COMPARATIVE EVALUATION OF THE EFFICACY OF COMMERCIAL AND LOCALLY FORMULATED VITAMIN – MINERAL PREMIXES IN THE DIETS OF EGG – TYPE CHICKENS

BY

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DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria under the supervision of Professors I. I. Dafwang, J.J. Omage and F.O. Abeke. The work of other Authors referred to in this study is acknowledged by means of references. No part of this dissertation has been previously submitted for the award of higher degree.

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CERTIFICATION

This dissertation titled ***"Comparative Evaluation of the Efficacy of Commercial and Locally Formulated Vitamin – Mineral Premixes in the Diets of Egg – Type Chickens"*** by Maikano Amos meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D) of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This research work is dedicated to God the Omniscience and to all Animal Scientists who are interested in unveiling the secrets behind the use of organic systems for poultry production in order to produce ‘’natural’’ and ‘’ welfare friendly’’ meat and eggs.

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ABSTRACT

A comparative study on the evaluation of the efficacy of commercial vitamin – mineral premix (CVMP) and two locally formulated vitamins - mineral premixes 1 and 2 (LFVMP1 and LFVMP2) in egg – type chicken diets was investigated. Locally and naturally sourced feedstuffs were used in formulating locally produced vitamins – mineral premix 1 and 2, where locally formulated vitamins - mineral premix 1 was used as a template. Proximate compositions of the premixes were carried out in the Biochemistry laboratory of Department of Animal Science, Ahmadu Bello University Zaria and the Central laboratory of National Animal Production Research Institute, Shika – Zaria. Samples of the premixes were analyzed for vitamins and minerals using Ultraviolet Visible Spectrophotometer (UV-S 6405) and Atomic Absorption Spectrophotometer (AA240FS) in the laboratory of National Research Institute of Chemical Technology (NARICT) Basawa, Zaria. Results obtained, showed that commercial vitamin – mineral premix was richer than locally formulated vitamin – mineral premixes in terms of proximate, vitamins and minerals compositions. Vitamins analyzed were vitamins A, B_2, B_6, B_9 and E while the minerals analyzed were calcium, phosphorus, sodium, potassium, copper, zinc, iron and manganese. The feeding trials were conducted using Lohman Brown Pullets at day old in a Completely Randomized Design (C.R.D). Seven diets were formulated containing CVMP, LFVMP1 and LFVMP2 at 0.00, 0.125, 0.25, 2.30, 4.60, 2.30 and 4.60% respectively. Reasons for these levels of inclusion are: recommended level of inclusion of CVMP by the manufacturer is 0.25% (full dose). The study tested 0.125% (half dose) inclusion level to see whether it will not be detrimental to the production parameters and also minimize costs in order to maximize profit. For LFVMP1which was used as a template for LFVMP2, the producer recommended 4.60% and was also tested at half dose. The appropriate diets were fed to the pullets from 0 – 8, 9 – 20, 20 – 32 and 32 – 56 weeks of age. In the chick phase, results obtained indicate that in terms of final weight and weight gain chicks fed on CVMP diets were significantly ($P < 0.05$) better than chicks on LFVMP based diets. The addition of premixes at half or full doses resulted in better growth performance than the control. Growth performance on full dose was significantly ($P < 0.05$) better than on half dose for all the premixes. Growth performance was best for CVMP followed by LFVMP 2 and LFVMP 1. The feed cost per kg gain was better on CVMP 0.25% followed by LFVMP 2 (4.60%). During the grower phase, performance in terms of final weight, weight gain of birds fed CVMP based diets were significantly ($P < 0.05$) better than those of LFVMP2 and LFVMP1 based diets respectively. Feed consumption was higher at full doses of premixes diets than half dose premixes diets for all the treatments, except treatment 1. Feed to gain ratio improved in chicks fed CVMP based diets than those on LFVMP2 and LFVMP1 respectively. At the early laying phase of the birds, results indicate that the final weight and weight gain increased as the levels of the premixes increased to full doses across the treatments. Chicks fed on CVMP based diets performed better than those on LFVMP2 and LFVMP1 respectively. CVMP based diets had significant ($P < 0.05$) effects on feed cost / dozen, hen egg – day and hen egg – housed production, percentage production at peak and income above feed expenses. At the laying phase, appropriate diets were fed to the birds from 32 – 56 weeks of age. Final weight, percent change in body weight and feed intake were significantly ($P < 0.05$) different across the treatments. Feed cost/kg, feed cost/dozen eggs, hen egg day and hen housed egg productions, percentage production at peak of lay and

income above feed expenses were significantly ($P < 0.05$) different across the treatments. Laying hens on full doses of CVMP premixes diets had higher profit margin than those on half doses and control diets. Performance on half dose of CVMP was similar to full dose of CVMP and full doses of LFVMP 1 and 2. Therefore, performance was same on full dose for all the three premixes which means that for laying hens any of the premixes can be used in the absence of CVMP. CVMP diets fed to laying hens produced eggs with superior external and internal qualities than the LFVMP1 and control diets. LFVMP2 diets fed to laying hens produced eggs that compares favourably with CVMP diets. All premixes resulted in eggs that had better quality than the control diet even at half dose. All premixes at full dose gave rise to eggs of higher quality than half dose. Egg weight, shell thickness and Haugh unit were best for all eggs from hens fed CVMP, followed by LFVMP 2 and LFVMP 1. The final study was on the determination of cholesterol in egg yolk of eggs laid based on the different treatments on subsequent performance of layers and at the laying phase was investigated. CVMP fed hens laid eggs with higher total cholesterol than LFVMP. Eggs from hens fed the control diet also had high levels of total cholesterol; this is because of the importance of cholesterol which is use in synthesing steroid hormones, body membranes and other important structures in the body.The animal body produces cholesterol daily to meet minimum requirements. A standard egg had 186 milligrammes of cholesterol and the standard blood cholesterol in humans is 200 milligrammes per deciliter or 2.2 millimole per liter. CVMP fed hens tended to have higher triglycerides.Normal triglyceride in the blood is less than 1.7 millimole per liter, eggs laid by hens on CVMP and LFVMP 1 diets had higher triglycerides than that of the control and LFVMP 2 but within the desirable levels. However, eggs from CVMP fed hens had higher high density lipoprotein (HDL) cholesterol but less low density lipoprotein (LDL) cholesterol than eggs from LFVMP fed hens, which is desirable. The levels are comparable to the standard of 1.6 millimole per liter in the blood. Hens fed diets with 0.00, 0.125 and 0.25% CVMP producedeggs with low cholesterol while hens fed diets with LFVMP1 and LFVMP2 at 2.30 and 4.60% levels of inclusion produced eggs with high low density lipoprotein cholesterol at the early laying and laying phases based on the National Cholesterol Education Programme ranking. The values are not as high as 2.6 – 3.3 millimole per liter for the level near ideal in human blood. The probable reasons were because blood meal and whole hard eggs which are known to be high in cholesterol were used as ingredients in formulating LFVMP1 and LFVMP2. CVMP at 0.25% and LFVMP2at 4.60% compared favourably in terms of laying performance, internal egg quality characteristics, feed cost per kg, income above feed expenses, egg production characteristics, egg yolk cholesterol and triglycerides values. It is concluded that in the absence of CVMP, LFVMP2 may be used as an alternative.

CHAPTER ONE

1.0 INTRODUCTION

Poultry production is one of the major enterprises enjoying a high rate of increase in production globally (Aduku, 2004). Africa accounts for only a little over 4% of world egg production. However, its rate of growth, averaging 3.4% a year from 2000 – 2010, easily outstripped the global figure of 2.2%. Looking ahead, production in this region will continue to increase with output reaching at least 2.8 million tonnes in 2012 and possibly topping 3 million tonnes by 2015. Global egg output should come close to 65 million tonnes in 2012 of which, Africa could produce 2.8 million tonnes or 4.3%. The leading producer in the region is Nigeria where output exceeded 153 million tonnes in 2010. South Africa is the second largest producer, annual output having risen by some 135,000 tonnes or 43% from 2000 – 2010 (Terry, 2012).

In Nigeria, between 1990 and 2003, egg production increased from 337,000 tonnes to 548,000 tonnes while poultry meat production increased from 57,000 tonnes to 121,000 tonnes indicating more than 112% increase over a period of 14 years and clearly showing the prime importance of poultry both as a source of protein and as a source of employment or income generation (Alimi*et al*., 2006). The two most important factors responsible for the phenomenal increase in poultry production in Nigeria were identified as its profitability and the quick return on invested capital (Dafwang and Odiba, 1993; Ogundipe and Sanni, 2002).

Poultry is a major commodity in World food production with the highest proportion of manufactured feed of all species. The dynamic growth and success of the poultry industry is

based on a high degree of vertical integration, improved production efficiency standards such as increased market weights, reduced days to market, improved feed conversion and greatly automated processing combined with successful marketing such as low fat, high protein products and changed consumer habits such as health considerations (National Research Council, 1994).

Some thirteen vitamins are required by poultry and are often incorporated into poultry feeds. A fourteenth vitamin, vitamin C is now being considered in overall vitamin supplementation programmes. Vitamin C is synthesized by poultry and is not considered a required dietary nutrient. However, there is evidence of a favourable response to vitamin C by birds under heat stress (Abidin and Khatoon, 2013).

Vitamins are always added to feeds in amounts that meet minimum dietary requirements. This ensures that birds consume plenty of vitamins for proper health and performance. Higher levels are not usually harmful but extra vitamins are unnecessary and expensive. They are usually added as vitamin – mineral premix, it is important that adequate amounts of all vitamins are provided. It may be necessary to add extra amounts of some vitamins to achieve minimum levels for other vitamins. This may increase the cost of the complete feed but is better than creating vitamin deficiencies that can be detrimental to production (Bert and Dianne, 2011).

During periods of stress caused by disease, shipping or sudden changes in the environment, it is recommended that extra vitamins and electrolytes be provided in the drinking water

until the stressing condition is corrected. Like vitamins, adequate levels of minerals must be provided to all birds,for instance chicks, growers, layers and breeders (Avitech, 2007).

Minerals in breeder feeds are especially important. Laying hens require higher levels of minerals for egg shell formation. Chicks require high levels of minerals for proper bone formation and development. Breeder feeds are fed only to laying birds. Trace minerals are those minerals required at very low levels for good growth and production. Most feed ingredients provide some of these minerals but sometimes they may contain less than adequate quantities. Many of these minerals are contained in commercial vitamin – mineral premixes (Bert and Dianne, 2011).

A premix is a mixture of vitamins, trace minerals, medicaments, feed supplements and diluents. A premix is a value added solution for feeds with sustainable safety and quality (Leslie, 2007). A vitamin – mineral premix is the combination of vitamins and minerals which is added to the formulated diet to meet up the requirements of vitamins and minerals that may be deficient in the formulated diet. Inclusion of vitamin-mineral premix in the formulated diet has become an indispensable practice because feed ingredients do not contain all essential vitamins and minerals at the right amounts needed for chickens (Asaduzzaman *et al*., 2005).

Critical vitamins namely choline, folic acid, pantothenic acid, pyridoxine, riboflavin, Vitamin A, Vitamin D_3 and Vitamin E and minerals namely calcium, phosphorus, copper, iodine, iron, manganese, sodium and zinc should be checked carefully in the diet. Minerals

and vitamins contribute only 10% of the total cost of feed (Singh and Panda, 1988). Economizing on these can lead to reduction in safety margin and restrict performance of birds leading to heavy losses.

Premixes solve two major problems in feed milling namely: that of weighing relatively small quantities of many ingredients and the possibility that the small quantities being weighed can be lost in one corner of the mixer if added individually. The use of a carrier allows addition of a large quantity of the premix, although, the final concentration of the individual nutrient in the diet is usually very small. Vitamin and mineral requirements are very much important in poultry production. Many pharmaceutical companies market vitamin-mineral premixes using different trade names. Few marketed vitamin-mineral premixes packages do not contain name and origin of products but attributed to appreciative quality of the products. Many poultry farmers observed that among the many commercial vitamin-mineral premixes, few are not quality products; most probably due to adulteration by businessmen. Registration and analysis of quality of every product is obligatory (Asaduzzaman *et al*., 2005).

Vitamin – mineral premixes are sold throughout the country without any established standards. The registration of feed additives is based on three criteria: the claimed effects of a product on the farm animals terms of performance, disease prevention, antioxidant effect, or pigmentation which must have been clearly demonstrated through experimentation. Absence of undesired side effects must be well documented and safety for human beings, animals and environment must be guaranteed (Wenk, 2000).

Vitamin nutrition is a dynamic input for the production and marketing of poultry meat and eggs because it must be updated regularly to accommodate improvements in production and marketing methods, changes in conditions on the farm and new vitamin nutrition knowledge. Continued improvement of genetic potentials of poultry demands that we determine if increased vitamins levels may be needed to adequately meet these higher performance levels. The dynamics of vitamin nutrition for poultry are demonstrated by changes in the NRC vitamin requirements of growing chickens (0 – 8 weeks of age) that have been made during the past four decades. These changes were primarily due to advances in knowledge of vitamin nutrition, improvements in vitamin product forms and extensive changes in commercial broiler production methods that have occurred since the last publication (National Research Council, 1994).

The world is advocating for organic materials utilization in animal feeds. The organic materials come in packages with vitamins, enzymes and minerals that control the way the body recognizes, metabolizes and uses them to make what it needs (Avitech, 2007). Natural vitamins and minerals are derived from plant and animal sources. However, more than 95% of the vitamin – mineral premixes being sold are manufactured synthetically with chemicals and do not come straight from their natural resources. Many synthetic vitamins and minerals lack the transporters and cofactors associated with naturally – occurring vitamins and minerals because they have been isolated. The Organic Consumers Association (O.C.A) emphasizes that isolated vitamins and minerals cannot be used or recognized by the body in the same way as the natural version (Global Healing Centre, 2013).

The use of organic systems for poultry production is growing due to consumer demand for specifically "natural" and "welfare friendly" meat and eggs (Dan*et al*.,, 2012). The most appropriate technology for developing tropical countries like Nigeria, with escalating ingredient prices is the development of premix formulations, which would allow locally and naturally available non – conventional premix ingredients to be exploited. Nigeria has potential of non – conventional feedstuffs that can be used in premix formulation (Avitech, 2007). However, limited studies have been conducted to assess the locally and naturally sourced feedstuffs nutritive value in poultry diets. In particular, fish meal, blood meal, wood ash, red pepper, poultry litter ash, egg and *Moringa oleifera*are known to be very rich in minerals and vitamins and are also locally available.

Commercial Vitamin – Mineral Premixes are compulsory in poultry diets, however they are very expensive and sometimes of doubtful potency. They may also not be readily available when critically needed. This research will therefore be of great importance as it is expected to lead to production of improved, readily available and cost – effective locally formulated vitamins and minerals for laying hens and invariably the provision of quality and affordable animal protein in Nigeria. In the same vein, Locally Formulated Vitamin – Mineral Premixes can easily be subjected to better quality control.

This study was therefore designed to compare the efficacy of a commercial vitamin – mineral premix and two different locally formulated vitamin – mineral premixes from natural sources in the performance of egg – type chickens.

Objectives of the Study

This study has the following specific objectives:

1. To determine the proximate, vitamin and mineral compositions of the two locally formulated vitamin-mineral premixes (LFVMPs) and commercial vitamin – mineral premixes (CVMPs).
2. To evaluate the comparative efficacy of LFVMPs and CVMPs on the growth, health and mortality of egg-type chicken.
3. To evaluate the effect of LFVMPs and CVMP diets on egg quality characteristics.
4. To evaluate the cholesterol and some other lipid contents of yolk of eggs laid by birds fed on diets with LFVMPs and CVMP.
5. To evaluate the economic implication of using LFVMPs and CVMP on growth and egg production of egg-type chicken.

Research Hypotheses

Experiments 1, 2 and 3

Ho: LFVMPs diets do not numerically have high nutrients, vitamins and minerals as that of CVMPs diets.

H_A:LFVMPs diets do numerically have high nutrients, vitamins and minerals as that of CVMPs diets.

Experiments 4 and 5

Ho: Feeding diets containing LFVMPs does not have significant effects on the growth performance of chicks and growing pullets as diets containing CVMP.

H_A: Feeding diets containing LFVMPs do have significant effects on the growth performance of chicks and growing pullets as diets containing CVMP.

Experiments 6 and 7

Ho: Feeding diets containing LFVMPs does not have significant effects on the growth, subsequent early laying, laying performance of egg type pullets; and the cost of production as diets containing CVMP.

H_A: Feeding diets containing LFVMPs do have significant effects on the growth, subsequent early laying, laying performance of egg type pullets; and the cost of production as diets containing CVMP.

