QUALITY ATTRIBUTES OF EGGS FROM HENS FED DIETARY *Moringa oleifera, Ocimum gratissimum* and *Vernonia amygdalina* LEAF MEAL INCLUSION

BY

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CERTIFICATION

I hereby certify that this work was carried out by ADETORO, Benedicta Omosalewa under my supervision in the Department of Animal Science, Faculty of Agriculture, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

This project work is dedicated to the Almighty God, my Father, my maker, my strength, my healer and above all the Saviour of my life for the opportunity and grace to begin and complete this research. Also, to my darling parents and siblings who God made pillars of support for me from birth till now as well as my lovely husband and children for standing by me and encouraging me. I love you so much.

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ABSTRACT

Green leafy vegetables like *Moringa oleifera*-MO*, Ocimum gratissimum*-OGand *Vernonia amygdalina*-VA are ubiquitous and contain bioactive compounds which may be used to improve quality indices of eggs. Golden yellow colour is an index of egg yolk that is most preferred by consumers and could be achieved by feeding leaf meals to laying hens instead of commercial yolk colourants. Previous researches on Dietary Leaf Meals (DLM) for hens were focused on performance and digestibility with scanty information on sensory attributes and quality of eggs. Therefore, quality characteristics of eggs from hens fed diets containing MO, OG and VA DLM were investigated.

Leaves of MO, OGandVA were harvested at week 20 of regrowth, air-dried, milled, sieved with 0.05 mm mesh and assayed for crude protein, ash and secondary metabolites using standard procedures. Four soybean-maize based diets were formulated: control T_1 (0% DLM) while T_2, T_3 and T_4 contained 2% MO, OG and VA, respectively. One-day old pullet chicks (n=240) were allotted to the diets. Control diet was fed to 24 chicks for 61 weeks while diets T_2, T_3 and T_4 were fed to six chickens per replicate from: weeks 0-61 (Regime 1); 9-61 (Regime 2) and 18-61 (Regime 3) using completely randomised design. Each treatment was replicated four times. Egg Weight-EW (g), Yolk Weight-YW (g), Yolk Colour-YC (Roche yolk-fan scale: very light yellow 1-14 orange), Albumen Weight-AW (g) and Haugh Unit-HU (%) were determined at Mid Lay-ML (53-58 weeks) using standard procedures. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

The MO, OG and VA leaves contained 19.6±0.0, 19.1±13.6 and 24.8±4.6% crude protein and; 3.8±1.7, 7.3±5.9 and 7.2±5.2% ash, respectively. Terpenoids, steroids and phenols were detected in the three DLM while only VA had saponins. The DLM type did not affect EW. Hens on regime 1 had significantly higher EW (58.3±5.3) than those on regime 2 (56.0±5.9) and regime 3 (57.2±5.6). Similar YW was observed in eggs from control(15.1±0.2) and OG (15.1±0.2) but higher than MO (14.9±0.2), while VA (15.5±0.3) was highest. Eggs from regime 1 had significantly higher YW of 15.4±2.3 thanregime 2 (15.1±3.1) which was also higher than regime 3 (14.8±2.2). Eggs of hens fed OG, VA and control diets had similar light yellow YC ratings (2.9±0.2, 3.0±0.2 and 2.9±0.1, respectively) but differed from MO (3.5±0.1, golden yellow).Feeding regime had no effect on YC. Albumen weight among DLM was similar but differed among regimes. Regime 1 had AW of 35.6±4.3,regime 2 and regime 3 had values of 33.7±5.2 and 34.6±4.6, respectively. Haugh unit of eggs was not affected by DLM while regime of feeding was significant. Eggs from Regime 1 had significantly higher HU (79.8±4.1) than Regime 2(278.8±5.4) and also higher than Regime 3 (77.8±5.5).

Dietary leaf meals used in this work improved egg weight of hens fed from one day old to week 61. Leaf meal inclusion resulted in healthier eggs with lower low density lipoprotein *Moringa oleifera* leaf meal enhanced golden yellow yolk colour of eggs.

Keywords: *Moringa oleifera, Ocimum gratissimum*, *Vernonia amygdalina*, dietary leaf meal, Egg quality.

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CHAPTER ONE

1.0 INTRODUCTION

Egg is the natural and major biological product of poultry that is laid by various species of female animals and meant for hatching into the young form of the particular specie for reproduction as in poultry and other egg laying animals (snails, lizards and fishes) specie. Poultry egg is contained in a shell that consists of the albumen and yolk which develops, when fertilized into an embryo and at the same time contains the food the embryo feeds on till it hatches (Abanikannda and Leigh, 2007). The most common of the poultry species particularly kept for egg production is the chicken eggs in which unfertilized chicken eggs are named table eggs as they cannot develop into a living embryo.

Egg is a very rich source of lutein and zeaxanthin which are carotenoids that play very vital roles in eye health and its intake has been confirmed to decrease eye muscular degeneration associated with aging (Johnson, 2014) and cataracts. Feeding of eggs to infants has been reported to improve their nutrient profile particularly protein, docosahexaenoic acid (DHA), α-linolenic acid, phosphorus, choline, vitamin B12, and lutein + zeaxanthin, (Papanikolaou and Fulgoni, 2018). Eggs are equally high in dietary choline which happens to be another essential nutrient that is usually not adequately consumed (USDA, 2019) and in α-linolenic-acid (octadecatrienoic acid) (Pitkin *et al*., 2000). Egg yolks are particularly rich in lipids, making it a good source of reserved energy.

There are little variations within the last few years on the consumption of table eggs and there isn't much information on the cause of this sluggishness, (Bertechiniand Mazzuco, 2013). The main challenge to egg consumption is the inadequate knowledge on its value in the feeding and health of man (Patil*et al*., 2005). Egg users need to be equipped with adequate information on the effectiveness of eggs, its compositions,

biological value and how it's involved in man's diet. It is a common belief that eggs from birds raised traditionally and without inorganic feed ingredients are of better qualities in terms of nutrient composition and this therefore determines the type of eggs they purchased (Bajaei*et al.,* 2011).

Consumption of eggs is determined by so many factors, among which are: age, income, level of education, ethnicity (Kant and Graubard,2012; Kirkpatrick *et al.,*2012; Conrad *et al*., 2017) and health status. A good number of people believe that eggs are only good for children to enhance their growth and proper development but not for adults because adults are believed to have completed their growth and so do not need so much nutrient rich food such as egg. An increase in income generation mostly affect people's choice of food, eggs can thus be added to the menu to create class/ status and/ improve their diets (Leung *et al.,* 2014). Level of education coupled with the information people are equ0ipped with greatly affects their feeding habits and diets. Those furnished with adequate information on egg compositions and especially its effect if consumed will gladly include it in their diets.

Development of different products from egg will boost the demand and consumption of eggs. This will encourage egg intake in diverse forms and so encourage both the producers of eggs to increase their output and at the same time, the consumers who would have so many ways to consume the same product and be more satisfied as their desires are met. Egg production and consumption are expected to increase the moment egg users discover the facts about the egg nutrient profile and how its intake affects their health as eating of eggs regularly improves the totality of human growth and development. Antioxidants are present in egg yolks and these have been said to prevent aging (Bertechiniand Mazzuco, 2013).

Egg production contributes significantly to the growth of a Country's economy by improving its commerce (USDA, 2018) since it is consumed throughout the entire world. It creates jobs for a large number of people and thereby reducing the problem of unemployment. Egg production interval is short once hens begin to lay and so encourages resource turnover. Table eggs are cheap, readily available and richer than most other animal protein source and so have been successfully used to combat malnutrition and protein deficiencies. Commercial egg production will successfully lower the unit cost of eggs. Table eggs can be easily processed and converted to more

durable products while still retaining the original nutrients of the egg if produced in excess and can be exported to less producing Countries as it is an important commodity in international trade. Table eggs are also raw materials of so many other companies while the undesirables can be fed to aquatic animals and pet foods. With so many inexhaustible usefulness of egg especially to humans, the production and quality of eggs should be improved and maintained to encourage its consumption and utilization.

Egg shell colour does not have any effect on the egg nutrient composition but a reflection of the plumage colour of the egg laying hen, though it is believed by most consumers that, egg shell colour has a great impact on the egg's nutrient composition. The essential nutrients of all eggs are the same, irrespective of the shell colour. Egg nutrient contents can be improved by feeding nutritionally fortified diets to laying hens and such eggs are sold separately as nutritionally improved eggs that are specially packaged and labelled. The nutrient content and generally the egg quality are equally altered by the health status of the hens. Colour of egg yolk is dependent on the ingredients used in compounding the hen's diet especially the carotenoids though deep yolk colouration is an egg quality that improves marketability of eggs by the producers and the purchasing quality of most consumers.

Quality of an egg encompasses everything that makes the egg suitable for consumption and other uses, both nutritionally and health-wise. The nutritional aspects are the compositions, the nutrient content of the egg (calorie, protein and other classes of nutrients) while the health aspect include the safety in consuming such eggs as they must be disease-free and not pose any form of threat to the consumers. These egg qualities can be assessed both internally and externally and both are dependent on the treatments (management and environment) the hens are subjected to before reaching the laying phase and how the eggs are handled after lay (Bertechiniand Mazzuco, 2013), that is, from production on the farms to consumption. Egg quality can be easily influenced by manipulation of the feed fed to the laying birds. Therefore this calls for a reason to find a way in order to achieve these qualities. Egg weight is economically considered and the most commonly measured quality parameter, while air cell, haugh unit and yolk index are vital tools adopted in egg freshness measurement (Samli *et al*., 2005).

Egg quality maintenance and improvement have therefore been a source of concern for both egg producers and the consumers and this has made room for values addition to eggs in many diverse ways while the consumer's safety and satisfaction are of utmost importance. Egg-laying hens' diets have been enriched with so many different supplements/ additives with the aim of improving the egg qualities. Some of the additives used in the past have been banned due to their residual effect on the egg consumers. Inclusion of antibiotics at sub-therapeutic level lowers the population of some undesired microbes, resulting in enhanced feed utilization and so are categorized as feed additives. These additives lower production cost by optimizing the efficiency of the laying birds and these additives are classified as organic (obtained from plants and used to boost animal performance (Nakatani, 2000) or inorganic (agrochemicals which include antibiotics) in poultry industry.

Antioxidants, emulsifiers, binders, pH control agents and enzymes, etc., have equally been utilized in poultry diets as feed additives. Growing concern about chemical products usually synthetic, especially, antibiotic growth promoters (particularly their residual effects in the products, meat and eggs) in animal nutrition has created efforts to use different alternative naturally occurring plant materials/ parts (seeds, leaves, stems and roots) having potentials to serve as growth promoting agents, antioxidants, inhibitors of anti-nutrients and or at least improving the quality of the feed fed to the animals as well as the produce/product from such animals. Among such plants are *Moringa oleifera, Ocimum grattisimum* and *Vernonia amygdalina.*

Moringa oleifera (MO)is a plant that has several uses that has given considerable fodder yield and thrives very well in tropical humid zone of Nigeria all year round (Fadiyimu*et al*., 2011) belonging to a single-genus family Moringaceae. Every single part of the plant can be used in feeding animals and since it can be easily cultivated, it has served as an inexpensive source of protein. This plant has been so researched and found to contain abundant antioxidants which makes it suitable for stabilizing fat-rich foods, (Dillard and Bruce, 2000) and has been widely used in poultry nutrition studies.

Ocimum grattisimum contains sweet smelling oil that is volatile and contains tannin an antibacterial agent (Elujoba, 2000)has been screened to possess alkaloids, citral, camphor, cinnamate, eugenol, flavonoids, linaol, methyl, saponins, thymol and triterpenes(Lemos *et al*.,2005). Eugenol possesses antihelminthic, nematicidal, and

insecticidal properties (Pessoa *et al*., 2002) with the oil being an excellent antimicrobial agent (Mbata and Saikia, 2009), a potent antidiabetic agent (Mohammed *et al*.,2007) and equally worked in the treatment of many disorders, including diarrhea, eye problems, fever, headache, pneumonia, skin disorders, upper respiratory tract infections (Ofem *et al*., 2012), spasm and hyperactivity of the gastrointestinal tract (Elujoba, 2000).*O. gratissimum* is good for flavoring foods (Mbata and Saikia, 2009) which makes it a suitable additive to improve poultry production and products.

Vernonia amygdalina (VA) have been put into several uses by scientists among which are the use of VA leaf extracts to treat coccidiosis (Dakpogan *et al*., 2018). The extract from the leaf was also used to treat bacilliary white diarrhea and bronchitis (Gbolade, 2009). Many experimental studies on VA have revealed that the plant possesses antibacterial and anti-parasitic property (Tadesse*et al.,* 1993) and possess active complex compounds that have pharmacological usefulness (Tadesse*et al.,* 1993). VA leaves increased feed utilization efficiency of cockerels but not their hematological assay (Olobatoke and Oloniruba, 2009) and provide antioxidant benefits (Erasto *et al.,* 2007).

This study sought to investigate the consequences of *Moringa oleifera, Ocimum grattisimum* and *Vernonia amygdalina* leaf meal (treatments) inclusion in poultry diet from one day old (Regime 1), grower stage (Regime 2) and point of lay (Regime 3) and to provide knowledge on the probable effects of the regime of introduction of the leaf meals to the birds on egg quality parameters (internal and external).

1.1 Justification of the study

Information on the beneficial effects of leaf meals on the overall performance and quality of eggs of laying hen should be well documented.

Enhancement of nutritional value of eggs may improve daily nutrient intake and eventually improve human health. Improved egg quality and value will encourage consumers and thereby improve egg marketability and egg farmers income.

Information on these three local leaf meals will be beneficial to poultry industry

Data generated on blood profile of pullet chicks will serve as guide for other researchers as such information is presently scanty especially in the tropics.

1.2 Objective of the study

1.2.1 General objective

1. To improve the quality of table eggs with the use of three common leaf meals and thereby promote egg consumption

1.2.2 Specific objective

To evaluate the:

2. effect of selected leaf meals on blood parameters of birds fed with diets containing *Moringa oleifera, Ocimum gratissimum* and *Vernonia amygdalina* leaf meals
3. growth performance of birds fed diets containing the leaf meals
4. laying performance of hens fed diets containing (*M. oleifera, O. gratissimum* and *V. amygdalina*) leaf meals.
5. Internal and external qualities of eggs from the hens fed diets containing the medicinal plants
6. Fatty acid and cholesterol profile of eggs from the hens fed diets containing the test ingredients.

1.3 Hypotheses

H_{o1}: There are no significant differences in the performance, blood parameters and quality characteristics of eggs from hens fed dietary *M. oleifera, O. gratissimum* and *V. amygdalina* leaf meal inclusion.

H_{A1}: There are significant differences in the performance, blood parameters and quality characteristics of eggs from hens fed dietary *M. oleifera, O. gratissimum* and *V. amygdalina* leaf meal inclusion.

H_{o2}: Interaction of leaf meals and the regime of introduction of the leaf meals to experimental birds are not significantly different on performance, blood parameters and quality characteristics of eggs from hens fed dietary *M. oleifera, O. gratissimum* and *V. amygdalina* leaf meal inclusion.

H_{A2}: Interaction of leaf meals and the regime of introduction of the leaf meals to experimental birds are significantly different on performance, blood parameters and quality characteristics of eggs from hens fed dietary *M. oleifera, O. gratissimum* and *V. amygdalina* leaf meal inclusion.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry production

Large proportions of farmers in rural and urban areas are involved in poultry farming either as small or medium scale business (Idowu *et al.,* 2005). In time past, Cock fighting was the main reason chickens were raised and later exhibition in the 19th century in some parts of Europe. Breeders in the poultry industry later in this same 19th century in Europe and America emphasized chicken meat and egg production more than any other poultry specie. Artificial incubation of eggs started in ancient China and Egypt and later in Nigeria where farmers gained interest in artificial incubation of eggs on commercial level of production around 1870s. Presently in Nigeria, application of scientific knowledge to poultry breeding and management has grossly improved both production and utilization of poultry products as food compared to what was obtained before World War II (Kaws, 1998).

New procedures were developed to improve poultry product distribution and storage and especially to boost its purchase and utilization as farmers specialize in broiler production and as such expand the poultry industry since 1945. In Nigeria, so much improvement has been speedily achieved in the last three decades and these include in quantity, efficiency and technical expertise owing to strictest norms. Poultry as a branch of agriculture has recently increased its importance in supplying these products for more than twenty five years which has resulted in a steady increase in the industry.First is the fact that poultry successfully utilize feed, turning it into food consumed by human. Secondly that the expansion of poultry industry to a commercial scale was also easily achieved even with cheap labour while it still provides considerable amount of meat and eggs both for consumption and sale (Fayeye *et al*., 2000)

Poultry is the production and rearing of avian species. Poultry originated from the wild jungle fowl but now include ducks, geese, turkeys, ostrich, pigeons, guinea fowl, quail and chicken. Different poultry species serve as companions, food (eggs, meat) and agro-industrial by-product (feathers) (Alikwe *et al.,* 2016). Birds have been reared and used for show and others at fair. Large scale poultry industries provide egg and meat, though feathers are utilized to manufacture fertilizer, mattress, pillows and milled as feed ingredient. Droppings of poultry are among the best manure used for crop production. Chicken meat is highly toothsome and is a major source of protein eaten by man its by-product has also been adopted as meat. Poultry meat is considered a white meat that is healthy for consumption

Poultry industries in a bid to improve the nutrition of people in the developing countries, functions in transforming grains and other ingredients /products to eggs and meat. Thereby providing high quality food to consumers at the most economically possible and reasonable cost as this is the main objective of agriculture. Due to the fact that these products are obtained from the poultry, the industry has recently become very important. Layer poultry farming involves management of birds that lay eggs with the aim of producing eggs for commercial utilization. These birds are reared from one day old and begin to lay from 18 to 19 weeks old until they are about 72 to 78 weeks old (Adeyemo, 2020). During their active moment, they are able to lay close to 1kg eggs on consuming 2.25kg feed.Hybrid layers are special breeds of birds that begin to lay between 18- 20 weeks old and reaches the height of egg production in the first production cycle (Jacobs *et al.,* 2018).

Production efficiency of laying birds however decreases as the birds grow older and becomes worse in the second production cycle. A good number of factors affect production efficiency of laying birds especially climate, (Oluyemi and Roberts, 2000), breed, mortality rate, body weight, illumination of the pen, feeding and culling (North and Bell, 1990).Environments with high temperature that are also humid, have their commercial hybrids lay between 180 and 200 eggs annually where about 250 and 300 eggs annually is achievable in temperate regions. Laying birds are usually culled after actively laying for a year and sold for slaughtering as meat though they have the ability to lay for longer periods (North and Bell, 1990) but due to market demands and disease while new set of pullets are reared for efficient egg production (Smith, 2007).

2.2 Commercial layers' characteristics

Commercial layer's egg production are determined by care of the birds and farm management as proper care of birds result in high production and profit. Characteristics displayed by birds during their periods of production include:

About 5% of birds start laying within the first 20 weeks of age.

About 10% birds begin to lay at 21 weeks of age.

High production at about 26 to 30 weeks of age (This may be strain dependent).

Mostly stop laying for some days after laying a maximum number of eggs.

Followed by slow reduction in their egg production

There is gradual increase in egg production efficiency and egg size.

Hens' growth last for about 40 weeks of age while their weight and size continue to increase until they are about 50 weeks old (Adeyemo, 2020).

2.3 Factors affecting egg production

Reproduction cycle of laying hens typically lasts just over one year (52-56 weeks), in which many factors determine egg production. Effective and efficient management of the cycle is therefore essential so as to provide optimum yield and profitability (Milinsk *et al*., 2003). Among the factors that encourage egg production are the following.

*Age.*Birds starts laying at twentieth or twenty-first week and laying continue to improve with better egg size till a little above one year but their laying efficiency gradually decrease with reduced egg quality as the hens approach the end of their production cycle (FAO, 2003).

Body weight: This is generally expected to be about 1.5 kg during the laying period, though variations may occur as a result of breed difference. Reduced weight and increased weight of birds result in lower rate. Appropriate management with adequate feeding are essential to obtain optimum body weight (FAO, 2003).

*Breed.*This determines to a great extent the quality of eggs laid, especially shell quality (texture and thickness), presence of blood stain in eggs, texture of the albumen and egg production (Jones, 2006).

Feed: This to a large extent influence the hens' growth, production efficiency and egg quality Leeson (2011) (especially, taste, yolk colour and shell colour). Potable water must be made available always, since a laying hen requires about a quarter litre of water daily.

Health of the flock: mortality reduces the flock size and subsequently the flock production rate. Disease/ Morbidity also reduce production and also the egg quality ranging from poor quality shell to reduction in internal egg quality (Jones, 2006). Adequate and timely vaccination should therefore be administered to either prevent or correct the situation as the case may be. Increased environmental temperature (Usayren *et al*., 2001) and predators should also be controlled.

*Laying house:*Climatic condition of the site where the poultry house is situated is of utmost importance while the laying birds' safety is of primary concern (Rostagno *et al*., 2005). The house should offer adequate protection of the hens from pilfering, predators, direct unfavourable weather, dirt, flunctuating temperature and encourage good ventilation. The design should promote easy cleaning and adequate care of the flock (FAO, 2003).

*Lighting schedule:*Daylight stimulates egg production; so the longer the hens are exposed to light, the more production increases. Laying hens needs about 16 to 17 hours exposure to illumination (natural or artificial) to achieve constant and maximum egg production. Effective day length must therefore be maintained and never reduced throughout the hens' period of laying (Smith, 1990).

Management: handling, feeding and general care given to the laying hens can greatly improve egg production. Timely collection of eggs can also reduce damages to the egg and pecking of eggs by the hens(Coutts and Wilson, 1990).

Culling: is the separation of undesirable (that are either sick or unproductive) hens, from the flock. Culling encourages the maintenance of optimal egg production, reduces feed wasted on undesired birds and curb flock morbidity (FAO, 2003).

Culling is of two methods:

- Mass culling: the complete removal of flock with complete replacement with new set and
- Selective culling, which is the removal of the unproductive or sick birds.

Climate: Optimum temperature for laying ranges between 11° and 26° C. While humidity level beyond 75% results in reduced egg laying. Table 2.1 shows how temperature affects egg production (FAO, 2003).

Increased temperature beyond 28°C results in reduced performance and reduced egg quality. Egg production decreases by around 10% with rise in seasonal temperature.

2.4 Egg formation Process

Egg is normally formed in the reproductive tract of hens but this tract can be separated into two main parts, ovary and oviduct (Jacobs, 2019). The ovary being the organ that produces the female reproductive cells (ova-plural/ ovum-singular) releases the yolk (ovum) from a ruptured follicle and drops in ovarian pocket where in few minutes once the yolk is matured for shedding drops into the infundibulum by the process referred to as ovulation. Infundibulum (funnel-shaped structure at the upper part of the oviduct) captures the matured ovum shortly after shedding and the site where fertilization occurs. About 15 minutes later, the fertilized ovum (yolk) passes through to a glandular structure Magnum, comprising layers of circular muscles which helps in moving the yolk along. This is the site where the albumen is produced and deposited round the yolk as it is formed in approximately 3hours. Yolk rotation occurs, twisting the albumenous fibers and results in the formation the chalazae.

Then the egg moves into theIsthmus, the site of formation of the two shell membranes and this takes about 90 minutes. The developing egg then moves to the shell gland also called the Uterus where salt and water are added before the calcium carbonate ($CaCO_3$) shell with the colour is added. The egg reaches its full size and shape here and these last for 20-26 hours. After a few minutes, the egg that is now completely formed enters the vagina with the small edgefirst , then the uterus inverts through the vagina, the cloaca and then the vent to expel the egg from the body (oviposition) with its large end first (Jacobs, 2019). Another ovulation occurs between 15 to 60 minutes after oviposition.

Table 2.1: Temperature and its effects on egg production

Temperature (^{o}C)	Effects
11 – 26	Good production
26 – 28	Reduced feed intake
28 – 32	Reduced feed intake, increased water intake and reduced egg size with thin shell
32 – 35	Slight panting
25 – 40	Heat prostration sets in (pen house must be cooled)
40 and above	Heat stress results in mortality.

