemical studies**

Blood samples were collected at the 8th week of the experiment from 4 rabbits per treatment through the neck slitting using syringe and placed in the specimen bottles with and without EDTA (Ethylene Diamine Tetra Acetate) for haematological and serum biochemical studies, respectively. Blood was analyzed within three hours of collection for haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), platelets, packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), neutrophils, basophils, eusinophils, lymphocytes and monocytes, using standard methods (Monica 1984). Serum biochemical indices analyzed were total proteins, glucose, cholesterol, calcium, sodium, potassium, chloride, and carbonate. Procedures for analyses were as in layer trial above.

3.3.2.6 Carcass evaluation

At the end of the 56 day feeding trial, four rabbits from each treatment were randomly selected and starved overnight to clean up the gut. Their live weights were recorded before stunning and slitting at the jugular vein. They were bled up to 50% and skinned by hanging them on a hook and the fur skin pulled downwards after being cut at the neck to make for easy pulling. Evisceration was then carried out. The kidney, heart, liver, were removed and weighed. The weight of the organs were expressed as percentage of the live weight.

3.3.2.7 Histological studies

The internal organs (liver, heart and kidney) after being carefully weighed were preserved in 10% formalin and taken to the laboratory for histopathological examination. Histographs of organ tissues were also taken.

3.3.2.8 **Statistical analysis**

Data collected were subjected to statistical analysis using the analysis of variance (ANOVA) according to Snedecor and Cochran (1978). Where analysis of variance indicated significant treatment effects, means were compared using Duncan's New Multiple Range Test (DNMRT) as outlined by Obi (1990).

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 **Mineral and vitamin composition of bitter kola**

The mineral and vitamin C composition of bitter kola used for the studies are shown in table 4.1. The value 0.012mg/g for Mn, 0.28mg/g for Mg, 0.25mg/g for Ca, and 1.61mg/g for K were close to the values, 2.01mg/100g for Mn, 0.42mg/100g for Mg, 0.80mg/100g for Ca and 2.50mg/100g for K respectively, reported by Okwu (2005). The value of zinc (0.03mg/g) tended to agree with the value reported by Okwu (2005) and Odebunmi *et al.* (2009). The values for P, Na, Fe and Cu varied from the values obtained by Okwu (2005) and Odebunmi *et al.* (2009). The variation in values of the mineral contents of bitter kola could be attributed to the method of processing the bitter kola, the season when the bitter kola was harvested, the storage method, the length of time it stored before processing for analysis, the length of time the powdered seed meal stored before analysis and the method of analysis. Vitamin C value (11.43mg/100g) was close to the value 23.10mg/100g obtained by Okwu (2005).

4.2 **Performance of the experimental birds**

The performance of the experimental laying hens fed graded levels of bitter kola seed meal is presented in table 4.2.

4.2.1 **Average initial weight**

The average initial body weights of the laying hens ranged from 1503.00g to 1643.33g. There were no significant differences ($P>0.05$) in the initial body weights of the laying hens.

Table 4.1: **Mineral and vitamin C composition of *Garcinia kola* seeds (dry weight basis)**

Minerals	Amount (mg/g)
Macro elements	
Magnesium	0.28
Calcium	0.25
Potassium	1.61
Phosphorus	0.057
Sodium	0.282
Chlorine	0.400
Sulphur	0.073
Micro elements	
Fe	0.02
Zinc	0.003
Cupper	0.05
Manganese	0.012
Vitamin	(mg/100g)
Vitamin C	11.43

Table 4.2: Performance of the experimental laying hens

Parameters	Dietary levels of *Garcinia kola* seed meal (%)				SEM
	T_o	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Initial body weight (g)	1643.33	1560.00	1503.00	1520.50	24.78
Av. final body weight (g)	1754.33[a]	1683.33[ab]	1634.00[b]	1710.00[a]	19.75
Av. body weight changes (g)	111.00[b]	123.33[b]	130.67[b]	190.00[a]	12.87
Av. daily feed intake (g)	133.02	132.38	135.84	136.74	0.78
Mean hen-day egg production (%)	67.43[a]	54.83[b]	63.73[a]	59.00[ab]	3.25
Egg weight (g)	58.54[b]	60.27[a]	60.78[a]	59.55[ab]	0.36
Feed conversion ratio (g feed/g egg)	2.27	2.20	2.23	2.30	0.02
Mortality	0.00	0.00	0.00	0.00	-

[ab]Means within the same row with different superscripts are significantly different ($P<0.05$).

4.2.2 **Average final body weight**

Average final body weights of the layers ranged from 1634.00g to 1754.33g. There were no significant differences (P>0.05) in the final body weight among the treatment groups and between the control and the bitter kola groups except at 5% dietary inclusion levels. However since no significant differences existed between the control and 7.5% dietary level which was the highest inclusion level, it indicated that the bitter kola posed no adverse effect on the weight of the birds.

4.2.3 **Average body weight change**

Average body weight changes ranged from 111.00g to 190.00g. The control had the least average body weight change. $T_{7.5}$ had the highest average body weight change and was significantly different (P<0.05) from the control. This is contrary to the findings of Esiegwu and Udedibie (2009) where 2.5% dietary level of *Garcinia kola* seed meal recorded significantly (P<0.5) heavier body weight gain in similar experiment with broilers. These findings may suggest that bitter kola seed meal has no depressive effect on body weight of laying hens.

4.2.4 **Average daily feed intake**

The average daily feed intake of the layers ranged from 132.38g to 136.74g. There were no significant differences (P>0.05) among the treatment groups. $T_{2.5}$ had the least feed intake of 132.38g whereas $T_{7.5}$ had the highest feed intake of 136.74g. This is contrary to the report of Uko *et al*. (2001) that water extract from *Garcinia kola* had a depressive effect on appetite. Esiegwu and Udedibe (2009) recorded no differences in feed intake of broilers fed *Garcinia kola* seed meal diets.

4.2.5 **Mean hen-day egg production**

The mean hen-day egg production ranged from 54.83 to 67.43. There was significant difference (P<0.05) between the hen-day egg production of the control and the $T_{2.5}$ group. The control recorded the highest mean hen-day egg production followed by $T_{5.0}$, $T_{7.5}$ and $T_{2.5}$ respectively.

4.2.6 **Egg weight**

The egg weights were 58.54, 60.27, 60.78 and 59.55 for the control, $T_{2.5}$, $T_{5.0}$ and $T_{7.5,}$ respectively. Significant differences (P>0.05) existed among the treatment groups. The egg weight of $T_{5.0}$ and $T_{7.5}$ were significantly (P<0.05) heavier than that of the control. This contradicts the report of Akpantah *et al.* (2005) that *Garcinia kola* seed extract reduced the weight of foetus.

4.2.7 Feed conversion ratio

The feed conversion ratios were 2.27, 2.20, 2.23 and 2.30 for the control, $T_{2.5}$, $T_{5.0}$, $T_{7.5}$ respectively. No significant differences ($P>0.05$) existed among the treatment groups. $T_{2.5}$ had the best feed conversion ratio. This finding agrees with the report of Esiegwu and Udedibie (2009) in a similar experiment with broilers placed on *Garcinia kola* seed meal where 2.5% dietary level had a superior feed conversion ratio.

4.2.8 **Economics of production**

The economics of production of the experimental laying birds is summarized in table 4.3.

4.2.9 **Feed cost (₦/Kg)**

The feed cost for the treatment groups (₦/kg) were 82.61, 109.26, 135.91 and 162.56 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5,}$ respectively. The cost per kg of feed increased as the *Garcinia kola* levels increased. The percent increases in value were 32.26, 64.52 and 96.78 for $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for the *Garcinia kola* group compared to the control.

4.2.10 **Feed cost per kg eggs (₦)**

The cost of feed per kg of eggs were 187.52, 240.37, 303.08 and 373.89 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. The cost of feed per kg of eggs increased as the dietary levels of *Garcinia kola* increased. The percent increases were 28.18%, 61.62 and 99.33 for $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for the *Garcinia kola* groups compared to the control.

Table 4.3: Economics of egg production of the experimental laying hens

Parameters	Dietary levels of *Garcinia kola* seed meal (%)				
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	SEM
Feed conversion ratio (kg feed/kg egg)	2.27	2.20	2.23	2.30	0.02
Feed cost (₦/kg feed)	82.61	109.26	135.91	162.56	-
Cost of production (₦/kg egg)	187.52	240.37	303.08	373.89	-

4.2.11 **Egg quality characteristics**

Egg quality characteristics of the experimental layers fed graded levels of *Garcinia kola* seed meal are shown in table 4.4.

(i) **Egg weight**

The egg weights ranged from 60.09 to 64.86. There was significant difference ($P<0.05$) between the control and the treated groups. The weight of the eggs of the bitter kola groups were heavier than the control.

The egg weights were close to the egg weights reported by Okonkwo and Oketola (1996) and Durunna *et al.* (2007) which ranged from 60.54 to 61.11. Birds fed diets $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ laid eggs with heavier weights. This may be due to the numerically lower production values observed.

Kuhl and Sullivan (1977) observed a similar effect and suggested that heavier egg weight was not a dietary effect but a function of egg number. That means that birds that lay fewer number of eggs tend to lay heavier eggs. The values of the weight of the eggs suggested that bitter kola had no negative effect on the weight of the eggs when compared to the 58 gm, average egg size reported by Oluyemi and Roberts (2000).

(ii) **Egg width, egg length and egg shape index**

The values ranged from 4.09 to 4.22 for egg width, 5.49 to 5.63 for egg length and 0.74 to 0.77 for egg shape index. There were no significant differences ($P>0.05$) in the egg width, egg length and egg shape index.

Table 11: Egg characteristics of the experimental laying

Parameters	Dietary levels of *Garcinia kola* seed meal (%)				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
External egg quality:					
Egg weight (g)	60.09^{b}	64.80^{a}	64.86^{a}	63.77^{a}	0.85
Egg width (cm)	4.14	4.22	4.18	4.09	0.03
Egg length (cm)	5.63	5.49	5.55	5.50	0.06
Egg shape index	0.74	0.77	0.75	0.74	0.01
Internal egg quality:					
Haugh unit (HU)	53.60	52.60	50.81	51.15	0.53
Albumen height (mm)	1.48	1.55	1.42	1.41	0.03
Albumen length (cm)	9.81	9.27	9.26	8.59	0.32
Albumen width (cm)	9.01	8.02	7.99	7.38	0.29
Albumen weight (g)	35.49^{c}	47.46^{a}	39.30^{b}	39.61^{b}	1.36
Percent albumen (% egg weight)	59.04^{b}	73.42^{a}	58.61^{b}	62.18^{b}	2.04
Yolk width (cm)	3.56	3.59	3.90	3.60	0.06
Yolk length (cm)	3.82	4.03	4.19	3.95	0.07
Yolk weight (g)	15.72^{a}	13.47^{b}	15.01^{ab}	15.85^{a}	0.37
Percent yolk (% egg weight)	26.17^{a}	20.77^{c}	23.18^{bc}	24.83^{ab}	0.70
Shell weight (g)	4.80	5.05	5.99	5.59	0.25
% egg shell (% egg weight)	7.99	7.78	9.26	8.73	0.41
Egg shell index	10.98^{b}	10.97^{b}	13.03^{a}	12.12^{ab}	0.55
Egg shell thickness (mm)	0.34	0.30	0.31	0.31	0.02
Egg specific gravity	1.08	1.07	1.08	1.08	0.00
Egg breaking strength	2796.56^{b}	3015.74^{a}	3018.23^{a}	2967.66^{ab}	39.73

^{abc}Means within the same row with different superscripts are significantly different (P<0.05)

Plate 4: Eggs from the experimental birds

The values of the egg shape index were very close to an index of 0.75 which is regarded as the most satisfactory when eggs are to be packaged in specialized containers for transportation (Smith, 1990). Belyavin and Boorman (1981) observed that elongated (low index) and heavier eggs were more prone to cracking. Good egg shape enhances marketing and profitability as round eggs do not show good appearances and long eggs are much likely to break during packaging.

From the values of the egg shape index obtained, *Garcinia kola* tends to produce eggs of good shape.

(iii) Albumen height, albumen length, albumen width and percent albumen

The values ranged from 1.41 to 1.55 for albumen height, 8.59 to 9.81 for albumen length, 7.38 to 9.01 for albumen width and 58.61 to 73.42 for percent albumen. There were no significant differences ($P>0.05$) in the albumen height, albumen length and albumen width, even though the values tended to decrease as the dietary treatment levels increased. Percent albumen weight, an indicator of egg quality was significantly affected at $T_{2.5}$. The values ranged from 58.61 to 73.42 with $T_{2.5}$ significantly higher ($P<0.05$) than the control and other treatment groups. The other treatments, T_0, $T_{5.0}$ and $T_{7.5}$ were not significantly different ($P>0.05$). Egg containing a large proportion of thick white is regarded as being of high quality (Harms and Hussein, 1993). Harms and Hussein (1993) also reported that albumen weight is more closely associated with egg weight than yolk weight. The values showed that bitter kola is capable of producing eggs of high quality without producing any deleterious effect.