Experiment 8

Ho: Feeding diets containing LFVMPs does not have significant effects on egg quality characteristics of hens as diets containing CVMP.

H_A: Feeding diets containing LFVMPs do have significant effects on egg quality characteristics of hens as diets containing CVMP.

Experiment 9 and 10

Ho: Feeding diets containing LFVMPs does not have significant effects on the cholesterol contents of yolk of eggs laid by hens compared with diets containing CVMP.

H_A: Feeding diets containing LFVMPs do have significant effects on the cholesterol contents of yolk of eggs laid by hens compared with diets containing CVMP.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Over the years the number of vitamins recommended by National Research Council (NRC) has increased and vitamin requirement values have been revised. The number of vitamins recommended by the NRC for growing chickens increased from nine in 1944 to thirteen in 1994. A fourteenth vitamin, vitamin C, is now being considered in vitamin supplementation programmes. Vitamin C is synthesized by poultry and is, accordingly, not considered a required dietary nutrient. The 1950 requirement values included margins of safety to compensate for vitamin losses during feed processing and storage and for other factors influencing the vitamin needs have commercially produced chickens (influencing factors). For instance, the 1944 NRC recommendation allowed a general 20% safety margin for all vitamins. Later on, it was recognized that the influencing factors varied considerably from farm to farm. Thus, in 1954, the margins of safety were excluded and the NRC vitamins requirements were reduced to minimum values. Therefore, the poultry nutritionists must use these minimum requirement values as a base, arrive at margins of safety and adjust vitamins allowances to compensate for the influencing factors occurring in his operation (Ward, 1993).

2.2 Vitamins Requirement of the Chicken

Vitamins are active substances essential for life of humans and animals. They belong to the micronutrients group and are required for normal metabolism in animals. Vitamins are essential for optimum health as well as normal physiological functions such as growth,

development, maintenance and reproduction. As most vitamins cannot be synthesized by poultry in sufficient amounts to meet physiological demands, they must be obtained from the diet.

Vitamins are present in many feedstuffs in minute amounts and can be absorbed from the diet during the digestive process. If vitamins are absent from the diet or improperly absorbed or utilized, specific diseases or deficiency syndromes occur. Classically, vitamins have been divided into two groups based on their solubility in lipids or in water. The fat soluble group includes the vitamins A, D, E and K; while vitamins of the B complex: B_1(thiamine), B_2 (riboflavin), B_6 (pyridoxine), B_{12} (cyanocobalamine),B_3(niacin), B_5 (pantothenic acid), folic acid, choline and biotin as well as vitamin C are classified as water – soluble. Fat – soluble vitamins are found in feedstuffs in association with lipids. The fat – soluble vitamins are absorbed along with dietary fats, apparently by mechanisms similar to those involved in fat absorption. Uptake of water – soluble vitamins occurs usually via simple diffusion. Fat – soluble vitamins may be stored in the animal body, in contrast to that of water soluble vitamins which are not stored because excesses are rapidly excreted (Weber, 2009).

2.2.1 Fat – soluble vitamins: The fat – soluble vitamins are A, D, E and K

2.2.1.1 Vitamin A (Retinol)

Retinol can only be found in animal tissues such as fats, fish liver oils, butter, cheese, milk, while the provitamin A, beta-carotene occurs predominantly in vegetables such as carrots, sweet potatoes, pumpkins, spinach, tomatoes, bananas, peaches and plums . Dietary retinyl-

esters are hydrolyzed in the intestinal lumen and the alcoholic form of vitamin A is actively absorbed in association with low density lipoproteins. In the intestinal wall, retinol is re-esterified and transported via the blood stream to the liver and the kidneys were it is stored in the form of vitamin A esters. From there it is mobilized through hydrolysis, distributed to the cells as retinol via the blood stream (bound to retinol binding protein) and transformed into the active compound, retinoic acid. Vitamin A is essential for normal vision; it supports the development and maintains the integrity of epithelia as well as of the skeleton. In farm animals, vitamin A is important for normal feed intake, growth and performance. Furthermore, it supports an optimum immune response and thereby reduces the susceptibility to infection (Weber, 2009).

Vitamin A deficiency induces loss of appetite, growth retardation and can result in death. Affected birds develop a dry and scaly skin, rough plumage and metaplasia of respiratory epithelia, which increases the risk of infections. Keratinization of epithelia in the digestive tract depresses gland activities and results in poor absorption of nutrients. In laying hens a severe degeneration of the ovary can occur, inducing a marked decline in egg production. In a series of experiments, Richter *et al*. (1990) established that a minimum of 5000 IU vitamin A per kg was necessary to allow adequate feed intake and laying performance during lay. However, no benefits could be demonstrated if higher than this dietary vitamin A level was fed, probably due to the limited genetic potential of those hens at that time. The performance of laying hens fed on vitamin A deficient diets was shown to decline significantly, but egg weight and shell thickness were not consistently changed. Vitamin A

levels of egg yolk were found not to be related to dietary level and therefore were not useful to predict future vitamin A deficiency in laying hens (Squires and Naber, 1993).

In heat – stressed commercial layers it was demonstrated that a high level of dietary vitamin A (12,000 IU/kg) improved the laying performance and immune functions of hens (Lin *et al*., 2002). Feed intake and laying rate was beneficially influenced and the egg weight of heat – stressed hens increased. A good supply of vitamin A was essential when rearing replacements for future laying flocks. When pullets fed with low levels of vitamin A diets, plasma concentrations of retinol dropped considerably, body weights were lower than that of the respective controls and more of the vitamin A deficient birds died (Beynen *et al*., 1989).

Since poultry under commercial husbandry conditions were always more vulnerable to infectious diseases, the effect of vitamin A on the immune response was further investigated. Coskun *et al*. (1998) investigated dietary levels of up to 24,000 IU of vitamin A per kg feed in laying hens over one year, but could not find any beneficial effects on various parameters of immune responses. In contrast to that finding, a high level of dietary vitamin A (12,000 IU/kg) increased the antibody titre against Newcastle disease' virus (NDV) of heat – stressed hens (Lin *et al*., 2002). Vitamin A is usually low in poultry diets; however, addition of other vitamins to poultry diets is a good insurance to protect birds from deficiency diseases and disorders (Wenk, 2000). It is important to note that; turkey requirement for vitamin A was twice that of chickens (Robinson, 2010).

2.2.1.2 Vitamin D

This consists of several forms and of these, vitamin D_2(ergocalciferol) and vitamin D_3 (cholecalciferol) are the important ones. They help to maintain a proper calcium-phosphorus balance in the body by regulating the absorption of calcium and phosphate from the intestine. Consequently, they are necessary for proper bone formation and development. Vitamin D also prevents a condition often known as egg paralysis that accompanies the production of soft shelled eggs in laying birds. Consideration should be taken to supplement vitamin D, calcium and mineral contents of the ration. Their deficiency causes rickets in chickens, a condition in which the bones fail to harden and the ribs become mis-shaped due to incomplete calcification of bones. These results in the bones becoming curved when subjected to pressure and the chest becoming deformed, causing the condition known as 'Pigeon chest'(Cunningham, 2008; Robinson, 2010).

A chick deficient in vitamin D has weak legs, ruffled feathers and general unthrifty conditions are typical. In this particular individual even the beak is soft and out of shape, which is not always a symptom accompanying leg weakness. These symptoms normally show up between the 4^{th} and 11^{th} weeks of growth. This might happen if you are brooding large group chicks in ratio to one feed dish. If a chick is not feisty or strong enough to fight through to the dish before it is empty or he's pushed out of the way he will lack the amount of feed and vitamins necessary to sustain his needs. Be aware that these same symptoms can be attributed to what is known as perosis or slipped tendon in which the legs are bowed or twisted in spite of the fact that the bones are well calcified and hard (Robinson, 2010).

Natural sources of vitamin D are fish liver oil especially halibut liver oil, cod liver oil and sardine oil are particularly rich in vitamin D, fortified milk, egg yolk and butter. It may interest you to know that direct contact with the ultra-violet rays of the sun enables chickens to produce within themselves the necessary vitamin D, enabling them to make the best use of their feed. Birds kept in an enclosed barn, coop or other places that do not have direct sunlight available are the birds that might require this vitamin supplement most. In comparison to other species, chickens are more susceptible to this vitamin deficiency because gut flora of chickens provide very little vitamin synthesis but compete with the host for dietary vitamins and intensively kept chickens undergo many stresses. Vitamin D is usually low in poultry diets and completely absent from diet based on corn and soybean meals (Wenk, 2000).

The active form of vitamin D is not widely distributed in nature, but vitamin D precursors are present in many vegetables. Poultry can only utilize vitamin D_3 therefore D_2 has no physiological value in this species. Cholecalciferol is absorbed from the intestinal tract in conjunction with fats and so the presence of bile salts is needed for absorption. Vitamin D_3 is first taken up by the liver and finally deposited in adipose tissue. In the liver, Cholecalciferol is converted to 25-hydroxy-cholecalciferol ($25(OH)_2D_3$) and then further hydroxylated in the kidney to 1,25-dihydroxy-cholecalciferol ($1,25(OH)_2D_3$). The last transformation step to the active hormonal form of vitamin D_3 is under the control of the parathyroid hormone. Vitamin D regulates the homeostasis of calcium (Ca) and phosphorus (P), for instance it increases the absorption of calcium and phosphorus from the small

intestine as well as the re-absorption of these minerals in the renal tubules and influences the calcification process by increasing the uptake of minerals by the bones (Weber, 2009).

Clinical deficiency signs include inhibition of growth, loss of weight, reduced or lost appetite and high mortality. The epiphyses are enlarged with the bones of the extremities, vertebral column and cranium becoming deformed and brittle. The beak of rachitic chicks is typically soft and pliant. Animals move stiffly and hesitantly due to the high incidence of lameness and muscular weakness. In layers, egg production decreases and eggs with thin shells are laid (Weber, 2009).

The fundamental effects of vitamin D_3 in laying and breeding poultry have mainly been elucidated by study of the adverse effects of deficiency. In adult laying hens, vitamin D_3 deficiency produces osteomalacia. This is characterized by a decreased concentration of Ca and P in the bone matrix. Furthermore, vitamin D_3 deficiency reduces egg production and egg weight and increases the occurrence of thin shells, cracks and deformities. When laying Japanese quails were fed a vitamin D deficient diet, their egg shell weights and egg production rate were reduced. The calcium content of the medullary bone of the femur decreased markedly with the progress of vitamin D deficiency, whereas that of the cortical bone remained unchanged. Administration of vitamin D_3 to deficient quail has been shown to increase the mineralization of medullary bone (Takahashi *et al*., 1983).

Egg shell breakages, which is one of the various types of egg shell problems in the industry today, is directly and inversely related to the level of Cholecalciferol in the diet (Goodson – Williams *et al*., 1986). When supplemental vitamin D_3 was removed from the feed of White

Leghorn hens, egg weight, egg specific gravity, shell weight, percent shell, shell thickness and plasma calcium were reduced (Grunder and Tsang, 1984). Only four weeks is required on a vitamin D_3 deficient diet to reduce the thickness of the shell and induce the production of numerous thin – shelled and soft – shelled eggs. The outer layers of the shell may be reduced or absent, but the inner mammillary layer is always present suggesting that the hens stop laying before Calcium concentrations in blood become too low for the formation of the mammillary knobs. Uncalcified portions of the shell organic matrix were not found, suggesting that calcium deposition and matrix formation were inhibited simultaneously (Narbaitz *et al*., 1987).

Withdrawal of cholecalciferol from a layer diet drastically reduced blood $25(OH)D_3$, $1,25(OH)_2D_3$ and egg specific gravity within two weeks, which was followed by a decrease in blood total Ca. Doubling the vitamin D_3 supplement in the control diet to 1,100 IU/kg almost linearly increased the circulating concentration of $25(OH)D_3$ without raising the concentration of $1,25(OH)D_3$or of calcium (Tsang and Grunder, 1993).

In laying hens kept under commercial conditions, clinical signs of vitamin D_3 deficiency, such as cage – layer fatigue or thin – shelled eggs are frequently observed, indicating insufficient utilization of the dietary vitamin D_3. Supplementing an already activated form of vitamin D_3, such as one of the D_3-metabolites, has been proposed to counteract such problems and $25(OH)D_3$ representing the first metabolite in the cascade of vitamin D mobilization, has become commercially available to the poultry industry under the trade name Hy.D®. This $25(OH)D_3$form had been demonstrated to support the homeostatic

function of vitamin D_3 for the absorption of calcium and phosphorus in order to provide sufficient minerals for the development of the bone and the egg shell (Soares *et al*., 1995). Practical studies have shown that replacing vitamin D_3 partly by 25(OH)D_3 improved laying performance, decreased the number of broken eggs and increased egg weight, egg mass as well as the number of extra-large eggs (Soto-Solanova and Hernandez, 2004). In addition egg yolk susceptibility to oxidation was decreased and the loss of egg mass during storage was numerically reduced. Likewise, under commercial conditions a numerical improvement of laying performance with 25(OH)D_3 supplementation was observed (Soto-Solanova and Schliffka, 2007).

2.2.1.3 Vitamin E (Tocopherol)

Tocopherol is believed to be concerned with reproduction. It has been found that its absence in certain animals causes sterility and recurring abortions, therefore promotes infertility. Beside these, it serves as antioxidant, play vital roles in regulation of oxidation reactions and support cell membrane stabilization. In chickens, deficiency of tocopherol causes encephalomalacia (crazy chick disease), muscular dystrophy, exudative diasthesis, enlarged hocks and hatchability problems (Nicholas, 2008).

Young chicks grown on vitamin E deficient diets from the time of hatching develop a condition of imbalance and loss of muscular control about the third week. These chicks are found staggering around the pen or lying on their sides. Natural sources of tocopherol are green vegetables such as lettuce, polyunsaturated plant oils such as soybean, corn and canola oils, wheat germ, sunflower seeds, tofu avocado, sweet potatoes, shrimp, cod and the oil of

certain seeds such as wheat grains. The most concentrated source of vitamin E is wheat germ oil and will undoubtedly prove beneficial in increasing hatchability (Robinson, 2010). Among the richest sources of vitamin E are cereal germs and certain oilseeds. Within the group of tocopherol, alpha-tocopherol is the most effective vitamin E compound for poultry. Uptake is linked to fat absorption, facilitated by the presence of bile salts. There is a negative interaction with the absorption of vitamin A (high vitamin A levels depress E absorption). Transport from the intestine to the systemic circulation runs via chylomicrons of the lymph. In plasma, vitamin E is bound to lipoproteins; storage of vitamin E occurs in the liver (Weber, 2009).

Vitamin E (alpha-tocopherol) is nature's most powerful fat-soluble antioxidant and as such is important to protect the phospholipids of cellular and sub-cellular membranes from destruction by lipid oxidation and accordingly to maintain the morphological integrity and functionality of cells and tissues of the organism. Furthermore, as an essential micronutrient, vitamin E optimizes performance and reproduction of farm animals. In poultry, vitamin E protects the ovarian follicles from oxidative damage and there is evidence that it also facilitates the release of vitellogenin as a precursor of the yolk from the liver and thus has an important function in egg production. Due to its modulatory effect on the immune system (e.g. the activation of macrophages and production of antibodies) alpha – tocopherol has proven effective for the prevention and resistance against various diseases such as New castle, Coccidiosis (Puthpongsiriporn *et al*., 2001).

Signs of clinical vitamin E deficiency include muscular myopathy, exudative diathesis (abnormal permeability of the capillary walls) and disturbance of the nervous system (encephalomalacia in chicks). More often subclinical vitamin E deficiency occurs, which is manifested as slow growth, reduced productivity, frequent health problems and diminished fertility. In animal nutrition, vitamin E is usually supplied in its stable ester form as alpha-tocopheryl acetate (Weber, 2009).

Several attempts have been made to determine the vitamin E requirement of laying hens. More than 20 years ago, Richter *et al*. (1986) performed a series of experiments with laying hens supplemented with vitamin E at 20 mg/kg against an unsupplemented control. Due to absence of any measurable effects of vitamin E on laying performance, feed efficiency or mortality, the authors concluded that the natural content (> 1mg alpha-tocopherol/kg) of the diet was sufficient for an adequate performance of laying hens.

Later, Scheideler and Froning (1996) demonstrated that a dietary supplementation of hens with 50 mg/kg instead of 27 mg/kg improved egg production, which proved the essential function of vitamin E for optimizing performance of laying hens. In a study investigating immune response, the combination of 65 ppm vitamin E and 1,000 ppm vitamin C during heat stress showed the highest lymphocyte proliferative responses to concanavalin A and *Salmonella typhimurium* lipopolysaccharide in comparison to treatments with lower E supplementation (Puthpongsiriporn *et al*., 2001). This result indicates an important function of vitamin E for maintenance of health of laying hens.