Source:Kekeocha (1985)

2.5 Composition of eggs

An egg is composed of many parts mainly, the albumen, yolk, chalaza, shell membrane and shell. Albumen also called egg white is usually clear in its raw state but becomes white once it's cooked. It is composed of two parts, the thick and the thin parts. The thick part is closer to the yolk. Yolk is the most visible part of the content of an egg close to the center of the egg. Chalaza is usually located in the thick albumen and it's the twisted part of the albumen which prevents the yolk form being moved from the center of the egg thereby preventing it from sticking to the inner part of the egg shell. Shell membrane are two in number they are thin membranes usually differentiated as the internal and the external shell membranes, these surrounds the egg contents. Shell is the outermost part of the egg which encloses every other part of the egg and holds them all together. Fresh eggs are mainly composed of 58% egg white, 32% yolk and 10% shell (Leeson, 2006).

2.5.1 Composition of egg albumen

The albumen of an egg is made up of 88% water with 10% proteins (albumins, globulins and mucoproteins) dissolved in the water and below 1% carbohydrate (Tiefenbacher, 2019, Wikipedia) and minerals. The albumen is normally alkaline in nature, usually of 7.6-7.9 pH which upon storage losses carbon dioxide through diffusion through the pores of the shell with a rise of pH to 9.7 due to aging (Tiefenbacher, 2019). Albumen have an excellent foaming property which has been attributed to the presence the following proteins arranged in order of importance, globulins, ovalbumin, ovotransferrin, lysosome, ovomucoid and ovomucin (Mine, 1995). The albumen have four main structures, the chalaza which is usually next to the yolk makes about 3%, then the internal thin layer that envelops the chalaza, and makes about 17% of the albumen followed by the thick layer which binds the thin albumen and the yolk adhering to the shell membrane at the two ends and forms 57% of the egg white (USDA, 2000).

2.5.2 Composition of egg yolk

Yolk is mainly comprised of about 36% fat (of which about 25-31% fractions are triacylglycerols, phospholipids and 4-5% cholesterols), 17% protein and 65-70% water (Kasperrs, 2016). Protein portion consist of α-livetin (14%; = serum albumen), β-

livetin (41%; = glycoproteins) and γ-livetin (45%; = Immunoglobulin Y (IgY) while carbohydrates, inorganic compounds and vitamins are below a percentage of the egg yolk (Kaspers, 2016). Xanthophylls are the carotenoid responsible for egg yolk colouration (Nys and Guyot, 2011). Liver is the organ in which yolk fats and proteins (except IgY) are produced by the induction of estrogen and testosterone secreted from maturing ovarian follicles (Kaspers, 2016).

The fatty portion is carried in the blood as very low density lipoproteins (VLDL) which binds to certain receptors designated LR8 seriously reflected on the membrane of the oocyte and are subsequently endocytosed into the yolk proteins and protein associated vitamins are also carried with the VLDL. Infertility resulting from fat not being transferred into the oocyte and hyperlipidemia are associated with LR8 receptor mutations (Nimpf and Schneider, 1991).

2.6 Quality of eggs

The quality of an egg could be described as "totality of the nature and compositions of egg that makes it appealing and the choice of consumers". The nature of the egg entails the structure or conformity which must be appropriate, that is, without alteration while the composition takes care of the various constituents of the egg. The reason an egg is preferred depends majorly on the use for which the egg is intended and the likely additional value possessed by such eggs (Bajaei *et al*., 2011).

2.6.1 Factors affecting egg quality

i. Egg Shell Quality:

Desirable shell Qualities include that the shell must;

a) be without visible dirt

b) be without visible cracks even on candling

c) be without developing embryo, spoilage/ putrefaction, and conspicuous blood clots

d) not have undergone incubation

e) have been handled and stored under optimal conditions that maintains its composition.

Both shell quality (external) and internal egg qualities are vital in deciding on egg quality.

ii. Egg shell integrity: All forms of alterations to the shell of the eggs especially cracks, thin shell and shell-less eggs reduce consumer's preference for such eggs and eventually reduces the profit of the farmer. Cracks can result from mechanical damage to the eggs by the birds or egg handlers if not properly managed and especially when eggs have weak shell. Shell weakness also exposes the eggs to contamination especially with microbes. Size of eggs greatly influences the strength of the shell as small eggs are stronger than large eggs since hens are able to deposit specific quantity of calcium on both large and small size egg (Butcher and Miles, 2003).

iii. Age of the hen:Age of the hens influence shell strength as young hens usually have shell glands that are not so matured and so makes the hens to lay eggs without shell or thin shelled eggs, these can however be prevented by extending the sexual maturity of the hens by 1-2 weeks(Coutts and Wilson, 1990).

iv Stress:Anything that disrupts the peace of can alter egg formation process for a number of and this disruption may also alter egg quality (Coutts and Wilson, 1990).

v. High temperature:High (above 25^{o}C) temperature in and around the birds' house usually result in the reduction of feed consumption of the birds which eventually reduces their calcium intake leading to poor shell quality (Butcher and Miles, 2018a).

vi. Quality of feed and water: The feed supplied to the birds should be carefully formulated with appropriate nutrients. Attention should be paid to minerals and vitamins which are vital to achieving good eggshell quality. Water is also very essential for optimal performance of the stock as inadequate intake of water increases shell defects (Coutts and Wilson, 1990).

vii. Genetics:This also have effect on the texture of the egg shell like calcium deposition on egg shell p (Butcher and Miles, 2003a; Coutts and Wilson, 1990).

viii. Management: Inappropriate spacing of hens, inconsistent lighting and inadequate ventilation can all increase egg shell defects (Coutts and Wilson, 1990).

ix. Colour*:* Egg shell colour is dependent on the genetic composition of the hens, while hens with whitefeathers lay white shelled eggs, brown eggs are laid by brown feathered hens (Jacob *et al.*,2000).

2.7 Internal egg quality

Internal egg quality starts to reduce once the egg is laid. Aside the hens' nutrition and management, handling and storage also have very crucial effect on egg quality. It is not so easy to assess internal quality of unbroken eggs as the external qualities. The fact that eggs are used in many diverse ways makes its internal quality very important. Attention has recently shifted to improving the nutrient content of eggs especially by manipulating the hens' diets. Egg internal quality defects can be grouped into three main categories which are: Whole egg quality defects, albumin quality and yolk quality defects but they have been further listed into nine classes.

2.7.1 Yolk quality

Yolk quality can be evaluated using its colour, firmness, smell and texture (Jacob *et al.*, 2000).

i. Yolk colour (YC): is usually the main factor on which most egg users judge egg quality, egg users' preference for YC is greatly subjective and differ from a country to another. Xanthophyll, the pigment responsible for egg yolk colouration is obtained from plants used as feed ingredients during formulation of the hens' diets. This therefore make possible the manipulation of egg yolk colouration by adding xanthophylls (either natural or synthetic) to the feeds fed to laying hens and this can boost consumers satisfaction. Whatever affects pigments absorption from the diet or pigments deposition in egg yolk can cause yolk paleness (Coutts and Wilson, 1990).

ii. Yolk firmness: Though fresh egg's yolk is usually round and firm (Jacob*et al*., 2000), it flattens with time and experiences degeneration of the vitelline membrane resulting from the water movement into the yolk from the albumen.

iii. Yolk texture: Refrigeration or freezing of shell eggs may cause the yolk to become elastic in nature. Feeding of unrefined cottonseed oil or seeds of certain weeds can also denature egg yolk.

2.7.2 Albumen quality

This depends on the consistency, appearance and functional properties. Haugh Units (HU) is then term used to evaluate albumen quality using albumen height and egg weight (Coutts and Wilson, 1990) with the least HU of 60 getting the consumers. Egg should have between 75 and 85 HU before leaving the farm. Albumen consistency is affected by laying hens'age, their genetic makeup, length of egg storage egg storage conditions and diseases (like egg drop syndrome, infectious bursitis and new castle disease, ILT) (Coutts and Wilson, 1990; Jacob *et al.*, 2000).

2.7.3 Overall quality

i. Blood spots*:* results from rupturing of the ovary's small blood vessels during the release of the egg yolk. Although blood spots are usually observed in the yolk, it sometimes diffuses through the albumen and can also be on the yolk surface or in the entire yolk. Blood spots occurrence varies with hens' strain, rises with hens'age and more in brown egg layers (Coutts and Wilson, 1990).

ii. Meat spots:most at times affect the albumin and not the yolk, often consisting of tiny pieces of body tissues. (Coutts and Wilson, 1990)

iii. Off odours / flavours: These can be prevented by proper storage (Coutts and Wilson, 1990), since eggs easily absorb strong odours or flavours. Length of storage and high temperatures also cause off odours or flavours. Feed ingredients with strong flavours should be avoided when formulating feed (Coutts and Wilson, 1990).

2.8 Poultry nutrient requirements

Layers as with all animals need complex nutrients in their diets. The nutrients are specific chemical compounds/ elements that are needed in the diet of layers to support their production and play a key role in maintaining egg quality (King'ori, 2012). These are usually categorized into six classes somewhat based on their function and chemical nature. Birds require a continuous provision of energy, protein, essential amino acids, essential fatty acids, minerals, vitamins and water (Butcher and Miles, 2018bs).

i. Water: Cool, clean water should always be supplied (*ad libitum*) and is therefore often not given due consideration by some nutritionist (Kirk,

2015)and farmers. It is a major requirement for growth and body function. Water is required in large amount, the source of water could be well, borehole, river, dams, tap and rain. They also said that water should be free from salt because of its laxative effect. The water intake increases with temperature of the area where the birds are kept. The ratio of dry matter intake to water intake by layers in temperate environments is normally 1:2 but does depend to some extent on the diet.Deprivation of water beyond 12hours adversely affect poultry growth and egg production of layers (Mitchell, 2016).

ii. Energy requirement of layers:Birds eat mainly to meet their energy requirements, once other essential nutrients are present in the right proportions in the diet. Feed intake of birds is therefore dependent mainly on the diet's energy content. Carbohydrate is required for energy to maintain body temperature and for synthesis of fat stored in the body as structural in the egg. The energy in the poultry diets is derived mainly from cereals, those available in Nigeria include maize, millet, sorghum, wheat, rice and while non-cereals carbohydrates include cassava which can be up to 50 – 60% in growers and layers diet without detrimental impact on the birds' performance. The author reported that the unit of energy in the feed stuffs utilized in poultry is normally expressed as 'metabolizable energy' per unit weight (Smith, 1990).

Smith (1990) also stated that metabolizable energy (ME) refers to the proportion of feed available to birds for the production of muscle and maintenance of vital functions.. Birds are able to digest sugar in their feed but not insoluble one, because their stomach does not secrete enzymes capable of breaking down substances like cellulose (Oladele *et al., 1993*). Long (1988) carried out a study on the apparent and true ME content of some ingredient usually incorporated into poultry feed in the tropics and found that true ME value on dry matter basis ranged between 15.9 and 16.6 for cereals. Olomu and Offiong (1980) reported that energy level of 2800 – 3000kcalME/kg ME is optimum for layers and finisher diet in the tropics. (Aduku, 1992) also recommended 2800kcalME/kg for finisher and 3000kcalME/kg for layers.

iii. Protein and amino acids: Dietary protein provides the amino acids used for maintenance, muscle growth and synthesis of egg protein (Elwinger *et al*.,

2016) in which these syntheses of muscle and egg proteins depend on 20 amino acids, (required physiologically). 10 out of the 20 amino acids are either left unproduced or are produced sluggishly and as such must be supplied in the diet to sooth metabolic requirement, these are designated “essential amino acids”. Ten others can be synthesized from other amino acids and are designated dietary non-essentialamino acids since they are not usually given consideration when formulating feeds(Ravindran, 2019).

Protein sources are divided into two:

1. Animal protein sources include fish meal, blood meal, milk powder and meat meals.

2. Plant protein sources include groundnut cake (G.N.C), palm kernel cake (P.K.C), soya bean meal, cotton meal seed

Studies have stressed the importance of physiological optimum qualities and quantities of protein in the diet of layers. (Fetuga, 1997) protein is of great nutritional significance, due to their function in the tissue like building and replacement of worn-out like tissues and damaged cell. In respect of the nutritional advantage of poultry meat, so many households keep poultry at the backyard either for consumption or as an additional sources income. The protein requirements were put at 23% from 0-3 weeks and 20% from 3-6 weeks and 18% from 5 weeks to maturity or point of lay.

iv. Fat requirement of layers is important for increasing the energy density of ration especially those of layers. Fat also functions as source of essential fatty acids acting as solvent. Soluble vitamins and fat are the forms in which energy is stored in eggs and meat. There are many sources of fats, among which are Soya bean oil, corn oil, cotton seed oil, palm oil and vegetable oil.

v. Vitamins requirement of Layers: The discovery of vitamins revolutionized the poultry industry intensively. Vitamins are organic compound not synthesized by the body tissues and are required in very small amount in the diet. He further stated that vitamins are not structural component of the body but rather they mostly commonly function as co-enzymes regulators of metabolism required by birds in minute quantities and functions in all biochemical pathways. Vitamins may either be fat-soluble or water-soluble and all except

vitamin C have to be supplied in the diet. Vitamin C are easily synthesized by birds and so are is not essential but are supplemented in the diet during heat stress (Roberts, 2004; Mitchell, 2016).

vi. Minerals:These function in the formation, growth and maintenance of the skeletal system, egg shell, general health and osmoregulation of the body of the birds. Calcium (Ca) and phosphorus (P) being the most abundant mineral elements in the body, are grouped as macro-minerals together with sodium, potassium, chloride, sulphur and magnesium since they are needed in concentrations above 100 mg/kg. Ca and P must be in a ratio of 2:1 in the feed of growing birds to enable appropriate absorption of both elements (Kirk, 2015) but 13:1 in laying hens diets to meet their Ca need for good shell integrity. Electrolyte balance of the body depends entirely on three elements sodium (Na), potassium (K) and chloride (Cl) which must be present in adequate proportions to maintain the body pH. Copper, iodine, iron, manganese, selenium, zinc and cobalt are required in very small quantities (Elwinger *et al*., 2016) and so are called ‘’Trace minerals’’ but function as components of larger molecules therefore they are referred to as co-factors of enzymes in different metabolic reactions. Both major and trace minerals must be added to poultry feeds (Chang 2020) since most of the cereals used in poultry feed do not contain them (Ravindran, 2019).

2.9 Blood examination:

Blood examination (Haematological and Serum biochemistry) provides a clear picture to investigate metabolites and other constituents present in the body (Alagbe, 2017). Presence of these numerous blood metabolites and constituents reveals in a simple way the relationship between the diets of an animal and its effect on blood parameters which are either beneficial (physiological), improving animal health or harmful (pathological) (Etim *et al.*, 2014). The fact that blood constituents change with normal development and maturation makes them useful natural instrument the body of animals to monitor changes that occur due to aging. Numerous factors such as age, breed, environmental condition, feed quality, nutrition, sex, physiological status and management can result to varied haemato-biochemical parameters (Jiwuba *et al*.,

2016). Deviation from the normal values of blood components reveals the animal's metabolic state and quality of the diets fed to the animal.

2.9.1 Haematology:

Study of structure and normal functions of blood and the organs concerned with blood formation with their defects. Of great importance are the indices referred to as "Haematological indices" which are valuable pointers to the physiological condition of animals (Etim, 2010) and are basis for comparing deficiency of nutrients (Etim *et al*., 2014a). The haematological indices include:

i. Red Blood Cell (RBC) gives adequate knowledge of the RBC size and the blood's haemoglobin content. Alteration of value connotes anaemia (a condition of RBC shortage) and its type (Gernsten, 2009). RBC is a vital diagnostic tool to investigate the reason for anaemia, which may be affected by the cells sizes (MCV) or quantity of Hb (MCH). RBC carries Hb that equally carries oxygen to the cells and RBC value varies in various animals. Increased RBC value can result from congenital heart disease, dehydration, (hypoxia), polycythemia vera and movement of an animal to a higher altitude, can equally raise RBC level for a good number of weeks (Gernsten, 2009; Bunn, 2011). RBC reduction can be caused by bone marrow infections, kidney infections, haemolysis, malnutrition, nutritional deficiencies (of vitamins and minerals that boost blood formation of RBCs), dehydration, pregnancy and drugs (Gernsten, 2009; Bunn, 2011). Loss of blood due to parasitic organisms, cuts and diarrhea can result in chronic anaemia (Chineke *et al*., 2006).

ii. Packed Cell Volume (PCV): Otherwise called haematocrit, is the volume percentage (%) of RBCs in a particular blood sample (Purves *et al.*, 2004; Demoranville and Best, 2013). PCV reduction can occur with diseases that affect hepatic and renal organs, inappropriate nutrition, iron and folic acid deficiencies with vitamin B12 and pregnancy. PCV can be determined using (MCV) also with red cell distribution width (RDW), (Etim *et al.*, 2013). Increased PCV can be caused by polycythemia resulting from hypoxia or from multiplication of cells that produce blood in the bone marrow (Polycythemia vera) (Demoraville and Best, 2013).

iii. Mean Corpuscular Volume: is a part in a standard complete blood count representing blood cell volume calculated by the division of total PCV by the total number of RBCs then multiplying the answer by 10, (Etim *et al.*, 2013). MCV helps to classify the type of anaemia present which may be microcytic anaemia (MCV that is less than normal limit), normocytic anaemia (normal MCV limit), macrocytic (MCV beyond normal limit).

iv. Haemoglobin (Hb): Hb is the RBC content which contains iron and functions in the transportation of oxygen (Maton *et al.*, 1993) from the organ responsible for respiration in an animal to all body cells to digest the necessary nutrients to supply energy and the removal of carbon dioxide through the same organ of respiration. Hb in mammals can pick 4 oxygen molecules (Costanzo, 2007) which can be modified by altering blood pH or Co_2. Normal respiration involves binding of oxygen to the heme content of the protein haemoglobin (RBC protein) to form 'Oxyhaemoglobin'. Various molecules could be used by animals to bind Hb thereby changing its love and ability of transporting oxygen when conditions are not favourable (Etim *et al.*, 2013).

v. Mean Corpuscular Haemoglobin (MCH): being mean volume of Hb in individual RBC found in a blood sample (MedicineNet, 2012). MCH can be evaluated through division of the total weight of Hb by RBC of a blood sample and its value decreases in hypochromic anaemia (Gersten, 2009).

vi. Mean Corpuscular Haemoglobin Concentration (MCHC): was said by MedicineNet (2012) to be the quantity of Hb that is concentrated in a volume of packed RBC. MCHC is vital in blood deficiency investigations and especially gives important information on the ability of the bone marrow to produce RBCs. It is calculated by the division of HB by PCV (Gernsten, 2009) and measured in percentage (%).

vii. White Blood Cell (WBC): functions in the body's immunity that helps it to resist infections, pathological abnormalities and other strange substances (Medline Plus, 2012) with the production of antibodies in response to immunity Jiwuba *et al.*, (2016). WBC consist of about 5 different types (Brooks, 2008) all produced from the same cell in the bone marrow referred to as hematopoietic stem cell. WBCs have a life span of three to four days in both

animals and human. The quantity of leucocytes in a blood sample reveals the presence or absence of disease. WBC in a mature and healthy animal is about 1% of the whole blood volume (Alberts, 2005).

Leucocytosis is the term used to describe a rise in WBCs above the upper limit while leucopenia signifies a fall beyond the lower limit. Leucocytosis can be as a result of allergy, diseases, inflammation, infection, stress, trauma (Etim *et al.*, 2013), anaemia, bone marrow tumour, infections, inflammations, severe physical stress, tissue damage like burns (Dinauer, 2008; Dugdale, 2011). Leucopenia can result from reduced or failed bone marrow, hepatitis, spleen diseases and exposure to radiation (Dinauer, 2008; Dugdale, 2011). There are two main classes of WBCs though with numerous similarities but with specific form and function. The two classes are the granulocytes (those with cytoplasmic granules) and the agranulocytes (those without granules) but contain lysosomes. Granulocytes include basophils, eosinophils and neutrophils while agranulocytes include lymphocyte, monocyte and macrophages.

2.9.2 Serum biochemistry

This is the chemical analysis of blood serum (the remaining part of plasma after blood has been coagulated that is the part of the blood that looks like water without cells). Components of serum are biomarkers of several conditions like diseases and infections. Blood urea nitrogen (BUN) and creatinine indicates renal intake, metabolism and excretion of creatinine. Uric acid is directly dictated by protein catabolism extent and it's the final product of purine decomposition of poultry's nucleic acid. An increase of which depicts amino acid imbalance of the diets fed to the birds, therefore it is a vital indicator of amino acid requirement and utilization which could reduce with increase lysine content of the diets (Yang *et al*., 2010) Liver function- alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (AP), gamma-glutamyltransferase (GGT), total and direct bilirubin.

Serum proteins: are complex mixtures of proteins in the blood plasma. They function in protein synthesis and in transporting lipids, hormones, metals, minerals and vitamins through the circulatory system and cellular activity regulation, blood clotting coordination of the immune system and as enzymatic activities.They are total protein, albumin, fibrinogen globulin, alpha, beta and gamma globulins separated through

protein electrophoresis.Lipids- these are cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons. These function basically in stabilizing cell membrane integrity and regulate transportation of substances through the membrane. Cholesterol functions in hormone synthesis (like bile acids which is essential in fat metabolism) and a precursor of several hormones including adrenal and sex hormones.

Triglycerides supplies and stores energy, they usually have light weight and stored in adipose cells though transported in combination with proteins as lipoproteins which are converted through a the process of lipolysis to fatty acids and glycerol. HDL, LDL and VLDL are lipoproteins whose main objective is the transportation of fat (hydrophobic lipids) molecules in fluids (like the plasma and extracellular fluids). Lipoproteins usually have two ends in which one is hydrophilic and the other end is hydrophobic which is located in the membrane of the cell.

2.10 *Moringa oleifera*

Moringa oleifera (MO) is the most common specie (Price, 2007) with 13 other species of the genius Moringaceae. *Moringa oleifeira* also known as horseradish or drum stick tree due to its numerous uses is cultivated throughout the tropical belt. *Moringa oleifera* has several use, among which is integration into animal feeds to boost its quality and quantity since it has high protein that is equally cheap, readily available, rich in nutrients (especially protein and fibre) and improve digestibility of feed. *Moringa* leaves abound in protein and can be fed to poultry, pigs and cattle (Ojukwu 2012) hence, since high feed cost especially of protein and energy sources (Abbas, 2013) seriously threatens poultry industry in developing countries, *Moringa oleifera c*an be utilized as fodder crop (Nouman *et al*., 2013) to bridge this gap and combat the challenge of high feed cost.

2.10.1 M*oringa oleifera's* active ingredients

Moringa oleifera leaves are wealthy stores of carotenoids, vitamins, minerals, amino acids, alkaloids, flavonoids also with an uncommon association with phenolic compounds, including zeatin, quercetin, kaempferol, apigenin, with a good number of phyto-constituents which offer essential and nutrients that prevent human diseases (Karthivashan *et al.*, 2015). Quercetin is a flavonol found at high concentrations of about 100 mg/100g of dried *MO* leaves (Lako *et al.*, 2007), has several therapeutic properties (Bischoff 2008) and a powerful antioxidant (Zhang *et al.*, 2011).

2.10.2 *Moringa oleifera's* nutrient content

Analysis of 100g leaves and pods of *Moringa Oliefera* contain vitamins (vitamin A, B and C) minerals (calcium, copper, sulfur and iron but low in phosphorus) fat, carbohydrates and amino acids that are essential and make them a healthy diet. Table 2.2 shows the nutrient content of *Moringa oleifera* though MOLM composition is affected by location and stage of the harvested leaves.

Table 2.2: Nutrient content and digestibility of *Moringa oleifera* plant parts

Plant parts(g/kg)	DM	Ash	CP	EE	CF	Digestibility
Seeds	950.0	34.8	391.7	388.0	48.0	-
Flowers	892.5	112.1	314.8	68.0	170.0	-
Pods	940.0	97.1	71.2	20.0	490.0	430.7
Leaves	930.0	138.9	267.9	64.0	210.0	790.5
Stems	940.0	101.1	112.3	32.0	430.0	521.7
Whole plant	914.0	123.7	200.0	24.0	279.0	760.9

Source: Mabruk *et al.* (2010)

2.10.3 Uses of *Moringa oleifera*

The *MO* leaf abounds in crude protein, which therefore makes it suitable for being added to animal feed as an ingredient as it is rich in nutrients. *M. oleifera* leaves has been used in place of other commercial sources of protein for ruminant production (Nouala *et al*. 2006). Utilization efficiency of concentrates has evidently been improved with *M oleifera* leaves. Phytochemical analysis showed that cyanide, oxalates, phytates, saponins, tannins and trypsin inhibitors are anti-nutrients found in *M. oleifera* leaves (Ogbe and John, 2012). The fact that is rich in nutrients makes it suitable ingredients in poultry nutrition to boost growth and the health of the birds.