(iv) **Haugh unit**

Haugh unit is the measure of egg white or albumen quality. The Haugh units were 53.60, 52.60, 50.81 and 51.15 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences ($P>0.05$) in Haugh unit among the treatment groups. The Haugh unit values of the treatments indicated that they were of good quality since they have values higher than 40% which depicts that an egg is of good quality (Brand *et al.,* 1991; Ayorinde *et al.*, 1999), and below it (40%) is regarded as inferior quality egg.

(v) **Yolk Width, Yolk Length and Percent Yolk**

The values ranged from 3.55 to 3.90 for yolk width, 3.82 to 4.19 for yolk length, 13.47 to 15.85 for yolk weight and 20.77 to 26.17 for percent yolk. No significant differences ($P>0.05$) existed among the treatments in yolk width and yolk length. The yolk weight values obtained in the trial (13.47 – 15.85g) were close to the values (14.65 – 15.14) recorded by Adedeji *et al.* (2008) and the values (14.50 – 15.00g) by Fafiolu *et al.* (2006). The yolk percent for the control was significantly ($P<0.05$) better than $T_{2.5}$ and $T_{5.0}$.

(vi) **Shell weight, percent shell weight, egg shell index and shell thickness**

No significant differences ($P>0.05$) existed among the treatment groups in shell weight, percent shell weight, egg shell index and shell thickness. The shell weight ranged from 4.80 to 5.99 and 7.78 to 9.26 for percent shell weight. The values as compared to the control indicated that bitter kola had no deleterious effect on egg shell weight and percent shell.

The values for egg shell index ranged from 10.97 to 13.03 which is higher than the values (8.4 – 9.09) reported by Idowu *et al.* (2006) when laying hens were fed cassava root wastes. T_0 and $T_{2.5}$ were similar in egg shell index. $T_{5.0}$ and $T_{7.5}$ recorded significantly ($P<0.05$) higher values. This tends to suggest that bitter kola seed meal tends to enhance calcium and phosphorus utilization in egg shell formation.

The shell thickness (0.30 – 0.34) reported in this study is in agreement with the values (0.30 – 0.36mm) reported by Oluyemi and Roberts (2000) and close to the range (0.33 – 0.36) reported by Durunna *et al.* (2007). This level of shell thickness may prevent the egg from cracking easily during transportation. However, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ had values lower than 0.34mm reported for the tropical environment (Oluyemi and Roberts 1988). Tion and Orga (2004) also observed that reported values for shell thickness in Nigeria vary considerably but are generally within the 0.35mm – 0.40mm suggested as adequate for shell thickness values (USDA as cited by North, 1981). Only the control had shell thickness value up to the recommended range.

There were no significant differences among the treatments in egg specific gravity ($P>0.05$). However eggs from bitter kola seed meal groups had significantly ($P<0.05$) higher egg breaking strength than the control.

4.2.12 **Haematological indices of the experimental layers**

Data on the haemotological indices of the experimental layers fed graded levels of bitter kola seed meal are presented in table 4.5.

(i) **Haemoglobin counts**

The values of the haemoglobin for the treatment groups were 9.40, 10.05, 10.15 and 9.10g/dl for T_o, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. There were no significant differences ($P>0.05$) between the control and those of the bitter kola groups and also amongst the bitter kola groups.

Table 4.5: Haematological parameters of the experimental laying hens

Parameters	Dietary levels of Garcinia kola seed meal (%)				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Hb (g/dl)	9.40	10.05	10.15	9.10	0.28
PCV (%)	26.45	28.15	28.75	25.20	0.85
RBC ($x10^6$/ul)	2.70	2.53	3.02	2.64	0.10
MCV (fl)	97.40	100.25	95.10	95.35	1.31
MCHC (g/dl)	35.65^{ab}	34.80^{b}	36.30^{a}	36.05^{ab}	0.25
MCH (pq)	34.70	36.35	34.55	34.40	0.36
WBC (mm^3)	36550	40900	41100	37250	1799.60
Lymphocytes (%)	94.00	90.00	90.50	89.50	1.25
Neutrophils (%)	5.50	9.50	9.50	10.50	1.31
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00
Monocytes (%)	0.00	0.00	0.00	0.00	0.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00
Platelets (mm^3)	36000^{a}	22000^{ab}	14500^{b}	13500^{b}	3932.47

^{ab}Means within a row with different superscripts are significantly ($P<0.05$) different.

These values were in agreement with the range of values (9.65 - 11.65%) reported by Emenalom *et al.* (2009) and are within normal range (Merck Veterinary Manual, 1997) and indicates a normal health condition of the birds.

(ii) **Packed cell volume (PCV)**

The values for the packed cell volume for the treatments were 26.45, 28.15, 28.75 and 25.20% for T_o, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences (P>0.05) among the treatments. The values observed in this study (25.20 – 28.75) were within the range (25.00 – 31.00) recorded by Emenalom *et al* (2009); and is within normal range (Mitruka and Rawnsey, 1977).

The values, however, are lower than the mean value (30.7%) reported by Oyewale (1987) for adult female Nigerian domestic chickens and the value (35 - 55%) for caged birds as reported by Campbell and Coles (1986) who also noted that a PCV less than 35% indicates anaemia. However, no symptoms of physiological anaemia were observed during the study.

(iii) **Red blood cell (RBC) (x10^6/ul)**

The RBC values for the treatments were 2.70, 2.53, 3.02 and 2.64 (X10^6/ul) for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5,}$ respectively. There were no significant differences (P>0.05) among the treatment groups. The values (2.53 – 3.02 x $10^{6/}$ul) observed in this study fall within the normal range (2.0 – 4.0 x 10^6ul) reported by Mitruka and Rawnsey (1977) and Heath and Olusanya (1985) who reported a mean value of 3.0 x 10^6/ul.

(iv) **Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).**

The values were 97.40, 100.25, 95.10, and 95.35(fl) for MCV, 34.70, 36.35, 34.55 and 34.40(pg) for MCH and 35.65, 34.80, 36.30 and 36.05g/dl for MCHC. There were no significant differences ($P>0.05$) in the MCV and MCH and their values were within the normal range. The values had no pattern. There was a significant difference ($P<0.05$) in the MCHC between $T_{2.5}$ and $T_{5.0}$ but no significant difference ($p>0.05$) among the control, $T_{2.5}$ and $T_{7.5}$. The values were within normal range.

(v) **Platelets**

The values for platelets were 36000, 22000, 14500 and 13500 mm^3 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively.

The platelet values decreased significantly ($P<0.05$) from the control as the level of bitter kola inclusion increased except in $T_{2.5}$.

Platelets are blood cells concerned with the clotting of blood, especially at the site of an injury to reduce loss of blood (Frandson, 1974; Heath and Olusanya, 1985). These results showed that bitter kola seed meal may obstruct thromboplastin formation (essential for clot formation), vitamin k metabolism and consequently delay blood clotting time when high doses of it are consumed.

(vi) **White blood cells (x10^3/mm)**

The values for white blood cells were 36550, 40900, 41100 and 37250 mm^3 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5,}$ respectively. No significant differences ($P>0.05$) existed between the control and the bitter kola groups. However, the values for the bitter kola groups were consistently higher than the control. These values, varied and non-significant to each other were

within normal WBC range values (Mitruka and Rawnsey, 1977; Heath and Olusanya, 1985). The values of WBC are indication of intact immune system. The values in this study showed that bitter kola has the tendency to boost the phagocytic action of the WBC.

(vii) Lymphocytes, monocytes, neutrophils, eosinophils and basophils

The values were 94.00, 90.00, 90.50 and 89.50% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for lymphocytes, 5.50, 9.50, 9.50 and 10.50% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively for neutrophils. No significant differences ($P>0.05$) occurred. There were no traces of eosinophils, monocytes and basophils in all the treatments. Neutrophils function primarily as phagocytes at sites of inflammation and infection; eosinophils in circulation increases in parasitic diseases and in allergic reactions and monocytes play a role in processing foreign materials (antigens) for anti-body production by plasma cells and lymphocytes (Heath and Olusanya, 1985). Graczyk *et al.* (2003) noted that differential leucocytes were used as indicators of stress response and sensitive biomarkers crucial to immune functions and Jean (1993) reported that bacterial and vital illness affects the number of white corpuscles and the ratio between the different types of white corpuscles and the percentages of the various types in healthy animals vary little but are greatly modified in sick animals.

The value for lymphocytes and neutrophils in this study were within normal range. Generally, the values of the lymphocytes, monocytes, eosinophils, neutrophils and basophils showed that the birds were in good health condition including the control. Both diets were adequate and good for good health of the birds.

4.2.13 **Serum biochemical indices of the experimental laying hens**

Data on the serum biochemical indices of the experimental laying hens fed graded levels of bitter kola seed meal are presented in table 4.6.

(i) **Total proteins**

The serum total protein levels were 6.50, 4.85, 5.50, 6.50 g/100ml for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences ($P>0.05$) between the control and the groups the treated groups. There was no pattern in the values of total proteins.

Serum proteins have been implicated as a pointer to strong amino acid metabolism (Schukle and Pachaurii, 1995). Wilson and Brigstoke (1981) reported that high total proteins is an indicator of sufficient protein intake. The values of serum proteins obtained in this study showed that bitter kola did not inhibit protein metabolism. There was a progressive increase of serum protein among the bitter kola groups as the dietary bitter kola increased. However, no significant increase ($P>0.05$) was observed.

This observation supported the report of Esiegwu and Udedible (2009) that plasma proteins tended to increase as the bitter kola dietary levels increased and contradicted the report of Uko *et al.* (2001) that bitter kola extract decreased total plasma proteins.

(ii) **Serum albumin and serum globulin**

The values were 2.50, 2.15, 1.85 and 2.50% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for albumin and 4.25, 2.70, 3.65 and 4.00% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively for globulin.

Table 4.6: Serum biochemical indices of the experimental laying hens

Parameters	Dietary levels of *Garcinia kola* seed meal				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Total proteins (g/100ml)	6.50	4.85	5.50	6.50	0.38
Albumin (%)	2.50	2.15	1.85	2.50	0.16
Globulin (%)	4.25	2.70	3.65	4.00	0.30
Glucose (mg/dl)	296.00[a]	226.00[b]	340.00[a]	302.00[a]	24.70
Cholesterol (mg/dl)	136.50[a]	82.00[b]	73.00[b]	141.00[a]	14.61
Calcium (mg/dl)	7.18[b]	7.76[b]	7.06[b]	10.78[a]	0.61
Sodium (mmole/l)	138.05	137.30	137.50	150.90	2.67
Potassium (mmole/l)	6.51[a]	3.72[b]	4.30[ab]	4.75[ab]	0.51
Chloride (mmole/l)	129.00[ab]	111.00[b]	100.50[b]	153.00[a]	8.09
Carbonate (mmole/l)	23.50	24.50	22.00	25.00	0.56

[ab]Means within a row with different superscripts are significantly ($P<0.05$) different.

There were no significant differences (P>0.05) in both parameters either between the control and the bitter kola groups or within the bitter kola groups. Hoffenberg *et al.* (1966) reported that serum protein and serum albumin depended on availability of protein.

(iii) **Serum glucose**

The serum glucose were 296, 226, 340 and 302mg/dl, for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{2.5}$ respectively. No significant differences (P>0.05) were observed within the bitter kola groups and between the control and the bitter groups. Bitter kola did not reduce the serum glucose contrary to the report of Iwu *et al.* (1990) that kolaviron from bitter kola seeds reduced the blood sugar in normoglycaemic and alloxan diabetic rabbits. There was no dietary trend increase. However, $T_{5.0}$ and $T_{7.5}$ were relatively higher than $T_{2.5}$ and T_0 in value. The result of this study supports the earlier report of Esiegwu and Udedibie (2009) that bitter kola seed meal had no significant effect (P>0.05) on serum glucose compared to the control.