In layers subjected to high ambient temperatures, basic vitamin E requirements were found to be higher. Under conditions of chronic heat stress, vitamin E supplementation of 500 mg/kg was necessary to alleviate the adverse effects on egg production and egg weight (Bollengier – Lee *et al*., 1998). If the supplemented diet was provided before, during and after heat stress, a reduced dietary dose of 250 mg vitamin E per kg was found to be optimum for alleviating the adverse effects of chronic heat stress in laying hens (Bollengier – Lee *et al*., 1998).

Vitamin E is a particularly safe micronutrient, as supplementation up to 20,000 mg/kg did not impact on performance of hens. As expected, a dose – dependent increase in alpha – tocopherol concentration in plasma, liver, muscle and egg yolk was found (Sunder and Flachowsky, 2001). Higher dietary supplementation levels of vitamin E have been used to improve the oxidative stability of the egg yolk, particularly when polyunsaturated fatty acids (PUFAs) were used. Grune *et al*. (2001) added fish oil to the diet of laying hens, which were supplemented from 0 up to 160 mg vitamin E per kg diet. Egg yolks contained more n – 3 PUFAs, mainly due to accumulation of docosahexaenoic acid. To prevent the increase of lipid peroxidation during production and storage of n – 3 PUFA enriched eggs; a high vitamin E supplementation with at least 80 mg/kg was needed. Further processing of eggs increases the requirement for stabilization of lipids.

When eggs enriched with PUFAs were submitted to an atomization process followed by spray – drying, the oxidation values increased 10 to 20 fold over the values of fresh eggs. Only supplementation of hens with 200 ppm vitamin E significantly reduced lipid oxidation

in comparison to the standard level of 50 ppm. Accordingly, vitamin E contributes substantially to the quality of eggs during processing(Galobart *et al*., 2001).

When alpha – tocopherol and beta – carotene, two antioxidants which are associated with health benefits for humans and animals, were supplemented in laying hen diets at levels of up to 400 mg/kg each, their concentration in chicken egg yolks could be significantly improved. Alpha-tocopherol increased from the control level of 144 µg/g of yolk to 477 µg/g of yolk (at 400 ppm vitamin E), and yolk retinol levels increased from11.6 µg/g in controls to 13.9 µg/g at 200 ppm beta-carotene. Beta-carotene content in the yolk also increased from 0.14 µg/g of yolk in controls to 5.19 µg/g of yolk, when supplied at levels of 200 mg/kg diet. Supplemental beta-carotene markedly decreased the yolk deposition of alpha-tocopherol when the two compounds were fed together (Jiang *et al*., 1994).

When feeding very high doses of vitamin E (100, 1000, 10,000 and 20,000 mg/kg) over a period of 20 weeks, no significant influence on health or performance of hens was observed. Vitamin E content of eggs increased from 1 to 4, 21, 46 and 51 mg per egg with vitamin E supplementation, which represents a considerable enrichment of eggs with vitamin E (Sunder *et al*., 1999).

In order to overcome dietary deficits, multiple – enriched eggs have been obtained by feeding laying hens with linseed, minerals, vitamins and lutein. These eggs have greater nutritional value than standard eggs, containing six times more omega – 3 fatty acid (ALA), three times more DHA, three times more vitamin D, four times more folic acid, six times

more vitamin E, six times more lutein and zeaxanthine, two and a half times more iodine and four times more selenium. Furthermore, these eggs are rich in vitamin B_{12} and A, plus vitamin B_2, vitamin B_5 and phosphorus (Bourre and Galea, 2006). Supplementing tocopherol in well balanced diets has shown increase of humoral immunity for monogastric species (Langweiler *et al*., 1983;Wuryastuti*et al*., 1993).

2.2.1.4 Vitamin K

The absorption of vitamin K happens in association with dietary fats, facilitated by the presence of bile salts. Vitamin K_3 (menadione) is the main source in animal nutrition. Following absorption, vitamin K is metabolized in the liver. Very little is stored; which results in rapid depletion, probably within one week, if dietary supply is low. Vitamin K regulates the production of certain coagulation factors in the blood plasma, for example prothrombin and clotting factors VII, IX and X, preventing uncontrolled bleeding from wounds. These factors are proteins, produced in the liver and their synthesis depends on the presence of minute quantities of vitamin K. Deficiency of this vitamin increases blood clotting time, resulting in haemorrhagic diseases in most tissues and organs (Wuryastuti *et al*., 1993).

In young poultry general weakness, rough plumage and paleness as well as changed in the colouration of the comb, the wattles and eye – lids as a result of anaemia have been described. Subcutaneous and intramuscular haemorrhages and bloody faces due to bleeding in the crop and in the caeca can occur. The specific activity of vitamin K could also be of importance in laying hens, which are injured by management practices (beak trimming) or

nervous behaviour (cannibalism). Having an optimum blood clotting, hens might avoid problems due to blood loss and enable faster healing as a consequence of such events (Weber, 2009).

Vitamin K is also important in relation to bone formation and bone re-modelling. Osteocalcin, one of the main bone proteins, is dependent on vitamin K. Osteocalcin is found in bone, the uterus and egg shell. Low levels of osteocalcin can interfere with bone mineralization during skeletal development and egg shell formation. Furthermore, a link between sub-optimal vitamin K supply and osteoporosis in laying hens has been proposed by Fleming (2008).

The supplementation of laying feeds with 2 to 3mg menadione per kg diet is recommended. In order to determine the effect of vitamin K deficiency on indices of skeletal metabolism in laying hens, the birds were fed a vitamin K – deficient diet for 28 weeks, which resulted in impaired blood clotting and reduced bone gamma – carboxyglutamic acid concentration compared with vitamin K sufficient hens. However, this treatment did not influence egg production, egg shell deposition or other reproductive performance criteria (Lavelle *et al*., 1994). In conflict with these findings, Fleming *et al*. (2003) found that additional vitamin K_3 (10 mg/kg) resulted in higher proximal tarso-metatarsus cancellous bone volumes at 15 weeks and throughout the laying period compared with controls. Vitamin K also prolonged the modeling period of bone formation or the inhibition of medullary bone loss during the first phases of lay. Plasma osteocalcin concentrations, however were unaffected by vitamin K_3 supplementation during growth.

Vitamin K is known to preserve the clotting power of blood by synthesizing blood – clotting proteins and regulates blood calcium. In the absence of this vitamin, chicks bleed to death from any injury causing a rupture of the blood vessel walls. Deficiency of vitamin K is not visible to the naked eye and is extremely rare. Natural sources of vitamin K are Brussel sprouts, leafy green vegetables, spinach, broccoli, cabbage and liver. Vitamin K is generally added to poultry diets more than to those for other species becausebirds have less intestinal synthesis because of a shorter intestinal tract and faster rate of food (Wenk, 2000).

2.2.2 Water – soluble vitamins

These groups of vitamins are soluble in water and include vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (Niacin), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxine), vitamin B_{12} (cyanocobalamine), Choline, Folic acid, Biotin and vitamin C.

2.2.2.1 Vitamin B

Vitamin B complex is any group of substances that are essential for the working of certain enzymes in the body that are not chemically related but are grouped together merely because they occur in the same plant and animal foods. The B vitamins have very important functions in avian metabolism, as most of them represent coenzymes which fuse with larger enzyme molecules in order to activate or accelerate various metabolic processes. Vitamin B_{12} and folic acid exert their activity on growth and cell maintenance. It is important to consider that B – vitamins that are involved in particular metabolic effects interact with each

other, which makes it difficult to determine individual requirements for each vitamin within the B – group (Weber, 2009).

Thiamine

All vertebrates are almost completely dependent on dietary thiamine intake. Although bacteria are able to synthesize some thiamine in the intestinal tract (mainly in the caeca), this material is only accessible via coprophagy and accordingly caged laying hens cannot benefit from that. Thiamine is present in considerable concentrations in yeast, cereals, soya and oil seeds, but is not always entirely available to poultry. Thiamine is rapidly and actively absorbed from the small intestine and then transformed by phosphorylation into the active co-enzyme thiamine pyrophosphate. The body is incapable of storing free thiamine, but small amounts of the phosphorylated form is found in all animal cells. Dephosphorylation can occur in the kidney and excess quantities of the free vitamin are excreted in the urine or as uric acid in the case of birds (Olkowski and Classen, 1996).

The active co-enzyme of thiamine is thiamine pyrophosphate (TPP). As a co-enzyme TPP is involved in oxidative decarboxylation of pyruvic acid and ketoglutaric acid. These reactions yield acetyl-coenzyme A (Co A) and succinyl – CoA and have a central function in the citric acid cycle, leading to complete degradation of carbohydrates. Accordingly, thiamine is important for the metabolism of carbohydrates, proteins and fats. Deficiency symptoms in poultry include loss of appetite, weight loss, general weakness and death. More particularly, fatty acid degeneration and necrosis of heart fibres, bradycardia, heart failure (sudden death syndrome) and haemorrhages of the liver. Mucosal inflammation, ulcers and haemorrhages

of the intestinal tract as well as skin oedema and cyanosis have been observed. Progressive paralysis may ultimately leads to death of the birds. In laying hens atrophied ovaries were observed, resulting in reduced egg production (Weber, 2009).

The main investigations into the thiamine requirements of poultry are quite old and, based on this information; the current recommendations by NRC (1994) are very low at 0.7 mg/kg. Studies on requirement for broiler chicks shows that a thiamine supplementation level above 2 mg/kg is necessary to avoid a decline in blood thiamine concentrations. This finding could be taken as an indication that modern birds, selected for fast growth have a superior requirement for thiamine (Olkowski and Classen, 1996).

Riboflavin

Riboflavin is widely distributed in all leafy vegetables, in the flesh of warm blooded animals and fishes. This vitamin is stored in small quantities in liver, spleen, kidney and cardiac muscle. These depots are maintained even in severe deficiency states at a steady level (Squires and Naber, 1993). Riboflavin is eliminated in the urine and the daily losses can amount up to 30% of intake. Riboflavin is phosphorylated in the intestinal mucosa to flavin mononucleotide (FMN) during absorption and then converted to Flavin-Adenin Dinucleotide (FAD) in the liver. Riboflavin is an essential factor of flavin enzymes (flavoproteins). These enzymes are involved in the transport and transfer of hydrogen within the respiratory chain and thus contribute to energy production (Squires and Naber, 1993).

Furthermore, they are important for both synthesis and degradation of fatty acids, which explains why vitamin B_2 requirement is increased on a high-fat diet. B_2-containning amino acid oxidases also catalyze the oxidative catabolism of amino acids. Obvious deficiency signs in poultry include slow growth and reduced appetite. The mouth, nasal mucous membranes and skin of the extremities tend to get inflamed and muscular debility, trembling, spasms and paralysis, due to myelin degeneration of peripheral nerves (curled toe paralysis) can occur. Vomiting, resorption disorders and diarrhea due to inflammation of the mucous membranes of the digestive tract, have been described and in hens, egg laying is disturbed (Weber, 2009).

Squires and Naber (1993) investigated the relationship between dietary riboflavin supplementation of laying hens (1.55 – 8.8 mg/kg) and egg production as well as riboflavin content in eggs. Egg production, egg weight, hatchability and hen weight were all significantly depressed by the two lower riboflavin levels, when compared with the higher levels. Results indicated that egg riboflavin concentrations were related to important production parameters that could be used to predict future dietary riboflavin inadequacies. A study with breeding hens, supplemented with 2.5 to 12.5 mg riboflavin per kg and kept under conditions of a humid tropical environment, confirmed this finding. Out of the various productive parameters analyzed, the only one which responded was egg production, which increased significantly, when the dietary riboflavin level was 8.5 mg/kg (Arijeniwa *et al.*, 1996).

Niacin (Nicotinic acid)

Nicotinic acid is active in metabolism as nicotinamide and represents an indispensable component of hydrogen – carrying co-enzymes; NAD (Nicotinamide Adenine Dinucleotide) and NADP (Nicotinamide Adenine Dinucleotide Phosphate). Nicotinamide participates directly in the transfer of hydrogen, which is of utmost importance in the intermediary metabolism. These biochemical functions are important for normal tissue integrity, particularly for the skin, the gastrointestinal tract and the nervous system. An important part of niacin, present in cereals and their by-products is bound and not available for absorption by poultry. The bioavailability of niacin from other feed sources is not clearly documented. Thus, it is obvious, that a dietary supplementation of hens with niacin is necessary. The minimum requirement, indicated by NRC (1994), is 10 mg niacin per kg feed, based on studies by Ingrose *et al*. (1965). In a review carried out by Whitehead (2001) the practical level of supplementation for layers was found to be 50 ppm niacin. Based on such information, the recommendation for to modern laying hens under commercial conditions is considered to be higher.

Jensen *et al*. (1976) failed to show a significant effect of 44 mg niacin per kg feed, either alone or in combination with biotin, on egg production, egg weight, feed consumption or body weight changes observed over a 12 week period. In another study with commercial egg-type hens, their response to various levels of niacin supplementation of the diet was investigated by Quart *et al.*, (1987) and egg production, feed conversion, nervousness, fertility and hatchability were observed, but no significant differences were found among the dietary treatments.

Leeson *et al*. (1991) evaluated dietary niacin supplementation of 22, 44, 66 or 132 mg/kg feed in laying hens through a 364 – day trial periods. Hens fed 66 or 132 mg/kg supplemental niacin produced significantly more eggs than birds fed 22 mg/kg. Niacin supplementation was demonstrated to affect shell quality which was measured as the degree of shell deformity. A very high dose of up to 1,022 mg/kg showed neither positive nor negative effects on layer performance or health, indicating that niacin is well tolerated, even at supra-nutritional levels.

Pantothenic Acid

Pantothenic acid occurs in all animal and plant tissues. Rich sources include yeast, liver, kidney, egg-yolk, cereals and green plants. The absorption of pantothenic acid and its alcohol-derivative in the intestine occurs by passive diffusion. The conversion to co-enzyme A (CoA), which represents the conjugated nucleotide form of pantothenic acid, occurs within the tissues. CoA acts as a carrier mechanism for carboxylic acids which when bound to CoA becomes active, for instance, have a high potential for transfer to other groups. The most important form is acetyl-CoA, which appears in the citric acid cycle when fat, carbohydrate and certain amino acids are degraded. Acetyl CoA is also a precursor for the biosynthesis of long chain fatty acids, phosphatides, cholesterol, steroid hormones and bile acids (Weber, 2009).

Clinical signs of deficiency include reduced growth, decrease in appetite, poor feed utilization, rough feathering and depigmentation of the feathers. In severe cases, crusts at the corner of the beak as well as exudate on eye-lids can be seen and fatty degeneration of

the liver occurs. Particularly to layers, impaired egg production has been reported. Requirement of this vitamin depend on its interaction with other vitamins such as vitamin C, biotin and vitamin B_{12}, as well as on the fat content of the ration. Low levels of vitamin B_{12} and high levels of fat increase the requirements of pantothenic acid, while the presence of vitamin C may reduce dietary needs (Olkowski and Classen, 1996).

But overall, insufficient information is available to draw final conclusions on the current pantothenic acid requirement of layers. For an adequate supplementation, the genetic improvement of the breeds towards higher yield have to be considered and due to the relationship of pantothenic acid with energy metabolism, must be adjusted to the energy content of the diet. The current recommendation guidelines of the vitamin manufactures already consider all these factors (Leeson *et al*., 1991).

Pyridoxine

Vitamin B_6 in its main forms pyridoxal and pyridoxamine are widely distributed in low concentrations in all animal and plant tissues. In a glycosylated form, however, it is not entirely bioavailable. Absorption of pyridoxine occurs in the proximal jejunum, after which it is phosphorylated in the liver by pyridoxal kinase and released into the blood. Both pyridoxal phosphate and pyridoxamine phosphate are bound to albumen and distributed throughout animal tissues in form of co-enzymes. A large portion of absorbed pyridoxine can be excreted in urine as inactive metabolite called 4-pyridoxic acid. Pyridoxal phosphate and pyridoxamine phosphate are co-factors of various enzymes such as transaminases, deaminases, desulphydrases and amino acid decarboxylases. Thus, are essential for the

metabolism of amino acids, which explains an increased requirement of pyridoxine on high protein diet (Herbert *et al*., 2005).

Furthermore, pyridoxine is involved in both synthesis and transformation of glycogen and markedly influences the fatty acid metabolism via conversion of linoleic to arachidonic acids. Thus, pyridoxine is an essential element for energy production, fat metabolism, central nervous system activity and haemoglobin production. In pyridoxine deficiency, loss of appetite, slow growth and poor feed utilization in poultry have been observed. Dermatitis, rough and deficient plumage, inflated oedema of eyelids, anaemia and ascites can also occur. Through demyelinisation of peripheral nerves, movements can appear uncoordinated, resulting ultimately in muscular convulsions, followed by paralysis. In laying hens, impaired egg laying performance, fertility and hatchability are induced by deficiency (Weber, 2009).