2.10.4 Moringa in chicken diets

Moringa leaves won't be readily ingested by chickens and so it must be presented in a form that will be consumable to the chickens while the nutritive content of the Moringa leaves must be intact and its protein concentrated (Price, 2007). Moringa leaf is high in protein and can have negative effect if used raw in chicken's diet and so care must be taken to prevent birds from consuming it excessively (Gaia, 2005). Moringa has also been said to enhance digestibility of other feed ingredients due to its ability to curb moulds and pathogenic bacteria thereby assisting the birds to fully exhibit their inherent genetic potential (Gaia, 2005). Kakengi *et al*. (2007) evaluated and reported up to 10% exchange of sunflower seed meal replacement by *MO* leaf meal (MOLM) on laying hens' performance. Olugbemi *et al*. (2010c) reported reduced egg cholesterol on including MOLM in cassava-based diets fed to laying hens thereby suggesting MOLM to be possess cholesterol reducing ability.

Ayssiwede *et al*. (2011) evaluated the outcome of including Moringa leaves in the diets of growing indigenous Senegal chickens up to 24% devoid of detrimental effect on the birds' activities, carcass with organs attributes and mortality though Onu and Aniebo (2011) reported up to 7.5% inclusion in the diets of broilers starter with absolutely no detrimental impact on their growth and blood characteristics. Abou-Elezz *et al*. (2011) recorded reduced feed intake, egg laying rate and egg mass of Rhode Island Red hens with increased MOLM from 0 -15% at 5% interval and submitted that the hens could accept MOLM up to 10%. Banjo (2012) recommended an inclusion of 2% MOLM to the diets of Anak broilers for weight enhancement but

not feed intake or feed conversion ratio of the broilers. Moreki and Gabanakgosi (2014) found that addition of 5 to 20% MOLM to broilers' diets and 10% MOLM in laying hens' diets enhanced their performance both growth and egg production, egg size inclusive. These two authors added that MOLM could be added up to 20% in laying hens' diets if MOLM is without cost and the cost of egg encouraging.

2.11 *Ocimum gratissimum*

Ocimum gratissimum belongs to Kingdom 'Plantae', an angiosperm, Eudicots, Asterids, of Lamiacs order, Lamiaceae family, genus *Ocimum*, and *s*pecies *O. gratissimum* with its full nomenclature of *Ocimum gratissimum*.

2.11.1 *Ocimum gratissimum's* active ingredients

Ocimum gratissimum, (OG) is just one of the several *Ocimum*species, equally called clove basil or African basil, and a member of Lamiaceaefamily. *Ocimum gratissimum* is common and naturally found in so many places all over the world. Lamiaceae is a family in plant kingdom used in folk medicinefor several centuries and has been used to treat so many diseases. OG has been found to contain tannins and a volatile oil with sweet smell which has been said to be an antibacterialagent (Elujoba, 2000). *Ocimum gratissimum* contains several chemicals and active ingredients including Camphor, Cinamate, Eugenol and Thymol (Odoemelam *et al*., 2017) all of which confer on it its effective antimicrobial properties. It has also been said to contain alkaloids, citral, flavonoids, linaol, methyl cinnamate, saponins,, triterpenes and thymol. Oil from OG has been reported to stop spasm anddiarrhea by reducing the contract rate of the stomachmuscles and the gastrointestinal tracts(Elujoba, 2000) and destroying a good number of other disease causing bacteria. Eugenol is known to have anti-helminthic, insecticidal, and nematicidal abilities. (Chatterje, *et al*.,1982; Chavan and Nikam1982; Pessoa, *et al*., 2002). Table 2.3 showed the nutritional composition of *Ocimum gratissimum* as determined byIdris *et al.,* 2011.

Table 2.3: Nutritional composition of the leaves and stem of *Ocimum gratissimum*

Parameters (%)	**Leaves**	**Stem**
Moisture	82.60±0.01	82.60±0.11
Crude protein	3.33±0.07	1.65±0.02
Crude fibre	9.52±0.01	19.65±0.03
Crude lipid	8.50±0.04	3.00±0.15
Ash	13.67±0.13	13.67±0.02
Available carbohydrate	64.98±0.01	62.03±0.04
Energy Kcal/100g	343.08±0.01	278.42±0.011

Source: Idris *et al*., 2011

2.11.2 *Ocimum gratissimum* in chicken diets

Improved stability of blood components and reduced blood micro-organisms of finishing broilers have been achieved with the use of *Ocimum gratissimum* leaf extracts (Nweze and Ekwe, 2012). Another species of tulsi (*Ocimum sanctum*) leaf meal in broiler diet for 42 days at the rate of 1% was reported to reduce blood cholesterol levels of broiler. (Lanjewar *et al*., 2004). Ekwe *et al*. (2017) reported an improvement in red blood cells of broiler chickens fed *Ocimum gratissimum* leaf extracts. Umukoro and Ashorob reported a reduction in haemoglobin and packed cell volume of male mice fed *O. gratissimum* leaf extracts, Red blood cells, white blood cells and haemoglobin concentration of Wister rats fed 40% *Ocimum gratissimum* leaf extracts have also been reported to have increased (Mohammed *et al*., 2007)

Green plants contain Xanthophylls (a type of carotene) but its source, availability and value in laying hens' feed determines the extent of egg yolk pigmentation (McGraw and Hill 2006; Adegbenro *et al*., 2020). *Ocimum gratissimum* leaf meal inclusion in feed has been reported to improve carcass quality and egg shell thickness of chicken. Better proximate composition of eggs of hens in terms of protein and mineral content which is of nutritious advantage to the consumers of such eggs has equally been reported (Azeezah*et al*., 2019). *Ocimum gratissimum* leaf meal like *MOLM* is hypocholesterolemic having flavonoids (an antioxidant) that reduces cholesterol formation by producing a reductase enzyme that lessens formation of cholesterol (Ghasi *et al*., 2000; Rao and Agarwal, 2000 ; Partama *et al*., 2018; Adegbenro *et al*., 2020). Adegbenro(*et al*., 2020) also reported increased yolk colouration which he said resulted from the presence of carotene in an experiment to examine the ability of a composite leaf mealas a replacement for standard premix in laying bird diets.

2.12 *Vernonia amygdalina*.

2.12.1 Botanical Classification and origin of *Vernonia amygdalina*

Is a plant of the Kingdom Plantae, an angiosperm, that belongs to Asterales order, Asteraceae family, of genus *Vernonia*, and *s*pecies *V. amygdalina* with its full nomenclature as *Vernonia amygdalina.Vernonia amygdalina* (bitter leaf), a little shrub that is common in tropical Africa and all over sub-Saharan Africa but for its nutritional

importance planted in Nigeria (Igile *et al*, 1995). Though it's of about 1–3 meters high in so many places, it is found to be above 6 meters high in Nigeria.

2.12.2 *V. amygdalina's* nutritional compositions and isolated compounds

Different researchers documented different values of crude protein for *V. amygdalina* leaf meal (VALM). Okoli *et al.,* (2003); Fajemisin (2009) and Owen *et al.,* (2009) recorded crude protein (CP) range of 18 - 21.50% for the extracts of *V. amygdalina* . Table 2.4 showed the nutritional composition of *Vernonia amygdalina* as determined by Okeke *et al.,,*(2015). Chemical components with active biological characteristics have been separated and categorized from *Vernonia amygdalina* leaves. Some of these components as identified by Tona *et al.,* (2004) are sesquiterpene lactones, flavonoids, steroid, glycosides, and vernonioside A, A1- A4, and B, B2- B3. The leaves also contain alkaloids, glycosides saponins, and tannins which are anti-nutritive agents and are responsible for its bitterness.

Table 2.4: Nutritional composition of *Vernonia amygdalina*.

Parameters (%)	**Leaves**	**Stem**	**Root**
Moisture	67.16±0.02 d	44.86±0.00 e	31.42±0.01 f
Dry matter	32.84±0.00 d	55.14±0.01 e	69.58±0.00 f
Protein	22.05±0.00a	5.95±0.01d	2.80±0.00e
Crude fibre	10.86±0.01 a	36.94±0.01 b	56.22±0.01 c
Fat	2.72±0.00 c	0.32±0.01 b	0.22±0.02 b
Ash	9.24±0.01 d	7.22±0.00 c	9.18±0.02 d
CHO (NFE)	55.13±0.03 d	49.57±0.00 e	31.80±0.01 f
Energy Kcal/g	320.44±0.01 d	224.96±0.03 e	153.84±0.00 f

Source: Okeke *et al.,* 2015

2.12.3 Uses of *V. amygdalina* plant

Leaves of *V. amygdalina* are usually washed and consumed as green leafy vegetable and in soups while its aqueous extracts have been used as tonic in treating various ailments. *Vernonia amygdalina* have been tested and documented to have several health importance due to its bioactive components (vitamins, carotenoids and phenolics and so on) and rich nutrients. Since *V. amygdalina* is mostly used as vegetables, it has been documented to have anti-oxidative abilities which enables it to hunt reactive oxygen species, chelate metal ions, hinder nitrosation and regulate some particular enzymatic reactions (Ola *et al*., 2009). Leaves of this valuable plant have been reported to diverse crude protein by different authors.

Though leaves of *V. amygdalina* are considerably cheap and abounding in a good number of nutrients particularly β-carotene and vitamin C that play vital roles in the health of humans, they are equally blessed with minerals like iron, phosphorus, calcium, potassium (Oshodi *et al.,* 1992; Musa *et al.,* 2011), proteins, folic acid, dietary anemia factors (Abosi and Rasoreta, 2003).

2.12.4 *Vernonia amygdalinain* chicken's diet

Vernonia amygdalina atinclusion rate of 200 g - 400 g/ 150 kg feed was reported to have had no adverse effect on the growth and haematological parameters of broiler chickens (Banjoko *et al*., 2019). Tokofai *et al*., (2020) reported that VALM did not affect haemoglobin and Red blood cells but increased packed cell volume (though the values were within the normal range of 25%-45%) and total white blood cells of broiler chickens. He equally reported that VALM reduced serum cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein. VALM had no regular effect on serum total protein, it increased the serum albumin and high density lipoprotein (Tokofai *et al*., 2020) and he concluded on 2% inclusion level of VALM for optimal growth performance and physiological responses of broiler chickens.

One of the benefits of*Vernonia amygdalina* in poultry is its anti-oxidative ability (Erasto *et al*., 2006) which made it a suitable replacement for anti-biotics and other drugs that have been barned because of their residual effects on eggs and meat. It has been reported to contain alkaloid, carbohydrate, tannin, saponin, flavanoids and non cyanogenic glycosides (Olobatoke and Oloniruha, 2009) which makes its aqueous leaf extract to exhibit hepatoprotective activity due to its antioxidant property attributable to its flavonoid content, as a result of the sesquiterpene lactone present in the leaves (Babalola *et al*., 2001; Arhoghro *et al*., 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The experiment was carried out at the Teaching and Research Farm of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. Average ambient temperature ranged from 21.3 to 31.2 ^{o}C. The site is located in the rain forest zone of South- western Nigeria on longitude 7^{O} 23N and latitude 3^{O} 51 C and 160m above the sea level. The climate is humid with an average temperature of 34.7^{o}C (Aderonke and Gbadegesin, 2013).

3.2 Sources and preparation of test ingredients

The *M. oleifera* leaves used were harvested from Oke-Arungbo Estate in Osogbo, Osun State. *Ocimum gratissimum* was obtained from Ede in Osun State, while *Vernonia amygdalina* leaves were obtained from Imalefalafia, off Ring road, Ibadan.
The leaves were handpicked and rinsed to remove sand and all the foreign materials. After cleaning, the leaves were air-dried individually in a well ventilated roomat room temperature for an average of 10 days, ground to meals using an electric grinder, sieved with 0.05mm size sieve and stored in air proof containers respectively until required for compounding feed.

Study 1

3.3 Management of experimental birds

One day old Isa brown pullet chicks (n=300 randomly picked from a total of 357) sourced in Ibadan from a reputable farm were used for this experiment.Chicks were assigned randomly to 4 dietary treatments of 36 birds per treatment and further replicated in quadruplicates of nine birds per replicate. Deep liter system of housing was adopted. The chicks were weighed at inception and after each phase of the trial. Routine management activities with vaccination and medication according to the

hatchery's specifications were adhered to. Feed and water were supplied *ad libitum* while the study lasted for 61 weeks.

3.4 Design of the experiment

This was a 3 x 3 + 1 factorial arrangement of a completely randomised design (CRD). The two factors were: three leaf meals (*Moringa oleifera*, *Ocimum gratissimum* and *Vernonia amygdalina*) and three regimes (Chick phase, Grower phase and Laying phase) of introduction of the leaf meals to the birds. Chick mash was fed for the first eight weeks, followed by growers mash for another eight weeks followed by layers mash for the remaining 45weeks of the experiment.

3.4.1 Regime: 1

144 of the chicks wereassigned randomly to 4 dietary treatments (T1-control; T2, T3 and T4) of 36 chicks per treatment which was further separated into 4 replicates of 9 birds each after adjustment for weight in a CRD. While the remaining 213 chicks were fed separately without leaf meals.

3.4.2 Regime: 2

A total number of 84 fifty seven days (*i.e.* 9 weeks) old Isa brown pullets (growers from the 213 left from regime 1) wereassigned randomly to dietary treatments (T5, T6 & T7) of 28 birds per treatment and further separated into 4 replicates of 7 birds per replicate after adjustment for weight in a CRD. These were added to the first four treatments to make seven treatments. The growers mash was fed to the pullets for another eight weeks (week 9-16).While the remaining 129 chicks were fed separately without leaf meals.

3.4.3 Regime: 3

A total number of 72 one hundred and thirteen days (*i.e*. 17 weeks) old Isa brown birds (pre-laying hens from the 129 left from regime 2) were assigned randomly to 3 dietary treatments (T8, T9 & T10) of 24 birds per treatment and further separated into 4 replicates of 6 birds per replicate after adjustment for weight in a CRD. These were added to the seven treatments to make ten treatments. The layers mash was fed to the hens from day 127 (1^{st} day of the 17^{th} week).

Factorial model

$$Y_{ij} = \mu + \alpha i + \Sigma_{ij}$$

Where:

Y_{ij} = observation j in treatment i

μ = Overall mean

αi = Fixed effect of treatment i

Σ_{ij} = Random error with mean o and variance σ^2

3.5 Experimental diets

Four experimental diets were compounded and fed to each experimental group. Diet 1 (control) was devoid of leaf meals, diet 2 contained 2% of *Moringa oleifera* leaf meal (MOLM), diet 3 contained 2% *Ocimum gratissimum* leaf meal (OGLM) and diet 4 contained 2% *Vernonia amygdalina* leafmeal (VALM). The chick mash was formulated to be iso-caloric and iso-nitrogenous and were fed to the chicks for the first eight weeks while the grower and layer mashes were also similarly formulated. The four dietary treatments were formulated as follows:

T1 (control) waswithout leaf meal supplementation (LMS)
T2 contained 2kg MOLM (*Moringa oleifera* leaf meal)/100kg feed
T3 contained 2kg OGLM (*Ocimum gratissimum* leaf meal) /100kg feed
T4 contained 2kg VALM (*Vernonia amgydalina* leaf meal) /100kg feed.

Tables of the experimental layout and basal diets are shown on Tables 3.1- 3.6

Table 3.1: Layout of the study

			LEAF MEALS	
Regime/	CONTROL	2%MOLM	2%OGLM	2%VALM
1 (chick)	TI	T2	T3	T4
2 (grower)	TI	T5	T6	T7
3 (layer)	TI	T8	T9	T10

(T1: Diet without leaf meal; T2 had 2kg MOLM (*Moringa oleifera* leaf meal)/100kg feed; T3 had 2kg OGLM (*Ocimum gratissimum* leaf meal) /100kg feed;T4 had 2kg VALM (*Vernonia amgydalina* leaf meal) /100kg feed) from day old to 61 weeks; (T5 had 2kg MOLM (*Moringa oleifera* leaf meal)/100kg feed; T6 had 2kg OGLM (*Ocimum gratissimum* leaf meal) /100kg feed; T7 had 2kg VALM (*Vernonia amgydalina* leaf meal) /100kg feed) from nine weeks old to 61 weeks; (T8 had 2kg MOLM (*Moringa oleifera* leaf meal)/100kg feed; T9 had 2kg OGLM (*Ocimum gratissimum* leaf meal) /100kg feed; T10 had 2kg VALM (*Vernonia amgydalina* leaf meal) /100kg feed) fed to the hens from 18 weeks to 61 weeks;

Table 3.2: Gross composition of chick mash

Ingredients (%)	T1	T2	T3	T4
Maize	55.00	55.00	55.00	55.00
Soybean	28.00	28.00	28.00	28.00
Fish meal	3.00	3.00	3.00	3.00
Wheat offal	11.00	9.00	9.00	9.00
MOLM	-	2.00	-	-
OGLM	-	-	2.00	-
VALM	-	-	-	2.00
DCP	2.25	2.25	2.25	2.25
Lysine	0.10	0.10	0.10	0.10
Methionine	0.15	0.15	0.15	0.15
Chick premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Energy kcal/kg	3016.72	3021.93	3014.77	3016.61
Crude protein (%)	21.25	21.30	21.29	21.40
Crude fiber (%)	4.44	4.43	4.63	4.66

MOLM - *Moringa oleifera* leaf meal, OGLM-*Ocimum grattisimum* leaf meal, VALM-*Vernonia amygdalina* leaf meal, DCP-Di Calcium Phosphate

Premix composition: Vitamin A-12.5×10^6IU, Vitamin D3-2.5×10^6 IU, Vitamin E-4 ×10^4mg, Vitamin K3-2×10^3 mg, Vitamin B1 3×10^3 mg, Vitamin B2 -5.5×10^3 mg, Vitamin B6 -5.0×10^3 mg, Vitamin B12-25mg, Niacin -55×10^3 mg,, Caicium Pantothenate-11×10^3 mg, Cholin chloride -5.0×10^6 mg,, Folic acid-1000mg, Biotin-80mg, Manganeze -120×10^3 mg,, Iron -100×10^3 mg, Zinc-80 ×10^3 mg, Copper -8.5×10^3 mg, Iodine -1.5×10^3 mg,, Colbalt-300mg, Selenium-120mg, Anti-Oxidant- -120×10^3 mg,

Table 3.3: Gross composition of grower mash

Ingredients (%)	T1	T2	T3	T4
Maize	57.00	57.00	57.00	57.00
Soybean	17.20	17.20	17.20	17.20
Wheat offal	22.00	20.00	20.00	20.00
MOLM	-	2.00	-	-
OGLM	-	-	2.00	-
VALM	-	-	-	2.00
DCP	3.00	3.00	3.00	3.00
Lysine	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15
Grower premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Energy kcal/kg	2832.04	2857.49	2850.33	2852.17
Crude protein (%)	16.38	16.43	16.42	16.54
Crude fiber (%)	4.86	4.85	5.04	5.07

MOLM- *Moringa oleifera* leaf meal, OGLM-*Ocimum grattisimum* leaf meal, VALM-*Vernonia amygdalina* leaf meal, DCP-Di Calcium Phosphate

Premix composition:Vitamin A-10×10^6IU, Vitamin D3-2×10^6 IU, Vitamin E-2×10^4mg, Vitamin K3-2×10^3 mg, Vitamin B1 3×10^3 mg, Vitamin B2 -5×10^3 mg, Vitamin B6 -4.0×10^3 mg, Vitamin B12-20mg, Niacin - 45×10^3 mg,, Caicium Pantothenate-10×10^3 mg, Cholin chloride -3.0×10^6 mg,, Folic acid-1000mg, Biotin-50mg, Manganeze -300×10^3 mg,, Iron -120×10^3 mg, Zinc-80 $\times10^3$ mg, Copper -8.5×10^3 mg, Iodine -1.5×10^3 mg,, Colbalt-300mg, Selenium-120mg, Anti-Oxidant--120×10^3 mg,

Table 3.4: Gross composition of layer mash

Ingredients (%)	T1	T2	T3	T4
Maize	55.00	55.00	55.00	55.00
Soybean	19.00	19.00	19.00	19.00
Wheat offal	16.20	14.20	14.20	14.20
MOLM	-	2.00	-	-
OGLM	-	-	2.00	-
VALM	-	-	-	2.00
DCP	2.00	2.00	2.00	2.00
Oyster shell	7.00	7.00	7.00	7.00
Lysine	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15
Layer premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Energy KCal/Kg	2703.54	2728.99	2721.83	2723.67
Crude protein (%)	16.01	16.06	16.05	16.16
Crude fiber (%)	4.34	4.33	4.52	4.55

MOLM- *Moringa oleifera* leaf meal, OGLM-*Ocimum grattisimum* leaf meal, VALM-*Vernonia amygdalina* leaf meal, DCP-Di Calcium Phosphate

Premix composition:Vitamin A-10×10^6IU, Vitamin D3-2×10^6 IU, Vitamin E-2.3 $\times10^4$mg, Vitamin K3-2×10^3 mg, Vitamin B1 3×10^3 mg, Vitamin B2 -6×10^3 mg, Vitamin B6 -5.0×10^3 mg, Vitamin B12-25mg, Niacin - 50×10^3 mg,, Caicium Pantothenate-50×10^3 mg, Cholin chloride -4.0×10^6 mg,, Folic acid-1000mg, Biotin-50mg, Manganeze -120×10^3 mg,, Iron -100×10^3 mg, Zinc-80 $\times10^3$ mg, Copper -8.5×10^3 mg, Iodine -1.5×10^3 mg,, Colbalt-300mg, Selenium-120mg, Anti-Oxidant- -120×10^3 mg.

3.6 Data Collected

3.6.1 Growth Performance Characteristics

i. Feed intake (FI): Feed consumption per phase was obtained by weighing a known feed quantity for a particular replicate in a well labeled container on the first day. The remnant at the end of each phase was obtained and used to calculate feed consumed for the corresponding phase by difference.

ii. Body weight gain (WG): The birds' initial weight was recorded at the onset of the study. Subsequently, body weight was noted at the end of each growth phase and the difference in mean weights for the two phases was calculated to attain average weight gain of birds for each phase of growth.

iii. Feed Conversion Ratio (FCR): obtained by a ratio of feed intake and weight gain

$$\textit{Feed conversion ratio} = \frac{\textit{feed intake}}{\textit{Weight gain}}$$

$$\textit{Average daily feed intake} = \frac{\textit{Total feed intake per phase/ replicate}}{\textit{Number of birds per replicate x 63}}$$

Where

63 = No of days per phase

3.7 Collection and analyses of blood sample

On the last day of week 8, 16 and 24 of the experiment, hypodermic needle and syringe were used to bleed 2 birds per replicate. For haematological assay blood was put in EDTA (an anticoagulant) pretreated bottles. For serum biochemical assay blood was put in bottles (without anticoagulant) that were carefully labelled. These blood samples were loaded in the centrifuge and spurn at 3,000 rpm after which the clear portion was decanted and stored in a freezer. RBC, WBC, PCV, Leucocyte differential count (monocyte, lymphocyte, eosinophil, basophil and heterophil), platelets, Hb concentrations were assayed haematologically. Total protein, albumin, ALP, ALT, globulin, albumin- globulin ratio, triglycerides, AST, cholesterol, creatinine, glucose, uric acid, LDL and HDL were assayed biochemically.

3.8 Laying performance

Data, such as average daily FI, daily egg production (DEP) and egg weight (EW) were documented during laying phase. Internal and external egg qualities measured included: egg (length, width, weight), shell (weight, thickness), yolk (weight, height, width, colour, index), albumen (weight, height) and Haugh unit. Eggs collection was twice daily (6:30am and 6:00pm). Feed weighing was done at the start of each phase and remnant weighed at the end of each phase and this was used to calculate the average daily FI of each bird.