(iv) **Serum cholesterol**

The serum cholesterol values were 136.50, 82.00, 73.00 and 141.00mg/dl for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$. T_0 was significantly (P<0.5) higher than $T_{2.5}$ and $T_{5.0}$ but statistically similar to $T_{7.5}$. It tended to decrease at the bitter kola levels of 2.5 and 5.0% but rose again at $T_{7.5}$, very similar to the report of Esiegwu and Udedibie (2009). This trend could indicate that high dosage of bitter kola consumption could increase the serum cholesterol contrary to the report of Adaramoye *et al.* (2005) that kolaviron (a *Garcinia kola* seed extract) administered to hypercholesterolaemic rats reduced plasma cholesterol levels and relative weight of the heart in cholesterol fed animals. It is worthy of note that Schukle and Pachaurri (1995) reported

that reduced serum cholesterol concentration implies impaired transport of and metabolism of lipids.

(v) **Serum calcium**

The serum calcium values were 7.18, 7.76, 7.06 and 10.78mg/dl for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. The values were variable and of no pattern and were not significantly different ($P>0.05$) from one another. The values showed that bitter kola did not influence plasma calcium negatively. Reichmann and Cornor (1977) reported that as the level of dietary calcium increases, plasma calcium also increases significantly in laying hens with the resultant decrease in alkaline phosphate level.

Simkis (1967) reported that the blood cells are almost devoid of calcium but the serum and plasma contain 9 - 12mg/dl in most species when not in reproductive activity. Hurwitz (1973) reported that plasma calcium increases from around 10mg (typical of non laying hens) to over 20mg (typical of laying hens).

(vi) **Serum potassium, serum sodium and serum chloride**

The values were 138.05, 137.30, 137.50 and 150.90 mmole/l for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for serum sodium, 6.51, 3.72, 4.30 and 4.75 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for serum potassium, and 129.00, 111.00, 100.50 and 153.00 mmole/l for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for serum chloride. There were no significant differences ($P>0.05$) in serum sodium and potassium. The values were also variable with no pattern. For serum chloride, significant difference ($P<0.05$) existed between $T_{7.5}$ and the control. These three elements are electrolytes and play important role in maintaining osmotic pressure in both intracellular and extracellular fluid as well as maintaining the acid base balance of the body system.

(vii) **Serum bicarbonate**

The values were 23.50, 24.50, 22.00 and 25.00mmole/l for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences (P>0.05) among the various treatment groups. Chlorine acts with bicarbonate to balance electrically the sodium of the extracellular fluid.

4.2.14 Carcass and internal organ weights of the experimental laying hens

The effects of bitter kola on live weight and internal organ weights of the experimental laying hens are shown in table 4.7.

(i) **Live weight (g)**

The live weights of the layers were 1877.50, 1832.50, 1717.50 and 1875.00 for T_0, $T_{2.5}$ $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences (P>0.05) in the live weights of all the treatment groups.

(ii) Dressed weight

The dressed weight ranged from 920.00 to 1022.50gm and dressing out percentage from 52.39 to 55.34 respectively. There were no significant differences (P>0.05) among the treatments. The varying and non-significant nature of the weights indicated that *Garcinia kola* had no damaging or specific influence on the weights of the birds.

(iii) Liver

The absolute values were 35.00g, 33.75g, 25.00g and 40.00g or 1.84%, 1.82%, 1.45% and 2.07% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively.

Table 4.7: Internal organ weights of the experimental laying hens

Parameters	Dietary Levels of *Garcinia kola*				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Live weight (g)	1877.50	1832.50	1717.50	1875.00	43.81
Dressed weight (g)	1022.50	1007.50	920.00	980.00	25.68
Dressed weight (% Lw)	54.46	55.34	53.80	52.39	0.77
Liver (g)	35.00	33.75	25.00	40.00	3.56
Liver (% Lw)	1.84	1.82	1.45	2.07	0.15
Gizzard (g)	37.25	33.75	40.00	31.35	2.01
Gizzard (% Lw)	2.02	1.85	2.35	1.67	0.13
Kidney (g)	5.00^{a}	2.75^{b}	2.00^{b}	1.75^{b}	0.38
Kidney (% Lw)	0.27^{a}	0.15^{b}	0.12^{b}	0.10^{b}	0.02
Heart (g)	9.38^{a}	8.00^{ab}	6.75^{ab}	6.00^{b}	0.55
Heart (% Lw)	0.50^{a}	0.44^{ab}	0.40^{ab}	0.32^{b}	0.03
Abdominal fat (g)	57.50	33.75	27.50	41.75	9.33
Abdominal fat (% Lw)	2.88	0.62	1.50	2.17	0.41
Intestinal weight (g)	62.50	70.00	50.00	50.00	4.05
Intestinal weight (% Lw)	3.37	3.82	2.94	2.63	0.22
Intestinal length (cm)	170.00	176.50	178.50	166.00	2.67
Caecum length (cm)	16.13^{b}	21.28^{a}	21.00^{a}	17.25^{b}	0.93
Crop (g)	6.83^{b}	8.61^{a}	8.91^{a}	9.51^{a}	0.34
Crop (% Lw)	0.37^{b}	0.47^{ab}	0.52^{a}	0.51^{a}	0.02

^{ab}Means within a row with different superscripts are significantly ($P<0.05$) different.

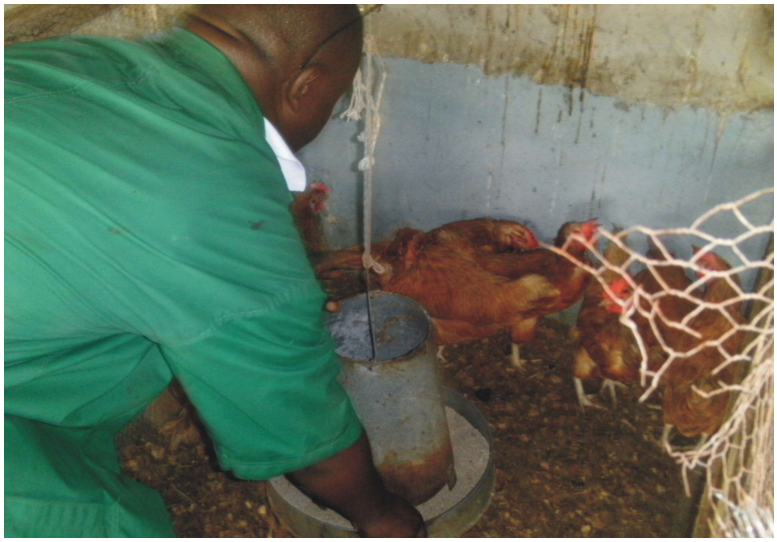


Plate 5: Experimental laying hens selected for carcass evaluation

Plate 6: Carcass of an experimental bird on weighing scale

There were no significant differences in the weights. This finding supports the work of Uko *et al.* (2001) that water extract from *Garcinia kola* did not significantly influence the organ weights of experimental rats. However, Esiegwu and Udedibie (2009) in a similar experiment with broilers observed heavier livers of the experimental broilers as compared to the control. The non significant nature of the values is a pointer to balanced availability of proteins to all the groups.

In other words, the *Garcinia kola* with its associated antinutritional factors especially tannin that forms complexes with protein (Vaithiyanathan and Kumar, 1993), posed no serious effect on protein availability as reported by Horigone *et al.* (1988) that condensed tannin caused reduced protein availability.

Haslam (1989) noted that tannin complexes with protein is soluble if protein is in excess. If protein is available, the liver has less metabolic work in terms of making proteins available to the body from the system, hence the liver will be bearing normal weights. From the haematologicl studies, there were no significant differences ($p>0.05$) in the available total serum proteins for all the treatment groups to justify the liver weight status.

(iv) Gizzard

The values for the gizzards were 37.25g, 33.75g, 40.00g and 31.25g or 2.02%, 1.85%, 2.35% and 1.67% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. Their were no significant treatment ($p>0.05$) effects on the weights of the gizzard. This shows that the fibre content of the treatment diets were within normal range.

(v) Kidney

The weights of the kidneys were 5.00g, 2.75g, 2.00g and 1.75g or 0.27%, 0.15%, 0.12% and 0.10% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. The weight of the kidney of the control was significantly ($P<0.05$) higher than the treated groups. The kidney functions to remove waste products of protein metabolism such as urea and maintains the acid base balance of the animal's internal environment. As the level of *Garcinia kola* increased, the kidney weights decreased as compared to the control. This might indicate the inability of the kidney to remove waste products of metabolism especially nitrogen as reported by D'Mello and Acamovic (1989) that condensed tannin in *Leucaenia leucocephala* caused poor nitrogen retention and low apparent metabolizable energy in poultry.

(vi) Heart

The weights of the hearts ranged from 6.00g to 9.38g or 0.32% to 0.50%. There was a significant difference ($P<0.05$) between the control and the treated groups, at $T_{7.5}$ dietary level. There was a dose related decrease of the heart weights as the *Garcinia kola* increased. This contradicts the report of Uko *et al.* (2001) that *Garcinia kola* extract induced no significant influence ($P>0.05$) on any of the organs of experimental rats.
Adaramoye *et al.* (2005) reported that kolaviron (a bitter kola seed extract) reduced plasma cholesterol levels in rats and the relative weight of the hearts in cholesterol fed animals.

(vii) Abdominal fat

The values ranged from 27.50g to 57.50g or 0.62% to 2.88%. There were no significant differences ($P>0.05$) among the treatments in abdominal fat weights. The abdominal fat of the control was relatively higher than those

of the *Garcinia kola* groups. But because of the non-significant nature of the values one can say that *Garcinia kola* has no real effect on fat deposition in the body of laying hens.

(viii) Intestinal weight and length

Intestinal weights ranged from 50.00 to 70.00gm or 2.63% to 3.82% while the lengths ranged from 166.00 to 178.50cm. Caecum length ranged from 16.13 to 21.25cm. There were no significant differences ($P>0.05$) among the treatments. The varying and non-significant nature of the values indicated that the rate of movement of feed materials in the gastro-intestinal tracts were normal.

(ix) Crop

The values were from 6.81g, 8.61g, 8.91g and 9.51g or 0.37%, 0.47%, 0.52% and 0.51% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively.

The values for the *Garcinia kola* groups were significantly higher ($P<0.05$) than that of the control group. The weights of the crops increased as the *Garcinia kola* seed meal in the diet increased. The enlargement of the crop for the bitter kola groups could be attributed to the longer time feed stayed in the crop before passing out. Probably, digestion or movement of feed materials was slow in the gastro-intestinal tract of the *Garcinia kola* group leading to delayed release of ingested materials from the crop. The enlargement of the crop was a response to the length of time it took for feed materials to move out of the crop.

4.2.15 **Parasitological observations**

The data on parasitological studies are shown in tables 4.8 and 4.9.

During the clinical evaluation of the droppings at the beginning of the experiment, larvae of *Ascaridia lumbricoides* and oocyst of *Isospora bellies* were present in the intestines of the laying hens. At the end of the experiment, a second clinical investigation of the droppings carried out showed total absence of ova, larvae or cysts of protozoa. The absence of any of these parasites is an indication that bitter kola was lethal to them, supporting the report that *Garcinia kola* is anti-microbial (Ebana *et al.*, 1991; Akoachere *et al.*, 2002).

4.2.16 **Bacteriological observations**

Data on bacterial analysis of the intestinal droppings are shown in table 4.10 and 4.11.

At the beginning of the experiment, *Salmonella spp* were present in all the treatment groups whereas *Escherichia coli* were absent during the clinical evaluation of the intestinal droppings. At the end of the experiment, *Salmonella spp* were found to be absent in $T_{5.0}$ and $T_{7.5}$ but present in T_0 and $T_{2.5}$. *Escherichia coli* that were absent during the beginning of the experiment were present in all the treatments. This result indicated that bitter kola had a lethal effect on *Salmonella spp* but no effect on *Escherichia coli*, as earlier reported by Esiegwu and Udedibie (2009).

Table 4.8: Parasitological observations at first week of experiment

Parasites	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Ascaridia lumbricoides	-	+++	-	++
Oocyst of Isospora bellies	-	-	++	-

(+) Means presence of parasite

(-) Indicates no ova, larvae or cyst of protozoa seen

Table 4.9: Parasitological observations at the twelfth week of experiment

Parasites	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Ascaridia lumbricoides	-	-	-	-
Oocyst of Isospora bellie	-	-	-	-

(+) Means presence of parasite

(-) Indicates no ova, larva or cyst of protozoa seen

Table 4.10: Bacteriological observations at first week of experiment

Bacteria	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Salmonella spp.	+	+	+	+
Escherichia coli	-	-	-	-

(+) Indicates presence of bacteria

(-) Indicates absence of bacteria

Table 4.11: Bacteriological observations at twelfth week (end) of experiment

Bacteria	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Salmonella spp.	+	++	-	-
Escherichia coli	++	++	++	++

(+) Indicates presence of bacteria

(-) Indicates absence of bacteria

4.2.17 **Histopathological observations**

Data on histopathological observations of laying hens fed graded levels of *Garcinia kola* seed meal are shown in table 4.12.