In a classical experiment with breeding hens, the pyridoxine requirement was estimated at 1.5 – 2 mg per kg of feed, based on the reproductive characteristics of the hens which included laying performance (Abend *et al*., 1977). A certain benefit of higher pyridoxine supplementation levels of the hens was observed, when taking performance characteristics into account. It has to be mentioned that such requirement studies are usually carried out under optimum husbandry conditions. Stress and disease challenge may substantially increase the vitamin needs of farm animals. Accordingly, the practical recommendations are usually higher than the minimum requirements.

Cyanocobalamine (Vitamin B_{12})

Vitamin B_{12} usually does not get a lot of attention in nutrition, since it is required at very low concentrations in feed. As cyanocobalamine is synthesized by micro-organisms, coprophagy may potentially fulfill part of the intake demand in poultry. However, laying hens in cages cannot access this source and thus are entirely dependent on a dietary supply. Cyanocobalamine occurs widely in animal tissues such as meat, chicken, shell fish, milk, egg and has important physiological functions (House *et al*., 2002).

Cyanocobalamine interacts with the metabolism of folic acid, participates in the biosynthesis of labile methyl groups and is necessary for the biosynthesis of purine and pyrimidine bases, representing the essential constituents of nucleic acids. Furthermore, it is involved in the biosynthesis of methionine and choline. The most obvious signs of deficiency are impaired performance, defective feathering, leg weakness, perosis, pernicious anaemia, embryonic mortality in poultry and gizzard erosion (Weber, 2009).

Unlike the other B group vitamins, cyanocobalamine can be accumulated in the tissues, mainly in the liver, but also, to a lesser degree, in the kidney, muscles, bone and skin. The capacity of the hen to deposit reserves of vitamin B_{12} is disputed, and there remains an unresolved issue regarding how long body reserves in laying hens may support egg laying until they are depleted. An investigation with graded amounts of dietary vitamin B_{12} (4, 8, 16 µg/kg) given to breeding hens, showed that egg yolk vitamin B_{12} concentrations were high at the outset, but decreased markedly within 2 weeks in hens fed the two lowest dietary levels (Squires and Naber, 1992). After 12 weeks on the diets, egg concentrations of

vitamin B_{12} stabilized and were proportional to the amount of vitamin added to the diet. Maximum egg production, egg weight, hen weight and hatchability were obtained when the diet contained 8 µg/kg of vitamin B_{12}.

Choline

Choline, formerly known as vitamin B_4 was discovered as an indispensable feed additive in poultry. Choline is a water soluble essential nutrient; layers like broilers have an essential requirement for choline. A major use is in the formation of the phospholipid lecithin, a component of egg yolk. Current NRC recommendations for choline allowances in laying hens are 105 mg/day for white egg layers and 115 mg/day for those laying brown eggs. At feed intake levels of 100 and 110 g/day for the two types respectively, this implies a choline dietary concentration of approximately 1100 mg/kg. A number of factors may influence a hen's requirement for choline, for instance age, feed intake and dietary crude protein or methionine levels. It is generally accepted that dietary requirement declines with age, possibly associated with an increase in feed intake (Workel *et al*., 2002).

Methionine is the first limiting amino acid for egg production and given the common function with choline in methyl group donation, interactions between the two nutrients may be anticipated. Several trials have investigated these interactions: Parsons and Leeper, (1984), Keshavarz and Austic, (1985), Miles *et al*, (1986) and Miles, (1998) reported that where diets are low in crude protein and or marginal in total sulphur amino acids, responses to both methionine and choline supplementation occur. However, both nutrients can

increase egg production in these circumstances, only methionine appears to exert a positive influence on egg size (Keshavarz and Austin, 1985).

Emmert and Baker (1997) reported the bioavailability of choline in natural content of feedstuffs as follows: 83% of soybean contains 2,218 ppm, 24% of rapeseed contains 6,198 ppm and 76% of peanut contains 1,685 ppm. They further reported that choline chloride is a common supplement to poultry feed. Using a choline deficient basal diet, showed an almost linear response to incremental addition of choline chloride up to 1115 mg/kg feed in chicks from 10 – 22 days of age was observed. Increasing cholinechloride to 2000 mg/kg resulted in further weight gain, but to a lesser extent, levels in excess of this had no beneficial effects. Choline deficiency results to poor growth, fatty liver and decreased egg production.

Folic acid (Pteroylglutamic acid)

Folic acid is present in all green-leaved vegetables, liver, kidney and muscle as well as in milk and cheese. The term 'folates' embraces a group of biologically active compounds which are structurally derived from folic acid. The absorption of folic acid occurs at the jejunum and thereafter it is transported and attached to amino acidsthen to the liver. Limited quantities were present in the liver and otherwise there was no storage capacity for folates in the body (Weber, 2009).

Enzymatic transformation of folates results into tetrahydrofolicacd (THF), which is an active co-enzyme. THF is involved in various biochemical processes namely: methylation

reactions and amino acid metabolism, as well as biosynthesis of purines and pyrimidines. The fact that folate activity interacts with many compounds of carbon atom metabolism has to be considered for practical recommendations of folic acid fortification in layer diets.

The clinical picture of folic acid deficiency becomes manifest as a result of disturbances of amino acid metabolism and protein synthesis. Rapidly growing tissues such as epithelia of the gastrointestinal tract or skin and bone marrow are particularly affected. Retarded growth, diminished appetite, rough plumage and feather depigmentation have also been described. In severe cases cervical paralysis, leg weakness (perosis) and white watery diarrhea can occur (House *et al*., 2002).

When hens were fed a purified folate-deficient diet (0.07 mg/kg) egg production was reduced (Sherwood *et al*., 1993). Folates in egg yolk were concentrated approximately 43 fold relative to the blood plasma from which they were derived. Yolk and plasma folate concentrations became saturated with increasing dietary intake. Hens fed a commercial, folate sufficient diet (0.72 mg folate/kg) produced eggs with slightly less than half of the maximal folate content. However, when laying hens were supplemented with 0, 2, 4, 8, 16, 32, 64 or 128 mg/kg of crystalline folic acid for 21 days, laying performance was not affected (Hebert *et al.*, 2005).

Considering plasma homocystein concentrations, a strain-specific requirement for folic acid could be deduced as the higher egg mass producing strain benefited from increased folic acid through a reduction in plasma homocystein concentrations. The main objective of the study above was the enrichment of eggs with folic acid in light of evidence supporting a

need for humans to increase their dietary folate intakes. Significant main effects of folate supplementation were observed for egg folate content and plasma folate, which was increased. House *et al*. (2002) reported that, in terms of its nutritional value, one large egg collected from a folic acid-supplemented hen (4 mg/kg) provided approximately 12.5% of the recommended dietary allowance (RDA) for adult humans (RDA = 400 mg/day).

Biotin

Biotin is widely distributed in small concentrations in animal and plant tissues, but is almost entirely unavailable to laying hens from wheat (0%) or sorghum (10 – 20%) as reported by Buenrostro and Kratzer (1984). Biotin is absorbed in the small intestine, transported in the blood stream to the various tissues, but inadequately metabolized. Excess biotin is excreted in both urine and faeces. Biotin represents important co-enzymes in the intermediary metabolism of carbohydrates, proteins and fats. It is particularly important for carboxylations, since biotin-containing carboxylases take up CO_2 and transfer it to a suitable, and a possible effect of purine and pyrimidine synthesis has been put forward (Weber, 2009).

Biotin deficiency results in retarded growth, reduced appetite and poor feed conversion. Rough and brittle feathers, dry skin, dermatitis of foot pads and deformation of the beak can occur. Fatty liver and kidney syndrome (FLKS) as well as perosis develop under biotin insufficiency. In breeder hens, poor hatchability of eggs and malformation of the embryonic skeleton have been observed (Puthpongsiriporn *et al*., 2001).The influence of biotin supplementation on performance of laying hens has been evaluated in two studies: Jensen *et*

al. (1976) provided hens with biotin either alone or in combination with niacin, but could not observe a beneficial effect on egg production, egg weight, feed consumption or body weight over a twelve week period. Likewise, Whitehead (1980) found that biotin supplementation during lay did not have beneficial effects upon egg number, egg size, feed intake and feed conversion efficiency. However, internal egg quality, as reflected by albumen height, was slightly improved by biotin. As to safety, excess feeding up to 25-times the recommended level had no adverse effect on growth rate, liver weight or liver lipid content of laying hens (Balnave, 1975).

Ascorbic Acid

This vitamin is widely distributed in high concentrations in plants, particularly in citrus fruits and green vegetables. Birds are capable of synthesizing ascorbic acid in their kidneys but young chickens show insufficient synthesis and for growing poultry, endogenous production does not meet requirements under intensive husbandry conditions. Ascorbic acid is readily absorbed in the intestine at a high rate of up to 80%. Excess ascorbic acid or its metabolites are excreted with the urine. Ascorbic acid is concentrated in the adrenal and pituitary glands, in the brain, the eye lens and in leucocytes (Rostagno *et al*., 2000).

Vitamin C is a strong antioxidant and is able to quench free radicals and reactive oxygen. It is essential for the formation of intercellular substances namely connective tissues, bones, cartilage and supports wound healing via its key function, the hydroxylation of proline to hydroxyproline. Furthermore, due to its involvement in corticosteroid synthesis it has an

anti-stress activity and it stimulates defensive mechanisms such as phagocytic activity of leucocytes and formation of antibodies (Weber, 2009).

Classical vitamin C deficiency does not occur in poultry, but supplemental ascorbic acid was demonstrated to have beneficial effects under stressful conditions. In laying hens kept at high temperatures and in overcrowded conditions, supplemental vitamin C at 200ppm level reduced mortality slightly increased shell weight per unit surface area and improved Haugh units (Cheng *et al*., 1990). Vitamin E and C, both potent antioxidants, have been demonstrated to act synergistically in heat-stressed laying hens when monitored as serum levels of tri-iodothyronine (T_3) and thyroxine (T_4). These parameters were increased by supplementation; however adrenocorticotropic hormone (ACTH) was decreased (Sahin *et al*., 2002).

Vitamin C can exert beneficial effects under low ambient temperature conditions. Sahin *et al*. (2002) reported that supplemental vitamin C, either alone or in combination with chromium, significantly increased live weight change, egg production and improved feed efficiency in cold-stressed hens. Egg production and egg weight were also greater in each supplemental group compared with the unsupplemented control group. The benefits of vitamin C for health of laying hens were also demonstrated in combination with vitamin E (Puthpongsiriporn *et al*., 2001). Ascorbic acid, supplied as 1,000 ppm/kg feed during heat stress, generated the highest lymphocyte proliferative responses to *concanavalin A* and *Salmonella typhimurium* lipopolysaccharide in comparison to treatments with lower vitamin

supplementation. For these reasons, practical recommendations for commercial husbandry conditions should include vitamin C supplementation for laying hens.

Ascorbic acid plays important roles in the formation of connective tissue, bone and dentine; collagen synthesis, amino acid metabolism, aid in iron absorption and immunity antioxidant. Deficiency of this vitamin results in scurvy, which is characterized by swelling of the joints and gums, loosening of the teeth and haemorrhages of the skin and mucous membranes. Vitamin C is synthesized by poultry and is, accordingly, not considered a required dietary nutrient. However, there is evidence of a favourable response to vitamin C by birds under stress (Pardue, 1987; Nockels, 1988). Natural sources of this vitamin are fresh fruits and fresh leafy vegetables such as spinach, tomato juice, mango, orange, grape fruit juice, straw berries, snow peas, red bell peppers and broccoli.

2.3Requirements of the Various Vitamins for Chicken

Vitamins are always added to feeds in amounts that meet minimum dietary requirements. As intake and utilization of vitamins from natural sources is unpredictable due to differing content of vitamins in the feedstuffs and variable vitamin bioavailability, it is safer to cover the entire vitamins requirement of poultry through dietary supplementation (Weber, 2009).

Table 2.3.1 The NRC (1994) Vitamin Requirements' for Chickens and Turkeys

Animal	D (IU)	E (IU)	K (IU)	Thiamine	Riboflavin (mg)	Niacin (mg)
Leghorn Chickens						
Growing						
0 - 6 weeks	200	10	0.5	1	3.6	27
6 - 12 weeks	200	5	0.5	1	1.8	11
12 - 18 weeks	200	5	0.5	0.8	1.8	11
18 weeks to 1st egg	300	5	0.5	0.8	2.2	11
Broilers						
0 - 3 weeks	200	10	1	1.8	3.6	35
3 - 6 weeks	200	10	0.5	1.8	3.6	30
6 - 8 weeks	200	10	0.5	1.8	3	25
Turkeys						
0 - 4 weeks	1,100	12	1.75	2	4	60
4 - 8 weeks	1,100	12	1.5	2	3.6	60
8 - 12 weeks	1,100	10	1	2	3	50
12 - 16 weeks	1,100	10	0.75	2	3	50
16 - 20 weeks	1,100	10	0.75	2	2.5	40

Animal	**Pantothenic acid (mg)**	**Biotin (mg)**	**Folic acid (mg)**	**B_{12} (mg)**	**Choline (mg)**	
Leghorn Chickens						
Growing						
0 - 6 weeks	10	0.15	0.55	9	1,300	
6 - 12 weeks	10	0.1	0.25	3	900	
12 - 18 weeks	10	0.1	0.25	3	500	
18 weeks to 1st egg	10	0.1	0.25	4	500	
Broilers						
0 - 3 weeks	10	0.15	0.55	10	1,300	
3 - 6 weeks	10	0.15	0.55	10	1,000	
6 - 8 weeks	10	0.12	0.5	7	750	
Turkeys						
0 - 4 weeks	10	0.25	1	3	1,600	
4 - 8 weeks	9	0.2	1	3	1,400	
8 - 12 weeks	9	0.125	0.8	3	1,100	
12 - 16 weeks	9	0.125	0.8	3	1,100	
16 - 20 weeks	9	0.1	0.7	3	950	

Per Kg of diet

Source: DSM Nutritonal Products North America (2012)

Table 2.3.2 below gave an overview on the dietary levels recommended by the National Research Council (NRC, 1994), a German Trade Association (AWT, 2002) and a vitamin manufacturer (DSM, 2006). Table 2.3.3 gave the vitamin profile of the ingredients used in producing locally formulated vitamin mineral premixes (LFVMPs), which were used in the diet formulation. Table 2.3.4 gave the vitamin profile of dried chicken egg which was one of the materials used to compound locally formulated vitamin mineral premix 2.

Table 2.3.2 Recommended levels of all vitamins by NRC (1994), AWT (2002) and DSM (2006)

Vitamins	NRC[1] (1994)	AWT[2] (2002)	DSM[3] (2006)
Vitamin A (IU/Kg)	2,500 - 3,750	8,000 - 12,000	8,000 - 12,000
Vitamin D_3 (IU/Kg)	250 - 375	2,000 - 3,000	2,500 - 3,500
Vitamin E (mg/Kg)	6 - 8.	20 - 30	15 - 30
Vitamin K_3 (mg/kg)	0.4 - 0.6	2.0 - 3.0	2.0 - 3.0
Vitamin B_1 (mg/Kg)	0.6 - 0.9	2.0 - 3.0	15 - 30
Vitamin B_2 (mg/Kg)	2.1 - 3.1	5.0 - 8.0	4.0 - 7.0
Vitamin B_6 (mg/Kg)	2.1 - 3.1	3.0 - 5.0	3.0 - 5.0
Vitamin B_{12} (mg/kg)	0.004	0.015 - 0.025	0.015 - 0.025
Niacin (mg/Kg)	8.3 - 12.5	25 -40	20 - 50
Pantothenic acid (mg/kg)	1.7 - 2.5	8 - 10.	8 - 10.
Folic acid (mg/Kg)	0.2 - 0.3	1	0.5 - 1
Biotin (mg/Kg)	0.08 - 0.13	0.05 - 0.08	0.10 - 0.15
Vitamin C (mg/Kg)	0	100 - 150	100 - 200

[1]NRC Values are estimates of the minimum vitamin requirement in terms of total dietary (available) concentr based on a daily feed intake in the range of 80 - 120g per day.

[2] Commercial values are supplemental amounts to be added above the natural vitamin content of the diet based on a daily feed intake in the range of 80 - 120g per day.