3.8.1 Egg production performance

Records of DEP were kept as replicates from the point of lay to 61 weeks. Weekly egg production pooled, were expressed as percentage Hen-day production (HDP). At a given time the HDP was calculated as the percentage of egg laid to the number of hen days.

$$\%\text{HDP} = \frac{\text{No of egg laid per replicate}}{\text{No of birds per replicate} \times 7} \times \frac{100}{1}$$

Where

7 = Number of days per week

3.9 Vaccination and medications

Table 3.5: Vaccination/ Medication schedule of the birds

Vaccination/ medication	Period
Vitamin	Day 1
Antibiotics	Day 2-5
1st NDV Lasota (orally)	Day 6
1st Gumboro (IBDV)	Day 10
2nd NDV lasota (orally)	Day 16
2nd Gumboro (IBDV)	Day 21
Antibiotics (orally)	Day 22-24
Coccidiostat	Day 26-27
3rd NDV Lasota (orally)	Day 28

Other routine management practices were strictly monitored and provided.

3.10 Statistical analysis

Data obtained were subjected to a one-way analysis of variance ANOVA of the General linear model (GLM) of SAS (1999) package and means were separated using Duncan's (1955) multiple range tests of the same software at $\alpha_{0.05}$.

Study 2

3.11 Experimental birds and management

Nineteen weeks old Isa brown point of lay pullets (n=240) out of the 300 from study one were pulled out accordingly as allotted to their treatments (TI-No leaf meal and T2-T10 for the three leaf meals at three different regimes). Each treatment was replicated four times with 6 birds each.The conventional 3 tier battery cage system was used to house the pullets, each compartment measured $50\times45\times40cm^3$ with a floor space. Vaccination and medication were routinely practiced, feed and water were provided *ad libitum*, while the study lasted 61 weeks.

3.12 Design of the experiment

The experimental design was a 3 x 3 + 1 factorial arrangement in a completely randomised design. The two factors were: three leaf meals (*Moringa oleifera*, *Ocimum gratissimum*and *Vernonia amygdalina*) and three regimes (Chick phase, Grower phase and Laying phase) of introduction of the leaf meals to the birds at three different times (regime 1: chick phase, regime 2: grower phase, regime 3: layer phase of the experiment.

Factorial model

$$Y_{ij} = \mu + \alpha i + \Sigma_{ij}$$

Where:

Y_{ij} = observation j in treatment i

μ = Overall mean

αi = Fixed effect of treatment i

Σ_{ij} = Random error with mean o and variance σ^2

3.13 Experimental diets

Four diets were compounded and fed to each of the experimental group. Diet 1 (control) was devoid of leaf meals, diets 2, 5 and 8 contained 2% each of *Moringa oleifera* leaf meal (MOLM), diets 3, 6 and 9 contained 2% each of *Ocimum gratissimum* leaf meal (OGLM) and diets 4, 7 and 10 each contained 2% *Vernonia amygdalina* leafmeal (VALM). The layers mash was iso-caloric and iso-nitrogenous, this was fed to the pullets through this study. Treatments were formulated as follows:

T1 (control) waswithout leaf meal supplementation (LMS)
T2, T5 and T8 contained 2kg MOLM (*Moringa oleifera* leaf meal)/100kg feed
T3, T6 and T9 contained 2kg OGLM (*Ocimum gratissimum* leaf meal) /100kg feed
T4, T7 and T10 contained 2kg VALM (*Vernonia amgydalina* leaf meal) /100kg feed.

Tables of the experimental layout and experimental basal diets are shown in Tables 4.1 and 4.4.

3.14 Data collection

Twelve (12) freshly laid eggs from each treatment were selected weekly for six weeks at the early (14th- 19th week) and mid (33rd – 38thweek) laying phases of the laying period were sampled for internal and external egg characteristics. Egg quality assessment was done within 36 hours of lay for both internal and external qualities mentioned in 3.8.

3.14.1 Internal and external egg characteristics

i. Egg weight: This was determined with a digital scale (Saltex® electronic balance) to the nearest 0.01g.
ii. Length: The length of egg was linearly measured from the narrow to the broad end with a Vernier calliper (of 0.1mm accuracy).
iii. Egg breadth: The cross- section of the widest region of the egg was measured with a Vernier calliper as its breadth.
iv. Shell weight: shells were air- dried for 72 hours in trays, individually weighed with Saltex® electronic scale.
v. Shell thickness: measured to the nearest 0.01mm with micrometer screw gauge.

vi. Percentage shell weight: This was derived from the ratio of shell weight to egg weight and expressed in percentage.

$$\%\ shell\ weight = \frac{Shell\ weight\ \times 100}{Egg\ weight}$$

vii. Albumin height: The egg white height was derived away from the chalazae at a point above midway and circumference of the thick white with a P6085 spherometer (with 0.01mm accuracy).

vii. Haugh Unit (HU): values obtained above were used in the calculation of the HU (Haugh, 1937) as:

$HU = 100\ log_{10}\ (H + 7.57 - 1.7W^{0.37})$ where

H= albumen height (mm)

W= egg weight (gm)

viii. Yolk height: measured at a point midway between the egg yolk with P6085 Spherometer (0.01mm accuracy).

ix. Yolk width: The diameter of the widest cross- sectional region of egg yolk was taken as the yolk width. It was measured using Vernier calliper to the nearest 0.1mm.

x. The ratio of yolk height to yolk width was used to obtain yolk index according to Oluyemi and Robert (1979)

$$\text{Yolk index} = \frac{\text{Yolk height}}{\text{Yolk width}}$$

xi. Yolk was separated from egg white with a plastic egg separator to give the yolk weight measured with a digital electronic balance (Saltex® electronic scale with sensitivity of 0.01g).

xii Yolk weight percentage is the ratio of the yolk weight to egg weight, derived from

$$\%\ \text{Yolk weight} = \frac{\text{Yolk weight}}{\text{Egg weight}} \times 100$$

xiii. Albumen weight: given as the difference of egg weight and the combined weight ofyolk and dry egg shell for individual egg sample while albumen

weight percentage is the ratio of the albumen weight calculated from formulae:

$$\%\text{Albumin weight} = \frac{\text{Albumen weight}}{\text{Egg weight}} \times \frac{100}{1}$$

xiv. Yolk colour: The DSM Roche yolk colour fan was used to measure yolk colour.

3.14.2 Egg cholesterol profile

Egg yolk preparation for analyses

Raw eggs were put in a pot of water at room temperature and boiled for 12 minutes over stove. The well cooked eggs were cooled and their weights recorded. Egg shells were hand peeled and weighed while the albumen was carefully removed. Yolk samples were cautiously separated, weighed and crumbled. Each yolk sample (1g) was homogenized in 15ml of chloroform-methanol solution in ratio 2:1 and filtered. The filtrate was used to determine (Total cholesterol, HDL-cholesterol, Total triglyceride) concentrations using the respective RANDOX cholesterol assay kits (Elkin *et al.,* 1999) while LDL-cholesterol was estimated according to the equation of Friedewald (1972):

$$\text{LDL-Cholesterol} = \frac{\text{Total cholesterol} - \text{Triglyceride} - \text{HDL-cholesterol}}{5}$$

3.14.3 Eggs' fatty acid profile

Two grams/ millilitre of the yolk was weighed into a beaker and 20ml of Benzene and 10ml of Ethanol added to the yolk, thoroughly shaken and separated with separating funnel. Phenolphthalein (few drops) was added to 5ml of the aliquot put in a beaker, thoroughly shaken and titrated with 0.01M Sodium hydroxide (NaOH)*i.e.* 4g of NaOH pellet into 1litre of distilled water. The titre value was then used to determine specific fatty acid content using formulae:

$$\frac{\text{TV} * 0.1\text{M(NaOH)} * \text{STD}}{\text{Volume of sample used}} \times \frac{1000}{1}$$

Where

TV = titre value

0.1M (NaOH) = molarity of NaOH

STD= Fatty acid standard

3.15 Chemical analysis

Test ingredients (*M. oleifera*, *O. gratissimum* and *V. amygdalina* leaf meals) and compounded feed were analysed for proximate compositions as described by A.O.A.C.(1990).

3.16 Statistical analysis

Data were subjected to one-way analysis of variance ANOVA of the General linear model (GLM) of SAS (1999) package and means were separated using Duncan's (1955) multiple range tests of the same software at $\alpha_{0.0}$

CHAPTER FOUR

4.0 RESULTS

The proximate analysis of the test ingredients (Table 4.1) showed that dry matter value ranged between 91.38% and 92.72%.The crude protein value ranged between 19.10% and 24.77%. The crude fibre value of *Moringa oleifera* leaf of 9.60 was lower than that of *Ocimum grattissimum* leaf of 13.43% and *Vernonia amygdalina* leaf of 13.70%. The ash content of the test ingredients ranged from 3.78-7.28% and ether extract from 5.30% to 7.31% with 7.31% in *Moringa oleifera* leaf, 5.3% in *Ocimum grattissimum* leaf and 6.28% in *Vernonia amygdalina* leaf. The nitrogen free extract was 51.26% in *Moringa oleifera* leaf, 46.27% in *Ocimum grattissimum* leaf and 40.75 % in *Vernonia amygdalina* leaf.

Proximate analysis of chick mash (Table 4.2) showed that dry matter ranged between 90.58-91.43%. The crude protein content (CP) of feed samples varied from 21.15% – 25.90% and that of crude fibre from 4.90 -9.61%. The ether extract of the feed samples ranged between (5.46 – 6.88) %, ash content between (5.60– 10.20) % and nitrogen free extract (40.14 – 51.38) %.

The proximate analysis of the grower mash (Table 4.3) showed the dry matter ranged between 90.93 - 91.73%. The CP varied considerably between 15.05% – 21.00%. Diets 2, 3 and 4 (with 19.08, 20.48 and 21.00) % respectively were higher than the recommended values for growing pullets. The crude fibre contents of the feed samples ranged between 6.44% -8.60% while the ether extract of the feed samples ranged between (4.38% in the control diet and 10.51% in diet 3 with 2% *Ocimum gattisimum* leaf meal inclusion. The ash content of the diets ranged from 6.25- 8.33% . The nitrogen free extract of the control 5780% was higher than the values obtained for the other treatments that ranged between (45.19 – 52.19)%.

Table 4.1: Proximate composition of the test ingredients

Parameters (%)	*Moringa oleifera* leaf	*Ocimum grattissimum* leaf	*Vernonia amygdalina* leaf
Dry matter	91.55	91.38	92.72
Crude protein	19.60	19.10	24.77
Crude fibre	9.60	13.43	13.70
Ether extract	7.31	5.30	6.28
Ash	3.78	7.28	7.23
Nitrogen free extract	51.26	46.27	40.75

Table 4.2: Proximate analysis of feed samples (chick phase)0-8 weeks

Parameters (%)	Control	2%MOLM	2%OGLM	2%VALM
Dry matter	91.43	91.14	90.93	90.58
Crude protein	25.20	25.90	21.15	22.93
Crude fibre	9.61	4.90	7.35	8.52
Ether extract	6.08	6.88	5.46	5.50
Ash	8.67	6.78	5.60	10.20
Nitrogen free extract	40.14	46.68	51.38	43.45

KEY:

Control- (without leaf meal), 2% MOLM-diet with 2% *Moringa oleifera* leaf meal, 2% OGLM -diet with 2% *Ocimum gratissimum* leaf meal, 2% VALM -diet with 2% *Vernonia amygdalina* leaf meal

Table 4.3: Proximate analysis of feed samples (grower mash) 9-16 weeks

Parameters (%)	Control	2%MOLM	2%OGLM	2%VALM
Dry matter	91.23	91.73	90.93	91.33
Crude protein	15.05	19.08	20.48	21.00
Crude fibre	06.80	08.60	06.44	06.83
Ether extract	04.38	05.62	10.51	06.00
Ash	07.20	06.25	08.33	07.06
NFE	57.80	52.19	45.19	50.47

KEY:

Control- (without leaf meal), 2% MOLM-diet with 2% *Moringa oleifera* leaf meal, 2% OGLM -diet with 2% *Ocimum gratissimum* leaf meal, 2% VALM -diet with 2% *Vernonia amygdalina* leaf meal, NFE-Nitrogen free extract

The proximate analysis of layers mash (Table 4.4) shows the dry matter ranged between 91.45% – 92.02% and the CP varied considerably between 15.40% – 19.95%. Crude fibre of the feed samples ranged between 4.95% -13.45% while the ether extract ranged from 5.51% -6.08% and ash from 9.71% - 14.30%. The nitrogen free extract had a range of 44.14% -51.35%.

Table 4.4: Proximate analysis of feed samples (layer mash) 17-61 weeks

Parameters (%)	Control	2%MOLM	2%OGLM	2%VALM
Dry matter	91.45	92.02	91.46	91.61
Crude protein	15.40	18.55	19.76	19.95
Crude fibre	04.95	13.45	07.23	06.82
Ether extract	06.01	06.08	05.51	06.08
Ash	13.75	09.71	12.27	14.30
Nitrogen free extract	51.35	44.14	46.68	44.46

KEY:

Control- (without leaf meal), 2% MOLM-diet with 2% *Moringa oleifera* leaf meal, 2% OGLM -diet with 2% *Ocimum gratissimum* leaf meal, 2% VALM -diet with 2% *Vernonia amygdalina* leaf meal

Table 4.5 shows the growth performance of experimental birds at the chick phase where the initial weights were not significant while the final weight and weight gain both of which were significant had the same pattern where birds on diet 3 (T3) had the least values of (319.79g and 284.04) respectively. The least feed intake (1151.04g) was documented in birds on *MOLM* diet. The FCR was similar for birds on control diets and those on diets with 2% *Vernonia amygdalina* leaf meals (treatments 1 and 4) while those fed2% *MOLM* diet from chick phase had the best performance. The mortality was similar in treatments 1, 2 and 3 that is, birds on the control, *Moringa oleifera*and O. *gratissimum* leaf meal based dietswith a value of 0.50% respectively while treatment 4 that is birds on the diets with 2% *V. amygdalina* leaf meal had a value of 1.50%.

Growth performance of the pullets at the grower phase, (Table 4.6) shows significant differences between treatments for the initial weight, feed intake and feed conversion ratio. Pullets on the diets with 2% VALM from one day old hadthe highest initial weight and the least was recorded in pullets on 2% OGLM from one day old but the least feed intake of 1766.11g was observed in pullets on 2% MOLM from one day old and the highest in those on 2% OGLM from 9weeks. Pullets on the control diets with those on 2% OGLM from one day old had similar FCR values of 3.05 and 3.04 respectively. While the pullets fed *OGLM* based diet from nine weeks (T6) performed the least (4.18). Treatments 2, 4, 5 and 7 were not significantly different. There was no mortality at this phase.

Table 4.5: Growth performance of the experimental birds (0-8weeks)

PARAMETERS	T1	T2	T3	T4	SEM
Initial weight(g)	34.91	35.26	35.75	39.89	1.26
Final weight (g)	452.78^{a}	450.69^{a}	319.79^{b}	520.31^{a}	24.19
Weight gain (g)	417.86^{a}	415.43^{a}	284.04^{b}	480.43^{a}	23.49
Feed intake (g)	1526.74^{ab}	1151.04^{c}	1328.13^{bc}	1760.42^{a}	67.84
FCR	3.72^{b}	2.77^{c}	4.73^{a}	3.69^{b}	0.21
Mortality %	0.50	0.50	0.50	1.50	

^{abc}Means along the same row with different superscripts are significantly different ($P \leq 0.05$);
KEY:

T1- chicks fed control diet, T2- chicks fed diet with 2% *Moringa oleifera* leaf meal, T3- chicks fed diet with 2% *Ocimum gratissimum* leaf meal, T4- chicks fed diet with 2% *Vernonia amygdalina* leaf meal, SEM- Standard error of mean, FCR- Feed conversion ratio

Table 4.6: Growth performance of the experimental birds (9-16 weeks)

PARAMETERS	T1	T2	T3	T4	T5	T6	T7	SEM
Initial weight(g)	452.78^{ab}	450.69^{ab}	319.79^{c}	520.31^{a}	442.86^{ab}	401.79^{bc}	389.29^{bc}	14.87
Final weight (g)	1090.97	969.10	974.83	1041.67	1003.57	967.86	957.14	19.81
Weight gain (g)	638.19	518.40	655.03	521.35	560.71	566.07	567.86	17.22
Feed intake (g)	1943.20^{bc}	1766.11^{c}	1943.20^{bc}	1958.13^{bc}	2137.14^{b}	2351.43^{a}	2065.71^{b}	40.54
FCR	3.05^{b}	3.53^{ab}	3.04^{b}	3.79^{a}	3.83^{a}	4.18^{ab}	3.67^{ab}	0.10
Mortality %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^{abc}Means along the same row with different superscripts are significantly different ($P \leq 0.05$);

KEY:

T1- pullets fed control diet, T2- pullets fed diet with 2% *Moringa oleifera* leaf meal, T3- pullets fed diet with 2% *Ocimum gratissimum* leaf meal, T4- pullets fed diet with 2% *Vernonia amygdalina* leaf meal,from day 1,(T5- pullets fed diet with 2% *Moringa oleifera* leaf meal, T6- pullets fed diet with 2% *Ocimum gratissimum* leaf meal, T7- pullets fed diet with 2% *Vernonia amygdalina* leaf meal) from week 9,SEM- Standard error of mean, FCR- Feed conversion ratio

Main effect of additives and their period of introduction on average egg production (AEP) of the experimental birds (20th- 61st week), shown in Figure 1. The interaction effect of additives and period of introduction on AEP record of experimental birds from the 20th- 61st week of lay shown on Table 4.7 showed no significance (P>0.05) in AEP of the experimental hens.

Main effect of additives and their period of introduction on average hen day production of the experimental birds (20th- 61st week), shown in Figure 2 indicated no significance (P>0.05) in average hen day production of experimental hens.

Interaction effect of additives and its period of introduction on the average egg production and hen day production of hens (20th- 61st week) is presented in Table 4.7. None of the parameters measured was significant, that was, no interaction of the additives and the period of introduction of the additives on egg production and hen day production of the birds.

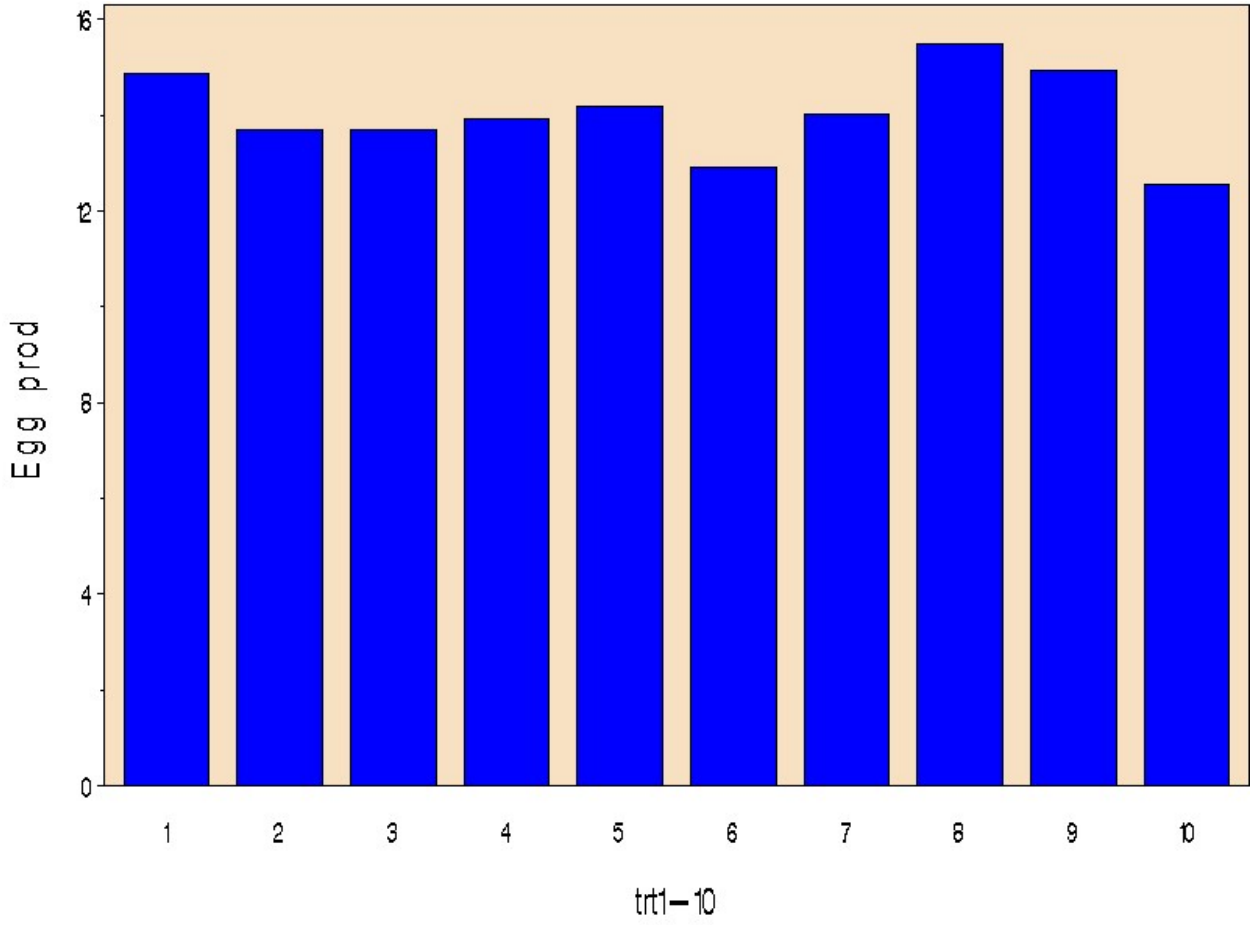

Figure: 1 Average egg production of the experimental birds 20th- 61st week

KEY:

trt: Treatment

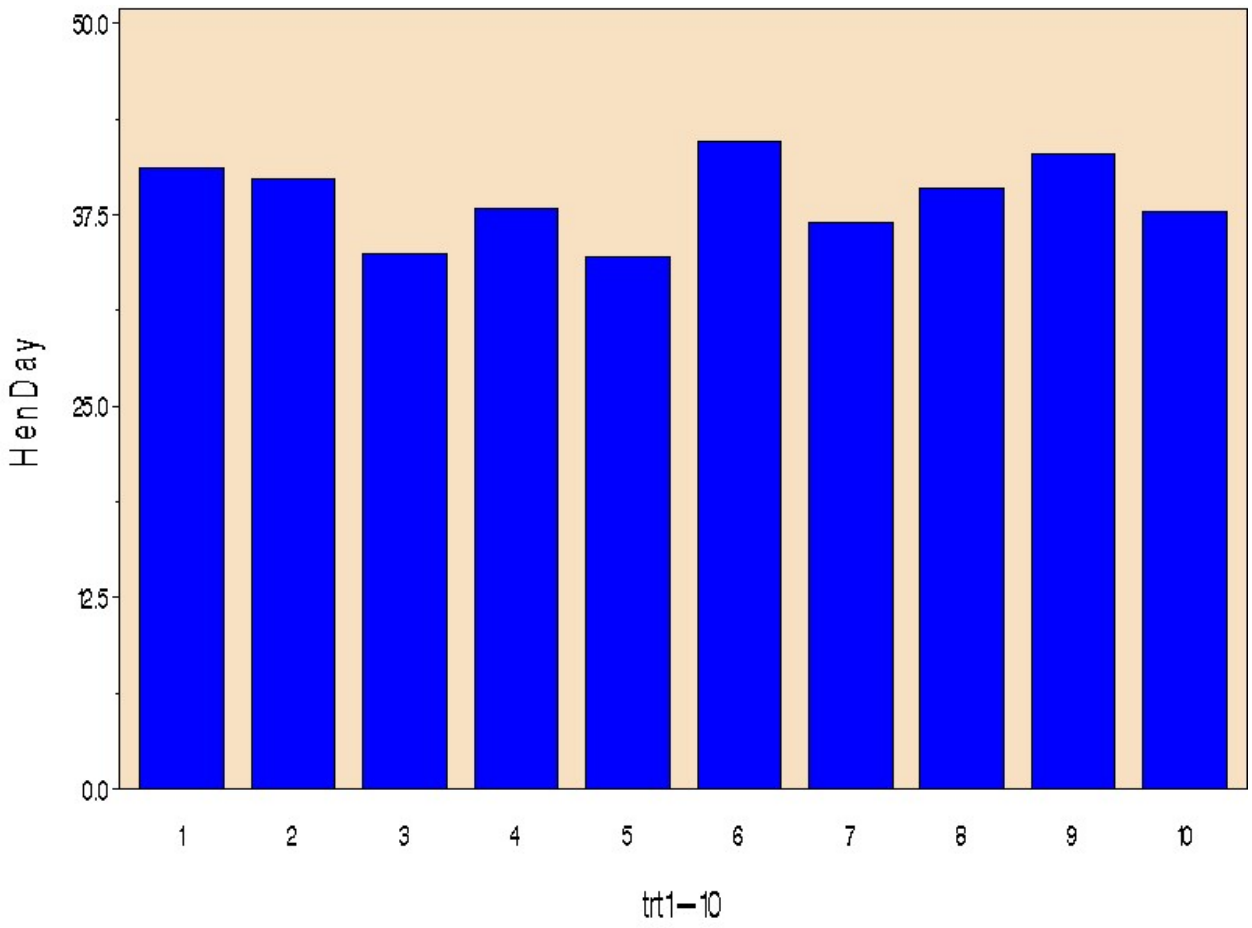

Figure: 2 Average hen day production of experimental birds (20th- 61st week)

KEY:

trt: Treatment

Table 4.7: Interaction of additives and their period of introduction on the average egg production and hen day production of the birds (20th- 61st week)

	ADDITIVE				REGIME			
	CONT	A1	A2	A3	CONT	R1	R2	R3
TOTAL EP	609.00	592.83	567.75	553.58	609.00	565.00	561.92	587.25
AV. EP	14.85	14.46	13.85	13.50	14.85	13.78	13.71	14.32
TOTAL HDP	1662.61	1555.10	1622.42	1538.46	1662.61	1540.50	1558.20	1617.27
AV.HDP	40.55	37.93	39.57	37.52	40.55	37.57	38.01	39.45

[abc]Means along the same row with different superscripts are significantly different ($P \leq 0.05$);

KEY:

A1- *Moringa oleifera* leaf meal, A2-*Ocimum gratissimum* leaf meal, A3- *Vernonia amygdalina* leaf meal, Regime: R1- from chick phase, R2- from grower phase, R3- from laying phase, EP- Egg production, AV.EP-Average egg production, HDP-Hen day production, AV.HDP-Average hen day production

The haematology of the experimental birds at the 8th week (chick phase) is shown in Table 4.8 where parameters considered were insignificant (P>0.05), except eosinophils with its highest value of 3.50% documented for chicks on diets with *VALM* then chicks on the diet with *MOLM* with a value of 2.87% chicks on control diet of 2.12%. The birds on *OGLM* diet had the lowest value of 2.00% which was insignificant from chicks fed control diets. Other parameters had no particular trend in their variation.