There were no observable changes in the livers of $T_{2.5}$ and $T_{5.0}$ compared to the control. The liver of $T_{7.5}$ suffered distortion of general tissue architecture with scanty stroma. There were no observable changes in the hearts of all the treatments. The kidney of $T_{2.5}$ appeared normal but $T_{5.0}$ had tissue stroma proliferation with presence of slit-like channels while $T_{7.5}$ showed proliferation of tissue stroma with hypertrophy and necrosis of the kidney. In the gizzard, there were no observable changes in $T_{2.5}$ and $T_{5.0}$ compared to the control while $T_{7.5}$ showed presence of oedema, tissue stromal proliferation with presence of slit-like channels. These observations indicated that bitter kola diets altered the cellular structure of the liver, kidney and gizzard. These findings support the report of Braid and grill (1990) on some histological alterations in the liver, kidney and duodenum of rats fed diets containing 10% (w/w) dry powdered bitter kola for six weeks and contradicts the report of Uko *et al.* (2001) that the organs (testes, kidney, liver, heart, lungs and brain) did not show any microscopic alterations for all the treatment groups when rats were fed water extract from bitter kola. Esiegwu and Udedibie (2009) observed a similar histological alterations of the gizzard, liver and heart of broilers fed graded levels of bitter kola seed meal for eight weeks.

Table 4.12: Histopathological observations of internal organs of the experimental laying hens

Parameters	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Liver	Normal	No observable changes, normal.	No observable changes, normal.	Distortion of general tissue architecture with scanty stroma.
Heart	Normal	No observable changes, normal.	No observable changes, normal.	No observable changes, normal.
Kidney	Normal	No observable changes, normal.	Tissue stroma proliferation with presence of slit-like channels.	Proliferation of tissue stroma with hypertrophy and necrosis.
Gizzard	Normal	No observable changes, normal.	No observable changes, normal.	Presence of oedema, tissue stromal proliferation with presence of slit-like channels.

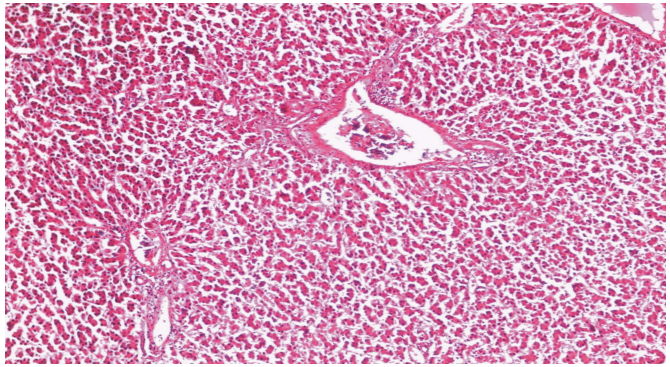


Plate 7: Heart (T_0). Normal

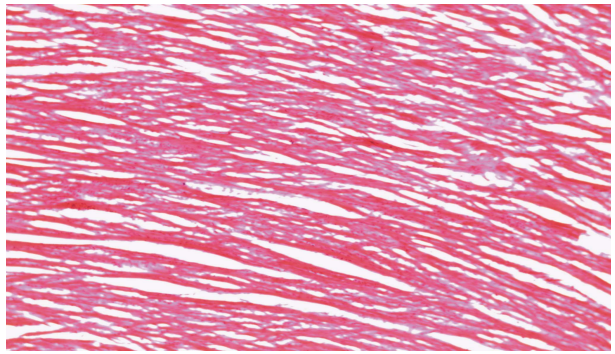


Plate 8: Heart ($T_{2.5}$). No observable changes

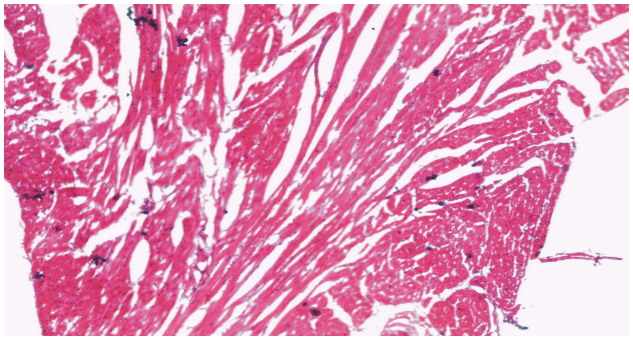


Plate 9: Heart ($T_{5.0}$). No observable changes

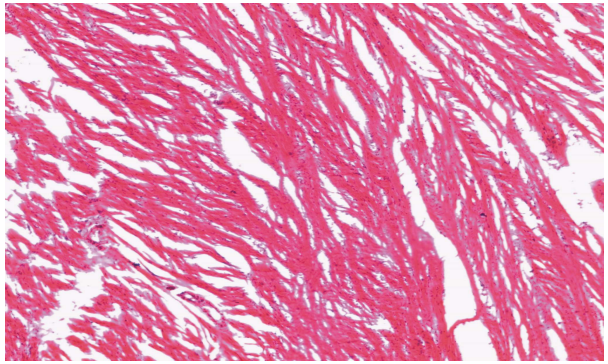


Plate 10: Heart ($T_{7.5}$). No observable changes

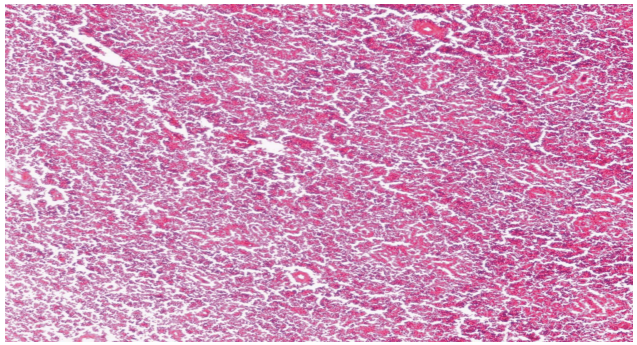


Plate 11: Kidney (T_0). Normal

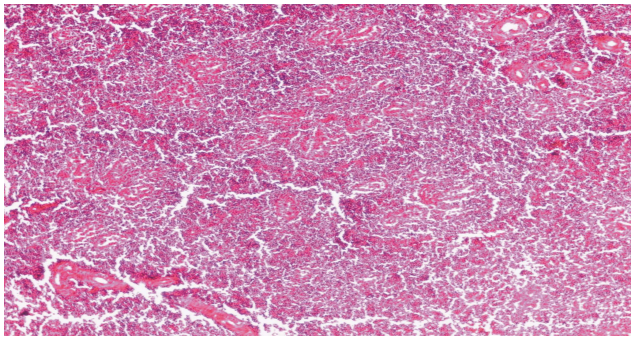


Plate 12: Kidney ($T_{2.5}$). Normal

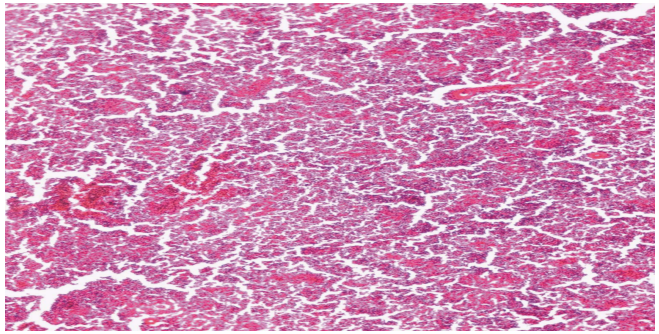


Plate 13: Kidney ($T_{5.0}$). Not normal

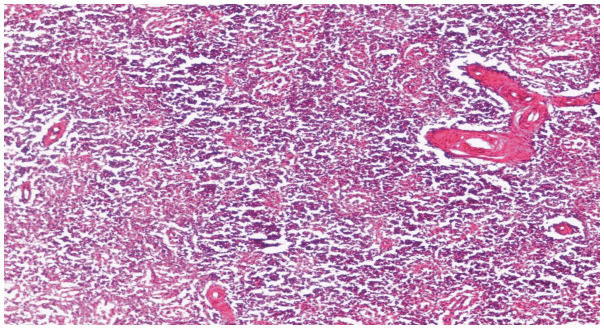


Plate 14: Kidney ($T_{7.5}$). Not normal

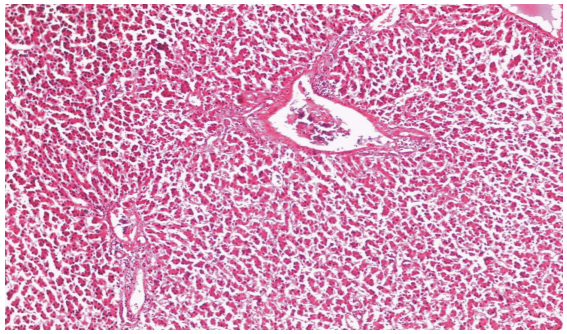


Plate 15: Liver (T_0). Normal

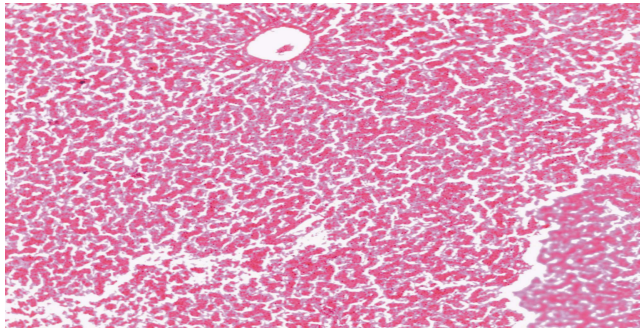


Plate 16: Liver ($T_{2.5}$). Normal

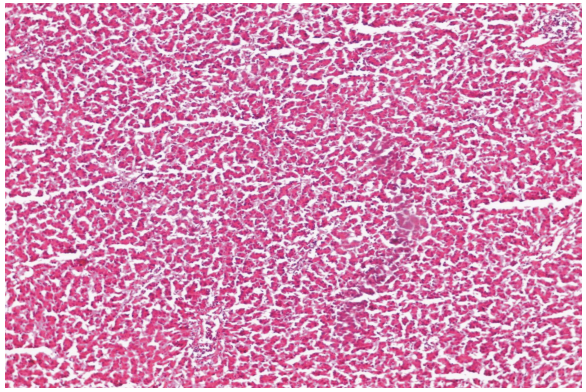


Plate 17: Liver ($T_{5.0}$). Normal

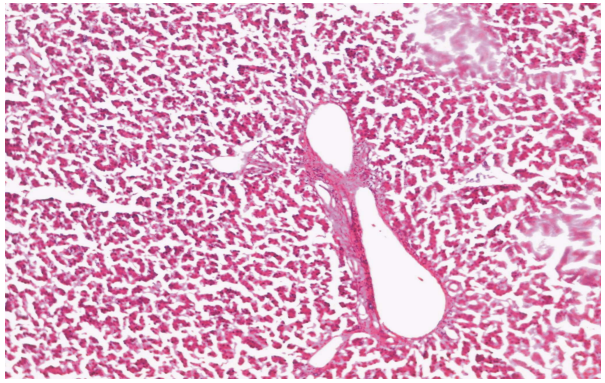


Plate 18: Liver ($T_{7.5}$). Not normal

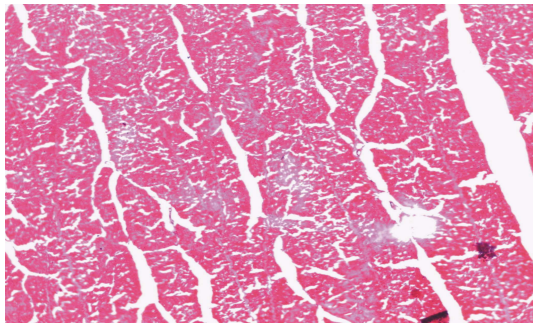


Plate 19: Gizzard (T_0). Normal

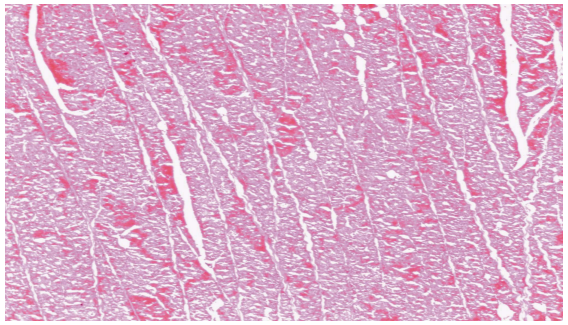


Plate 20: Gizzard ($T_{2.5}$). Normal

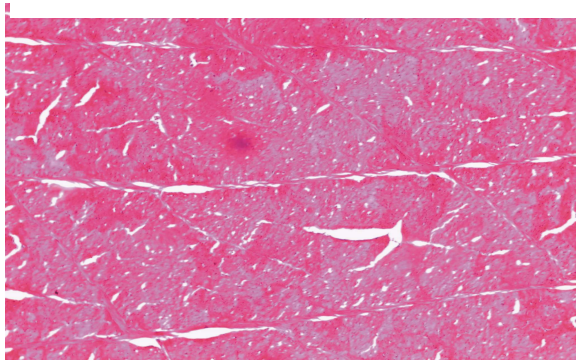


Plate 21: Gizzard ($T_{5.0}$). Normal

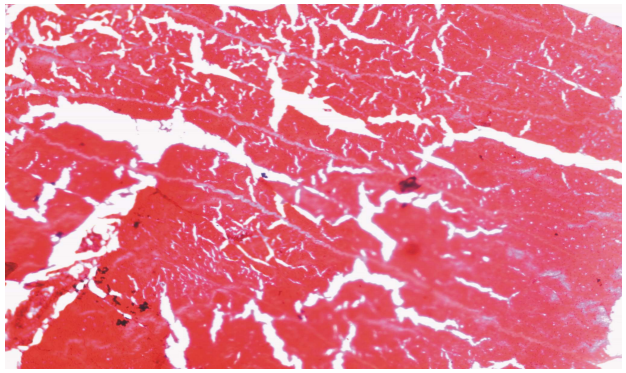


Plate 22: Gizzard ($T_{7.5}$). Not normal

4.3 **Performance of the experimental grower rabbits**

Data on the performance of grower rabbits fed graded levels of *Garcinia kola* seed meal are shown in table 4.13.