Source: Weber (2009)

Table 2.3.3 Vitamin profile of the ingredients used in producing Locally Formulated Vitamin and Mineral Premixes (LFVMP)

Vitamins	Blood meal	Fish meal	Red pepper	Moringa o.	Whole hard egg
Thiamine (B_1)	0.40 ppm	0.7 ppm			0.066 mg
Riboflavin (B_2)	2.0 ppm	4.5 ppm	0.03 ppm		0.5 mg
Cobalamine (B_{12})	0.044 ppm	0.15 pp,			
Biotin	0.08 ppm	0.5 ppm			
Choline	750 ppm	3000 ppm			225 mg
Folic acid	0.10 ppm	0.2 ppm			44
Pyridoxine (B_6)	4.4 ppm	5.0 ppm	0.248 mg		
Pantothenic acid	1.1 ppm	7.0 ppm			1.4 mg
Vitamin E		10.0 ppm	0.69 mg	7.4 mg	
Niacin		38.0 ppm	0.5 mg	0.8 mg	
Vitamin A		4411.7 I.U/kg	5700 I.U		140
Pro-vitamin A				11,300 I.U	
Vitamin C				220 mg	
Vitamin B				120 mg	

Sources: USDA (1968), Price (2007), Aduku (2008)

Table 2.3.4 Vitamin profile of Egg (Dried)

Vitamins	Whole egg	Yolk	Stabilised white
Niacin (mg)	0.34	0.08	0.77
Riboflavin (mg)	1.98	1.26	3.71
Cobalamine (mcg)	3.39	6.02	0.18
Pantothenic acid (mg)	5.55	9.06	0.67
Vitamin A (I.U)	500	973	< 100
Thiamine (mg)	0.18	0.39	< 0.05
Pyridoxine (mg)	0.494	0.842	0.04
Folic acid (mg)	0.119	0.209	0.022
Vitamin E (I.U)	3.24	7.17	< 0.500
Vitamin D (I.U)	125	75.1	< 0.90
Lutein (mg)	0.572	0.737	< 0.02
Zeaxanthin (mg)	0.263	0.396	< 0.02

Source: USDA (1999)

2.4 Minerals in Poultry Diets

These are inorganic salts that regulate the metabolism of the body and are essential constituents of many vital substances within the body. They play vital roles in the health and well-being of poultry. They are divided into two groups based on the amounts needed by chickens, those require in large amounts are called macro-minerals namely sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^+), phosphorus (P) and magnesium (Mg). While those minerals required in small amounts are called micro or trace minerals namely zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), selenium (Se) and Iodine (I).

2.4.1 Macro-minerals

2.4.1.1 Calcium (Ca^+)

Calcium is the primary mineral that makes up egg shells and when not supplied in the diet, the hen does not have the basic minerals for shell formation. Calcium deficiency occurs worldwide in all species of poultry especially layers. Some of the clinical symptoms of deficiency are rickets in young birds, osteomalacia or cage layer fatigue in older layers. Vitamin D_3 is needed for proper metabolism of calcium and phosphorus and in the formation of normal skeleton, hard beaks and claws, and strong egg shells. Cage layer fatigue is a related condition observed in caged laying hens, usually around peak egg production that may also be associated with osteoporosis, a condition causing brittle bones as a result of reduced bone density (Huyghebaert *et al*., 2005).

Rickets is caused by a deficiency or imbalance of circulating Ca, vitamin D_3 or P, it can be caused by an imbalanced or deficient diet, some medications and some mould toxins. Cage

layer fatigue is thought to be caused primarily by depletion of body stores of Ca as a result of delay in feeding high Ca feeds during high egg production or a metabolic malfunction that impairs calcium absorption or bone calcification during this production stage. These nutritional deficiencies can be prevented and treated as follows: for normal bone calcification, Ca and P need to be supplied not only in adequate amounts but in a ratio to each other of about 2:1. Thin shells are observed when Ca, P and vitamin D_3 are not provided in the diets at adequate levels. It is more often observed during periods of hot weather because Ca is conserved and retained within the hen's body less efficiently(Schwartz, 1994).

The quality of the shells is improved by feeding a complete laying ration as the only diet. This diet supplies all nutrients in the proper proportions so the hen can produce good shells. If thin egg shells become a problem, it is advisable to add 9.072×10^{-4}metric unit of oyster shells to every 4.54×10^{-2}metric unit of complete layer ration. It is also advisable to add a vitamin supplement to the drinking water while oyster shell is being added to the feeds. This will help ensure that Ca and P in the diet is being properly absorbed through the digestive system and will be available for deposition as shell on the egg (Anonymous[1], 2010). These minerals play vital roles in the formation of bones, egg shell and enhance hatchability in breeders and support blood clotting in chickens. Deficiency of these minerals results to poor egg shell quality, poor hatchability and rickets in poultry. Natural sources of Ca^+ are milk, yogurt, cheddar cheese, Swiss cheese, tofu, sardines, green beans, spinach and broccoli (Scheideler, 2008).

2.4.1.2 Phosphorus (P)

Phosphorus and calcium are sister minerals, phosphorus is vital in the formation of cells, bones, teeth and egg shell. They also maintain acid – base balance in poultry. The main source of the birds P ration comes from plants, in the form of phytate Phosphorus. Research on Ca utilization is more common than P in growing birds despite the importance of P in the diet. Phytate is a compound found in many common feed ingredients that decreases nutrient availability in animal diets. The main anti-nutritional effect of phytate is that it makes phytate phosphorus unavailable for digestion and absorption by non-ruminants such as swine and poultry (Carlyle, 2005).

Phytate also has negative effects on digestive enzymes, trace minerals, calcium, protein and amino acids and carbohydrates. Phytase is an enzyme that breaks down phytate. Phytase is produced in limited amounts in animals but is commercially available. When added to swine or poultry diets, the levels of phosphorus and calcium can be reduced by approximately 0.10% of the diets. Commercially, the primary use of phytase is to increase the availability of phosphorus, which reduces the amount of inorganic phosphorus added to the feed and consequently reduces amount of phosphorus in the manure (Carlyle, 2005).

Phytase (Nutri-phytase-5000) as an enzyme is capable of breaking down phytate in feeds to release inorganic P and inositol as well as protein, amino acids, trace mineral and othernutrients chelated with phytate. Phytase can reduce or eliminate the supplementation of inorganic P in feeds for monogastric animals and improve the utilization efficiency of these nutrients contained in feedstuffs. As much as 90% of the total P in cereals and oil

seeds can be locked up in the form of phytate, which is virtually indigestible form of P in plants used in animal feeds(Leytem *et al*., 2007).

Nutri – phytase 5000 obtained from *Aspergillusniger*, is a 3 – phytase, meaning the phosphates are released from the phytate beginning at the carbon – 3 of the inositol ring which efficiently breaks down phytate. Phytase lowers the P excreted in manure by releasing the phytate-bound P found naturally in grains and soybeans. Like poultry, swine lack sufficient intestinal phytase, by adding the enzyme to the diet more P become available to the animal. This lowers the amount of supplemental P required and the amount that goes undigested. Deficiency of phosphorus results in rickets, poor egg shell quality and hatchability. Natural sources of this mineral are meats, fish, eggs, milk; besides other animal foods(Huyghebaert *et al*., 2005).

2.4.1.3 Sodium (Na^+)

Food intake and growth rate of broilers kept at high temperatures can be improved by supplementing the diet or drinking water with sodium bicarbonate. The response appears to be due to the bicarbonate ion and is associated with an increase in water consumption (Ryssen and Ndlovu, 2004). Male broiler chicks were fed all plant protein diets with soybean meal or a mixture of soybean and canola meals as the source of supplementary protein from 0 – 4 weeks of age. Supplementation of the diets with 0.25% sodium chloride did not meet the requirements of the chicks for maximum growth. Growth was accelerated when the dietary sodium chloride was increased to 0.50 and 1.00% (March, 1984). Sodium maintains fluid and electrolyte balance of chickens, supports muscles contraction and nerve

impulse transmission. Natural sources of sodium are salt, soy beans, sauce, bread, milk, meats.

2.4.1.4 Potassium (K^+)

Potassium is the third most abundant element of the animal body and the main intracellular cat ion (McDowell, 1992). It participates in the processes that are essential to the body homeostasis such as acid-base equilibrium or balance, osmotic pressure regulation, development of membrane potentials of cells, activation of numerous intracellular enzymes, glucose and amino acids absorption and transport (Rhinehart *et al*., 1968; Reece, 1996; Leeson and Summers, 2001). Potassium does not work alone, the correct balance between sodium, potassium and chloride is necessary for best animal performance, bone development, egg shell quality and amino acid composition (NRC, 1994).

According to Murakami (2000), an EB between 150 and 350 mEq/kg is recommended in commercial diets for maximum bird performance. Leeson and Summers (2001) considered 250 mEq/kg as an appropriate level for adequate poultry development. Hooge and Cummings (1995) stated that the 1994 NRC recommendation on potassium dietary requirements for growing poultry were considerably lower than the levels typically present in commercial diets. No increases in potassium requirements were indicated when environmental temperature, water intake or stress conditions were increased or vice versa.

Studies using broilers from 7 – 21 days estimated 0.824% potassium as the K requirement with 0.15 – 0.17% Na in a practical type diet, which indicates approximately a 1:1 Na:Kratio

as optimal and with EB around 242 mEq/kg (Hooge and Cummings, 1995). Thus, this information suggested that the K requirement for maximum body weight gain (BWG) could be higher than the 0.30% level recommended by 1994 NRC. Rostagno *et al.* (2000) suggested that 0.501, 0.471 and 0.454% of K were adequate for EB in the periods from 1 – 21, 22 – 42 and 43 – 49 days respectively. Potassium maintains fluid and electrolyte balance, cell integrity, muscle contractions and nerve impulse transmission in poultry. Natural sources of potassium are potatoes, acorn squash, artichoke, spinach, broccoli, carrots, green beans, tomato juice, avocado, grape fruit juice, water melon, banana, straw berries, cod and milk.

2.4.1.5 Chloride (Cl^-)

It is well known that excessive intakes of sodium chloride are toxic to poultry. Hamilton and Thompson (2003) conducted a 3 x 6 factorial experiment involving 3 strains of single comb white Leghorn hens and 6 dietary levels of Na plus K to Cl (Na + K) / Cl) of 0.40, 0.91, 1.92, 2.83, 4.04 and 7.69 using 108 individually caged hens. Six corn – soybean meal – brewers' dried grains diets included comparisons among low and high levels of Na and Cl. Feed and distilled water were provided *ad libitum* from 403 – 487 days of age. Egg and shell measurements were taken on eggs laid between 403 – 487 days. At the completion of the experiment, blood pH,partial carbon dioxide ($pCo_{2)}$, partial oxygen(pO_2) and plasma Na, K, Cl, Ca, P and Mg were measured for each hen. Blood bicarbonate was calculated from the pH and pCo_2 values. Much of the variation in blood gas levels and 485 day body weight could be attributed to the effects of the 0.40% diet. The effects of dietary sodium and chloride levels appeared independent of each other with decreasing levels of Na and

increasing levels of Cl reducing blood pH, pCo_2 and bicarbonate. These ions had the reverse effects on plasma K. Significant differences in feed intake, feed efficiency and egg production were apparently, due to extremely poor performance of birds fed the 0.40% diet.

Differences among strains were significant for feed intake, productivity, egg and shell weight, % shell, shell weight per unit surface area and egg specific gravity,but were not significant for blood, gas and plasma inorganic ion levels, nondestructive deformation, compression fracture force and interior quality. Increasing dietary Cl levels significantly reduced deformation and fracture force. There were few dietary (Na + K) / Cl level x strain interaction. Partial correlation coefficients indicated very relationships (r = - .19 to .14) between blood pH or bicarbonate and specific gravity, deformation or fracture force. The results of this experiment indicate that the acid – base balance of laying hens was influenced by the (Na + K) / Cl level of the diet. There was no relationship between acid – base balance and egg shell strength (Leytem *et al*., 2007).

Chloride maintains fluid and electrolyte balance of poultry and also aid in digestion. Natural sources of chloride are salt, soysauce, milk, eggs and meats. Na^+, K^+ and Cl^- are closely related in the body and occur as ions in the body fluids; Na^+ in the extracellular fluids and K^+ intracellularly. Together with other salts, those of Na^+ and K^+ regulate the acid-base balance. Chlorine is a constituent element of hydrochloric acid, which is important in digestion. Na^+, K^+ and Cl^- occur in practically all natural foods. In most foods, however, there is more K^+ than Na^+(Weber, 2009).

2.4.1.6 Magnesium (Mg)

Magnesium is involved in many biochemical processes including activation of phosphates and in carbohydrate metabolism. Magnesium supports bone mineralization, protein synthesis, muscular contraction, nerve impulse transmission and immunity (Reddy *et al*., 1973). Its action is closely associated with Ca and P (Chou *et al*., 1979). In formulating a daily ration for broiler chickens younger than 28 days, the amount of Mg has been found to be increased especially when additional Ca and P are fed. Disregarding this interaction retards growth and causes malformation of the legs as well as high mortality (Lee *et al*., 1980).

Atteh and Leeson (1983) suggested that even the hardness and Mg content of the drinking water should be considered to ensure a proper balance of these elements in young broilers.
Mg supplementation of the diet of chickens and pigs has benefits when fed at different periods of age and of production or before a situation causing stress to the animals. The precise determination of Mg requirements of farm animals is necessary, depending on the stage of growth, performance and reproduction of the animals. Excess Mg may alter their Ca and P, just as it moderates Ca surplus (Pointillart, 1989). It is assumed that the new breeds of high producing farm animals or hybrids require more nutrients and minerals than the former races (Thielscher, 1990). Mg supplementation has remarkably improved the digestibility of feed. Deficiency of Mg results to sudden death. Natural sources of mg are spinach, broccoli, artichokes, green beans, tomato juice, navy beans, pinto beans, black-eyed peas, sunflower seeds, tofu, cashews and halibut(Price, 2007).

2.4.2 Trace Minerals

Trace mineral nutrition has a rich history of discovery and research in the field of poultry nutrition. Many of the early basic nutrient metabolism studies were conducted in chicks and then related to other livestock species and humans. The trace minerals of primary concern in poultry diets and having recommended levels of supplementation by the 1994 NRC Nutrient Requirements of Poultry include zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), selenium (Se),iodine (I) and cobalt (Co). The trace minerals typically supplemented in poultry premixes include Zn, Mn, Cu, Fe and I. Selenium is very often supplemented either in the premix or separate from the premix formulation. Trace mineral premixes should be formulated and supplemented to poultry feeds separate from the vitamin premix due to potential vitamin oxidation by the trace minerals (Scheideler, 2008).

2.4.2.1 Zinc (Zn)

Microbial phytase supplementation of a low phosphorus diet increased growth and relative retention of total calcium, phosphorus, copper and zinc and improved bone mineralization in broiler chickens. Dietary zinc stimulates immunity and supports skeletal development, feathering and skin strength. Feeding highly bioavailable sources of zinc such as zinc complexes to broilers showed improvements in weight gain and feed conversion. Zinc is an essential trace element in all living systems from bacteria, plants and animals to humans. The biological function was first understood in 1934; it took another 20 years before naturally occurring zinc deficiency was recognized as the cause of parakeratotic (hardening and cracking) lesions of the skin of pigs, slow growth, poor feathering and abnormal skeletal development in poultry (Sebastian *et al*., 1996).

Wiebe (2011) reported that zinc deficiency in poultry was associated with footpad lesions and poor carcass quality due to scratches and skin damage. This made poultry producers and nutritionists realize that adequate dietary zinc supplementation is critical in poultry production. Zinc is primarily absorbed in the small intestine; it is known that binding forms and other dietary ingredients can influence the bioavailability of zinc. In monogastric animals, phytate for example decreases zinc absorption.Dietary calcium, magnesium, phosphorus, nickel, copper and iron also affect zinc bioavailability,because of the low zinc content in some feed ingredients with varying levels of bioavailability, it is necessary to add zinc to poultry diets. In the European Union, up to 150 mg/kg feed of zinc is allowed. These levels cover largely the recommended requirements for most animals, especially when highly bioavailable zinc complexes are supplemented. When feeding zinc complexes within these legal limits, it helps decrease the severity of footpad and skin lesions as well as improves feathering condition and skeletal development. Trials conducted in various parts of World clearly indicate that zinc complexes have a positive effect on body weight and feed conversion in broilers, as well as egg production and shell quality in laying hens. These effects are more notable during heat stress (Sebastian *et al*., 1996).