Table 4.8: Haematology of the birds at the chick phase (8th week)

Treatment	T1	T2	T3	T4	SEM	*Reference value
PCV (%)	27.75	25.88	27.38	28.25	1.55	23.00-55.00
Haemoglobin (g/dl)	9.21	8.10	8.94	9.05	0.55	7.00-18.60
RBC ($\times 10^6 \mu l$)	3.09	2.95	3.02	2.98	0.25	1.52-4.50
WBC ($\times 10^3$ µl)	17.51	16.54	16.48	16.48	1.11	9.00-32.00
Platelets ($10^3/mm^3$)	123.00	135.25	140.13	132.88	0.61	13.00-70.00
Lymphocytes (%)	66.25	68.38	64.88	67.50	1.99	29.00-84.00
Heterophils (%)	28.50	26.12	29.50	6.50	2.22	15.10-50.00
Het:Lym	0.43	0.39	0.46	0.40	0.03	
Monocyte (%)	2.87	2.25	3.25	2.37	0.42	0.05-7.00
Eosinophils (%)	2.12^{b}	2.87^{ab}	2.00^{b}	3.50^{a}	0.32	0.00-16.00
Basophils (%)	0.20	0.37	0.37	0.12	0.12	0.00-8.00
MCV(FL)	91.00	88.24	91.63	95.93	4.39	90.00-140.00*
MCHC (%)	33.18	31.04	32.02	32.10	0.77	26.00-35.00*
MCH (pg)	30.18	27.35	29.95	30.82	1.51	33.00-47.00*

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

Normal range Source: Mitruka and Rawnsley (1997)

*Bounous and Stedman (2000)

KEY:

SEM- Standard error of the mean, T1-control, (T2-diet with 2% *Moringa oleifera* leaf meal, T3-diet with 2% *Ocimum gratissimum* leaf meal, T4-diet with 2% *Vernonia amygdalina* leaf meal) from day 1, PCV-Packed cell volume, RBC- Red blood cell, WBC- White blood cell, MCV-Mean Cell Volume, MCHC-Mean Cell Haemoglobin Concentration, MCH-Mean Cell Haemoglobin.

The haematology of the experimental birds at the16th week (growing phase), shown in Table 4.9 proved that all parameters considered were significantly different ($P<0.05$) except for the RBC and mean cell heamoglobin concentration. The PCV, haemoglobin, MCV and MCH, all had similar order and were highest in treatment 7 (T7) where the birds were fed diets containing 2% *Vernonia amygdalina* followed by birds on OGLM from day old with those on MOLM and OGLM from nine weeks old with (30.88, 10.03, 86.61 and 28.13) for birds on OGLM from day old, (30.00, 9,84, 86.00 and 28.26) for birds on MOLM from nine weeks old and (30.25,9.60, 87.07 and 27.63) for birds on OGLM from nine weeks old for PCV, HB, MCV and MCH respectively. While birds on the control diet (28.88, 9.21, 81.24 and 25.92), MOLM (29.50, 9.51, 82.97 and 26.76) and VALM (28.38, 9.16, 87.47 and 28.25) from day old for PCV, HB, MCV and MCH respectively had the lowest value and were statistically similar. MCHC was not significant.

Highest white blood cell was observed in chicks on control diets ($26700.00\times10^3\mu$) and OGLM ($26687.25\times10^3\mu$) from nine weeks while the least value was observed in those on OGLM ($20288.50\times10^3\mu$) from one day old. Other treatments were not significant from each other. Highest platelet was seen in T6 (birds on OGLM from nine weeks old while the least was seen in birds on T3 andT7 that is, birds on OGLM from day old ($217875\times10^3/mm^3$) with those on VALM from nine weeks old ($217000\times10^3/mm^3$). The highest Lymphocyte was seen in T5 (68.25%) that is, birds on MOLM from nine weeks old followed by birds on control diet, VALM from both day old and nine weeks old and OGLM from nine weeks old, all of which were statistically similar and ranged from 62.63% to 67.25% . Birds on MOLM (60.38%) and OGLM (61.13%) from one day old both had the least value and were insignificant from one another. Heterophils was high in birds on MOLM (33.00%) from one day old, similar in other treatments with a range of 20.00% to30.50%while he least was found in birds on MOLM (24.25%) from nine weeks old.

The highest platelet was seen in birds on MOLM (0.56) from one day old while the least was seen in birds on MOLM (0.36) from nine weeks old. The highest monocyte was seen in birds on MOLM (3.75%) and OGLM (3.63%) both from one day old and the least was seen in birds on the control diet. Eosinophils and basophils had similar pattern where highest values were seen in the control diet 5.00% and 0.50%, respectively and the least was seen in birds on MOLM (0.56)

from one day old for Eosinophils and (2.50 and 0.00) OGLM from nine weeks old for both eosinophils and basophils, respectively.

Table 4.9: Haematology of the birds at the growing phase (16^{th} week)

Treatment	T1	T2	T3	T4	T5	T6	T7	SEM	*Reference value
PCV (%)	28.88^{b}	29.50^{b}	30.88^{ab}	28.38^{b}	30.00^{ab}	30.25^{ab}	32.63^{a}	0.85	23.00-55.00
HB (g/dl)	9.21^{b}	9.51^{ab}	10.03^{ab}	9.16^{b}	9.84^{ab}	9.60^{ab}	10.79^{a}	0.29	7.00-18.60
RBC ($\times 10^{6}\mu l$)	3.56	3.56	3.57	3.28	3.50	3.48	3.54	0.11	1.52-4.50
WBC ($\times 10^{3}$ µl)	26700^{a}	26125^{ab}	20288.50^{b}	25369.50^{ab}	247.50^{ab}	26687.25^{a}	23057.50^{ab}	1275.23	9.00-32.00
Platelet($10^{3}/mm^{3}$)	238875^{ab}	260250^{ab}	217875^{b}	235750^{ab}	226812^{ab}	269500^{a}	217000^{b}	1.56	13.00-70.00
Lymphocytes(%)	62.63^{ab}	60.38^{b}	61.13^{b}	64.50^{ab}	68.25^{a}	66.24^{ab}	67.25^{ab}	2.15	29.00-84.00
Heterophils (%)	29.38^{ab}	33.00^{a}	30.50^{ab}	29.63^{ab}	24.25^{b}	28.00^{ab}	26.00^{ab}	1.78	15.10-50.00
Het:Lym	0.48^{abc}	0.56^{a}	0.50^{ab}	0.47^{abc}	0.36^{c}	0.42^{bc}	0.39^{bc}	0.04	
Monocyte (%)	2.50^{b}	3.75^{a}	3.63^{a}	3.50^{ab}	3.50^{ab}	3.38^{ab}	3.00^{ab}	0.33	0.05-7.00
Eosinophils (%)	5.00^{a}	2.63^{b}	4.00^{ab}	3.75^{ab}	3.75^{ab}	2.50^{b}	3.50^{ab}	0.35	0.00-16.00
Basophils (%)	0.50^{a}	0.25^{ab}	0.25^{ab}	0.13^{ab}	0.25^{ab}	0.00^{b}	0.13^{ab}	0.13	0.00-8.00
MCV(fl)	81.24^{b}	82.97^{ab}	86.61^{ab}	87.47^{ab}	86.00^{ab}	87.07^{ab}	92.31^{a}	2.81	90.00-140.00*
MCHC (%)	31.89	32.250	32.48	32.26	32.85	31.74	33.09	0.55	26.00-35.00*
MCH (pg)	00025.92^{b}	26.76^{b}	28.13^{ab}	28.25^{ab}	28.26^{ab}	27.63^{ab}	30.52^{a}	1.05	33.00-47.00*

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

KEY: SEM- Standard error of the mean, T1- hens fed control diet, (T2- hens fed diet with 2% *Moringa oleifera* leaf meal, T3- hens fed diet with 2% *Ocimum gratissimum* leaf meal, T4- hens fed diet with 2% *Vernonia amygdalina* leaf meal) from day 1,(T5 hens fed -diet with 2% *Moringa oleifera* leaf meal, T6- hens fed diet with 2% *Ocimum gratissimum* leaf meal, T7- hens fed diet with 2% *Vernonia amygdalina* leaf meal) from week 9, PCV-Packed cell volume, HB-Haemoglobin, RBC- Red blood cell, WBC-White blood cell, MCV-Mean Cell Volume, MCHC-Mean Cell Haemoglobin Concentration, MCH-Mean Cell Haemoglobin.

Haematology of the experimental birds at the 24th week (growing phase) is shown in Table 4.10 in which parameters considered were significant ($P<0.05$) but for the platelets, lymphocytes, monocytes and mean cell heamoglobin that were insignificant ($P>0.05$). The highest PCV was seen in birds on control diet (24.13%), OGLM diet (24.75%) from nine weeks old, VALM diet (24.63%) from nine weeks old and VALM diet (24.63%) from nineteen weeks old while the least value was seen in hens on MOLM diet (19.38%) from nineteen weeks old. Haemoglobin content of birds on VALM diet (8.53 g/dl) from nine weeks old was the highest but lower in birds on VALM diet (7.58 g/dl) from one day old and the least was recorded in birds on MOLM diet (6.48 g/dl) from nineteen weeks old. RBC had similar trend as haemoglobin in which birds on VALM diet (2.86×10^6µl) from nine weeks old had the highest value and the least was recorded in birds on MOLM diet (6.48×10^6µl) from nineteen weeks old.

The WBC ranged from (21725 to 29306) $\times 10^3$ µl, where the birds on VALM diet (29306×10^3µl) from nine weeks old had the highest value and birds on OGLM diet (21725×10^3µl) from nineteen weeks old. Other treatments had similar WBC. Heterophils and heterophil, lymphocyte ratio had similar trend where the highest value birds on VALM diet (50.75% and 1.22) from nineteen weeks old and the least was recorded in birds on VALM diet (40.25% and 0.78) from one day old for heterophils and heterophil, lymphocyte ratio respectively. Eosinophils was high in birds on MOLM diet from nine weeks (4.63%) and nineteen weeks (4.50%) old respectively, similar in other treatments but low in birds on VALM diet (2.50%) from nine weeks old.Basophil was high in birds on MOLM diet from nineteen weeks (0.63%), the least in birds on OGLM diet (0.00%), MOLM (0.13%) diet, VALM diet (0.13%) all from one day old and OGLM diet (0.13%) from nineteen weeks old. Other treatments have similar values of basophils. The highest MCV was observed in birds on VALM diet (138.10fl) from nineteen weeks old and the least was recorded in birds on control diet, OGLM diet (96.32fl) and VALM diet (93.97fl) from nine weeks old. Other treatments have similar values of MCV. The highest MCHC was found in birds on MOLM diet (33.69%) from one day old and VALM diet (34.54%) from nine weeks old while the lowest value was in birds on VALM diet (30.06%) from nineteen weeks old. Other treatments have similar values of MCHC.

Table 4.10: Haematology of the birds at the laying phase (24th week)

	CON	R1			R2			R3			
Parameters	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
PCV (%)	24.13^{a}	22.75^{ab}	22.88^{ab}	22.75^{ab}	23.75^{ab}	24.75^{a}	24.63^{a}	19.38^{b}	23.13^{ab}	26.63^{a}	1.58
Haemoglobin(g/dl)	8.10^{ab}	7.65^{ab}	7.59^{ab}	7.58^{b}	7.73^{ab}	8.00^{ab}	8.53^{a}	6.48^{c}	7.73^{ab}	7.60^{ab}	0.33
RBC ($\times10^{6}\mu$l)	2.21^{ab}	1.97^{ab}	2.19^{ab}	2.44^{ab}	2.03^{ab}	2.57^{ab}	2.86^{a}	1.67^{b}	2.21^{ab}	1.96^{ab}	0.32
WBC ($\times10^{3}$ µl)	26588^{ab}	22419^{ab}	27013^{ab}	25744^{ab}	23525^{ab}	22775^{ab}	29306^{a}	23785^{ab}	21725^{b}	23481^{ab}	2426.72
Platelet(10^{3}/mm^{3})	235125	237875	278500	272125	246250	229833	286000	241875	217875	239625	0.98
Lymphocytes (%)	44.88	47.13	50.38	52.38	48.00	47.25	49.50	46.25	45.25	43.75	3.03
Heterophils (%)	47.63^{ab}	46.63^{ab}	44.50^{ab}	40.25^{b}	44.75^{ab}	47.00^{ab}	44.12^{ab}	46.75^{ab}	47.00^{ab}	50.75^{a}	2.76
Het: Lym	1.07^{ab}	1.05^{ab}	0.93^{ab}	0.78^{b}	0.94^{ab}	1.01^{ab}	0.91^{ab}	1.02^{ab}	1.05^{ab}	1.22^{a}	4.33
Monocyte (%)	2.75	2.00	3.38	3.50	3.38	2.50	3.50	2.88	3.33	2.00	0.12
Eosinophils (%)	4.25^{ab}	4.13^{ab}	3.13^{ab}	3.88^{ab}	4.63^{a}	3.75^{ab}	2.50^{b}	4.50^{a}	4.13^{ab}	3.25^{ab}	0.53
Basophils (%)	0.25^{ab}	0.13^{b}	0.00^{b}	0.13^{b}	0.38^{ab}	0.13^{b}	0.38^{ab}	0.63^{a}	0.25^{ab}	0.25^{ab}	0.65
MCV(fl)	95.44^{b}	122.11^{ab}	105.52^{ab}	116.08^{ab}	117.84^{ab}	96.32^{b}	93.97^{b}	116.86^{ab}	106.58^{ab}	138.10^{a}	0.15
MCHC (%)	33.62^{ab}	33.69^{a}	33.16^{ab}	33.34^{ab}	32.59^{ab}	32.33^{ab}	34.54^{a}	33.43^{ab}	33.45^{ab}	30.06^{b}	13.53
MCH (pg)	39.02	41.46	34.94	38.42	38.40	31.13	32.26	39.04	35.76	39.34	1.24

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

KEY: CON- Control, R-Regime (R1-Chick phase, R2-Grower phase, R3-Laying phase), SEM- Standard error of the mean, T1- hens fed control diet, (T2,T5, T8- hens fed diets with 2% *Moringa oleifera* leaf meal, T3,T6, T9- hens fed diets with 2% *Ocimum gratissimum* leaf meal, T4, T7, T10- hens fed diets with 2% *Vernonia amygdalina* leaf meal), PCV-Packed cell volume, RBC- Red blood cell, WBC-White blood cell, Het: Lym- Heterophils, Lymphocyte ratio, MCV-Mean Cell Volume, MCHC-Mean Cell Haemoglobin Concentration, MCH-Mean Cell Haemoglobin.

Interaction effect of additives and its period of introduction on haematology of the experimental birds at 24th week (growing phase)

There was an interaction effect of additives and its period of introduction on the haemoglobin of the birds as shown in Table 4.11 but no effect was seen on any of the other parameters (PCV, RBC, WBC, Platelet, Lymphocytes, Heterophils, Heterophils-Lymphocytes ratio, Monocyte, Eosinophils, Basophils, MCV, MCHC and MCH) considered. Birds fed control diet had the highest contents of haemoglobin (8.10g/dl) while birds on MOLM diets (7.28) had the least. Birds fed control diet (8.10g/dl) and birds from regime 2 (8.08g/dl) had the highest Interactive effect on haemoglobin compared with those from regime 3 with the least value (7.27g/dl).

Table 4.11: Interaction of additives and their period of introduction on the haematology of the birds

		ADDITIVE				REGIME			
TREATMENT	CON	A1	A2	A3	CON	R1	R2	R3	*Reference value
PCV (%)	24.13	21.96	23.58	24.67	24.13	22.79	24.38	23.04	23.0-55.0
Haemoglobin (g/dl)	8.10[a]	7.28[b]	7.77[ab]	7.90[ab]	8.10[a]	7.60[ab]	8.08[a]	7.27[b]	7.00-18.6
RBC ($\times 10^6 \mu l$)	2.21	1.89	2.32	2.42	2.21	2.20	2.49	1.94	1.52-4.50
WBC ($\times 10^3$ µl)	26588.00	23243.00	23838.00	26177.00	26588.00	25058.00	25202.00	22997.00	9.00-32.0
Platelet($10^3/mm^3$)	235125.00	242000.00	242069.00	265917.00	235125.00	262833.00	254028.00	233125.00	13.0-70.0
Lymphocytes (%)	44.88	47.13	47.63	48.54	44.88	49.56	48.25	45.08	29.0-84.0
Heterophils (%)	47.63	46.04	46.17	45.04	47.63	43.79	45.29	48.17	15.1-50.0
Het:Lym	1.07	1.00	1.00	0.97	1.07	0.92	0.95	1.09	
Monocyte (%)	2.75	2.75	3.07	3.00	2.75	2.96	3.13	2.73	0.05-7.00
Eosinophils (%)	4.25	4.42	3.67	3.21	4.25	3.71	3.63	3.96	0.00-16.0
Basophils (%)	0.25	0.38	0.13	0.25	0.25	0.08	0.29	0.38	0.00-8.00
MCV(fl)	95.44	118.93	102.80	116.05	95.44	114.57	102.71	120.51	90.0-140*
MCHC (%)	33.62	33.23	32.98	32.64	33.62	33.39	33.15	32.31	26.0-35.0*
MCH (pg)	39.02	39.63	33.94	36.67	39.02	38.27	33.93	38.04	33.0-47.0*

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

KEY:

CON- Control, A1- *Moringa oleifera* leaf meal, A2-*Ocimum gratissimum* leaf meal, A3- *Vernonia amygdalina* leaf meal, Regime: R1- hens fed \additives from chick phase, R2- hens fed \additives from grower phase, R3- hens fed \additives from laying phase, PCV-Packed cell volume, RBC- Red blood cell, WBC-White blood cell, MCV-Mean Cell Volume, MCHC-Mean Cell Haemoglobin Concentration, MCH-Mean Cell Haemoglobin.

The parameters measured for serum biochemistry at the 8th week (chick phase) Table 4.12 showed insignificance (P>0.05) of all the parameters except for globulin, albumin - globulin ratio (A:G) and cholesterol, where the highest value 3.16 g/dl seen was in hens fed control diet and the least value 2.69mg/dl in hens on 2% MOLM diets from the chick phase. The highest AG ratio of 0.66 was seen in hens on diets with 2% MOLM and the least (0.48) in hens on diets with 2% VALM. Cholesterol in which the highest value 177.88mg/dl was seen in hens fed control diet and lowest value 139.25mg/dl in hens on diets with 2% OGLM.

Table 4.12: Serum biochemical indices of the birds at chick phase (8th week)

Parameters	T1	T2	T3	T4	SEM	Reference value
Total protein(g/dl)	5.14	4.88	4.56	4.55	0.21	5.00-700
Albumin (g/dl)	1.66	1.86	1.70	1.55	0.11	1.80-3.50
Globulin (g/dl)	3.16[a]	2.69[b]	2.83[ab]	3.00[ab]	0.15	2.00-4.00
AG ratio	0.51[ab]	0.66[a]	0.60[ab]	0.48[b]	0.05	
AST(I.U./L)	168.00	149.75	144.88	168.25	12.60	
ALT(I.U./L)	27.50	25.75	30.38	28.13	2.31	10.0-37.0
AP(I.U./L)	75.50	80.13	75.50	63.13	6.03	
BUN(mg/dl)	0.21	0.24	0.30	0.24	0.0c4	2.47-8.08
Creatinine(mg/dl)	0.54	0.90	0.60	0.58	0.19	
Cholesterol(mg/dl)	177.88[a]	162.63[ab]	139.25[b]	152.75[ab]	9.06	52.0-148.0
Glucose(mg/dl)	204.25	209.38	236.50	244.50	16.41	200.0-500.0
Triglyceride(mg/dl)	178.88	174.75	145.38	154.63	14.19	
HDL(mg/dl)	67.75	62.75	57.88	70.38	5.30	
LDL(mg/dl)	74.35	64.93	52.38	51.45	10.43	

[abc]Means along the same row with different superscripts are significantly different (P< 0.05)

KEY:

SEM- Standard error of mean, T1- chicks fed control diets, (T2- chicks fed diet with 2% *Moringa oleifera* leaf meal, T3- chicks fed diet with 2% *Ocimum gratissimum* leaf meal, T4- chicks fed diet with 2% *Vernonia amygdalina* leaf meal) from day 1, AG ratio Albumin Globulin ratio, AST- Aspartate amino transferase, ALT- Alanine amino transferase, Alanine phosphatase, BUN-Blood urea nitrogen, HDL-high density lipoprotein, LDL- low density lipoprotein

Serum biochemistry of experimental birds at 16th week (growing phase) Table 4.13 revealed that the albumin, A:G, ALT, alanine phosphatase (AP), AST, triglyceride (Trig), BUN, HDL, creatinine and LDL were significant ($P<0.05$) while total protein (TP), globulin, glucose and cholesterol were insignificant ($P>0.05$). Birds fed diets with 2% OGLM from the growing phase had the highest value of 2.60g/dl for albumin while birds fed diets with 2% MOLM and 2%VALM from the growing phase both had the least value of 2.00g/dl and 1.92g/dl respectively. Birds fed diets with OGLM (0.89) and VALM (0.88) from the chick phase had the highest value of AG ratio while birds fed diets with VALM (0.2) from the growing phase had the least value. Birds fed diets with OGLM and VALM from the growing phase had the highest value of 193.66I.U./L and 193.87 respectively for AST and the least value was obtained in birds on OGLM (173.37I.U./L) from the chick phase.