4.3.1 **Initial body weight (g)**

The average initial body weights of the rabbits were 566.67, 577.78, 555.56 and 533.33gm for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences ($P>0.05$) among the treatment groups in initial body weights.

4.3.2 **Final body weight (g)**

The average final body weights were 1864.47, 1609.18, 1710.26, and 1664.74gm for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were significant differences ($P<0.05$) in the final body weights of the treatment groups. The control gained significantly ($P<0.05$) heavier body weight than the bitter kola groups, $T_{2.5}$ and $T_{7.5}$ except $T_{5.0}$. There were, however, no significant differences ($P>0.05$) among the bitter kola groups.

4.3.3 **Body weight gain (g)**

The average body weight gains were 1297.80, 1031.40, 1154.70 and 1131.30gm for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. The control gained significantly ($P<0.05$) heavier body weight than the treated groups.

Table 4.13: **Performance of the experimental grower rabbits**

Parameters	Dietary levels of *Garcinia kola* seed meal (%)				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Initial body weight (g)	566.57	577.78	555.56	533.33	14.72
Av. final body weight (g)	1864.47^{a}	1609.18^{b}	1710.26^{ab}	1664.74^{b}	36.56
Av. body weight gain (g)	1297.80^{a}	1031.40^{c}	1154.70^{b}	1131.30^{bc}	32.14
Av. daily body weight gain (g)	14.42^{a}	11.46^{c}	12.83^{b}	12.57^{bc}	0.36
Av. daily feed intake (g)	64.99^{b}	77.99^{a}	85.59^{a}	84.55^{a}	3.19
Feed conversion ratio (g feed/g gain)	4.78^{b}	6.77^{a}	6.69^{a}	6.74^{a}	0.32
Mortality (number)	0.00	0.00	0.00	0.00	_

^{abc}Means within the same row with different superscripts are significantly different (P<0.05).

The reduced body weight gain by the *Garcinia kola* groups may be attributed to the effect of the presence of anti-nutritional factors in bitter kola, especially tannin. Condensed tannin from the browse plant Robin's pseudoacacia has been reported to cause reduced feed intake, reduced growth and coprophagy (Raharjo *et al*., 1990) and reduced protein digestibility (Horigone *et al*., 1988) in rabbits and rats. In this study feed intake was very normal, even far above the control. Reduced coprophagy is suggested as the possible cause because through coprophagy, a rabbit obtains a significant amount of water soluble vitamins and up to 20% of its crude protein (CP) requirement, 30% of its energy requirements as volatile fatty acids (VFA) and 18% of its daily dry matter (DM) intake (Lebas *et al*., 1997). These daily supplies through coprophagy is essential for increased growth rate.

4.3.4 **Daily body weight gain (g)**

The average daily body weight gains were 14.42, 11.46, 12.83 and 12.57gm. The control had significantly ($P<0.05$) higher daily body weight gain than all the bitter kola groups. However, $T_{5.0}$, was significantly ($P<0.05$) higher than $T_{2.5}$ but not significantly different ($P>0.05$) from $T_{7.5}$. Uko *et al.* (2001) in a similar experiment with rats fed water extract of *Garcinia kola* reported depressive effect on appetite and water intake with resultant poor feed utilization efficiency and weight gain of rats. The reduced growth rate of the *Garcinia kola* groups could still be attributed to reduced coprophagy and protein digestibility of the rabbits as a result of the presence of tannin in the *Garcinia kola*. D'Mello *et al.* (1987) showed that diet containing 100g leaf meals per kg diet significantly reduced growth without affecting dry matter intake.

4.3.5 **Daily feed intake (g)**

The average daily feed intake were 68.99, 77.99, 85.59 and 84.55gm for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. There were significant differences ($P<0.05$) among the treatment groups in feed intake. The treated groups consumed significantly ($P<0.05$) more feed than the control. Esiegwu and Udedibie (2009) reported no significant differences ($P>0.05$) in feed intake of broilers fed graded levels of dietary bitter kola. On the contrary, Uko *et al.* (2001) in a similar work reported depressive effect on appetite and water intake. These findings indicated that the saponins of *Garcinia kola* has no effect on voluntary feed intake of rabbit. Saponins of alfalfa caused retardation of growth rate primarily due to a reduction in feed intake in non-ruminant animals (poultry, rats, rabbits and swine) (Cheeke and Shull 1985). Reduction in feed intake by saponin is as a result of its unpalatability having an irritating effect on the membrane of the mouth and throat. All the four (4) diets could be said to be palatable and acceptable to the rabbits.

4.3.6 **Feed conversion ratio**

The feed conversion ratios were 4.78, 6.77, 6.69 and 6.72 for T_0, $T_{2.5}$, $T_{5.0}$, and $T_{7.5}$ respectively. There were no significant differences ($P>0.05$) among the bitter kola groups. The feed conversion ratio of the control was significantly ($P<0.05$) better than those of the *Garcinia kola* groups. Uko *et al.* (2001) reported poor feed utilization efficiency of rats fed water extract from *Garcinia kola*.

4.3.7 **Economics of production of the experimental growing rabbits**

The economics of production of the grower rabbits is summarized in table 4.14

4.3.8 **Feed cost (₦/kg)**

The feed cost for the treatment groups were ₦68.92, ₦95.57, ₦124.04 and ₦150.69 for T_0, $T_{2.5}$, $T_{5.0}$, and $T_{7.5}$ respectively. The cost per kg of feed increased as the bitter kola levels increased. The percent increases were 38.67, 79.98 and 118.64 compared to the control.

4.3.9 **Feed cost per kg body weight gain (₦)**

The cost of feed per kg of body weight gained were ₦329.44, ₦647.01, ₦829.83 and ₦1012.64 for T_0, $T_{2.5}$, $T_{5.0}$, and $T_{7.5}$ respectively. The cost of feed per kg of body weight increased as the dietary levels of bitter kola increased. The percent increases were 96.40, 151.89 and 207.38 for $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for the bitter kola groups compared to the control.

4.3.10 **Haematological indices of the experimental grower rabbits**

Data on the haematological indices of grower rabbit fed graded levels of bitter kola seed meal are shown in table 4.15.

(i) Haemoglobin (HB) (g/dl)

The values of the haemoglobin for the treatment groups were 11.95, 11.50, 11.70 and 12.00 (g/dl) for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. There were no significant differences among the treatments ($P > 0.05$). The non-

Table 4.14: **Economics of production of the experimental grower rabbits**

Parameters	Dietary levels of *Garcinia kola* seed meal				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Feed conversion ratio (g feed/g gain)	4.78[b]	6.77[a]	6.69[a]	6.72[a]	0.32
Feed cost (₦/kg feed)	68.92	95.57	124.04	150.69	-
Cost of production (₦/kg gain)	329.44	647.01	829.83	1012.64	-

[ab]Means within the same row with different superscripts are significantly different (P<0.05).

Table 4.15: Haematological indices of the experimental grower rabbits

Parameters	Dietary levels of *Garcinia kola* seed meal (%)				SEM
	T_0	$T_{2.50}$	$T_{5.0}$	$T_{7.5}$	
RBC ($x10^6$/ul)	6.45	6.10	6.20	6.97	0.21
Hb (g/dl)	11.95	11.50	11.70	12.00	0.26
PCV (%)	33.85	35.60	35.85	36.00	0.64
MCV (fl)	55.50	58.40	54.70	51.80	1.27
MCHC (g/dl)	33.30	32.20	32.75	33.37	0.54
MCH (pq)	18.45	18.80	17.85	17.30	0.45
WBC (mm^3)	10350	11950	12000	12133	911.04
Lymphocytes (%)	71.50^a	54.50^{ab}	52.00^{ab}	45.33^b	4.08
Neutrophils (%)	28.00^b	45.50^{ab}	48.00^{ab}	54.67^a	4.15
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00
Monocytes (%)	0.00	0.00	0.00	0.00	0.00
Platelets (mm^3)	292000	269000	330000	387667	34897.38

^{ab}Means within the same row with different superscripts are significantly ($P<0.05$) different.

significant differences of the values is an indication of the safety of the test material. The values obtained (11.50 - 12.00 g/dl) both for the control and the *Garcinia kola* groups compared favourably with the compilations from the literature of Hemograms for the rabbit as shown by Gardener (1947) and MacNamee and Sheely (1952) for which HB is 12.1 and 12.2 respectively. Johnes (1975) also reported that the haemoglobin of different breeds of rabbit ranged from 9.37g/dl to 17.50g/dl. The values from this work support the findings of Uko *et al.* (2001) and Adedeji *et al.* (2005) that water extract from *Garcinia kola* and bitter kola diet respectively caused no significant effects in all the blood parameters measured.

(ii) Packed cell volume (PCV) (%)

The values of the PCV were 35.85, 35.60, 35.85 and 36.00% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences among the treatments. This is in line with the findings of Uko *et al.* (2001) in a similar experiment with rats.

The values obtained in this work were within the normal range recommended by Mitruka and Rawnsley (1977). The values also fall within 28.75 - 35.50 and 30.50 - 37.75 reported for weaned rabbits by Adewumi *et al.* (2004) and Ahamefule *et al.* (2006) respectively. Gardener (1947) and MacNamee and Sheely (1952) reported PCV values of 36 - 48% and 42.4 respectively. The values obtained showed that the diets favoured adequate utilization of iron (fe) in the formation of haemoglobin.

(iii) Red blood cell (RBC) ($X10^6$/µL)

The values were 6.45, 6.10, 6.20 and 6.97 **($x10^6$/µl)** for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. There were no significant differences among the groups.

This is in agreement with the earlier report of Uko *et al*. (2001), Adedeji *et al.* (2005) and Esiegwu and Udedibie (2009) in similar experiment with rat and broilers, respectively. The values (6.10 to 6.97) were within the range of 3.8 to 10.0 reported by Katty (2003) for growing rabbits.

(iv) Mean corpurscular volume (MCV), mean corpurscular haemoglobin (MCH) and mean corpurscular haemoglobin concenteration (MCHC)

The values ranged from 51.80 to 58.40fl for MCV, 17.30 to 18.80pg for MCH and 32.20 to 33.37g/dl for MCHC. There were no significant differences (P>0.05) among the treatments in the 3 indices. The MCV values (51.80 to 58.40fl) fell a little bit below the normal physiological range of 58.8 to 66.5dl recommended by Mitruka and Rawnsey (1977). The relatively lower values could not be due to the diets since it also affected the control. The MCH values (17.30 to 18.80) in this study were close to the range (18.7 to 22.7) recommended by Mitruka and Rawnsey (1977) as the normal physiological values.