Zinc plays an important role in poultry, particularly layers, as a component of a number of metalloenzymes such as carbonic anhydrase which is essential for egg shell formation in the hens shell gland. Other important zinc metalloenzymes in the hen include carboxypeptidases and DNA polymerases. These enzymes play important roles in the hen's immune response, in skin and wound healing, and for hormone production (testosterone and

corticosteroids). Classic deficiency symptoms of zinc in poultry could include a suppressed immune, poor feathering and dermatitis, infertility and poor shell quality. Natural sources of this mineral are spinach, broccoli, green peas, green beans, tomato juice, lentils, oysters, shrimps, crabs, turkey (dark meat), lean ham, lean ground, beef, lean sirloin steak, plain yoghurt, Swiss cheese, tofu and ricotta cheese. Zinc from plant sources is not as available for use by the body as the zinc from animal sources (Touchburn*et al*., 1996).

2.4.2.2 Copper

Copper (Cu) is often added to poultry diets at prophylactic concentrations for its growth promoting effects. Cu from various compounds has often been added to poultry diets as an antimicrobial agent. Three experiments were conducted to study copper sulphate ($CuSo_4$) and tribasic copper chloride (TBCC) as sources of supplemental Cu for poultry. In experiment one, 252 chicks were fed the basal corn-soybean meal diet (26 ppm Cu) supplemented with 0, 150, 300 or 450 ppm from $CuSo_4$ or TBCC for 21 days. Chicks fed 450 ppm Cu from sulphate had lower ($P < 0.05$) feed intake than those consuming other diets (Sebastian *et al*., 1996; Miles, 1998).

Feeding supplemental Cu increased ($P < 0.0001$) liver Cu concentration linearly with increasing dietary Cu regardless of Cu sources. In experiment two, a 42 day floor pen study was conducted with 1,260 broiler chicks given the basal corn – soybean meal diet supplemented with 0, 200, 400 or 600 ppm Cu from either feed grade $CuSo_4$ or TBCC. Body weight and feed conversion did not differ in birds fed up to 400 ppm Cu from either source. Birds given 600 ppm Cu from either source had lower feed intake, poorer growth

and feed conversion ($P < 0.0001$). Liver Cu increased ($P < 0.0001$) linearly with increasing dietary Cu. In experiment three, Cu sources were added to broiler starter diets at concentrations of 25, 100 and 300 ppm Cu and diets were stored at an elevated temperature to examine the effect of particle size on oxidation (Miles, 1998).

Copper plays an important role in a number of enzyme functions in the bird, Cu is closely associated with iron metabolism as it is a part of ceruloplasmin which is an enzyme that plays an important role in the oxidation of ferrous to ferric iron, controlling the movement of iron from the reticulo-endothelum to liver and them plasma affecting red blood cell formation. Another important enzyme dependent on Cu is Lysyloxidase, which is an integral enzyme in elastin and collagen formation in birds. A deficiency of Cu can cause bone abnormalities due to abnormal collagen synthesis. Tibia dyschondroplasia is an example of a leg disorder in poultry that can be caused by Cu deficiency. Poor collagen and or elastin formation can also lead to cardiovascular lesions and aortic ruptures. Cu is vital for feather development as well as feather colour via its role in disulfide bond formation (Sheila, 2008).

2.4.2.3 Iron (Fe)

Iron is part of the protein haemoglobin which carries oxygen throughout body cells of chickens and other animals. The amount of iron absorbed from the diet depends on many factors: Iron from meat, poultry and fish such as haeme iron is absorbed 2 – 3 times more efficiently than iron from plants which is non-haeme iron. Foods containing haeme iron

(meat, poultry and fish) enhance iron absorption from foods that contain non-haeme iron,such as fortified cereals, some beans and spinach (Sheila, 2008).

Iron is present in all cells and has several vital functions, as a carrier of oxygen to the tissues from the lungs in the form of haemoglobin, as a transport medium for electrons within the cells in the form of cytochromes and as an integral part of enzyme reactions in various tissues. Any internal infection such as coccidiosis can also interfere with iron absorption and availability Iron deficiency results to microcytic and hypochromic anaemia and sideropenia or hypoferremia is one of the most common of the nutritional deficiencies. The natural sources of this mineral are artichoke, parsley, spinach, broccoli, green beans, tomato juice, tofu, clams, shrimps and beef liver (Miles, 1998; Sheila, 2008).

2.4.2.4 Manganese (Mn)

Manganese plays a significant role in the chicken's body in the formation of chondroitin sulphate. This mucopolysaccharide is an important component of bone cartilage. Excess dietary phosphorus can interfere with Mn availability in poultry. Deficiency of Mn in the diet of young growing chickens is one of the causes of perosis, thin-shelled eggs and poor hatchability. Calcium, phosphorus and vitamin D_3 can also cause these symptoms and chondrodystrophy. Prevention of perosis requires diets adequate in all necessary nutrients especially Mn, choline, niacin, biotin and folic acid. Deformities cannot be corrected by feeding more Mn but effects of Mn deficiency on egg production are fully corrected by a diet that contains Mn at 30 – 40 mg/kg, provided that the diet does not contain excess calcium and phosphorus (Leytem *et al.*, 2007).

2.4.2.5 Selenium (Se)

Selenium is a very unique trace mineral in the chicken's diet in that its inclusion rate is regulated and limited. Se has a special place among the feed-derived natural antioxidants, being an integral part of selenoproteins participating in the regulation of various physiological processes in the body. As part of glutathione peroxidase (GSH-PX) Se belongs to the first and second major levels of antioxidant defense in the cell. There are two major sources of Se for poultry organic selenium, mainly in the form of selenomethionine (SeMet), which can be found in any feed ingredient in varying concentrations and inorganic selenium, mainly selenite or selenite, which are widely used for dietary supplementation (Surai, 2002). Se deficiency and excess in modern poultry production are very rare, in general adequate Se supplementation is considered to be crucial factor in maintaining the high productive and reproductive characteristics of commercial poultry. Dietary Se works with vitamin E in boosting the immune status of poultry (Leytem *et al*., 2007).

2.4.2.6 Iodine (I)

Typically, poultry diets contain 1 – 2 mg I/kg, but higher concentrations are sometimes used to enhance the iodine content of eggs. In addition to an increased deposition of Iodine in the yolk, other often adverse responses occur, especially at exceptionally high concentrations. Excess iodine in grower's diets can prevent sexual maturity in male and female fowls and in layer's diets it will progressively reduce egg production. Beside these, ovulation is inhibited and egg production ceases (Lewis, 2004).

Most iodine accumulates in the thyroid gland and it is likely that the mechanism responsible for these reproductive disorders involves a modification of thyroid hormone activity. Simultaneously, with the declining rate of lay, feed intake declines, egg weight and yolk cholesterol contents decrease and the body weight increases. Whereas fertility is unaffected in female breeders, hatchability of fertile eggs is reduced, hatch time extended and embryonic mortality and dead in shell proportions increased. In contrast, male fertility is decreased because of an increased incidence of dead spermatozoa, although hatchability of eggs from normally fed hens is unaffected (Scheideler, 2008).

All reproductive variables together with feed intake and body weight are normalized within about 7 days of returning to a diet with normal iodine levels. Excess iodine suppresses growth in meat-type chickens, but does not affect feed conversion efficiency. There are transient increases in plasma iodine and cholesterol concentration during excess iodine intake in all types of birds. Sources of iodine are calcium iodate, seaweed, dairy products, poultry and beef (Lewis, 2004; Scheideler, 2008).

2.4.2.7 Cobalt (Co)

Cobalt increases the red cell mass in both man and animals by increasing the production of erythropoietin. Since meat – type chickens can develop pulmonary hypertension from increased erythropoiesis and polycythaemia. Feeding cobaltous chloride to broiler chicken at 500 parts/ 10^6 to meat type chickens from 1 day old to 42 days significantly increased haemoglobin content and to a lesser extent red blood cell count and haematocrit (Rostagno *et al*., 2000). No effect was observed on mean corpuscular volume, increased haemoglobin

content was linearly correlated with pulmonary hypertension as measured by the right ventricle weight to total ventricle weight ratio (RV: TV). Levels of malondialde – hyde in cardiac tissue were also correlated with the RV: TV ratio, suggesting that peroxidative damage may be related to ventricular hypertrophy. Chickens fed cobalt showed a significantly higher incidence of right ventricular hypertrophy and right ventricular failure and 18.3% developed ascites (Diaz, 1994).

Cobalt is not considered as an essential mineral for chickens although it may be as much as 4% of the composition of the molecule of vitamin B_{12}. Some authors consider cobalt addition for laying hens unnecessary and the mineral is supplemented only as vitamin B_{12} (NRC, 1994). Rostagno *et al.* (2000) recommended 0.2 ppm of cobalt in the diet for laying hens. However, there is no indication that this trace mineral is unnecessary for laying hens. Birds synthesize vitamin B_{12} using cobalt inside the caeca, but the levels are below the requirements and it must be supplemented. There is no consensus about cobalt supplementation in chicken diets. In practice, the industries of mineral supplement add on average 0.29g of cobalt per tonne of feed. Considering the high price of cobalt, adding it may represent an additional cost for poultry production(McDonald *et al*., 1975).

Cobalt concentration was determined in the yolk and liver by dry matter basis using Flame Atomic Spectrometry. Cobalt supplementation had no effect ($p > 0.05$) on egg quality. This indicated that cobalt supplementation was not necessary in order to improve internal and external egg quality (Aduku, 2004). There was no interaction ($p > 0.05$) between supplementation of cobalt and supplementation of vitamin B_{12} on egg quality parameters.

Cobalt supplementation in the diets of laying hens in the second cycle of production did not influence egg production, egg quality, blood characteristics and cobalt levels in the liver and yolk within the trial period; suggesting that there was no need for cobalt supplementation however vitamin B_{12} supplementation increased egg weight (Kato *et al*., 2003).

2.4.3 Mineral profile of the ingredients used in formulating local premixes

2.4.3.1 Macro - minerals

Minerals	Blood meal (%)	Fish meal (%)	Red pepper (mg)	Moringa oleifera (mg)	Cage layer litter (%)	Wood ash (g/kg dry ash)	Whole hard egg (mg)
Calcium	0.28	5.5	9	440	7.53	9.9	50
phosphorus	0.22	2.81	19	70	2.85		172
Sodium	0.32	0.9			0.18	2.5	
Chloride	0.3	0.6					
Potassium	0.09	0.7	177		1.53		126
Nitrogen					4.3		

Sources: USDA (1968), Materechera (2002), van Ryssen and Ndlovu (2004), Price (2007), Aduku (2008)

2.4.3.2 Micro - minerals

Minerals	Blood meal (ppm)	Fish meal (ppm)	Red pepper (mg)	Moringa oleifera (mg)	Cage layer litter (ppm)	Wood ash (g/kg dry ash)	Whole hard egg (mg)
Copper	10	11		110	67	246	
Iron	3700	540		7			1.2
Zinc	100	150			1151	175	
Manganese	5	35			1736	541	
Magnesium	2200	14	10			19.8	
Iodine	0	2					
Selenium	0.22	2.2					
Cobalt	0.08	0.2			67		

2.5 Vitamins and minerals in poultry diets

Bennett (2005) reported that, vitamin-mineral premixes provide a broader range and higher levels of vitamins and minerals than possible by using 'old fashioned' ingredients such as milk, green feed and fish oil. Many organic certifying organizations will not allow fish oils to be added to poultry diets even though they can be good sources of vitamins A and D, because it causes rancidity. Use of a commercial vitamin-mineral mix is strongly recommended. The manufacturer's recommendation for the amount of vitamin – mineral premix to add to the feed should always be followed. Some vitamin-mineral premixes contain iodine and will allow non-iodized salt to be used in the ration. He further reported that, phytase has been shown to increase the availability of some trace minerals such ascopper, manganese, iron and zinc. This was as a result of the positive effect of phytase on trace mineral utilization, commercial use may lead to removing trace minerals in diets where phytase is added.

Research has been conducted on the effect of phytase with or without the trace mineral premix in diets for chicks from hatch to 42 days and in diets for Pigs at different stages of growth. The trace mineral premixes used at the swine and poultry farms contain zinc, copper, iron, manganese, iodine and selenium. Research results indicated that removing the trace mineral premix from Poultry diets from hatch to 42 days has no effect on growth performance, but it does have negative effects on bone strength. This negative effect was not overcome with the addition of phytase, indicating that phytase may not be able to replace the trace mineral premix in diets for broilers(Ryssen and Ndlovu, 2004).

In comparison to other species, chickens are more susceptible to vitamin deficiency because gut flora of chickens provide very little vitamin synthesis but compete with the host for dietary vitamins and intensively kept chickens undergo many stresses (Anisuzzaman, 1993). Vitamin A, D, Riboflavin and B_{12} are usually low in poultry diets. However, addition of other vitamins to poultry diets is a good insurance to protect birds from deficiency diseases and disorders. Vitamins D and B_{12} are almost completely absent from diet based on corn and soybean meal. Vitamin K is generally added to poultry diets more than to those for other species because birds have less intestinal synthesis due to a shorter intestinal tract and faster rate of passage of food (Wenk, 2000).

Deficiency of vitamins and minerals cause various diseases such as xerophthalmia, cage layer fatigue and rickets. Anisuzzaman (1993) observed deficiency diseases of various natures in broiler flocks in spite of adding vitamin-mineral premix in the experimental diets. This was probably due to poor quality of premix used for the experimentation. Supplementing Vitamin E in well balanced diets has shown increase of humoral immunity for monogastric species (Langweiler *et al*., 1983; Wuryastuti *et al*., 1993). The importance of certain trace minerals in immune function has become increasingly evident. Selenium, copper, zinc and iron have been shown to alter various components of the immune system (Suttle and Jones, 1989).

Poultry under intensive production systems are particularly susceptible to vitamin-mineral deficiencies and it is a general practice to include all supplemental vitamins and minerals in a premix at the levels that will provide margins of safety which are adequate under all of the

various stress conditions to which chickens may be exposed (Scott *et al*., 1982). Especially birds in cages require more attention for supplying vitamin-mineral premix than those on floor housing because of more limited opportunity for coprophagy.

Islam *et al*.(2004) conducted a study on the effect of vitamin-mineral premix supplementation on body weight gain and certain haemato-biochemical parameters in 20 broiler chickens of Shaver star Bro strain, aged 20 days old. The chickens in three groups were fed with a commercial ration supplemented with vitamin-mineral premix (Provita$^{A®}$,Arifs Bangladesh Ltd at 1, 2 and 4% of total feed for a period of 21 days. Significantly higher body weight gain was recorded on 7th, 14th and 21st days of the experiment in all the supplemented groups in comparison to control. Among the treated groups, highest body weight gain was recorded in 4% vitamin-mineral supplemented group and lowest in 1% supplemented group at 21st day of experiment. It was therefore, suggested that supplementation of vitamin-mineral premix with commercial poultry ration is essential for proper growth and body resistance of poultry.

The NRC periodically publishes nutrient recommendations for poultry and other species. Controlled studies often do not include stresses often encountered in a commercial environment. Stress due to heat, feed processing, feed restriction in combination with vaccination, coccidiosis, *Escherichia coli* infection, mold and mycotoxins, peroxides and overcrowding can influence nutritional requirements and the NRC does not include overdoses to account for stress related requirement (Ward, 1993). Users of the NRC requirements should view the stated ‘requirements’ as working approximations. The

requirements for vitamins depend upon the measurements that define the need as well as the source of the birds and the environmental conditions under which they were raised. Some studies have shown that profitability for poultry grown under common stress conditions is greatly improved by increased vitamin supplementation. Vitamin supplementation as high as 25% above the 1994 NRC recommendations have resulted in higher profitability (Ward, 1993).

The dynamics of vitamin allowances (amount fed) have paralleled the dynamics of vitamin requirements (minimum amounts needed). An allowance today may become a requirement tomorrow. The dynamics of vitamin fortification – the best assurance for arriving at optimum vitamin allowances for poultry coincide with the dynamics of vitamins allowances. To ensure that poultry are fed the amounts of vitamins needed to prevent deficiencies and allow optimum performance, the vitamin fortification levels in poultry diets should be reviewed and (or) adjusted periodically in accordance with the latest meat and egg production and marketing methods, farm conditions and vitamin nutrition knowledge. A balanced vitamin fortification programme for meeting requirements of poultry under a wide range of feeds and different production systems will more than offset the cost of adding vitamins (NRC,1994).

Ceylan and Scheideler(1999), Monslave *et al.*(2004) and Lesson (2005) reported that trace mineral nutrition have a rich history of discovery and research in the field of poultry nutrition. Many of the early basic nutrient metabolism studies were conducted in chicks and then related to other livestock species and humans. The bulk of this work was conducted and

reported in the era from 1960 – 1980. Nutrient requirements were established for each species of poultry and functions of those nutrients, trace minerals were also researched into and reported. In the past 25 years, trace minerals role in immune function and related physiological roles have been studied. New organic sources of trace minerals have been patented and marketed providing a more available form of trace minerals for the chicken or turkey. The complexity of trace mineral nutrition requires a thorough review of functions, interactions and availability of sources from time to time by the poultry producers or nutritionists.