Birds fed diets with VALM from the chick phase had the highest value of 46.37I.U./L for ALT and the least value of 28.33I.U./L and 29.00I.U./L were seen in birds on OGLM and VALM respectively from the growing phase. 491I.U./L was obtained in birds on OGLM as the highest ALP value and the least was observed in hens on control diets. The BUN was observed to be highest in birds on VALM diet from one day old and the least in birds on the control (0.42 mg/dl) and those on MOLM diet from the chick phase. 0.72mg/dl was the highest creatinine and it was found in birds on VALM diet from one day old and the least in birds on MOLM diet (0.50), also from the chick phase. The highest value of 68.75mg/dl for triglyceride was seen in birds fed diets with MOLM from the growing phase while the least 40.75mg/dl was observed in birds on MOLM from the chick phase. 108.50mg/dl was the highest HDL and it was found in hens fed control diet and the least 82.38mg/dl was found in birds on VALM diet from one day old. The highest LDL 67.20mg/dl was recorded in birds on OGLM from the growing phase while the least 25.63mg/dl was observed in birds on MOLM diet also from the growing phase.

Table 4.13: Serum biochemical indices of the birds at the growing phase (16th week)

Parameters	T1	T2	T3	T4	T5	T6	T7	SEM
Total protein(g/dl)	7.08	5.03	5.07	5.30	4.88	0.06	0.81	4.59
Albumin (g/dl)	2.23^{ab}	2.18^{ab}	2.37^{ab}	2.45^{ab}	2.00^{b}	2.60^{a}	1.92^{b}	0.16
Globulin (g/dl)	2.81	2.75	2.73	2.71	2.76	2.76	2.83	0.10
A/G ratio	0.81^{ab}	0.76^{abc}	0.89^{a}	0.88^{a}	0.70^{bc}	0.81^{ab}	0.62^{c}	0.06
AST(I.U./L)	179.00^{bc}	188.87^{ab}	173.37^{c}	182.62^{abc}	178.75^{bc}	193.66^{a}	193.87^{a}	4.03
ALT(I.U./L)	31.87^{ab}	33.00^{ab}	32.12^{ab}	46.37^{a}	31.75^{ab}	28.33^{b}	29.00^{b}	5.00
AP(I.U./L)	351.38^{c}	358.75^{bc}	441.00^{ab}	369.75^{bc}	369.63^{bc}	491.00^{a}	408.00^{bc}	26.58
BUN(mg/dl)	0.42^{d}	0.43^{d}	0.64^{bc}	1.02^{a}	0.55^{cd}	0.81^{b}	0.46^{cd}	0.06
Creatinine(mg/dl)	0.57^{bc}	0.50^{c}	0.62^{ab}	0.72^{a}	0.57^{bc}	0.58^{bc}	0.57^{bc}	0.04
Cholesterol(mg/dl)	156.63	136.00	159.63	155.13	134.13	162.15	149.50	11.86
Glucose(mg/dl)	356.25	366.25	386.25	362.50	375.50	406.63	369.00	27.59
Triglyceride(mg/dl)	67.00^{ab}	40.75^{c}	60.62^{abc}	56.00^{abc}	68.75^{a}	47.25^{bc}	50.37^{abc}	6.51
HDL(mg/dl)	108.50^{a}	91.88^{ab}	95.63^{ab}	82.38^{b}	94.75^{ab}	85.50^{ab}	90.00^{ab}	7.43
LDL(mg/dl)	34.73^{ab}	35.98^{ab}	51.88^{ab}	52.93^{ab}	25.63^{b}	67.20^{a}	49.43^{ab}	12.19

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

KEY:

SEM- Standard error of mean, T1-pullets fed control diets, (T2- pullets fed diet with 2% *Moringa oleifera* leaf meal, T3- pullets fed diet with 2% *Ocimum gratissimum* leaf meal, T4- pullets fed diet with 2% *Vernonia amygdalina* leaf meal) from day 1,(T5- pullets fed diet with 2% *Moringa oleifera* leaf meal, T6- pullets fed diet with 2% *Ocimum gratissimum* leaf meal, T7- pullets fed diet with 2% *Vernonia amygdalina* leaf meal) from week 9, AG ratio Albumin Globulin ratio, AST- Aspartate amino transferase, ALT- Alanine amino transferase, Alanine phosphatase, BUN-Blood urea nitrogen, HDL-high density lipoprotein, LDL- low density lipoprotein

The serum biochemistry of the experimental birds at the 24th week (laying phase) shown in Table 4.14 reveals that the albumin, globulin, A:G, ALT, TP, AST, AP, creatinine, glucose and LDL were significant ($P<0.05$) while BUN, cholesterol, Trig and HDL were not significant ($P>0.05$). 7.21g/dl in birds on MOLM diet from the growing phase was the highest value recorded for TP and the least value of 6.01g/dl was seen in birds on MOLM diet from the chick phase. Albumin of the birds on diet with OGLM from chick phase (1.70) was significantly high ($P<0.05$) compared to albumin of other treatments, similar in treatments 1, 5, 7, 8, 9 and 10 but lower in birds on diet with MOLM (0.91g/dl) and VALM (1.05g/dl) from chick phase and those on MOLM (1.04g/dl) from the growing phase. The highest value for globulin was seen in birds fed MOLM (5.98g/dl) diets from the growing phase and the least seen in birds on OGLM (4.83g/dl) diet from the chick phase. The highest A/G ratio was seen in birds on OGLM (0.33) diet from the chick phase while the least (0.14) was seen in both birds on MOLM diet from the chick phase and OGLM diets from the growing phase. AST (232.63 I.U./L) and ALT (36.13 I.U./L) were very high in birds on OGLM diets from the laying phase but AST was low in birds on OGLM (181.44 I.U./L) diets from the growing phase and ALT in birds on MOLM (22.88 I.U./L) diet from the chick phase. Other treatments were similar. AP was high in birds on VALM diet (458.88I.U./L) and low in birds on OGLM diet (365.13I.U./L) both from the laying phase. BUN was high in birds on MOLM diet (13.20mg/dl) from the laying phase but similar in other treatments ranging from 0.65 to 0.80. The highest glucose was recorded in birds on VALM diet (422.50mg/dl) from the chick phase and the lowest glucose was recorded in birds on OGLM (204.38mg/dl) diets from the laying phase. The highest value for LDL was seen in birds on the control diets (99.35mg/dl) and in birds on VALM diet (90.88mg/dl) from the growing phase, the least in birds on MOLM (49.10mg/dl) diets from the growing phase.

Table 4.14: Serum biochemical indices of the birds at the laying phase (24th week)

	CON	R1			R2			R3			
Parameters	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
Total protein(g/dl)	6.90^{ab}	6.01^{b}	6.85^{ab}	6.28^{ab}	7.21^{a}	6.70^{ab}	6.85^{ab}	6.83^{ab}	6.99^{ab}	6.68^{ab}	0.36
Albumin (g/dl)	1.38^{ab}	0.91^{b}	1.70^{a}	1.05^{b}	1.24^{ab}	1.04^{b}	1.25^{ab}	1.33^{ab}	1.29^{ab}	1.28^{ab}	0.17
Globulin (g/dl)	5.44^{abcd}	5.10^{bcd}	4.83^{d}	4.86^{cd}	5.98^{a}	5.66^{abc}	5.48^{abcd}	5.38^{abcd}	5.70^{ab}	5.40^{abcd}	0.28
A:G	0.25^{ab}	0.14^{c}	0.33^{a}	0.16^{bc}	0.15^{bc}	0.14^{c}	0.19^{bc}	0.21^{bc}	0.19^{bc}	0.20^{bc}	0.34
AST(I.U./L)	196.50^{ab}	215.50^{ab}	217.25^{ab}	216.38^{ab}	210.38^{ab}	181.44^{b}	211.38^{ab}	215.25^{ab}	232.63^{a}	219.88^{ab}	15.62
ALT(I.U./L)	25.13^{bc}	22.88^{c}	29.13^{abc}	25.63^{bc}	29.25^{abc}	25.5^{bc}	26.13^{bc}	30.25^{b}	36.13^{a}	31.38^{b}	2.49
AP(I.U./L)	409.63^{ab}	390.63^{ab}	437.25^{ab}	424.25^{ab}	380.85^{ab}	404.38^{ab}	376.88^{ab}	423.38^{ab}	365.13^{b}	458.88^{a}	29.34
BUN(mg/dl)	0.61	0.58	0.65	0.58	0.59	0.64	0.59	0.56	0.58	0.63	0.04
Creatinine(mg/dl)	0.74^{b}	0.71^{b}	0.80^{b}	0.73^{b}	0.80^{b}	0.76^{b}	0.70^{b}	13.20^{a}	0.71^{b}	0.65^{b}	3.97
Cholesterol(mg/dl)	232.88	218.25	169.63	177.25	185.13	176.63	208.13	210.38	167.63	167.38	29.16
Glucose(mg/dl)	310.75^{bc}	371.25^{ab}	397.63^{ab}	422.50^{a}	301.50^{bc}	314.00^{b}	336.88^{ab}	344.88^{ab}	204.38^{c}	354.88^{ab}	37.07
Triglyceride(mg/dl)	45.75	50.75	55.63	46.75	56.25	48.63	61.25	47.38	63.00	51.38	6.39
HDL(mg/dl)	124.38	126.63	99.88	108.00	124.78	100.00	105.00	127.38	83.13	95.00	18.65
LDL(mg/dl)	99.35^{a}	81.48^{ab}	58.65^{ab}	59.95^{ab}	49.10^{b}	66.90^{ab}	90.88^{a}	73.53^{ab}	71.80^{ab}	61.60^{ab}	14.43

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

KEY: SEM- Standard error of the mean R-Regime (R1-Chick phase, R2-Grower phase, R3-Laying phase),, A-control, (T2,T5, T8- hens fed diets with 2% *Moringa oleifera* leaf meal, - T3, T6, T9- hens fed diets with 2% *Ocimum gratissimum* leaf meal, T4, T7, T10- hens fed diets with 2% *Vernonia amygdalina* leaf meal), A:G- Albumin Globulin ratio, AST- Aspartate amino transferase, ALT- Alanine amino transferase, AP-Alanine phosphatase, BUN-Blood urea nitrogen, Creat-Creatinine, Chol-Cholesterol, Trig-Triglycerides, HDL-high density lipoprotein, LDL- low density lipoprote

Interaction of additives and its period of introduction on serum biochemistry of the experimental birds at 24th week (laying phase) as shown in Table 4.15 revealed an interaction on the period of introduction of additives which was significant but the additives had no significant effect on globulin content of birds. A/G ratio had significant interaction effect on both additive and their period of introduction where the hens fed control diets (0.25) were the highest in value and the lowest was in hens on MOLM diets (0.17). ALT also had significant interaction effect on both additive and their period of introduction as hens fed control diet were of the highest value 30.25 (I.U./L) while period of introduction of the additives was highest in regime 3 (32.58 I.U./L). Additives significantly affected the interaction of cholesterol in which the period of introduction of the additives was insignificant. Hens on control diet (232.88mg/dl) were the highest in cholesterol and the least (171.29mg/dl) was seen in birds on OGLM based diets. Glucose was not affected by the additives but the period of introduction of the additives significantly affected glucose as Regime 1 (397.13 mg/dl) *i.e.* chick phase was higher than the others. LDL was significantly affected by the additives where birds on control diet were of the highest value of 99.35mg/dl while other treatments were similar.

Table 4.15: Interaction of additives and their period of introduction on the serum biochemistry of the birds at the laying phase

	ADDITIVE				REGIME			
Parameters	CON	A1	A2	A3	CON	R1	R2	R3
Total P(g/dl)	6.90	6.68	6.85	6.60	6.90	6.38	6.92	6.83
Album (g/dl)	1.38	1.16	1.34	1.19	1.38	1.22	1.18	1.30
Globu (g/dl)	5.48	5.44	5.40	5.24	5.44^{ab}	4.93^{b}	5.70^{a}	5.49^{a}
A:G	0.25^{a}	0.17^{b}	0.22^{ab}	0.18^{ab}	0.25^{a}	0.21^{ab}	0.20^{ab}	0.16^{b}
AST(I.U./L)	196.50	213.71	210.44	215.88	196.50	216.38	201.06	222.58
ALT(I.U./L)	30.25^{a}	27.46^{ab}	25.13^{b}	27.71^{ab}	25.13^{b}	25.88^{b}	26.96^{b}	32.58^{a}
AP(I.U./L)	409.63	398.28	402.25	420.00	409.63	417.38	387.37	415.79
BU(mg/dl)	0.61	0.58	0.62	0.60	0.61	0.60	0.60	0.59
Crea (mg/dl)	0.74	4.90	0.76	0.69	0.74	0.75	0.75	4.85
Chol (mg/dl)	232.88^{a}	204.58^{ab}	171.29^{b}	184.25^{ab}	232.88	188.38	189.96	181.79
Glu(mg/dl)	310.75	39.21	305.33	371.42	310.75^{b}	397.13^{a}	317.46^{b}	301.38^{b}
Trig (mg/dl)	45.75	51.46	55.75	53.13	45.75	51.04	55.38	53.92
HDL(mg/dl)	124.38	126.26	94.33	102.67	124.38	111.50	109.93	101.83
LDL(mg/dl)	99.35^{a}	68.03^{b}	65.78^{b}	70.81^{ab}	99.35	66.69	68.96	68.98

[abc]Means along the same row with different superscripts are significantly different (P< 0.05)

KEY:

CON- Control, Regime: R1- from chick phase, R2- from grower phase, R3- from laying phase, A1- *Moringa oleifera* leaf meal, A2-*Ocimum gratissimum* leaf meal, A3- *Vernonia amygdalina* leaf meal, SEM- Standard error of mean, Total P- total protein, Album- Albumin, Globu- Globulin A:G- Albumin Globulin ratio, AST- Aspartate amino transferase, ALT- Alanine amino transferase, AP-Alanine phosphatase, BU-Blood urea nitrogen, Crea-Creatinine, Chol-Cholesterol, GLU-Glucose Trig-Triglycerides, HDL-high density lipoproteiuin, LDL- low density lipoprotein

Qualities of eggs collected from the experimental birds at early laying phase (14th-19th week) Table 4.16 showed that major part of the parameters considered (Yolk weight (YW), Haugh unit (HU), yolk index (YI), yolk colIUour (YC) and shell thickness (ST)) were significantly different ($P<0.05$) while egg weight (EW), albumen weight (AW), Albumen &Yolk weight (AYW) and Shell weight (SW) were not significant ($P>0.05$). The best YW was seen in the eggs from birds fed VALMdiet (15.03g) from the chick phase and the lowest in the eggs from birds fed MOLM (13.00g) diet from the laying phase and similar in the other treatments. Birds on MOLM diet (3.49)from the chick phase had the best colour while the least was seen in hens fed control diet (1.11) and hens on OGLM diet (1.50) from the laying phase. The best yolk index of 0.41 was seen in birds on OGLM and VALMdiet both from the chick phase and the least in birds on OGLM diet from the laying phase.

The best haugh unit was seen in birds on VALM diet (80.34) from the growing phase and the least from birds on OGLM diet (75.54)from the chick phase while others were similar. Birds fed OGLM diet (0.20 mm)from the laying phase had the thickest shell and the least from birds on the control (0.10) and VALM diet (0.10) from the growing phase. The best %yolk was seen in birds on VALM diet (28.42%) from the chick phase and the least from hens fed control diet (25.28%) and MOLM diet (24.47%)from the laying phase.

Table 4.16: Egg qualities of birds at early laying phase (14th-19th week)

	CON	R1			R2			R3			
PARA	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
Egg weight(g)	53.77	54.05	53.91	53.06	56.20	53.45	53.37	54.09	53.96	55.16	0.82
Albumen W(g)	34.23	34.15	34.02	31.74	35.36	33.62	34.44	33.68	33.13	33.42	0.99
Yolk weight(g)	13.59^{ab}	14.11^{ab}	14.69^{ab}	15.03^{a}	14.44^{ab}	14.07^{ab}	13.78^{ab}	13.00^{b}	13.87^{ab}	14.63^{ab}	0.39
Yolk colour	01.11^{d}	03.49^{a}	1.75^{cd}	02.50^{bc}	03.17^{ab}	01.72 ^{cd}	02.94^{ab}	03.21^{ab}	01.50^{d}	02.63^{abc}	0.22
Albumen:Yolk	47.79	48.66	48.73	47.17	49.79	47.68	48.21	47.89	47.02	48.37	0.98
Yolk index	00.40^{ab}	00.40^{ab}	00.41^{a}	00.41^{a}	00.40^{ab}	00.40^{ab}	00.38^{ab}	00.37^{ab}	00.36^{b}	00.38^{ab}	0.01
Haugh Unit	77.67^{ab}	77.44^{ab}	75.54^{b}	76.58^{ab}	76.36^{ab}	79.16^{ab}	80.34^{a}	79.11^{ab}	79.2^{ab}	77.98^{ab}	0.97
Shell thickness (mm)	0.10^{b}	0.16^{ab}	0.12^{ab}	0.11^{ab}	00.12^{ab}	00.11^{ab}	00.10^{b}	00.13^{ab}	00.20^{a}	00.17^{ab}	0.02
Shell weight(g)	04.72	04.81	04.79	04.83	04.80	04.61	04.73	04.79	04.64	04.74	0.49
%Albumen	63.80	63.12	63.10	59.82	62.85	62.94	64.46	62.50	61.43	60.51	1.59
% Yolk	25.28^{b}	26.21^{ab}	27.33^{ab}	28.42^{a}	25.78^{ab}	26.30^{ab}	25.80^{ab}	24.47^{b}	25.79^{ab}	26.51^{ab}	0.68
%Shell weight	08.88	07.41	07.44	09.28	07.14	07.23	09.00	07.40	07.28	08.72	0.95
%Shell thickness	00.20	00.29	00.23	00.21	00.23	00.21	00.20	00.24	00.38	00.31	0.04

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

KEY: SEM- Standard error of mean, C-control, R-Regime (1- chick piuhase, 2-grower phase, 3-laying phase), %-Percentage

Interaction effect of additives and its period of introduction on the qualities of eggs collected from the experimental birds at the early laying phase (14th-19th week) shown on Table 4.17 revealed that period of introduction of additives to the hens' diet had significant ($P< 0.05$) effect on shell thickness but not the additives, the least value (0.12mm) was seen in hens fed control diet while the highest (0.22mm) was seen in birds on regime 1. Yolk weight was significantly ($P< 0.05$) improved by both additive and period of introduction of additives to the diet of birds with the least value recorded for birds fed control diet and the highest in hens on VALM (14.48g) diets for additives and regime 2 (14.46g) for the regimes. Yolk colour had similar pattern as YW with hens on the control diet with the least value both for additive (1.11) and the period of introduction of additives (1.55) to the diet of the birds. While the yolk colour was similar for the three regimes, MOLM had the highest value for the additives. Regime of introduction of additives to diet of the birds was significant ($P< 0.05$) on hens' YI and HU but not on the additives. Additives and the period of introduction of additives (regime) to the hens' diet had significant ($P< 0.05$) interaction on yolk percentage as the least values were obtained in hens fed control diets with highest for hens fed VALM diets for the additives and birds from regime 2 (that is, birds fed the leaf meals from the growing phase).

Table 4.17: Interaction of additives and their period of introduction on egg qualities of birds at early laying phase (14th-19th week)

		ADDITIVE			REGIME			
PARA	CON	A2	A3	A4	CON	R1	R2	R3
Egg weight(g)	53.77	54.78	53.78	53.87	54.06	54.36	54.50	55.48
Shell thickness(mm)	0.10	0.14	0.14	0.13	0.12^{b}	0.22^{a}	0.16^{ab}	0.18^{ab}
Shell weight(g)	4.72	4.80	4.68	4.77	5.87	5.90	5.92	5.63
Albumen weight (g)	34.23	34.40	33.59	33.20	33.18	32.39	33.21	31.80
Yolk weight(g)	13.57^{b}	13.85^{ab}	14.21^{ab}	14.48^{a}	13.17^{b}	13.88^{ab}	14.46^{a}	14.17^{ab}
Yolk colour	1.11^{d}	3.29^{a}	2.69^{b}	1.66^{c}	1.55^{b}	3.17^{a}	3.17^{a}	3.08^{a}
Albumen:Yolk	47.80	48.78	47.81	47.92	46.71	47.09	47.68	48.49
Yolk index	0.40	0.39	0.39	0.39	0.39^{ab}	0.42^{ab}	0.38^{b}	0.43^{a}
Haugh Unit	77.67	77.64	77.98	78.30	79.72^{ab}	76.94^{b}	78.46^{ab}	80.57^{a}
%Albumen	63.80	62.82	62.49	61.60	61.39	59.52	60.93	57.27
% Yolk	25.28^{b}	25.49^{b}	26.48^{ab}	26.91^{a}	24.33^{b}	25.53^{ab}	26.57^{a}	25.52^{ab}
% Shell weight	8.88	7.32	7.32	8.99	8.88	8.04	7.79	7.80

[abc]Means along the same row with different superscripts are significantly different (P< 0.05)

KEY: SEM- Standard error of mean, C- control, A-Additive (1-*Moringa oleifera* leaf meal, 2-*Ocimum gratissimum* leaf meal, 3-*Vernonia amygdalina* leaf meal), R-Regime (1- chick phase, 2—grower phase, 3-laying phase), %-Percentage

The qualities of eggs collected from the experimental birds at the mid laying phase (33rd-38th week) Table 4.18 showed that all parameters considered (EW, AYW, AW, YW, HU and %Y) were significantly different ($P<0.05$). Best weight (59.52g) was seen in the eggs from hens fed OGLM dietfrom chick phase (T3) and the lowest (55.66g) in eggs from hens on VALM diet from growing phase (T7). The albumen was high in birds in birds on MOLM (36.62g) and OGLM (36.77g) diets both from the chick phase and low in birds on MOLM (33.87g) and OGLM (33.53g) diets both from the growing phase and OGLM (34.04g) diets from the laying phase respectively. Yolk weight was high in birds OGLM (15.86g) diets and VALM (15.85g) diet both from the chick phase and low in birds on OGLM (14.50g) diet from the laying phase. Albumen: Yolk was found to be higher in OGLM (52.58) dietfrom the chick phase and the lowest in the eggs from birds fed MOLM (48.65) and VALM (48.40) diets both from the chick phase and in birds on OGLM (48.41) diets from the laying phase. The best Haugh unit was seen in eggs from birds on OGLM (80.30) diets and VALM (80.12) diet both from the chick phase and the least in hens on VALM (76.09) diet from the laying phase. Percentage yolk was high in eggs of hen on VALM diet (28.18%) from the growing phase while the least was seen in eggs of birds on MOLM (25.72%) dietfrom the chick phase, OGLM (25.84%) diets and VALM (25.86%) diet both from the laying phase respectively. YC, YI, ST, SW, %Albumen, %SW and %ST were in significant ($P>0.05$).