(v) Platelets (mm^3)

The values for the platelets were 292000, 269000, 330,000 and 387667mm^3 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences (P>0.05) among the treatments. The values were variable and non significant which showed that bitter kola had no effect on blood platelets of rabbit serum. Although not significantly (>0.05) higher, the values of platelets for $T_{5.0}$ and $T_{7.5}$ were relatively higher than the control. The values suggest that bitter kola may not interfere with the blood clotting mechanism in rabbits. The findings in this study agreed with the report of Esiegwu and Udedibie (2009) in a similar experiment with broilers.

(vi) **White blood cell (WBC) (mm3)**

The values for the white blood cells were 10350, 11950, 12000, and 12133mm^3. There were no significant differences (P>0.05) among the treatments. This is in agreement with the earlier report of Esiegwu and Udedibie (2009). The values of WBC in the study are within the range of 2.6 - 12.5 reported by Katty (2003).

(vii) Lymphocytes, eosinophils, neutrophils, basophils and monocytes (%)

The values were 71.50, 54.50, 52.00 and 45.33% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for lymphocytes and 28.00, 45.50, 48.00 and 54.67% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for neutrophils. Eosinophils, basophils and monocytes were not observed. The absence of eosinophils, basophils and monocytes in circulation was an indication of the absence of infection in the blood of the animals. The lymphocytes of the control group were significantly higher (P<0.05) than $T_{7.5}$. The neutrophils of the control were significantly lower (P<0.05) than those of the bitter kola groups. The increase in lymphocytes and eosinophils in the T_0 and $T_{7.5}$ respectively could be an immune response to fight an infection.

4.3.11 **Biochemical indices of the experimental grower rabbits**

Data on the biochemical indices of grower rabbits fed graded levels of bitter kola seed meal are shown in table 4.16.

Table 4.16: Serum biochemical indices of the experimental grower rabbits

Parameters	Dietary levels of *Garcinia kola* seed meal (%)				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Glucose	150.67[a]	57.00[b]	76.00[ab]	65.00[ab]	15.57
Cholesterol	96.67	86.67	96.67	110.00	6.64
Proteins	5.23[b]	6.53[a]	5.47[ab]	6.00[ab]	0.21
Albumin	4.40	4.20	4.63	4.97	0.21
Globulin	2.13	1.03	0.83	1.03	0.26
Potassium	6.18	5.27	6.75	6.41	0.27
Sodium	138.73	135.33	138.30	134.80	0.88
Chloride	109.00[ab]	104.03[b]	114.97[a]	105.77[b]	1.59
Bicarbonate	16.67	17.33	18.00	17.33	0.41
Calcium	11.67	11.47	13.80	11.47	0.63

[ab]Means within a row with different superscripts are significantly ($P<0.05$) different.

(i) Total proteins (g/100ml)

The serum total protein levels were 5.23, 6.53, 5.47 and 6.00g/100ml for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. $T_{2.5}$ was not significantly different ($P>0.05$) from $T_{5.0}$ and $T_{7.5}$ but significantly higher ($P<0.05$) than the control. There were no significant differences ($P>0.05$) among the control (T_0), $T_{5.0}$ and $T_{7.5}$. It showed that bitter kola did not interfere with protein metabolism and is in agreement with Esiegwu and Udedibie (2009), contradicting the report of Uko *et al.* (2001) that water extract from bitter kola decreased total plasma proteins. The values (5.23 – 6.53) for the serum total protein in this study were within the normal standard value of 5.0 - 8.0gldl as reported by CCAC (1980) for rabbits.

(ii) **Serum albumin and serum globulin (%).**

The values were 4.40, 4.20, 4.63 and 4.97% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for serum albumin and 2.13, 1.03, 0.83 and 1.03% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively for serum globulin. There were no significant differences ($P>0.05$) among the treatments. The values (4.20 - 4.97%) for albumin in this study were above the normal physiological range (3.58 – 3.95gldl) reported by CCAC (1980) for rabbits. The result showed that bitter kola did not affect negatively or interfere with serum albumin and globulin metabolism. This finding contradicts the report of Uko *et al.* (2001) that water extract from bitter kola decreased total albumin concentrations in rats.

(iii) Serum glucose (mg/dl)

The values for total serum glucose were 150.67, 57.00, 76.00 and 65.00mg/dl for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. The control was

significantly higher ($P<0.05$) than $T_{2.5}$ but not significantly ($P>0.05$) different from $T_{5.0}$ and $T_{7.5}$. The values indicated a lower sugar level in the treated groups.

(iv) **Serum cholesterol (mg/dl)**

The values were 96.67, 86.67, 96.67 and 110.00mg/dl for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant effects ($P>0.05$) among the treatments.

(v) **Serum calcium (mg/dl)**

The values for serum calcium were 11.67, 11.47, 13.80 and 11.47mg/dl for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were also no significant ($P>0.05$) differences among the treatments.

(vi) Serum potassium, serum sodium, serum chloride and serum bicarbonate

There were no significant differences ($P>0.05$) in serum potassium, sodium and bicarbonate values among the treatments. With regard to serum chloride, $T_{5.0}$ was significantly higher ($P<0.05$) than $T_{2.5}$ and $T_{7.5}$. The control was not significantly different ($P>0.05$) from the treated groups. Since the control was statistically similar to the other treatments, it could mean that bitter kola had no dietary effect on serum chloride.

It therefore implies that bitter kola did not interfere with serum electrolyte metabolism. Bicarbonate which was statistically the same for all the treatments combines with chloride to balance electrically the sodium of the extracellular fluid. The values indicated that bitter kola did not negatively influence the plasma electrolyte.

4.3.12 **Carcass evaluation of the experimental rabbits**

Data on carcass evaluation of rabbits fed graded levels of *Garcinia kola* seed meal are shown in table 4.17.

(i) Dressed weight

The dressed weights of the groups were 50.37, 46.41, 52.27 and 47.30% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. The percent dressed weight for the control was statistically the same with the treated groups. The dressing percents (46.41 – 52.27) obtained in this study were lower than the values (53.57 – 63.40%; 68.6 – 75.4) obtained by Ani (2009) with diets containing raw bambara nut waste, Togun *et al* (2009) reported that diets containing bovine rumen digesta-blood meal mixture (50:50) combination with 18% cooked mucuna bean meal respectively were similar to report of Ani, 2009 .

The low dressing percentage in the study could be attributed to the nutrient content of the ingredients used and the physiological health status of the rabbits. $T_{5.0}$ performed best both in live weights and in dressing percentage.

Table 4.17: Carcass evaluation of the experimental grower rabbits

Carcass (% of Lw)	Dietary levels of *Garcinia kola* seed meal				SEM
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$	
Live weight (g)	1482.50	1300.00	1520.00	1237.50	42.78
Dressed weight (% Lw)	50.37^{ab}	46.41^{b}	52.27^{a}	47.30^{ab}	0.96
Liver (% Lw)	2.24	2.54	2.44	2.31	0.06
Heart (% Lw)	0.26	0.27	0.25	0.30	0.01
Kidney (% Lw)	0.58	0.64	0.56	0.55	0.02
Skin (% Lw)	9.21^{a}	8.93^{a}	8.90^{a}	8.20^{b}	0.21
Lungs (% Lw)	0.46^{ab}	0.58^{a}	0.40^{b}	0.56^{a}	0.03
Abdominal Fat (% Lw)	2.01^{a}	0.38^{b}	1.29^{ab}	0.40^{b}	0.27

^{ab}Means within a row with different superscripts are significantly (P<0.05) different.

Plate 23: Experimental grower rabbits of Newzealand breed

Plate 24: Carcass display of the experimental rabbits

(ii) Liver

The weights of the livers expressed as percent of live weight were 2.24, 2.54, 2.44 and 2.31 for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. There were no significant differences (P>0.05) among the treatments. The percent liver values (2.24 – 2.54) obtained in this study were lower than the values 3.2 - 3.3% and 3.38 – 6.07% reported by Togun *et al.* (2009) and Ani (2009), respectively. The marked decrease in weight of the livers might not be an adverse effect of dietary treatments since it cut across all the treatments. It might be as a result of reduced protein synthesis in the liver due to balanced protein availability to the body system.

(iii) Heart

The values ranged from 0.25 to 0.30%. There were no significant differences (P>0.05) among the treatment groups. This indicated no dietary problems. The values (0.25 - 0.30) are higher than the value 0.20% recorded by Togun *et al.* (2009).

(iv) Kidney

The values were 0.58, 0.64, 0.56 and 0.55% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences (P>0.05) among the treatments. The values (0.55 - 0.64) are observed to be higher than the range (0.37 - 0.46%) reported by Ani (2009) and close to the range (0.7 - 0.9) reported by Togun *et al.* (2009).

(v) Skin

The weight of the skins were 9.21, 8.93, 8.90 and 8.20% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ respectively. There were no significant differences ($P>0.05$) among the treatments. However, at 7.5% dietary level, the weight of the skin was significantly ($P<0.05$) reduced.

(vi) Lungs

The weight of the lungs ranged from 0.40 to 0.58%. No significant differences ($P>0.05$) existed in the weight of the lungs of T_0, $T_{2.5}$ and $T_{7.5}$. The weight of the lungs of $T_{5.0}$ was significantly ($P<0.05$) reduced compared with those of $T_{2.5}$ and $T_{7.5.}$

(vii) Abdominal fat

The values were 2.01, 0.38, 1.29 and 0.40% for T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$, respectively. The control developed significantly more ($P<0.05$) abdominal fat than $T_{2.5}$ and $T_{7.5}$ but not significantly different ($P>0.05$) from $T_{5.0}$. The values for the bitter kola groups when compared to the control indicated that *Garcinia kola* could be used as defattener.

4.3.13 Parasitological observations of the faecal samples of the experimental rabbits

Data on parasitological studies are shown in tables 4.18 and 4.19.

Clinical evaluation of the faecal samples of the experimental rabbits at the beginning of the experiment revealed the presence of the parasite, *Isospora belli*. At the end of the experiment, the faecal samples still showed the presence of cyst of *Isospora belli*. *Ascaridia lumbricoides* were absent in both cases.

Table 4.18: Parasitological observations on the experimental rabbits (1st week of experiment)

Parasites	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Cyst of Isospora belli	+	++	+	+
Ascaridia lumbricoides	-	-	-	-

(+) indicates presence and levels of presence

(-) indicates absence

Table 4.19: Parasitological observations on the experimental rabbits (last week of experiment)

Parasites	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Cyst of Isospora belli	+	+	+	+
Ascaridia lumbricoides	-	-	-	-

(+) indicates presence and levels of presence

(-) indicates absence

4.3.14 **Bacterial observations of the faecal samples of the experimental rabbits**

Data on the bacteriological studies are shown in tables 4.20 and 4.21.

Clinical evaluation of the faecal samples at the beginning of the experiment revealed the presence of *Salmonella spp*, *Staphylococcus aureus, Escherichia coli* and *Streptococcus spp*.
At the end of the experiment, no *Salmonella spp* and *Streptococcus spp* were observed in the faecal samples which indicated that *Garcinia kola* may be bactericidal to the species, supporting the report of Ebana *et al.* (1991) and Akoachere *et al.* (2004) that the seeds of *Garcinia kola* have antibacterial and antimicrobial properties. *Garcinia kola* had no effect on *Staphylococcus aureus* and *Escherichia coli*.

4.3.15 **Histopathological examinations of the internal organs of the experimental rabbits**

Data on histopathological studies of the experimental rabbits are shown in table 4.22.

There were histological alterations of the liver and kidney in $T_{5.0}$ and $T_{7.5}$ compared to the control. There were no observable histologic changes for the kidney, liver and heart in $T_{2.5}$ as compared to the control. In $T_{5.0}$, the liver had a moderate oedema with tissue stroma proliferation while $T_{7.5}$ had a mild oedema with tissue stroma proliferation.
With regard to kidney, there was a glomeruli atrophy with marked cellularity within the tuft and tissue stroma proliferation in $T_{5.0}$.

Table 4.20: Bacterial observations on the experimental rabbits at the 1st week of experiment

Bacteria	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Salmonella spp	++	++	+	-
Staphylococcus aureus	+	-	++	+
Escherichia coli	+	++	++	+
Streptococcus spp	-	-	+	-

(+) indicates presence and levels of presence

(-) indicates absence

Table 4.21: Bacterial observations on the experimental rabbits at the 12th week of experiment

Bacteria	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Salmonella spp	++	-	-	-
Staphylococcus aureus	+	+	-	++
Escherichia coli	+	+	++	+
Streptococcus spp	-	-	-	-

(+) indicates presence and levels of presence

(-) indicates absence

Table 4.22: Histopathological examinations of the experimental rabbits

Internal organs	Dietary levels of *Garcinia kola* seed meal			
	T_0	$T_{2.5}$	$T_{5.0}$	$T_{7.5}$
Liver	Normal	No observable histologic changes	Moderate oedema with tissue stroma proliferation	Mild oedema with tissue stroma proliferation.
Kidney	Normal	No observable histologic changes	Glomeruli atrophy with marked cellularity within the tuft, tissue stroma proliferation.	Glomeruli atrophy with marked cellularity within the tuft, stroma proliferation, scanty stroma in some areas with cystically dilated spaces. Also observed as chicken wire appearance.
Heart	Normal	No observable histologic changes	No observable histologic changes	No observable histologic changes.