The objective of supplementation with trace minerals is to avoid a variety of deficiencies diseases. Trace minerals carry out key functions in relation to many metabolic processes, such as catalysts for enzymes and hormones, and are essential for optimum health, growth and productivity. Deficiency of trace minerals affect many metabolic processes and so may be manifested by different symptoms such as poor growth and appetite, reproductive failures, impaired immune responses and general ill-thrift (Ryan *et al*., 2002).From the 1950s to the 1990s, most trace minerals supplementation of animal diets was in the form of inorganic minerals and these largely eradicated associated deficiency diseases in farm animals. In these decades, global food and animal production has intensified and genetic potential for growth and yields has improved. As a result commercial tendencies have been focused to increase trace mineral supplementation, in order to allow for the greater mineral requirements of superior stock reared under industrial conditions. Increasing the concentration of inorganic minerals in animal diets has led to several problems (Atteh and Leesons, 1983).

The use of high Cu in poultry rations has caused accidental Cu poisoning in more sensitive animals, such as sheep grazing pastures fertilized with poultry manure. Secondly, inorganic minerals may form insoluble complexes with other dietary agents resulting in low absorption (Lee *et al*, 1980). Research in trace element nutrition has led to the development of more bioavailable organic minerals, including trace minerals derived from chelates (Ryan *et al*., 2002; Ao*et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of Study Area:

The study was carried out in Kuregu Wusasa, Zaria situated in Zaria Local Government Area of Kaduna state, Nigeria**.** The geographical coordinates are latitude $11^0$5´ north, longitude $7^0$41´east and altitude 747M (Nigeria Google Satellite Maps, 2013).

3.2 Premix Formulation

Simple hand mixing technique was used in the formulation of the local vitamin – mineral premixes. The chemical compositions of the feedstuffs for the initial formulation were derived from literature and consultation with researchers that have worked with such feedstuffs.

Locally Formulated Vitamin and Mineral Premix one (LFVMP1) contained the following ingredients blood meal, fish meal, wood ash and red pepper (*Capsicum annuum*) were all weighed in kilogrammes. Diet 4 in the trials contained 2.30 kg (half dose) of the LFVMP 1 while diet 5 contained 4.60 kg (full dose) of the LFVMP 1. LFVMP 1 was used as a template in formulating LFVMP 2 with varying ingredients from local sources namely blood meal, fish meal, whole hard egg, cage layer litter and *Moringa oleifera* leaves. Diet 6 in the trials contained 2.30 kg (half dose) of the LFVMP 2 and diet 7 contained 4.60 kg (full dose) of LFVMP 2.

Tables 3.1represent the compositions of premixes used in diets 4 and 5 while Table 3.2 represents what was used in diets 6 and 7. Diet 1 was the negative control; no premix was

included in the diet,while in diets 2 and 3 Commercial Vitamin and Mineral Premix (CVMP) was included at 0.125 kg (half dose) and 0.25 kg (full dose) in the diets. These diets (1 – 7) were subjected to the same treatments right from the chick, grower and laying phases of the birds.

Table 3.1: **Locally Formulated Vitamin and Mineral Premix (LFVMP) One**

	Weight in Kilogrammes	
Sources	**50% (Half dose)**	**100% (Full dose)**
Blood meal	1.30	2.60
Fish meal	0.50	1.00
Wood ash	0.25	0.50
Red pepper	0.25	0.50
Total	**2.30**	**4.60**

Table 3.2 Locally Formulated Vitamin and Mineral Premix (LFVMP) Two

	Weight in Kilogrammes	
Sources	**50% (Half dose)**	**100% (Full dose)**
Blood meal	0.65	1.30
Fish meal	0.50	1.00
Whole hard egg	0.65	1.30
Cage layer litter	0.25	0.50
Moringa oleifera	0.25	0.50
Total	**2.30**	**4.60**

3.3 Determination of Proximate Composition of Commercial Vitamin – Mineral Premix and Locally Formulated Vitamin Mineral Premixes.

The proximate composition of Commercial Vitamin and Mineral Premix and Locally Formulated Vitamin and Mineral Premixes were carried out according to the methods described by AOAC (1990). These samples were analyzed in two laboratories namely Animal Science Biochemistry Laboratory and Central Laboratory of National Animal Production Research Institute (NAPRI) Shika, Zaria.

3.4 Determination of Vitamins in Commercial Vitamin Mineral Premix and Locally Formulated Vitamin Mineral Premixes.

Five vitamins were determined out of thirteen vitamins required by poultry. Samples of CVMP and LFVMP 1 and 2 were taken to the laboratory of National Research Institute of Chemical Technology (NARICT) Basawa, Zaria for analyses. These samples were subjected to wet chemical digestion and the solutions were used for analyses (Amadi*et al*., 2004) . Colour reactions for vitamins were used to determine the presence of vitamins in the different premixes. Specifically the following vitamins were determined: vitamin A (retinol), vitamin B_2 (riboflavin), vitamin B_6 (pyridoxine), vitamin B_9 (folic acid) and vitamin E (tocopherol) using Ultraviolet Spectrophotometer (UV – S 6405) and were incubated for 5 minutes at 37^oC.

3.4.1 Drummond's Test for Vitamin A

In this test, concentrated sulphuric acid (H_2SO_4) as a dehydrating agent takes up water from retinol to give a coloured product. This constitutes the basis for the determination of vitamins in various natural materials. To five test tubes containing two drops of respective solutions of commercial vitamin mineral premix, locally formulated vitamin mineral premixes 1and 2 from digested solid samples, five drops of chloroform and 2 drops of concentrated sulphuric acid were added. A blue colouration of the solution was observed which gradually turned to brownish red (Amadi*et al*., 2004) . The absorbance of the solutions were read in a spectrophotometer at a maximum wavelength of 325.00 nanometer (nm), for vitamin A determination.

3.4.2 Determination of Riboflavin (Vitamin B_2)

Riboflavin forms an orange – red coloured complex on reaction with diazophenylsulphonic acid in an alkaline medium. To five test tubes containing two drops of commercial vitamin – mineral premix and locally formulated vitamin – mineral premixes 1 and 2 solutions, five drops of sulphanilic acid solution and five drops of sodium nitrite solution were added. The entire content of the test tubes were shaken and orange – red colour was observed (Amadi*et al*., 2004). The absorbance of the solutions was measured spectrophotometrically at a wavelength of 261.00 nm, to determine vitamin B_2content.

3.4.3 Determination of Pyridoxine (Vitamin B_6)

The reaction of pyridoxine with diethylthiocarbamate in an alkaline medium gives a blue – coloured complex.To five test tubes containing two drops of commercial vitamin – mineral premix and locally formulated vitamin – mineral premixes 1 and 2, eight drops of sodium diethylthiocarbamate and four drops of sodium hydroxide solutions were added. The test tubes were shaken and characteristic blue colouration was observed (Amadi*et al*., 2004). The absorbance of the solutions was measured spectrophotometrically at a wavelength of 332.80 nm, to determine vitamin B_6content.

3.4.4 Determination of Folic Acid (Vitamin B_9)

In this test, folic acid reacts with aniline hydrochloride to give a coloured product. Tofive test tubes containing two drops of commercial vitamin – mineral premix and locally formulated vitamin – mineral premixes 1 and 2, eight drops of sodium diethylthiocarbamate and four drops of sodium hydroxide solutions were added. The test tubes were shaken and characteristic blue colouration was observed (Amadi*et al*., 2004). The absorbance of the solutions was measured spectrophotometrically at a wavelength of 273.00 nm, to determine vitamin B_9content.

3.4.5 Determination of Tocopherol (Vitamin E)

This was based on the property of tocopherol to give red coloured compounds of quinoid structure under the action of strong oxidants such as concentrated nitric acid.To five test tubes containing two drops of commercial vitamin mineral premix and locally formulated vitamin mineral premixes 1 and 2, ten drops of concentrated nitric acid (HNO_3) was added

to each test tube and shake very well. Red colouration was observed and the absorbance of the solutions were measured spectrophotometrically at a wavelength of 285.00 nm, to determine vitamin E content (Amadi*et al*., 2004).

3.5 Determination of Mineralsin Commercial Vitamin Mineral Premix and Locally Formulated Vitamin Mineral Premixes

Eight minerals out of thirteen minerals required by poultry were determined. Samples of commercial vitamin mineral premix and locally formulated vitamin mineral premixes 1 and 2 were taken to the laboratories of National Animal Production Research Institute (NAPRI) Shikaand National Research Institute of Chemical Technology (NARICT) Basawa, Zaria for analyses. These samples were subjected to wet chemical digestion (ashing) for determination of various minerals, using Atomic Absorption Spectrophotometer (AA240FS). The minerals analyzed were calcium, phosphorus, sodium, potassium, copper, zinc, iron and manganese.

3.5.1 Equipments

The following equipment were used: fume hood constructed for safe exhaustion of perchloric acid fumes, hot plate or micro-digestion bench which was thermostatically controlled, micro-digestion flask (pyrex), volumetric flask (pyrex) 100ml capacity and glass funnel 1.5 cm diameter.

3.5.2 Reagents

The following reagents were used: digestion acid formed when 1 volume perchloric acid (60-62%) was added to 4 volumes nitric acid (69-71% HNO_3).

3.5.3 Procedure

One gram (1g) each of ground sample of commercial vitamin mineral premix and locally produced natural vitamin mineral premixes 1 and 2 were weighed into 250ml capacity micro-kjeldahl flask and 20 ml sulphuric acid and 3 glass beads were added to each. The flask was fixed on a clamp and kept overnight. When the initial reaction subsided, the temperature of hot plate or micro-digestion bench was increased slowly to 180^{o} – 200^{o}C. The digestion continued at this temperature with occasional swirling until there were no visible particles and the digestion acid was clear. The solution was allowed to darken when the volume reduced and the Kjeldahl flask was removed from the heating source. Two mls of HNO_3 was added and the digestion process continued. The temperature from the heating source was allowed to rise to 240^{o}C and the digestion acid was evaporated until dense white fumes were formed within the digestion flask.

After completing the digestion, the flask was removed from the heating source. The content of the flask was filtered through acid washed filter paper in a 100ml capacity volumetric flask using deionized water. At the end, suitable aliquot of digested material was transferred into washed polyethylene bottles and kept in a dust proof glass chamber.These aliquots were subjected to atomic absorption spectroscopy using Atomic Absorption Spectrometer (AA 240 FS). Required standards with known analyte content to establishthe relation between the measured absorbance and the analyte concentration was carried out. Each wavelength corresponds to only one element, and the width of an absorption line was only of the order of a few picometers (pm) which gave the technique its elemental selectivity.The elements determined were calcium, phosphorus,sodium, potassium, copper, zinc, iron and manganese.

3.5.4 Determination of Calcium by Atomic Absorption Spectroscopy (AAS).

Samples of commercial vitamin mineral premix and locally produced natural vitamin mineral premixes were taken to the laboratory of National Research Institute of Chemical Technology Basawa, Zaria (NARICT). These samples were subjected to wet chemical digestion for calcium determination. The procedure was described under method 3.5.3. Two important complications exist in determining calcium by AAS: calcium has a very low electronegativity and thus readily oxidized and if phosphorus is present in the sample, calcium can react with phosphorus in the flame to form calcium phosphate (a very stable ionic compound). The formation of calcium phosphate prevents the complete atomization of the calcium. Hence, phosphorus interferes with the detection of calcium.

3.5.5 Determination of Phosphorus by Electrothermal Atomic Absorption Spectrometry (ETAAS).

Samples of CVMP, LFVMP 1 and LFVMP 2 were taken to the laboratory of NARICT Basawa, Zaria. These samples were subjected to wet chemical digestion for phosphorus determination. The procedure was described under method 3.5.3. Three important complications exist in determining phosphorus by ETAAS. The practical inability of using phosphorus resonance lines, its complex chemical behaviours such as formation of volatile compounds and a tendency to interact with graphite, the overlap observed between molecular and atomic phosphorus lines, which makes it very difficult to obtain reliable results when using lines source.

3.6 Experiment 1:Response of Pullet Chicks to different dietary levels of Commercial Vitamin Mineral Premix (chick) and Locally Formulated Vitamin Mineral Premixes 1 and 2 diets (0 – 8 weeks).

This study was conducted to determine the effect of different levels of CVMP (chick), LFVMP1 and LFVMP 2 on the performance of pullet chicks from 0 – 8 weeks of age. The CVMP used for this experiment was purchased from Rebson agro-veterinary shop in Samaru, Zaria. While the ingredients used in formulating LFVMP1 and LFVMP 2 were purchased from Sabon – gari market in Zaria. The various ingredients were groundto powdered form and simple hand mixing of several ingredients was the technique used. Whereby 2.30 kg and 4.60 kg of LFVMP1 and LFVMP 2 were compounded respectively. The results of the chemical analyses of the premixes were reported in Table 4.1, vitamins in Table 4.2 and minerals in Table 4.3.

Seven isonitrogenous and isocaloric rations were formulated containing CVMP (chick), LFVMP 1 and LFVMP 2 at 0.0, 0.125, 0.25, 2.30, 4.60, 2.30 and 4.60 percent respectively (Table 3.3). Each diet constituted a treatment and each treatment was replicated three times. There were 15 birds per replicate. The design was a completely randomized design. Feed and water were provided *ad libitum*. The experiment lasted for 8 weeks. The average initial weights of the birds were taken before the commencement of the experiment. The birds were subsequently weighed every week to determine the weight gain. Feed weigh – back was also done weekly to determine weekly feed intake. The experiment lasted from day old to eight weeks of age. Routine vaccinations and management practices were administered as at when due. Mortality was recorded as it occurred. Data collected were subjected to the analysis of variance and test of significance (where appropriate) using Duncan Multiple Range Tests according to the General Linear Model of SAS (2002).

Table 3.3: Composition of Experimental Chick's Diets fed 0 - 8 weeks

	Diets						
Feedstuff	1	2	3	4	5	6	7
Maize	56.80	56.68	56.55	54.50	52.20	54.50	52.20
Groundnut cake	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Soybean meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Rice offal	3.85	3.85	3.85	3.85	3.85	3.85	3.85
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lime stone	1.65	1.65	1.65	1.65	1.65	1.65	1.65
Common salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
***CVMP**	0.00	0.13	0.25	0.00	0.00	0.00	0.00
***LFVMP 1**	0.00	0.00	0.00	2.30	4.60	0.00	0.00
***LFVMP 2**	0.00	0.00	0.00	0.00	0.00	2.30	4.60
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis							
M.E. Kcal/Kg	2776.00	2772.00	2768.00	2749.00	2723.00	2735.00	2700.00
Crude Protein (%)	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Crude Fibre (%)	4.75	4.73	4.75	4.69	4.63	4.69	4.63
Calcium (%)	1.34	1.34	1.34	1.34	1.34	1.34	1.34
Phosphorus (%)	0.71	0.71	0.71	0.70	0.70	0.70	0.70
Lysine (%)	1.22	1.22	1.22	1.22	1.21	1.22	1.21
Methionine (%)	0.52	0.52	0.52	0.52	0.52	0.52	0.52
Meth + Cystein (%)	0.85	0.85	0.85	0.84	0.84	0.84	0.84
Cost #/Kg	88.96	89.10	89.24	88.85	88.74	89.07	89.19

***Premixes supplied the following per Kg diet (mg/kg): CVMP** Vit. A 56.40, vit. B_2 1208.5, vit.B_6 690.08, vit. B_9 1.90, vit. E 186.13. Calcium 3.18, phosphorus 0.00,sodium 8.81 potassium 0.36, copper 2.42, zinc 1.68, iron 8.32, manganese 11.20, **LFVMP 1** vit. A 17.80 vit. B_2 0.04, vit. B_6 573.38, vit. B_9 1.78, vit. E 303.91. Calcium 15.04, phosphorus 0.01, sodium 1.02, potassium 0.62, copper 0.05, zinc 0.11, iron 0.61, manganese 0.16. **LFVMP 2** vit. A 19.70, vit. B2 0.05, vit. B6 635.79, vit. B9 1.78, vit. E 329.05. Calcium 29.62, phosphorus 0.03, sodium 1.14, potassium 1.06, copper 0.03, zinc 0.35, iron 20.19, manganese 0.23

3.7 Experiment 2: Effects of different dietary levels of CVMP (Grower), LFVMP1 and LFVMP 2 diets on the performance of Growing Pullets (9 – 20 weeks).