Table 4.18: Main effect of qualities of eggs from the mid laying phase (33rd-38th week)

	CON	R1			R2			R3			
PARA	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
Egg weight(g)	57.51^{abc}	58.93^{ab}	59..92^{a}	58.77^{ab}	56.46^{bc}	57.17^{abc}	55.67^{c}	57.05^{abc}	56.23^{bc}	58.64^{abc}	0.68
Albumen weight (g)	34.74^{ab}	36.62^{a}	36.77^{a}	35.53^{ab}	33.87^{b}	34.80^{ab}	33.53^{b}	34.84^{ab}	34.04^{b}	35.28^{ab}	0.57
Yolk weight(g)	15.16^{ab}	15.14^{ab}	15.86^{a}	15.85^{a}	14.96^{ab}	15.10^{ab}	15.55^{ab}	14.76^{ab}	14.50^{b}	15.12^{ab}	0.29
Yolk colour	02.63	03.36	02.76	003.04	03.22	02.56	02.67	03.03	02.65	02.58	0.23
Albumen:Yolk	49.90^{abc}	51.75^{ab}	52.58^{a}	51.21^{abc}	48.65^{c}	49.90^{abc}	48.40^{c}	49.41^{bc}	48.41^{c}	50.40^{abc}	0.64
Yolk index	00.23	00.22	00.21	00.21	00.22	00.22	00.22	00.23	00.23	00.22	0.01
Haugh Unit	79.12^{ab}	78.25^{abcd}	80.30^{a}	80.12^{a}	78.75^{abc}	76.31^{cd}	78.05^{abcd}	77.39^{bcd}	77.46^{bcd}	76.09^{d}	0.58
Shell thickness (mm)	00.39	00.41	00.41	00.42	00.40	00.39	00.40	00.40	00.40	00.39	0.01
Shell weight(g)	06.18	06.54	06.56	06.46	06.15	06.37	06.32	06.25	06.26	06.49	0.10
%Albumen	60.43	62.07	61.71	60.37	59.90	60.69	60.01	61.11	60.48	60.04	0.58
% Yolk	26.43$_{ab}$	25.72^{b}	26.77$_{ab}$	27.08^{ab}	26.62$_{ab}$	26.58$_{ab}$	28.18^{a}	26.07$_{ab}$	25.84^{b}	25.86^{b}	0.51
% Shell weight	10.78	11.15	11.07	11.02	10.96	11.18	11.40	11.03	11.19	11.12	0.17
% Shell thickness	00.68	00.70	00.70	00.72	00.71	00.68	00.73	00.71	00.72	0.067	0.02

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

KEY: SEM- Standard error of mean, C- control, R-Regime (1- chick phase, 2—grower phase, 3-laying phase), %-Percentage

The interaction effect of additives and its period of introduction on the qualities of eggs collected from the experimental birds at the mid laying phase (33rd-38th week) shown in Table 4.19 revealed that regime of introduction of additives to diets of the birds with significant (P< 0.05) effect on EW and ST with regime 2 having the highest (59.07g and 0.41mm) value but not the additives as the other regimes were similar the least value seen was in hens fed control diet. Shell weight had significant interaction on both additive and the period of introduction of additives to the diet of the birds. Birds fed diets with additives had higher shell weight than the control while birds on the control and regime 2 were least for the regimes. Period of introduction of additives to the birds' diets was significant (P< 0.05) for AW and YW as the highest values were recorded for eggs from birds from regime 1 and the least yolk weight in eggs of birds from regime 3 but was insignificant (P>0.05) for additive.

The YC was significantly affected (P< 0.05) by additives with the deepest colour in eggs from birds on MOLM diets (3.20), followed by those from birds on VALM diets and lastly by both those on OGLM diets (2.66) and control diet (2.63) but was insignificantly (P>0.05) affected by period of introduction of additives to the diet of the birds. Albumen-yolk ratio was significantly higher in eggs from birds from the regime 1 while others were similar. Haugh unit was significantly improved (P<0.05) by period of introduction of additives to the diet of the birds with control (79.12) and regime 1 (79.16) being higher than those on regime 2 (77.71) and regime 3(76.98) but not by the additives. Regime 1 had a higher albumen percentage (61.38%) than regime 2 (60.20%). Regime 2 (27.13%) had a higher yolk percentage than regime 3 (25.93%).

Additives and the period of introduction of additives to the diet of the birds had significant interaction effect on percentage Shell weight as the least values were seen in hens fed control diet while those with additives and regimes 1, 2 and 3 were similar but higher than hens fed control diet. The ST was not affected by both additives and the period of introduction of additives to the diet of the birds.

Table 4.19: Interaction of additives and their period of introduction on egg qualities at mid laying phase (33rd-38th week)

	ADDITIVE				REGIME			
PARA	CONT	A2	A3	A4	CONT	R1	R2	R3
Egg weight(g)	57.51	57.48	57.64	57.69	57.51^{b}	59.07^{a}	56.42^{b}	57.31^{b}
Shell thickness (mm)	00.39	00.40	00.40	00.41	00.39^{b}	00.41^{a}	00.40^{ab}	00.40^{ab}
Shell weight(g)	06.18^{b}	06.31^{ab}	06.40^{a}	06.42^{a}	06.18^{b}	06.52^{a}	06.28^{b}	06.33^{ab}
Albumen weight (g)	34.74	35.11	35.20	34.78	34.74^{b}	36.30^{a}	34.07^{b}	34.72^{b}
Yolk weight(g)	15.16	14.96	15.16	15.50	15.16^{ab}	15.62^{a}	15.60^{ab}	14.79^{b}
Yolk colour	02.63^{b}	03.20^{a}	02.66^{b}	02.76^{ab}	02.63	03.06	02.81	02.75
Albumen:Yolk	49.90	49.94	50.29	50.00	49.90^{b}	51.85^{a}	48.98^{b}	49.41^{b}
Yolk index	00.23	00.23	00.22	00.22	00.23	00.21	00.22	00.22
Haugh Unit	79.12	78.13	78.03	78.08	79.12^{a}	79.56^{a}	77.71^{b}	76.98^{b}
%Albumen	60.43	61.03	60.96	60.14	60.43^{ab}	61.38^{a}	60.20^{b}	60.55^{ab}
% Yolk	26.43	26.14	26.40	27.04	26.43^{ab}	26.52^{ab}	27.13^{a}	25.93^{b}
% Shell weight	10.78^{b}	11.05^{ab}	11.15^{a}	11.18^{a}	10.78^{b}	11.08^{ab}	11.18^{a}	11.12^{ab}
% Shell thickness	00.68	00.71	00.70	00.71	00.68	00.71	00.71	00.70

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

KEY: SEM- Standard error of mean, C- control, A-Additive (1-*Moringa oleifera* leaf meal, 2-*Ocimum gratissimum* leaf meal, 3-*Vernonia amygdalina* leaf meal), P-Period (1- chick phase, 2—grower phase, 3-laying phase), %-Percentage

Fatty acid profile (FAP) of eggs at early laying phase (14th-19th week) shown in Table 4.20 revealed that all the parameters measured (myristic (MA), palmitic (PA), palmitoleic (POA), stearic (SA), oleic (OA), olenic (OLA), linoleic (LA) and arachidonic acids (AA) of the eggs laid by the experimental birds were insignificant ($P>0.05$).

Interaction of additive and its period of introduction on FAP of eggs at mid laying phase (33rd-38th week) as shown in Table 4.21 revealed that there was interactive effect of both additive and their period of introduction in the diets on FAP of eggs laid by the birds with high significance ($P< 0.05$) as control had the highest value in all the parameters considered for fatty acid profile. For the additives, MOLM was next to the control as all the parameters were higher than those of birds on both OGLM and VALM diets but fatty acid profile of the birds on OGLM and VALM diets were similar. For the interactive effect of period (that is, regime) of introduction of the additives to the diets of the birds on eggs' fatty acid profile, eggs from hens fed control diet had the highest value, then, those of birds from regime 2 (that is, birds fed the additives from 9 weeks) followed by those from birds from both regimes 1 and 3, as regimes 1 and 3 are statistically similar.

Table 4.20: Fatty acid profile of eggs from early laying phase (14th-19th week)

	CON	R1			R2			R3			
PARAMETERS	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
14:0	4.37	2.85	2.66	3.04	4.18	3.04	2.66	3.23	2.66	3.04	0.65
16:0	4.90	3.19	2.98	3.41	4.69	3.40	2.98	3.62	2.98	3.40	0.72
16:1(Δ^9)	4.87	3.17	2.96	3.39	4.66	3.38	2.96	3.60	2.96	3.38	0.72
18:0	5.44	3.55	3.31	3.79	5.21	3.78	3.31	4.02	3.31	3.78	0.80
18:1(Δ^9)	5.41	3.52	3.29	3.76	5.17	3.76	3.29	3.99	3.29	3.76	0.80
18:2($\Delta^{9,12}$)	5.37	3.50	3.27	3.73	5.13	3.73	3.27	3.97	3.27	3.73	0.79
18:3($\Delta^{9,12,15}$)	5.32	3.47	3.24	3.70	5.09	3.70	3.24	3.93	3.24	3.70	0.78
20:4($\Delta^{5,8,11,14}$)	5.83	4.05	3.54	4.05	5.57	4.05	3.54	4.31	3.54	4.05	0.86

[abc]Means along the same row with different superscripts are significantly different (P< 0.05)

SEM- Standard error of mean, CON- control (T1), (T2,T5 T8-*Moringa oleifera* leaf meal, T3,T6 T9-*Ocimum gratissimum* leaf meal, T4, T7, T10-*Vernonia amygdalina* leaf meal), R-Regime (R1- chick phase, R2—grower phase, R3-laying phase)

Table 4.21: Interaction of additive and their period of introduction on fatty acid profile of eggs from early laying phase (14th-19th week).

		ADDITIVE			REGIME			
PARA	CON	A1	A2	A3	CON	R1	R2	R3
14:0	43.74^{a}	34.23^{ab}	27.89^{b}	29.16^{b}	43.74^{a}	28.52^{b}	32.96^{ab}	29.79^{b}
16:0	49.06^{a}	38.37^{ab}	31.26^{b}	32.69^{b}	49.06^{a}	31.98^{b}	36.96^{ab}	33.38^{b}
16:1(Δ^{9})	48.72^{a}	38.13^{ab}	31.07^{b}	32.48^{b}	48.72^{a}	31.78^{b}	36.72^{ab}	33.19^{b}
18:0	54.48^{a}	42.63^{ab}	34.74^{b}	36.32^{b}	54.48^{a}	35.53^{b}	41.06^{ab}	37.10^{b}
18:1(Δ^{9})	54.10^{a}	42.34^{ab}	34.50^{b}	36.06^{b}	54.10^{a}	35.28^{b}	40.77^{ab}	36.85^{b}
18:2($\Delta^{9,12}$)	53.71^{a}	42.04^{ab}	34.25^{b}	35.81^{b}	53.71^{a}	35.03^{b}	40.48^{ab}	36.59^{b}
18:3($\Delta^{9,12,15}$)	53.29^{a}	41.70^{ab}	33.98^{b}	35.52^{b}	53.29^{a}	34.75^{b}	40.16^{ab}	36.30^{b}
20:4($\Delta^{5,8,11,14}$)	58.31^{a}	45.63^{ab}	37.18^{b}	38.82^{b}	58.31^{a}	38.03^{b}	43.94^{ab}	39.72^{b}

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

KEY:

CON- control, A-Additive (1-*Moringa oleifera* leaf meal, 2-*Ocimum gratissimum* leaf meal, 3-*Vernonia amygdalina* leaf meal), R-Regime (R1-chick phase, R2—grower phase, R3-laying phase)

All the parameters measured for the fatty acid profile (FAP) MA, PA, POA, SA, OA, OLA, LA and AA) of eggs from hens at mid laying phase (33rd-38th week) Table 4.22 were significant ($P<0.05$) and had similar pattern as those by hens on both control and VALM diet (T10) from chick phase had the highest values while those on the diets with VALM diet (T10) from laying phase had the least value for all the parameters, in which other treatments had similar ratings and statistically indifferent ($P> 0.05$).

Table 4.23 revealed that there was interaction of both additive and their period (regime) of introduction in the diets on the FAP of eggs laid at mid laying phase (33rd-38th week) and they were highly significant ($P< 0.05$) as control had the highest value for all parameters considered for fatty acid profile for both additives and regimes of introduction of additives in the diets on the birds. The additives and regimes of introduction in the diets on the birds at mid laying phase had similar values in all the fatty acids considered.

Table 4.22: Fatty acid profile of eggs from mid laying phase (33rd-38th week)

	CON	R1			R2			R3			
Parameters	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
14:0	2.28^{a}	1.49^{ab}	1.77^{ab}	2.11^{a}	1.99^{ab}	1.91^{ab}	1.73^{ab}	1.77^{ab}	1.89^{ab}	1.14^{b}	0.19
16:0	2.56^{a}	1.67^{ab}	1. 99^{ab}	2.37^{a}	2.24^{ab}	2.14^{ab}	1.94^{ab}	1.99^{ab}	2.11^{ab}	1.28^{b}	0.22
16:1(Δ^{9})	2.29^{a}	1.65^{ab}	1.97^{ab}	2.35^{a}	2.22^{ab}	2.13^{ab}	1.92^{ab}	1.97^{ab}	2.10^{ab}	1.27^{b}	0.22
18:0	2.84^{a}	1.85^{ab}	2.21^{ab}	2.63^{a}	2.49^{ab}	2.38^{ab}	2.15^{ab}	2.20^{ab}	2.34^{ab}	1.42^{b}	0.24
18:1(Δ^{9})	2.82^{a}	1.84^{ab}	2.18^{ab}	2.61^{a}	2.47^{ab}	2.36^{ab}	2.13^{ab}	2.19^{ab}	2.33^{ab}	1.41^{b}	0.24
18:2($\Delta^{9,12}$)	2.80^{a}	1.82^{ab}	2.19^{ab}	2.61^{a}	2.45^{ab}	2.34^{ab}	2.11^{ab}	2.17^{ab}	2.31^{ab}	1.40^{b}	0.24
18:3($\Delta^{9,12,15}$)	2.78^{a}	1.81^{ab}	2.16^{ab}	2.57^{a}	2.43^{ab}	2.32^{ab}	2.10^{ab}	2.16^{ab}	2.29^{ab}	1.39^{b}	0.24
20:4($\Delta^{5,8,11,14}$)	3.04^{a}	1.98^{ab}	2.36^{ab}	2.82^{a}	2.66^{ab}	2.54^{ab}	2.30^{ab}	2.36^{ab}	2.51^{ab}	1.52^{b}	0.26

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

KEY:

SEM- Standard error of mean, CON- control (T1), (T2, T5, T8-*Moringa oleifera* leaf meal, T3, T6, T9-*Ocimum gratissimum* leaf meal, T4, T7, T10-*Vernonia amygdalina* leaf meal), R-REGIME (T2-T4: chick phase, T5-T7: grower phase, T8-T10: laying phase

Table 4.23: Interaction of additive and their period of introduction on fatty acid profileof eggs from mid laying phase (33rd-38th week)

Parameters	CON	A1	A2	A3	CON	R1	R2	R3
14:0	2.28^{a}	1.75^{b}	1.85^{b}	1.66b	2.28^{a}	1.79^{b}	1.88^{b}	1.60^{b}
16:0	2.56^{a}	1.96^{b}	2.08^{b}	1.86^{b}	2.56^{a}	2.01^{b}	2.11^{b}	1.79^{b}
16:1(Δ^{9})	2.29^{a}	1.95^{b}	2.06^{b}	1.85^{b}	2.29^{a}	1.99^{ab}	2.09^{ab}	1.78^{b}
18:0	2.84^{a}	2.18^{b}	2.31^{b}	2.07^{b}	2.84^{a}	2.23^{b}	2.34^{b}	1.99^{b}
18:1(Δ^{9})	2.82^{a}	2.16^{b}	2.29^{b}	2.05^{b}	2.82^{a}	2.21^{b}	2.32^{b}	1.98^{b}
18:2($\Delta^{9,12}$)	2.80^{a}	2.15^{b}	2.28^{b}	2.04^{b}	2.80^{a}	2.21^{b}	2.30^{b}	1.96^{b}
18:3($\Delta^{9,12,15}$)	2.78^{a}	2.13^{b}	2.26^{b}	2.02^{b}	2.78^{a}	2.18^{b}	2.28^{b}	1.95^{b}
20:4($\Delta^{5,8,11,14}$)	3.04^{a}	2.33^{b}	2.547^{b}	2.21^{b}	3.04^{a}	2.38^{b}	2.50^{b}	2.13^{b}

[abc]Means along the same row with different superscripts are significantly different (P< 0.05)

KEY:

CON- control, A-Additive (1-*Moringa oleifera* leaf meal, 2-*Ocimum gratissimum* leaf meal, 3-*Vernonia amygdalina* leaf meal), R-Regime (R1- chick phase, R2—grower phase, R3-laying phase)

The cholesterol profile of eggs laid at early laying phase (14th-19th week) Table 4.24 showed that all parameters considered (LDL, HDL, Trig and cholesterol-CHO) were significant ($P<0.05$) but in no particular order. Highest value recorded of LDL was seen in birds fed control diet while it was absent in the eggs from birds on the diet with VALM (T10) from the laying phase. For HDL, the least value (44.57mg/dl) was seen in hens fed 2% OGLM diets from the chick phase (T3) and the highest (95.24mg/dl) from birds fed diets with 2% MOLM from the chick phase (T2). The highest value (885.44 mg/dl) recorded for cholesterol was recorded for hens fed control diet (T1) with the lowest value (97.10 mg/dl) recorded in eggs from hens on 2% VALM diet from the laying phase (T10). The tryglyceride had the highest value of (352.01mg/dl) from birds fed diets with 2% MOLM from the laying phase (T8) and the least value of (107.66 mg/dl) from birds fed diets with 2% OGLM from the chick phase (T3).

Both additive and their period of introduction in the diets on LDL of eggs at early laying phase (14th- 19th week) presented in Table 4.25 had significantly different ($P<0.05$) interaction, with the highest in eggs from hens fed control diet which was low in eggs of hens fed diets containing additives. The HDL was not significant ($P>0.05$) but cholesterol had a similar pattern as LDL, it had significant ($P<0.05$) interaction on both additive and their period of introduction in the diets on the eggs laid by the experimental birds. Triglycerides was not affected by the additives but it was significantly affected ($P<0.05$) by period of introduction of additives to the hens' diets. The highest value of 301.99mg/dl was seen in eggs from birds from regime 3.

Table 4.24: Cholesterol profile of eggs from early laying phase (14th-19th week)

Parameters	CON	R1			R2			R3			
(mg/dl)	T1	T2	B3	T4	T5	T6	T7	T8	T9	T10	SEM
LDL	+++	++	++	++	+++	++	+++	++	++	-	
HDL	80.29^{ab}	95.24^{a}	44.57^{b}	63.26^{ab}	70.22^{ab}	77.13^{ab}	84.11^{ab}	87.54^{ab}	85.14^{ab}	91.89^{ab}	10.08
CHOL	885.44^{a}	662.46^{ab}	455.91^{ab}	336.61^{ab}	776.32^{a}	351.58^{ab}	838.95^{a}	572.63^{ab}	416.14^{ab}	97.10^{b}	120.37
TRYG	191.51^{bc}	234.43^{abc}	107.66^{c}	168.13^{bc}	148.00^{c}	219.44^{abc}	291.69^{ab}	352.01^{a}	346.44^{a}	207.53b^{c}	28.22

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

KEY:

CON- control (T1), (T2, T5, T8-*Moringa oleifera* leaf meal, T3, T6, T9-*Ocimum gratissimum* leaf meal, T4, T7, T10-*Vernonia amygdalina* leaf meal), R-Regime (R1- chick phase, R2—grower phase, R3-laying phase), SEM- Standard error of mean, LDL (low density lipoprotein), HDL (high density lipoprotein), TRYG (Triglycerides) and (CHOL) cholesterol

+++ = present in abundance (above 670mg/dl)

++ = present (above 230mg/dl)

+ = present in (below 225mg/dl)

- = not present

Table 4.25: Interaction effects of additive and its period of introduction on cholesterol profile of eggs laidby experimental birds atearly laying phase (14th- 19th week)

		ADDITIVE				PERIOD		
Parameters (mg/dl)	CON	A1	A2	A3	CON	P1	P2	P3
LDL	+++	++	++	++	+++	++	++	+
HDL	80.29	84.33	68.94	79.75	80.29	67.69	77.15	88.19
CHOL	885.40[a]	670.50[a]	407.90[b]	424.20[b]	885.40[a]	485.00[bc]	655.60[ab]	362.00[c]
TRYG	191.51	244.81	224.51	222.45	191.51[b]	170.07[b]	219.71[b]	301.99[a]

[abc]Means along the same row with different superscripts are significantly different (P< 0.05)

KEY:

CON- control, A-Additive (1-*Moringa oleifera* leaf meal, 2-*Ocimum gratissimum* leaf meal, 3-*Vernonia amygdalina* leaf meal), P-Period (1-chick phase, 2—grower phase, 3-laying phase), LDL (low density lipoprotein), HDL (high density lipoprotein), TRYG (Triglycerides) and (CHOL) cholesterol

+++ = present in abundance (above 670mg/dl)

++ = present (above 230mg/dl)

+ = present in (below 225mg/dl)

- = not present

Cholesterol profile of eggs laid by the experimental birds at the mid laying phase (33rd-38th week) shown on Table 4.26. Of all the parameters measured (LDL, HDL and Trig) were insignificant (P>0.05) but LDL was present in the eggs from the control, eggs from birds on MOLM from the chick phase and those on VALM from the laying phase and absent in the other treatments. The cholesterol was significantly different (P<0.05) with the highest value documented for cholesterol in eggs from birds on 2% MOLM diet from the chick phase (T2) and the laying phase (T8).

Interactive effects of additive and their period of introduction on cholesterol profile of eggs laid by the experimental birds at mid laying phase (33rd-38th week) is shown in Table 4.27. In which there was interaction effect of both additive and their period of introduction in the diets on LDL of the eggs with the highest value being in eggs from birds on the control diet which was reduced in eggs from birds fed diets with additives. HDL was equally significant (P> 0.05), both additive and their period of introduction to diets had significant interaction on the eggs laid by the experimental birds with the least value documented in birds on the control diets. Cholesterol had significant interaction effect of both additive and their period of introduction in the diets of the experimental birds. Triglycerides was unaffected by additives but was significantly influenced by period of introduction the additives in the diets of the birds. The highest value of 378.12mg/dl was seen in eggs from birds from period 1 and control.

Table 4.26: Cholesterol profile of eggs from mid laying phase (33rd-38th week)

	CON	R1			R2			R3			
Parameters (mg/dl)	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	SEM
LDL	++	+	-	-	-	-	-	-	-	+	
HDL	628.60	825.75	903.92	936.20	1022.76	933.58	1063.62	1107.46	1081.90	576.31	137.30
CHOL	950.60^{ab}	1032.59^{a}	769.82^{b}	859.91^{ab}	795.50^{b}	865.03^{ab}	803.15^{b}	1040.84^{a}	853.00^{ab}	792.99^{b}	55.43
TRYG	355.38	375.73	357.80	400.82	229.38	210.49	371.74	359.12	170.16	148.91	41.59

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

KEY:

CON- control (T1), (T2, T5, T8-*Moringa oleifera* leaf meal, T3, T6, T9-*Ocimum gratissimum* leaf meal, T4, T7 T10-*Vernonia amygdalina* leaf meal), R-Regime (R1- chick phase, R2—grower phase, R3-laying phase), SEM- Standard error of mean, LDL (low density lipoprotein), HDL (high density lipoprotein), TRYG (Triglycerides) and (CHOL) cholesterol

+++ = present in abundance (above 670mg/dl)

++ = present (above 230mg/dl)

+ = present in (below 225mg/dl)

- = not present

Table 4.27: Interaction of additives and their period of introduction on cholesterol profile of eggs from mid laying phase (33rd-38th week)

Parameters (mg/dl)	CON	ADDITIVE			CON	REGIME		
		A1	A2	A3		R1	R2	R3
LDL	++	-	-	-	++	-	-	-
HDL	628.60[b]	985.30[a]	973.10[a]	858.70[ab]	628.60[b]	888.60[ab]	1006.70[a]	921.90[a]
CHOL	950.60[a]	956.31[a]	829.28[b]	0818.18[b]	950.60 [a]	887.44[ab]	821.23 [b]	895.61[ab]
TRYG	355.38	321.41	246.15	0307.16	355.38[a]	378.12[a]	270.54[ab]	226.06[b]

[abc]Means along the same row with different superscripts are significantly different (P< 0.05)

KEY:

CON- control, A-Additive (1-*Moringa oleifera* leaf meal, 2-*Ocimum gratissimum* leaf meal, 3-*Vernonia amygdalina* leaf meal), R-Regime (R1-chick phase, R2—grower phase, R3-laying phase), LDL (low density lipoprotein), HDL (high density lipoprotein), TRYG (Triglycerides) and (CHOL) cholesterol

+++ = present in abundance (above 670mg/dl)

++ = present (above 230mg/dl)

+ = present in (below 225mg/dl)

- = not present

CHAPTER FIVE

5.0 DISCUSSION

Study 1

The values obtained for the proximate examination of *Moringa oleifera* leaves revealed percentage moisture of air dried leaves used was 9.35% , with the dry matter (DM) of 91.55%, crude protein (CP) was 19.60%, ether extract (EE) of 7.31% all of which were similar to the findings of Alikwe and Omotosho (2013), though others are not similar. This result was also coincidental with those of Sodamade *et al*. (2013), who reported 9.00% moisture, while they were lower than those by Nadeem *et al*. (2013) of 13.92% moisture. The values obtained by Alnidawi*et al*., 2016 for Ash, Fiber and Protein were 5.89, 17.41 and 25.37% respectively and as well similar to those by El-Massry *et al*. (2013). The differences reported for *Moringa oleifera* leavescould be attributed to different weather and soil conditions of the environment in which the plant was propagated and the stage of growth at which the leaves were harvested.