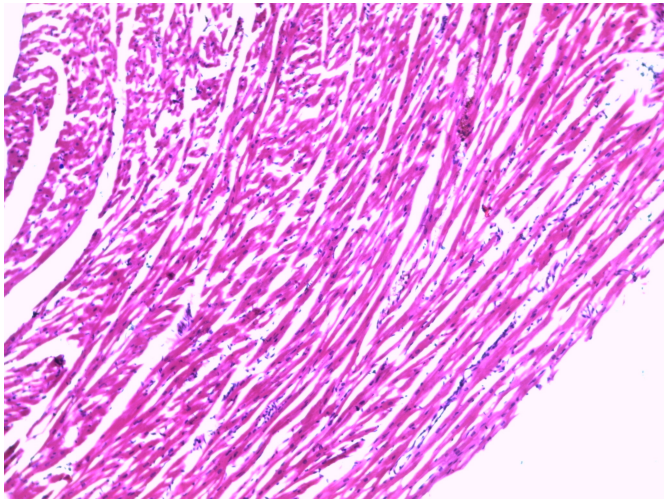


Plate 25: Heart (T_0). Normal

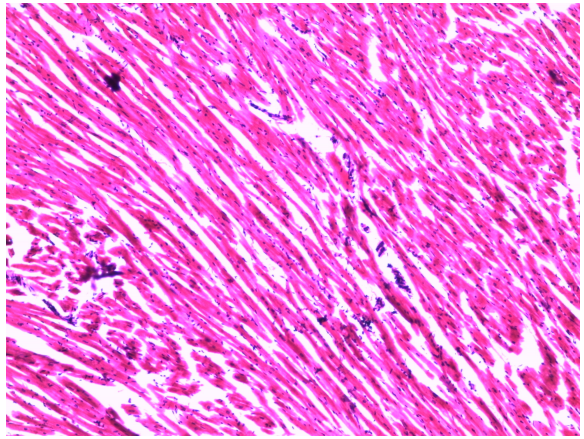


Plate 26: Heart ($T_{2.5}$). Normal

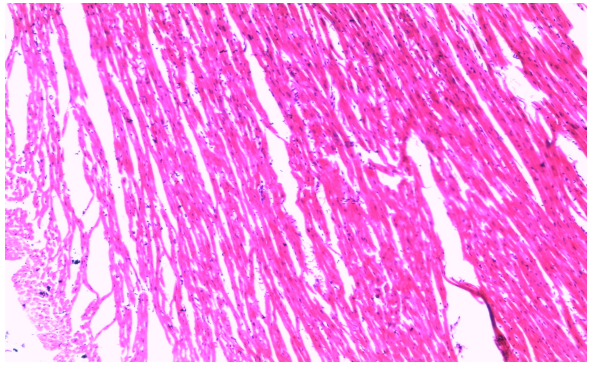


Plate 27: Heart ($T_{5.0}$). Normal

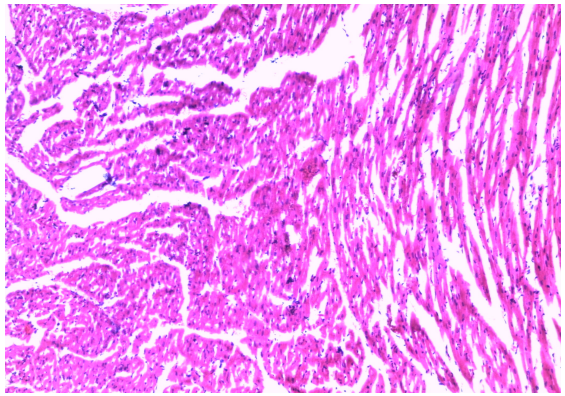


Plate 28: Heart ($T_{7.5}$). Normal

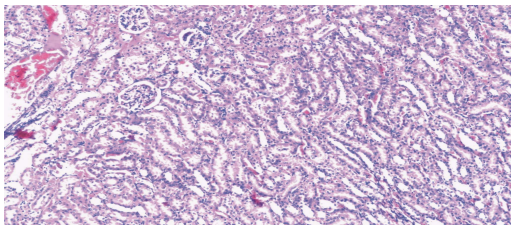


Plate 29: Kidney (T_0). Normal

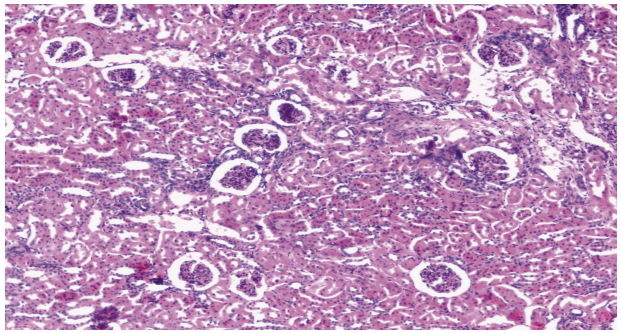


Plate 30: Kidney ($T_{2.5}$). Normal

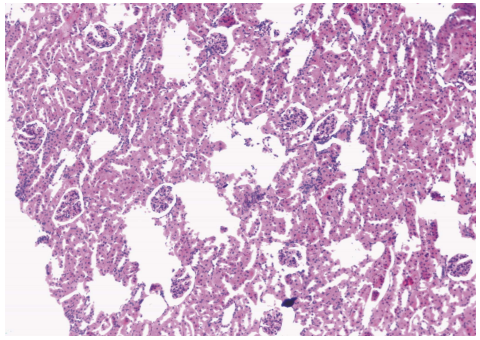


Plate 31: Kidney ($T_{5.0}$). Not normal

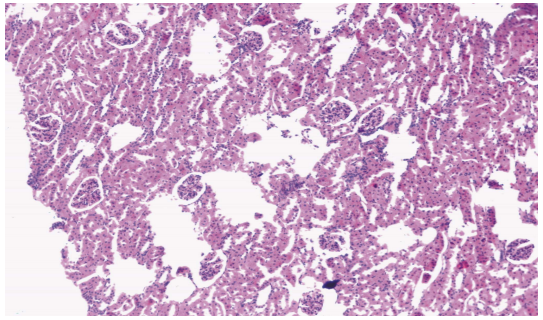
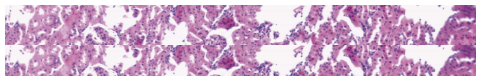


Plate 32: Kidney ($T_{7.5}$). Not normal



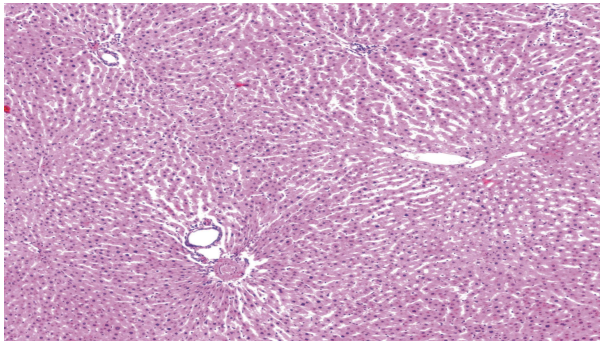


Plate 33: Liver (T_0). Normal

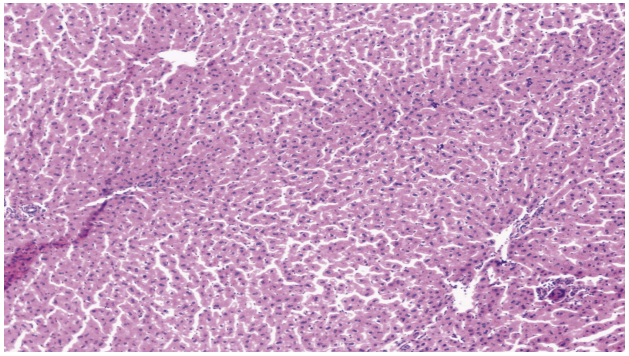


Plate 34: Liver ($T_{2.5}$). Not normal

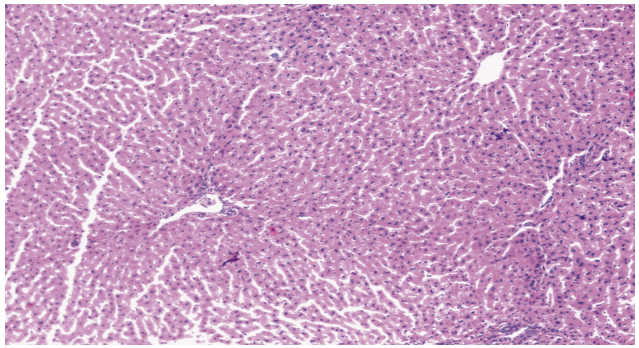


Plate 35: Liver ($T_{5.0}$). Not normal

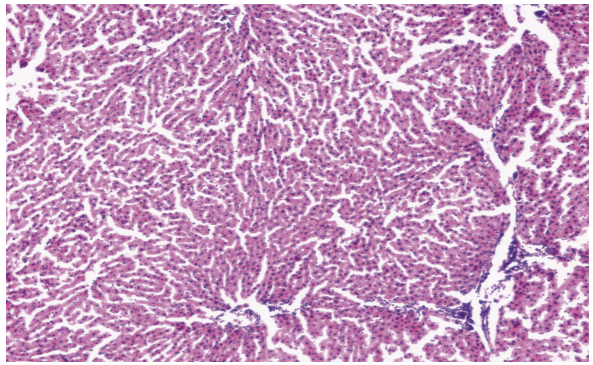


Plate 36: Liver ($T_{7.5}$). Not normal

Glomeruli atrophy with marked cellularity within the tuft, stroma proliferation, scanty stroma in some areas with cystically dilated spaces and chicken wire appearance were observed in $T_{7.5}$.

There were no observable histological changes in the heart for $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$ as compared to the control. This implied that *Garcinia kola* had no deleterious effect on the hearts of rabbits.

4.4 **Discussions**

Much work has not been done on the nutritional quality and antimicrobial activities of *Garcinia kola* for laying hens and growing rabbits. *Garcinia kola* contains nutrients such as crude protein, crude fibre, ether extract, crude ash and nitrogen free extract (Ibekwe *et al*, 2007; Esiegwu and Udedibie, 2009); phytochemicals such as phenols, alkaloids, tannins, saponins and cyanogenic glycosides (Okwu, 2005; Esiegwu and Udedibie, 2009); Vitamins (Okwu, 2005) and minerals (Okwu, 2005; Odebunni *et al.*, 2009). The presence of these nutritional and phytochemical substances gives it food and antimicrobial value. That is why Okwu and Ekeke (2003) reported that the protective effect of bitter kola is due to the presence of phytochemicals, vitamins and minerals.

A proximate analysis of bitter kola in this study showed that it contained minerals and vitamin C as earlier reported by Odebunmi *et al.* (2009) and Okwu (2005). However, most of their values were not in agreement with the values obtained in this study.

The feed intakes for both the layers and the rabbits in this study were similar. This is in line with the report of Esiegwu and Udedibie (2009) on broilers fed graded levels of *Garcinia kola* seed meal but contradicts the report of Uko *et al.* (2001) that water extract from bitter kola fed to rats had depressive effect on appetite and water intake. The results of this experiment showed that bitter kola seed meal had no significant effect on

feed consumption and the values indicated that *Garcinia kola* did not depress appetite.

There were no significant differences (P>0.05) in hen-day egg production. However, the value for the control was relatively higher than those of the *Garcinia kola* groups. This may be attributed to the anti-inflammatory property of bioflavonoids in bitter kola (Braid 1993) that may have affected ovulation, an inflammatory process. However, this finding contradicts the findings of Adediji *et al.* (2008) in a similar experiment with *Garcinia kola* on layers where the *Garcinia kola* group had significantly (P<0.05) higher hen-day egg production than the control. This study indicated that *Garcinia kola* seed meal had no deleterious effect on laying hens.