The leaves of *Ocimum grattissimum* investigated had 8.62% moisture and DM of 91.38%, CP was 19.10%, crude fibre (CF) of 13.43%, EE of 5.30%, ash of 7.28% and nitrogen free extract (NFE) of 46.27%which were identical to the findings of Ogbu and Amaefule (2015) who recorded DM of 86.32%, Moisture content 13.68%, CP was 19.68%, CF of 12.24%, EE of 3.48%, ash of 8.75 % and NFE of 42.20 %, Nworgu *et al.*(2012) reported DM 91.97%, Moisture content 8.03%, CP was 6.38%, CF of 18.52%, EE of 5.89%, ash of 12.24% and NFE of 48.94% while Adewole (2014) reported moisture content of 10.30%, CP of 16.51%, CF of 9.07%, EE of 2.78%, ash of 2.45% and NFE of 58.89%, The differences in the leaves of *Ocimum grattissimum* reported could be ascribed to

different weather and soil conditions of the environment in which the plant was propagated and the stage of growth at which the leaves were harvested.

Moisture content for air dried leaves of *Vernonia amygdalina* investigated was 7.28%, DM was 92.72%, CP was 24.77%, CF of 13.70%, EE of 6.28%, ash of 7.23% and NFE of 40.75% Asaolu *et al.,* (2012) reported moisture content of 10.02%, CP of 50.64%, CF of 12.08%, EE of 9.05%, ash of 9.56% and NFE of 8.65%, a result completely different from that of the *Vernonia amygdalina* leaf meals used in this present experiment. These differences in the proximate compositions of *Vernonia amygdalina* leaves could be blamed on the stage of growth at which the leaves were harvested and to different weather and soil conditions of the environment in which the plant was propagated.

Leaf meals' inclusion in the pullet chicks' diets was not in any way detrimental to weight gained (WG), though feed intake (FI) was slightly affected as seen in hens fed MOLM and OGLM respectively. The final weight (FW), WG andFCR (feed conversion ratio) of hens on MOLM were improved and this is in line with the report of Ayssiwede *et al.* (2011) that MOLM addition to growing indigenous Senegal chickens' diets up to 24% had no negative effect on live body weight, average daily WG, (FCR) and mortality in birds as against those on control diets. This also agreed with the report of Moreki and Gabanakgosi**(**2014) who concluded that 5 to 20% addition of MOLM to broilers' diets and 10% to layers' dietsimproved birds' performance that is, both growth and laying including egg size. Addition of OGLM in pullet chicks' diet significantly ($P<0.05$) improved FCR as submitted by Nworgu *et al*. (2012) though the figures recorded here are higher than the (2.53-2.76) documented by Nworgu *et al*. (2012), in an experiment with 5% addition of fresh *Ocimum gratissimum* leaves in the diets of growing pullet.

The birds on OGLM had the worst performance across the treatments as they were unable to favourably convert the feed consumed to flesh in other to gain better weight. This could probably be that OGLM conferred constringency since OGLM contained alkaloids, tannins, phenols, saponins and flavonoids (Edeoga *et al*. 2006). Though Nworgu *et al.* (2012) observed reduced intake of feed, when pullet growers were fed diets with graded quantity of wilted water leaves (WWLM) which he attributed to reduction of feed

palatability and consumption. All these benefits of spices can however be shielded if utilizeed in huge quantities as impacts of intrinsic anti-nutrients like tannins and saponins become obvious. These anti-nutrients were said to have had a negative impact on growth and nutrients maximization (Odoemelam*, et al., 2012*; Odoemelam *et al*.,2013b). Alanine absorption was said to be adversely affected with feeding of *Ocimum gratissimum* at higher dietary inclusions since *Ocimum gratissimum* is rich in Cinnamaldehyde and Eugenol (Lee *et al*., 2004 and Odoemelam *et al*., 2013b).

Odoemelam *et al*. (2013b)documented a reduction in WG with increased dietary addition of *O. gratissimum* above 1.0%. Feed intake was highest in birds on bitter leaf (*Vernoniaamygdalina*) leaf meal (VALM) based diets at the chick phase supported Owen*et al*. (2010) who recorded an increase in feed consumption across treatments on adding VALM to broiler finishers' diet as observed in an experiment of four weeks duration at 0%, 5%, 10% and 15% respectively while the performance in terms of FCR of the birds reduced as the quantity of VALM increased. However, Owen *et al*. (2010) recorded no mortality while there was mortality in this experiment and this could be due to difference in bird type and stage of growth of the birds since he used broiler finishers while the present study was done with pullet chicks and the difference in the quantity of VALM in the birds' diets in which Owen *et al*. (2010) used between 5-15%.

At the grower phase, Swain *et al*.(2017) recorded a reduced FI of Vanaraja laying hens fed 2% MOLM while Olugbemi *et al*. (2010) documented no effect in feed intake up to 10% addition of MOLM in Cassava chips based laying hens diets. Dietary bulkiness of MOLM could be responsible for the reduced intake and the fact that hens have simple digestive system which could not efficiently digest highly fibrous ingredients since birds lack the enzymes responsible for high fibre digestion (Swain *et al*., 2017). Feed intake was lower in birds on MOLM as against those fed control and OGLM diets. The improved FI observed in birds on OGLM diets from 56 days could be due to the palatability and the aroma of OGLM as reported by Alabi and Chime (2007) that *O. gratissimum* is rich in mineral and vitamin and able to enhance diets digestibility and also improve feed utilization.

The best growth performance recorded was in treatments T3 (birds on 2% OGLM based diet from day old) and control (T1) diets with the values of 3.04 and 3.05 respectively.This could be due to adaptability of the pullets to OGLM as those fed this same diet but from day 56 had the least performance thereby confirming the submission of Odoemelam, *et al*. (2013) of a better performance of broilers fed diets of varying quantities of OGLM up to1.0% and this improved performance could be ascribed to the fact that scent leaf is rich in mineral and vitamin and can enhance diets digestibility and also improve feed utilization (Alabi and Chime, 2007). However the decreased performance recorded for birds on 2% OGLM based diet from day 56 supports the result of Odoemelam, *et al*. (2013) that the least performance for birds with 2l% inclusion and thereby stating that the benefits of spices can however be shielded if utilized in huge quantities as impacts of intrinsic anti-nutrients like tannins and saponins become obvious.

The reduced feed intake recorded in birds on VALM could be caused by reduction in feeds' palatability due to bitterness of the leaves though Owen*et al*. (2010) recorded no adverse implication of VALM in pullet diets up to 20%. The poor performance of birds on VALM can therefore be blamed on the bitterness of the diets. Treatments 2, 4, 5 and 7 were not significantly different. No mortality was documented at this stage of the study.

Haematological parameters are measures and an implication of the impacts of diet on animals as regards the characteristics and quantities of FI and utilized for the animal's physiological, biochemical and metabolic necessities (Ewuola *et al*., 2004). RBC, HB and PCV are blood parameters that are most dependent on diets feeding level and feed quality, (Etim *et al.,* 2014a and Jiwuba *et al*., 2016). *Moringa oleifera (MO), Ocimum grattisimum (OG)* and *Vernonia amygdalina (VA)* wereused in this work to formulate diets for egg strain birds at the same inclusion level in which eosinophil was significant at 8^{th} week, all haematological parameters except RBC and MCHC at the 16^{th} week and at 24^{th} week PCV, Hb, RBC, heterophils, basophils and MCHC were notably enhanced in no particular order by the dietary treatments.

The values obtained from these parameters indicated that adding leave meal to the birds' diets had no adverse impact on them. This confirmed the report of Onu and Aniebo (2011) who evaluated the implications of MOLM on performance and blood chemistry of broiler

starters and reported up to 7.5% MOLM inclusion in broiler diets without adversely altering both performance and blood parameters of such broilers. Unaltered WBC, lymphocytes, monocytes, neutrophils and eosinophils is a proof that these parameters are not affected by feeding pattern (Ameen *et al.,* 2007). The haematological indices obtained for birds on OGLM based diet at 8 weeks is similar to the report of Fajohunbo (2010) in an experiment done with 4weeks old broiler birds at an inclusion level of 2% OGLM while the haematological indices obtained for *Ocimum grattisimum* at 16 weeks in this work resembled that obtained for 8 weeks old broiler birds also fed with 2%OGLM based diet.

Interaction effect of additives and its period of introduction on the haematology of the experimental birds at the 24th week (growing phase) had only the haemoglobin significant though the results are similar to those by Mitruka and Rawsley (1977).Hb and PCV concentration are generally affected by inadequate intake of energy and protein with lower values indicating anaemia. This means that the birds were adequately fed and not anaemic. When haematological parameters of an animal are within appropriate range, it is a proof of proper environmental, nutritional state and pathological of such animals (Afolabi*et al.,* 2010). haematological parameters helps to know if and when to increase or reduce certain nutrients in animals' feeding (Rafiu *et al.,* 2013; Etim *et al.*, 2014a). Haemoglobin, the component of RBC responsible for binding and transportation of oxygen had a slightly significant interaction effect, where additive 1 (MOLM) had a slightly lower value than those by Mitruka and Rawsley (1977) with Period 3 (laying phase) also having a value, less than the report of Mitruka and Rawsley (1977) and this may imply that the birds in Period 3 were just trying to adapt to the additives while those in the other two periods were already used to the additives.

Normal level of haematological constants is a measure of the transportation efficiency of oxygen and carbon dioxide within the animal's body. MCV, MCH and MCHC are key indicators used in confirming anaemia in animals (Saleh *at al*., 2014). MCH, an indicator of the oxygen transporting ability of the RBC was not significant but between the normal range documented by Mitruka and Rawsley (1977) and this implies efficiency in the birds' respiration. WBC are disease fighters, the result of this study indicate that birds have

similar immunity status. Animals having reduced WBC count can be easily infected by diseases, those with higher counts can generate antibodies in the process of phagocytosis and with better diseases resistance (Eheba *et al*., 2008 and Togun *et al.,* 2007)

Total protein and albumin of serum have been said to be directly influenced by protein intake and quality (Onifade, 1998). Serum proteins and WBC functions in the production of immunoglobins on which antibodies development depend (Mmereole, 2008) but the globulins have lower values compared to the report by Mitruka and Rawsley (1977) of 7.80-16.50g/dl for globulin range. This implies that protein attribute of the diet was not adequate to encourage normal protein reserves across the periods but not significant for the additives. Albumin-globulin ratio (A:G) though significant across both additives and periods were higher in the control compared with the treatments and so implies that birds fed diets with the additives were less stressed unlike A:G of hens on the control diets. Concentration of enzymes in serum enzymology is used in diagnosis of organ' health just as creatinine functions in liver evaluation and serum urea for renal function and this gives vital information on the level of damage to the organ(s) concerned (Belewu and Ogunsola, 2012).

The results for ALT is similar to those of Mitruka and Rawsley (1977) and this indicate that the hens didn't experience any heart, kidney and liver damage just like serum concentration of AST is an index that reveals the state of the heart and liver in animals (Ewuola *et al*.,2004). The birds successfully tolerated the anti-nutritional components in the leaf meals, since any inordinate rise in serum quantities of AST and ALT can result in liver damage (Yalcin *et al*., 2012). Creatinine measures the extent of muscular activity/ damage and the values observed were not up to those of Mitruka and Rawsley (1977). The quantity of creatinine in this study revealed that there was no muscular wastage due to availability of anti-nutritional agents in leaf meals used which meant that high creatinine revealed a very high muscular phospho-creatinie degradation to form creatinine.

The cholesterol at the 8th week reduced across the treatment this indicates that the leaf meals in the diets helped in this reduction while the values obtained at the 16th and 24th week were insignificant but interaction effect of the additives on serum cholesterol content was highly significant. This is in line with the findings of Michael (2005) that anti-

nutrients affirm a physiological effect on reducing plasma cholesterol concentration in the test animals. There was an interaction effect of the additives on serum cholesterol content but no interaction effect of the period as shown in Table 4.14, though the observed values were greater than 52-148mg/dl reported by Mitruka and Rawsley (1977).

Serum glucose was not significant at both 8th and 16th week which indicated that energy was available to the birds but was significant at 24thweek though without a particular order, there was no interaction effect of the additives on serum glucose between the treatment groups and the control which suggests nutritional adequacy and safety of the test materials but there was interaction effect of the period on serum glucose which was higher in period 1 than periods 2 and 3. Some plant proteins exhibit hypoglycemic or hyperglycemic effect in some experimental animals (Abdulazeez *et al*., 2016). There was an interaction effect of the additives on serum LDL content with the highest value seen in birds fed control diets meaning that leaf meals were able to reduce the LDL component of the eggs but no interaction effect of the period on LDL as shown in Table 4.14, which suggests that period had no influence on LDL.

The egg production expressed weekly had no particular order though some variations were recorded in some of the weeks (1, 4, 5, 7, 9, 14, 17, 21, 25 etc). This negates the report ofAlikwe*et al*. (2016) and Akangbe *et al.*(2011), that inclusion of 2% MOLM improved egg production but in line with Paguia*et al.*(2014) who recorded no obvious change in egg production with the incorporation of MOLM as vitamin source in laying hens' diets. Akangbe *et al.*(2011), recorded no improvement in egg production with the addition of 2% OGLM in laying hens diet. Though birds fed control diet initially produced eggs better till week four that birds on some of the other treatments caught up with the control at week five and since no particular order was maintained, it could be said that the leaf meals had no influence on egg production performance of the experimental birds and on the hen day production as well

Study 2

Total egg weight: Total egg weight was slightly improved by the MOLM as against the report of Kakengi *et al.* (2007) that MOLM addition to the laying hens' diets significantly

reduced the egg weight (EW) though Olugbemi *et al.*(2010a) reported decrease in total egg weight and average egg weight at higher levels beyond 10% of MOLM in layer's diet. Abou-Elezz *et al.* (2011) said that MOLM supplementation at levels up to 10% to laying hens diets did not significant impact while Ebenebe *et al.*(2013) recorded a rise in average EW with MOLM supplementation. Weight of eggs of the birds fed MOLM based diets from both chick and laying phases were not so much different from those on the control diets as those of Alikwe*et al*. (2016) and Paguia*et al.* (2014) in an experiment done using 2% and 3% MOLM in layers diets, in which egg weight was insignificantly affected and also that ration does not affect egg weight but those fed MOLM based diets from the growing phase had significantly higher weight than the control.

Egg shell thickness and proportion: Abou-Elezz *et al.*(2011) and Kaijage *et al*.(2004) submitted no adverse effect of MOLM on the shell integrity. The smaller eggs had stronger shells than large ones, since hens have definite ability to deposit calcium (Ca) in egg shell since equal quantity of Ca spread across a larger area (Butcher and Miles 2003). The Haugh unit is the major tool of quality assessment of table egg protein (USDA, 2000), determined with the height of egg white (albumen) (Monira *et al*., 2003). Haugh unit (HU) is said to be an empirical method of determining the relationship between the thick albumen weight and height (Haugh, 1937; Stadelman and Coterill, 1995). This thick albumen becomes thin as eggs age, resulting in a reduction in the height of thick albumen, a vital component in HU equation. The higher the HU value, the better the egg quality. Any egg grouped as AA has HU of 72or more, those grouped as A have HU that fall between 60 to 72 while those in B group range between 31 and 60 HU.

The highest were observed in the birds fed with *Ocimum* and *Vernonia* from the chick phase compared to those that were started from the growing and laying phases. This means that the duration of exposure of the birds to the test ingredients improved the Haugh unitvalue. Higher HU indicates superior egg quality (in terms of freshness and thicker egg whites). The YI and HU are primary pointers of internally lower concentration of cholesterol a relative egg quality (Isikwenu *et al*., 1999). Higher haugh unit and yolk index (Mudhar, 2011) makes egg better desired with better quality. Better haugh unit with lower YI implies a relatively lower concentration of cholesterol (Kaijage *et al*., 2004). The

least desirable Haugh unit value of whole egg for domestic consumption is 60 (Optimum egg quality, 2010).

Yolk colour is a major determinant in consumers' study that concerns egg quality (Jacob *et al.,* 2000). Though the values obtained for yolk colour had no specific order but were higher in the eggs laid by birds on *Moringa* fortified diets. Yolk colouration impacts consumer's preference but is dependent on the xanthophyll (a natural pigment of egg yolk, Nys, 2000; Sirri *et al.,* 2007) in hen's diet. This xanthophyll is the cause of the pigmentation of the egg yolk but it has little or no nutritive value. Etalem *et al*. (2013) concluded that MOLM improved yolk colouration while Akangbe *et al*.(2011) submitted that MOLM did not have any positive improvement on yolk colouration. Colour variation from light yellow in control and birds on OGLM based diets to dark yellow colour in VALM and light orange colour MOLM based diets of egg yolk from each treatment varied which negated Akangbe *et al*.(2011), that 2% OGLM in laying hens diet improved the yellow colour of egg yolk. Yolk colour was yellow in eggs of birds on VALM based diets and thick yellow in eggs from birds on MOLM based diets and this colouration between treatments persisted. This disagreed with Paguia*et al.* (2014) and Alikwe*et al*., (2016) that recorded similarity of yolk colouration of both treated and control groups. The result however corroborate the report of Etalem *et al*., (2014).

The higher the YI (Mudhar, 2011) and HU (Haugh, 1937; Mudhar, 2011), the more desirable the egg quality. Odunsi *et al.*(2002) recorded a slight rise in yolk index with MOLM inclusion in layer's diet and without adversely affecting the shell weight (Akande *et al.,* 2008). The low yolk index values obtained could be caused by ambient temperature which primarily affects the egg quality, as lower temperature of about 12^{o}C (refrigeration) is recommended for eggs to maximally retain its quality (Brake *etal.,* 1997).

The value obtained for all the parameters measured for eggs' fatty acid (FA) profile of the birds fed experimental diets at early laying phase were not significant. The values were lower than those documented by Cotterill and Glauert (1979) (in an experiment done with 30% flax seed in the diet of laying birds) except oleic which was lower and linoleic that had similar values. For the late laid eggs however the values were lower than those obtained for the mid lay and this may be due to the fact that the eggs were frozen for some

time (storage of the yolk for analysis). The values obtained for the late laying phase for palmitic acid and oleic were similar to those reported by Cotterill and Glauert (1979) while the others were higher than those reported by these researchers. Of great importance is the value of linolenic acid which is known as an inhibitor of free fatty acid release which usually occurs during acute coronary heart disease (CHD) which is one of many reasons people avoid/ reduce their level of egg consumption.

Linolenic is usually not adequate in the diets of most humans but present in plant tissues and chickens have been reported to have the ability of diverting greater quantities of the linolenic acid present in their diets into their eggs. The increase observed in the other unsaturated FA content of eggs will also reduce the saturated FA present. The FA and cholesterol profile of eggs became very important due to the consumers demand for healthier eggs *i.e.* eggs with especially, reduced content of cholesterol and total fat which has been tagged a culprit in several diseases especially CHD and arteriosclerosis. The present results are also like to those of Khan *et al.* (2015) in an experiment done also with flax seed to improve the Omega -3 contents of bio-fortified eggs except for linoleic acid that was considerably higher while others were lower in his work, except for Arachidonic acid that had similar values with his report. Leaf meals incorporation in the birds' diets did not improve the FA content of eggs as the values of these FAs in eggs from the control had higher values for all the fatty acids considered.

The parameters measured were higher in the late lay samples compared to the mid lay samples except for HDL which was lower in the mid lay egg samples. This could be explained to increase with the age of the birds. Cholesterol levels of eggs laid by hen fed the three leaf meals had no obvious variation which Milinsk *et al*. (2003) and Rowghani *et al*. (2007) reported to be slightly reduced in the omega-3 eggs. Olugbemi *et al*. (2010) observed reduced egg cholesterol content when MOLM was included in cassava based layer's diet due to its hypo-cholesterolemic effect. While this was true for the early lay it did not support the result of the mid laying phase.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

6.1 Summary

Haematology and Serum biochemical indices of the experimental birds had no particular order of variation for both the regime of feeding and additives (leaf meal). The haemoglobin content of the blood was however better in birds on the control diets for both the additives (leaf meal) and the regime of feeding of the additives (leaf meal) to the birds. Alanine amino transferase and glucose were higher in regimes 1 and 3 respectively.

Birds fed diets with 2% *Moringa oleifera* leaf meal from the chick phase (one day old) had the best growth performance while those on diet with 2% *Ocimum gratissimum* leaf meal from the chick phase (one day old) had the least growth performance. Mortality was high in birds on diet with 2% *Vernonia amygdalina* leaf meal. At the growing phase (9-16 weeks) birds on control diet and those on diets with 2% *Ocimum gratissimum* leaf meal from the chick phase however had the best performance.

There was no interaction of the leaf meals and their regime of feeding on both the egg production and the hen day production of the birds.

Some qualities (yolk colour and percentage yolk) of eggs laid by birds fed diets containing the medicinal plants were improved by the inclusion of the leaf meals (additives) at early laying phase 14th-19th week especially *Moringa oleifera (*yolk colour) and *Vernonia amygdalina* (percentage yolk) while regime of introduction of the leaf meals (additives) had no particular order. Yolk colour was however improved considerably at all the regimes. While at the mid laying phase (33rd-38th week), shell weight, yolk colour and percentage shell weight were improved with the leaf meals (additives)where regime of introduction of the leaf meals (additives) had no particular order on their effect the egg qualities

Fatty acid profile of the eggs at early laying phase were more in eggs of birds fed control diets and better in eggs of birds fed diets with *Moringa oleifera* but less in eggs from birds on diets with *Ocimum gratissimum* and *Vernonia amygdalina* leaf meals. Fatty acid profile of the eggs was also better enhanced in Regime 2. Both leaf meals (additives) and the regime of introduction of the leaf meals (additives) to the diets of the birds did not improve fatty acid profile of eggs at mid laying phase (33rd-38th week). Cholesterol profile of eggs of the birds fed the experimental diets had no particular order at both early and mid-laying phases though low density lipoprotein and triglycerides.

6.2 Conclusion

Inclusion of 2% *Moringa oleifera, Ocimum gratissimum* and *Vernonia amygdalina* Leaf meal the diets of hens at 3 different regimes improved egg weight of hens fed diets with leaf meal inclusion from one day old. *Moringa oleifera* leaf meal inclusion in the bird's diets greatly enhanced the golden yellow yolk colour of eggs.

6.3 Recommendation

It is recommended that 2% *Moringa oleifera* leaf meal inclusion in hen's diet from laying phase to enhance golden yellow yolk colour of eggs.

Further studies should also be carried out on the use of these leaf meals at varied levels of inclusion and at various stages of growth.

6.4 Contributions to knowledge

1. Theinclusion of *Moringa oleifera* in contrast to *Ocimum gratissimum* and *Vernonia amygdalina* leaf meals at 2% in chicken hen's diets does not impede the production of eggs as compared to non-leaf based diets.
2. *Moringa oleifera, Ocimum gratissimum,* and *Vernonia amygdalina* leaf meals at 2% in chicken hen's diets resulted in healthier eggs with lowered value ranges of between 9.17- 69.9% and 5.25- 61.91% for low density lipoprotein (LDL) and total cholesterol respectively during the early laying phase.

3. *Moringa oleifera* is a superior yolk colourant compared to *Ocimum gratissimum,* and *Vernonia amygdalina* leaf meals.
4. Feed conversion of chicken hens fed 2% *Moringa oleifera* leaf meal diets is more efficient than those of *Ocimum gratissimum,* and *Vernonia amygdalina* leaf meals at 2% inclusion level as well as non-leaf based diet.

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