There were no significant differences (P>0.05) in egg weight among the *Garcinia kola* groups; however, $T_{5.0}$ was significantly (P<0.05) heavier than the control. Adediji *et al.* (2008) observed no significant differences (P>0.05) in egg weight. The findings from this study contradicts the findings of Akpantah *et al.* (2005) that bitter kola seed extract reduced the weight of foetuses in treated rats. The bitter kola groups performed better than the control with regard to body weight changes with $T_{7.5}$ significantly (P<0.05) heavier than the control. This was a reversed case in the rabbits where the control performed significantly (P<0.05) better compared with treated groups. This may be due to the presence of tannin that causes reduced coprophagy in rabbits which is a process of getting some daily supplies of proteins, vitamins, volatile fatty acids and dry matter in rabbits (Lebas *et al.*, 1997). Uko *et al.* (2001) reported that water extract from *Garcinia kola* caused poor feed utilization efficiency and weight gain of rats. Esiegwu and Udedibie (2009) reported heavier body weight for $T_{2.5}$ and the control compared to other treatment groups.

There were no significant differences (P>0.05) in feed conversion ratios for the layers; however, for the rabbits, feed conversion ratio was

significantly (P<0.05) improved than the treated groups, possible due to lack of coprophagy caused by *Garcinia kola*.

Economics of production revealed that feed cost increased as the dietary levels of *Garcinia kola* increased. The percent increases in cost of kg *Garcinia kola* seed meal feed were 32.26% - 96.78% for layers and 38.67% - 118.64% for rabbits respectively, from 2.5% to 7.5% level of inclusion. This increase in cost of treated feed could be attributed to the high cost of *Garcinia kola* seed in the market as a result of imbalance in the forces of demand and supply in the market. The demand for *Garcinia kola* seed is high especially in the eastern part of Nigeria where it serves as a sign of welcome to visitors and is used in ceremonies for entertainments. Invariably, the price rises as the demand rises with limited supplies. Consequently, the cost of feed per kg of eggs and per kg body weight gain of rabbit rose from 28.18% to 99.33% and 96.40% to 207.38% respectively, from 2.5% to 7.5% dietary levels of *Garcinia kola* seed meal. In other words, the high cost of *Garcinia kola* seed increased the cost of production of kg of feed and consequently there was an abnormal rise in cost of production of kg eggs and kg body weight gain of rabbits. To reduce the cost of *Garcinia kola* seed, farmers should be encouraged to plant *Garcinia kola* in their farms, to increase its supply in the market and subsequently reduce the demand which consequently will bring about a drop in the price of *Garcinia kola* seed. This will eventually reduce the cost of production of *Garcinia kola* supplemented feed.

Khul and Sullivan (1977) suggested that egg weight was not a dietary effect but a function of number. The egg weights were within average egg size reported by Oluyemi and Roberts (2000). There were no significant differences (P>0.05) in the egg length, egg width, yolk length, yolk width, albumen height, albumen length and albumen width. $T_{2.5}$ had the albumen

weight and percent albumen significantly heavier (P<0.05) than the other treatments.

Harms and Hussein (1993) reported that eggs containing a large proportion of thick white albumen are regarded as being of high quality. This showed that *Garcinia kola* was capable of producing good quality eggs. The yolk weight and yolk percent of the control were significantly higher (P<0.05) than $T_{2.5}$ but not different from $T_{5.0}$ and $T_{7.5}$. This indicates that the eggs from the control birds were lower in quality compared with treated groups since heavier albumen indicates good quality eggs and the heavier the yolk the lower the albumen. It implied that the eggs of the *Garcinia kola* groups were higher in nutrients than the control.

There were no differences (P>0.05) in the shell weight and percent shell weight, shell thickness, egg shape index, egg shell index, and Haugh unit. These parameters were not affected due to *Garcinia kola*. Shell index indicated no marked effect due to treatment.

There were no significant differences (P>0.05) in the PCV, Hb, WBC and RBC of both the layers and rabbits. This is in line with the findings of Uko *et al.* (2001). There were also no significant differences in the MCV, MCHC and MCH of the rabbits and layers among the treatments. There were no significant differences (P>0.05) also in the differential counts, lymphocytes, monocytes, eosinophils, basophils and neutrophils. The absence of these parameters (basophils, eosinophils and monocytes) in the serum plasma indicated the good health status of the animals because these differential leucocytes multiply in the presence of infection or parasite invasion. The platelets responsible for blood clothing were not affected by the treatments (P>0.05) although, there was an increase of platelets as the dietary bitter kola level increased in the rabbits. This was quite different from the layers. The platelets of the layers on T_0 was significantly (P<0.05) higher than those of $T_{5.0}$ and $T_{7.5}$. This may suggest

the possibility that *Garcinia kola* could delay blood clotting time when high dosage of it is consumed by obstructing thromboplastin formation. Haematological indices are an index and reflection of the effect of dietary treatments on animal. Ojebiyi *et al.* (2007) reported that one of the ways of evaluating the health status of animals used in feeding trials is to evaluate the haemotological indices. Olorode *et al.* (1995) remarked that dietary components have been reported to have measurable effects on blood components. This study revealed that *Garcinia kola* did not alter the blood constituents of both the rabbits and layers compared to the control, hence it is safe and can be used as feed additive.

There were no significant differences ($P>0.05$) in the serum albumin and globulin of the layers and rabbits. This contradicts the report of Uko *et al.* (2001) that water extract from *Garcinia kola* decreased total albumin concentration in rats. There were no significant differences ($P>0.05$) in serum proteins of layers but in rabbits, serum proteins of the control was significantly lower ($P<0.05$) than $T_{2.5}$ but statistically the same with $T_{5.0}$ and $T_{7.5}$. However, it should be noted that serum proteins of the *Garcinia kola* groups tended to be higher than the control. This is in agreement with the findings of Esiegwu and Udedibie (2009) that *Garcinia kola* seed meal tended to increase plasma proteins as the dietary levels increased which contradicted the report of Uko *et al.* (2001) that water extracts from *Garcinia kola* decreased total plasma proteins of rats. The serum glucose of the layers were statistically the same unlike in the rabbits where the control was significantly ($P<0.05$) higher than $T_{2.5}$ and similar to $T_{5.0}$ and $T_{7.5}$. However, there was a steep drop in serum glucose value for the bitter kola groups. Iwu *et al.* (1990) reported that kolaviron from *Garcinia kola* seeds reduced blood sugar in normoglycaemic and alloxan diabetic rabbits whereas Esiegwu and Udedibie (2009) reported that *Garcinia kola* had no significant effect ($P>0.05$) on serum glucose of broilers compared

to control. The serum calcium of $T_{7.5}$ of layers was significantly higher ($P<0.05$) than $T_{5.0}$, $T_{2.5}$ and the control whereas in the rabbits, all the treatment groups were statistically the same. It therefore appeared that *Garcinia kola* increased plasma calcium level although the values had no pattern. Esiegwu and Udedibie (2009) in a similar experiment with broilers observed no significant differences but the *Garcinia kola* groups had a progressive increase in plasma calcium.

There were no significant treatment effects in plasma cholesterol in both rabbits and layers. The serum sodium, potassium and bicarbonate values of the layers and rabbits were also statistically the same. It implied that *Garcinia kola* did not interfere with the serum electrolyte metabolism. This is in agreement with the findings of Esiegwu and Udedibie (2009). The serum chloride of the control for the rabbits and layers were statistically the same with the *Garcinia kola* groups.

The ratio of dressed weight to live weight is used as a measure of meat production in farm animals, because there is a relationship between weight and physical characteristics of animals which is a reflection on feed efficiency and performance (Ijaiya and Fasanya, 2004). Significant differences did not exist in the dressing out percentage for layers but in the rabbits, $T_{5.0}$ performed best, but statistically the same with the control and $T_{7.5}$. Treatment effect did not reduce carcass weight of the animals. In other words, there was no negative effect on carcass due to treatment effects.

The weight of the liver was not affected in both the layers and rabbits. The weights of the kidney were statistically the same in the rabbits but in the layers the control was significantly higher ($P<0.05$) than the *Garcinia kola* groups. This might probably indicate that the *Garcinia kola* groups were not effective in removing the waste products of metabolism especially urea. Abdominal fat for the layers were not significantly different ($P>0.05$)

from one another. However, in the rabbits, the control developed significantly more ($P<0.05$) abdominal fat than the treated groups. This may indicate that *Garcinia kola* could be used to defatten animals especially when lean meat is required. The intestinal length, intestinal weight and gizzard of the layers did not show any significant treatment effects ($P>0.05$). It showed a balanced state of activity in the intestinal tract. The crops of $T_{5.0}$ and $T_{7.5}$ were significantly ($P<0.05$) heavier than that of the control. Probably, food stayed longer in the crops of the *Garcinia kola* groups due to delayed time of digestion in the gastrointestinal tract. Also the caecum lengths of the treated groups were more than the control but no significant differences existed. The weights of the lungs of the *Garcinia kola* groups of the rabbits were statistically the same compared to the control. The weights of the skins were also statistically the same. The hearts of the rabbits were also statistically the same but heavier in the control group of the layers. There was a progressive decrease in the weight of the heart as dietary *Garcinia kola* increased. Adaramoye *et al.* (2005) reported that kolaviron, an extract of *Garcinia kola* reduced plasma cholesterol levels and the relative weight of the heart. Plasma cholesterol in this study were non-significant and of variable values similar to the report of Esiegwu and Udedibie (2009).

Parasitological observations showed the presence of *Ascaridia lumbricoides* and *oocyst* of *Isospora belli* in the experimental animals at the beginning of the experiments. At the end of the experiments, *Ascaridia lumbricoides* and *oocyst* of *Isospora belli* disappeared in the layers but *oocyst* of *Isospora belli* were still present in the rabbits. The disappearance of *Ascaridia lumbricoides* and *oocyst* of *Isospora belli* in the layers could be attributed to the dietary treatments indicating that *Garcinia kola* has anti parasitic activity, supporting the report of Ebana *et al.* (1991) and Akoachere *et al.* (2002) that *Garcinia kola* is antimicrobial.

However, why the parasites in the rabbits did not respond to the dietary treatments may need further investigations.

Clinical evaluation of the intestinal droppings of the layers and the faecal samples of the rabbits revealed that bitter kola was bactericidal to *Salmonella spp* and *Streptococcus spp*. *Salmonella spp* and *Streptococcus spp* were present in the intestines of the experimental animals at the beginning of the experiments but disappeared at the end of the experiment in the $T_{5.0}$ and $T_{7.5}$. *Streptococcus* was present in the $T_{5.0}$ of the rabbit, although at a low concentration but disappeared at the end of the experiment. *Salmonella* was still present in the control and $T_{2.5}$.

Histopathological observations revealed no histological alterations of the heart for both the layers and the rabbits due to *Garcinia kola* dietary treatment. The gizzard of the laying hens were histologicaly altered at $T_{7.5}$. The kidney and the liver of the rabbits and layers were distorted at $T_{5.0}$ and $T_{7.5}$. The main cellular changes were proliferation of tissue stroma with hypertrophy and necrosis, presence of oedema and distortion of general tissue architecture. This report agrees with the findings of Braid and Grill (1990) who revealed some histological alterations in the liver, kidney and duodenum of rats fed diets containing 10% (w/w) dry powdered *Garcinia kola* for six weeks but contradicts the report of Uko *et al.* (2001) that there were no histological alteration of the liver and kidney of rats fed water extract of *Garcinia kola*.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 **Conclusions**

It is concluded that *Garcinia kola* contains both macro and micro minerals and vitamin C but at low levels. It can serve as a feed additive or ingredient for layers and rabbits in view of the comparative dressed weights obtained which is a good economic and unbiased indices of estimation of performance. It is, however, very expensive to use as feed ingredient for poultry or rabbit production.

The trial also showed that *Garcinia kola* had no depressive effect on appetite and feed consumption. In the contrary, it stimulates feed consumption. It had no deleterious or harmful effect on egg production and egg quality and tends to promote high egg quality.

The trial has also shown that *Garcinia kola* has no toxic effect on blood constituents of layers or rabbits and can serve as anti-parasitic and anti-bacterial agent at 5.0% and 7.5% levels of inclusion against cyst of *Isospora bellies*, *Salmonella and Streptococcus species.*

It, however, causes histological alterations of the kidney and liver of both rabbits and layers at 5% and 7.5% inclusion levels and the gizzard of layers at 7.5% dietary level.

5.2 Recommendations

Dietary inclusion of *Garcinia kola* seed meal for layers and rabbits should not exceed 5.0% since beyond this level there could be histological alterations of the kidney, liver and gizzard.

In view of the fact that it is effective as anti-parasitic and anti-bacterial agent only at 5% dietary levels and above, the level at which feed cost almost doubles, it cannot be recommended as feed additive or ingredient for layers and rabbits, since at this level, it also causes histological alterations in the kidney, liver and gizzard.

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