The objective of this experiment was to determine the effect of feeding different dietary levels of CVMP (grower), LFVMP 1 and LFVMP 2 in the diets of growing pullets. The source of the LFVMP and ingredients used in formulating LFVMP 1 and 2, vitamins and mineral analyses was described under materials and methods in experiment 5. Seven isonitrogenous and isocaloric diets were formulated to contain CVMP (grower), LFVMP 1 and LFVMP 2 at 0.0, 0.125, 0.25, 2.30, 4.60, 2.30 and 4.60 percent respectively (Table 3.4).

Chicks from pullet phase were fed common diet from 8 – 9 weeks and were used for the grower phase. 252 growing birds at ninth week of age were randomly allocated to seven dietary treatments with three replicates of twelve birds each having uniform weight in a completely randomized design. Feed and water were provided *ad libitum*. The experiment lasted for eleven weeks. The average initial weights of the birds were taken before the commencement of the experiment. The birds were subsequently weighed fortnightly to determine the weight gain. Feed weigh-back was also done fortnightly to determine feed intake. Mortality was recorded as it occurred. Routine vaccinations and management practices were administered as at when due.

Data collected were subjected to analysis of variance and test of significance (where appropriate) using Duncan's Multiple Range Test according to the General Linear Model of SAS.

Table 3.4: **Composition of Experimental Grower's Diets fed 9 - 20 weeks**

	Diets						
Feedstuff	1	2	3	4	5	6	7
Maize	61.25	61.13	61	58.95	56.65	58.95	56.65
Groundnut cake	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Rice offal	11.10	11.10	11.10	11.10	11.10	11.10	11.10
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
***CVMP**	0.00	0.13	0.25	0.00	0.00	0.00	0.00
***LFVMP 1**	0.00	0.00	0.00	2.30	4.60	0.00	0.00
***LFVMP 2**	0.00	0.00	0.00	0.00	0.00	2.30	4.60
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis							
M.E. Kcal/Kg	2757.00	2753.00	2748.00	2730.00	2703.00	2716.00	2700.00
Crude Protein (%)	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Crude Fibre (%)	7.20	7.20	7.19	7.14	7.08	7.14	7.08
Calcium (%)	1.04	1.05	1.05	1.05	1.04	1.05	1.04
Phosphorus (%)	0.89	0.89	0.89	0.88	0.88	0.88	0.88
Lysine (%)	0.99	0.98	0.98	0.98	0.97	0.98	0.97
Methionine (%)	0.48	0.48	0.48	0.47	0.47	0.47	0.47
Meth + Cystein (%)	0.74	0.74	0.74	0.73	0.73	0.73	0.73
Cost #/Kg	63.25	63.39	63.53	63.14	63.03	63.37	63.48

***Premixes supplied the following per Kg diet (mg/kg): CVMP** Vit. A 21.18, vit. B_2 1255.67, vit.B_6 789.94, vit. B_9 1.97, vit. E 53.85. Calcium 6.05, phosphorus 0.00,sodium 4.47 potassium 0.49, copper 3.42, zinc 2.20, iron 9.59, manganese 14.50, **LFVMP 1** vit. A 17.80 vit. B_2 0.04, vit. B_6 573.38, vit. B_9 1.78, vit. E 303.91. Calcium 15.04, phosphorus 0.01, sodium 1.02, potassium 0.62, copper 0.05, zinc 0.11, iron 0.61, manganese 0.16. **LFVMP 2** vit. A 19.70, vit. B2 0.05, vit. B6 635.79, vit. B9 1.78, vit. E 329.05. Calcium 29.62, phosphorus 0.03, sodium 1.14, potassium 1.06, copper 0.03, zinc 0.35, iron 20.19, manganese 0.23

3.8 Experiment 3: Effects of different dietary levels of CVMP (grower), LFVMP 1 and LFVMP 2 diets on the growth andsubsequent early laying performance of egg type pullets (20 – 32 weeks).

The objective of this experiment was to determine the effect of feeding different levels of CVMP (grower), LFVMP 1 and LFVMP 2 in the diets of growing pullets on their subsequent performance at the laying phase. The source of the CVMP (grower) and ingredients used informulating LFVMP 1, LFVMP 2, vitamins and minerals analyses was described under materials and methods in 3.4 and 3.5. Seven isonitrogenous and isocaloric diets were formulated to contain CVMP (grower), LFVMP1 and LFVMP 2 at 0.0, 0.125, 0.25, 2.30, 4.60, 2.30 and 4.60 percent respectively (Table 3.5). The design was a completely randomized design. Feed and water were provided *ad libitum*.

At 20 weeks of age, the final group weights of the birds were taken per replicate. This weight formed the initial weights of the birds for the subsequent – laying phase of the study. The birds were then fed on a normal layers ration containing 16.0% CP and 2600 – Kcal/kg metabolizable energy for the twelve weeks of the early laying performance phase. Feed and water were provided *ad libitum*. Mortality was recorded as it occurred.

Early laying performance parameters measured include: body weight at first egg, 10%, 50% and at peak of egg production, the weight of first egg, age in days at first egg, percent hen – day and hen – housed egg production. Feed consumed was measured and costs of dozen eggs were computed for each treatment. All data collected were subjected to the analysis of

variance using the SAS (2002), general linear model procedure was performed on final weight, weight gain, feed intake, feed cost and percent mortality. Differences between treatment means were separated using Duncan's Multiple Range Test (Steel and Torrie, 1980).

Table 3.5: **Composition of Experimental Layer's Diets**

	Diets						
Feedstuff	1	2	3	4	5	6	7
Maize	60.25	60.13	60.00	57.95	55.65	57.95	55.65
Groundnut cake	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Soybean meal	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Rice offal	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Bone meal	3.70	3.70	3.70	3.70	3.70	3.70	3.70
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine (%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
***CVMP**	0.00	0.13	0.25	0.00	0.00	0.00	0.00
***LFVMP 1**	0.00	0.00	0.00	2.30	4.60	0.00	0.00
***LFVMP 2**	0.00	0.00	0.00	0.00	0.00	2.30	4.60
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis							
M.E. Kcal/Kg	2600.00	2600.00	2600.00	2600.00	2600.00	2600.00	2600.00
Crude Protein (%)	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Crude Fibre (%)	4.82	4.81	4.81	4.76	4.69	4.76	4.69
Calcium (%)	3.74	3.74	3.73	3.70	3.70	3.70	3.70
Phosphorus (%)	0.89	0.89	0.89	0.88	0.88	0.88	0.88
Lysine (%)	0.94	0.94	0.94	0.93	0.93	0.93	0.93
Methionine (%)	0.47	0.47	0.47	0.46	0.46	0.46	0.46
Meth + Cystein (%)	0.72	0.72	0.72	0.71	0.70	0.71	0.70
Cost #/Kg	62.76	62.90	63.04	62.65	62.54	62.87	62.99

***Premixes supplied the following per Kg diet (mg/kg): CVMP** Vit. A 76.75, vit. B_2 1255.67, vit.B_6 746.38, vit. B_9 1.97, vit. E 186.13. Calcium 10.00, phosphorus 0.01,sodium 3.28 potassium 1.42, copper 1.16, zinc 1.29, iron 9.92, manganese 8.46, **LFVMP 1** vit. A 17.80 vit. B_2 0.04, vit. B_6 573.38, vit. B_9 1.78, vit. E 303.91. Calcium 15.04, phosphorus 0.01, sodium 1.02, potassium 0.62, copper 0.05, zinc 0.11, iron 0.61, manganese 0.16. **LFVMP 2** vit. A 19.70, vit. B2 0.05, vit. B6 635.79, vit. B9 1.78, vit. E 329.05. Calcium 29.62, phosphorus 0.03, sodium 1.14, potassium 1.06, copper 0.03, zinc 0.35, iron 20.19, manganese 0.23

3.9 Experiment 4: Evaluation of the laying performance and egg quality characteristics of laying hens fed diets containing different levels of CVMP (layer), LFVMP 1 and LFVMP 2 diets (32 – 56 weeks).

This study was conducted to determine the effects of feeding different levels of CVMP (layer), LFVMP 1 and LFVMP 2 on the performance of laying hens. The source of the CVMP (layer) and ingredients used in formulating LFVMP 1, LFVMP 2, vitamins and minerals analyses wasdescribed under materials and methods in 3.2. Seven isonitrogenous and isocaloric rations were formulated to contain CVMP (layer), LFVMP 1 and LFVMP 2 diets at 0.0, 0.125, 0.25, 2.30, 4.60, 2.30 and 4.60 percent respectively (Table 3.5). Each diet constituted a treatment and each treatment was replicated three times in a completely randomized design.

There were 10 layers per replicate. The birds used were Lohman brown purchased from Obasanjo's farm Otta. Feed and water were provided *ad libitum*. The experiment lasted for twenty - four weeks. The average initial weight of the birds per replicate was taken before the commencement of the feeding trials. They were again weighed at the end of the experiment to determine weight gain or loss. Data collected included feed intake which was measured monthly and egg production were recorded daily. All data collected were subjected to the analysis of variance using SAS (2002) general linear model procedure. Differences between treatment means were separated using Duncan's Multiple Range Test (Steel and Torrie, 1980).

3.9.1 Egg Quality Determination

Three freshly laid eggs were sampled per replicate, weighed, using the Citizon MP 600 electronic platform scale. The external egg quality characteristics measured included egg weight, egg length, egg diameter, egg shell weight and egg shell thickness. The internal characteristics measured included albumen height, albumen width (diameter), albumen weight, yolk width, yolk height and yolk weight. Egg length and diameter were measured with vernier caliper and egg shape index were calculated as egg diameter divided by the length. Egg shell thickness was measured with a micrometer screw gauge. This was done for 3 consecutive days in every four weeks or 28 days for the duration of the experiment.

The eggs were broken on a flat white plate (breakout method) to obtain yolk and albumen, the egg shells were carefully washed with water to remove adhering albumen, dried at room temperature ($22^{o}C$) and weighed to obtain egg shell weight. Yolk diameter, yolk height and albumen height were determined with a vernier caliper. Yolk index was calculated as yolk diameter/yolk height. The Haugh unit which is the most significant measure of egg internal quality was calculated from the measured height of thick albumen and weight of egg using the following formula proposed by Haugh (1937).

$$HU = 100\log_{10}(h - 1.7w^{0.37} + 7.6)$$

Where; HU = Haugh unit

h = observed height of the albumen in mm

w = weight of egg in g

The HU value normally ranges from 0 – 130 and were ranked as follows:

AA : 72 or more

A : 71 – 60

B : 59 – 31

C : 30 or less

The higher the value, the better the quality of eggs. All data obtained were subjected to analysis of variance using the SAS (1995) general linear model procedure. Differences between treatment means were separated using the Duncan Multiple Range Test (Steel and Torrie, 1980).

3.10 Determination of cholesterol contents of yolk of eggs laid by hens on different dietary levels of premixes.

Three fresh eggs per replicate laid by hens fed the seven diets containing CVMP (layer), LFVMP1 and 2 were sampled once in every four weeks for the determination of cholesterol contents. The yolks were extracted and placed in specimen bottles; they were taken to the Chemical Pathology Laboratory in Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria for analyses. The total cholesterol (T.C), triglyceride (TRIG), high density lipoprotein (HDL) were analyzed from the samples and the low density lipoprotein (LDL) was calculated from the data obtained from the analyses using this formula:

LDL = TC – (TRIG/2.2 + HDL)

3.10.1 Cholesterol Enzymatic End – point Method Using Randox (RX) Monza Analyzer

This technique is designed for the quantitative in vitro determination of cholesterol in egg yolk, serum and plasma on the Rx Monza Analyser.

3.10.1.1Materials

The following materials were used: test tubes, water bath, thermometer, spectrophotometer, egg yolk, Rx Monza Analyser, reagents.

3.10.1.2 Reagents

The reagent used contained the following: 4-aminoantipyrine 0.3mmol/l, phenol 6mmol/l, peroxidase 0.5u/ml, cholesterol esterase 0.15 u/ml, cholesterol oxidase 0.1u/ml, pipes buffer 80mmol/l, pH 6.8 and standard.

3.10.1.3 Method

A calibration in cuvette mode was performed using fresh deionized water (dd H_2O). Cholesterol in the run test screen was selected and water blank was carried out as described below. Five micro litre (5 µl) of deionized water which served as the reagent blank was pipette into a cuvette. Five micro litre (5 µl) of the standard solution; five micro litre (5 µl) of the yolk sample and the reagent which contained five hundred micro litre (500 µl) of reagent blank, five hundred micro litre (500 µl) of the standard solution and 0.5 mls of the yolk sample were all pipette into a cuvette.

These reagents were mixed and incubated for 5 minutes at 37^{o}C. Then inserted into the RandoxMonza flow cell holder and was read within the span of 60 minutes,using calibration standard provided in the kit. The absorbance of the sample (A sample) was measured at the

wavelength of 546 nm (Hg) against the reagent blank within 60 minutes. The formula used to calculate concentration of cholesterol in the sample was:

Conc. Of CHOL in sample = ⌂ A sample / ⌂ A sample x Conc. of standard.

3.10.2 Triglycerides Glycerol – 3 – Phosphate Oxidase – Phenol + Aminophenazone Method (TRIG GPO – PAP Method)

This technique was used for the quantitative in vitro determination of triglycerides in egg yolk, serum and plasma.

3.10.2.1 Materials

The following materials were used: test tubes, water bath, thermometer, spectrophotometer, TRIGS Randox Monza kit, buffer, enzyme reagent, egg yolk and standard.

3.10.2.2 Reagents

Randox1a buffer contained the following: buffer 40mmol/l, pH 7.6, 4 – chloro-phenol 5.5 mmol/l, magnesium ions 17.5 mmol/l. While randox1b enzyme reagent contained 4-aminophenazone 0.5 mmol/l, ATP 1.0 mmol/l, lipases 150u/ml, glycerol – kinase 0.4 u/ml, glycerol – 3 – phosphate oxidase 1.5u/ml and peroxidase 0.5u/ml.

3.10.2.3 Method

A calibration in cuvette mode was performed using fresh deionized water (dd H_2O). Triglycerides in the run test screen were selected and water blank was carried out as described below. Ten micro litre (10 µl) of the standard solution was pipette into a cuvette mode, ten micro litre (10 µl) of the yolk sample and one thousand micro litre (1000µl) of the

reagent blank, one thousand micro litre (1000µl) of standard solution and 1.00 mls of the yolk sample were all pipette into a cuvette mode.

These reagents were mixed and incubated for 5 minutes at 37^{o}Cand was inserted into the RX Monza flowcell holder. The reading was taken within the span of 60 minutes, using the calibration standard provided in kit. The absorbance of the sample (A sample) and standard (A standard) were measured at the wavelength of 546 nm (Hg) against the reagent blank within 60 minutes. The formula below was used to calculate TRIG concentration.

Triglyceride concentration = A sample / A standard x Standard concentration (mmol/l) = mmol/l

3.10.3 High Density Lipoprotein – Cholesterol Precipitant on the RX Monza Analyser

This technique was used for the quantitative in vitro determination of HDL – Cholesterol in egg yolk, serum and plasma.

3.10.3.1 Materials

The following materials were used: test tubes, water bath, thermometer, spectrophotometer, RX Monza analyzer, reagent, standard, egg yolk, HDL – Cholesterol precipitant, centrifuge tubes, pipettes.

3.10.3.2Reagents

The following reagents were used; phosphotungstic acid 0.55 mmol/l and magnesium chloride 25mmol/l.

3.10.3.3 Method

Two hundred micro litre (200 µl) of yolk sample, 200 µl of standard solution and five hundred micro litre (500µl) of diluted precipitant were pipette into centrifuge tubes. Clear supernatants were produced on centrifugation. These specimens were mixed and allowed to settle for 10 minutes at room temperature of 23^0C. It was centrifuged for 2 minutes at 12,000 revolutions per minute (rpm). The clear supernatant was separated off within 2 hours and the cholesterol content was determined by the cholesterol oxidase – phenol + aminophenazone (CHOD – PAP) method. The supernatant was stored for five days at 23^oC. A calibration in cuvette mode was performed using fresh dd H_2O.

High Density Lipoproteins (HDL) in the run test screen was selected and water blank was carried out as described below. Fifty micro litre (50 µl) of deionized water as reagent blank was pipette into a cuvette, the same procedures were followed for fifty micro litre (50 µl) of standard supernatant, fifty micro litre (50 µl) of sample supernatant. For the cholesterol reagent, five hundred micro litre (500 µl) of reagent supernatant, five hundred micro litre (500 µl) of standard supernatant and five hundred micro litre (500 µl) of sample supernatant were pipette.

These specimens were mixed and incubated for 5 minutes at 37^oC. Later on, inserted into the Randox Monza flow cell holder and was read within 60 minutes. The absorbance of the

sample (A sample) and standard (A standard) were measured at the wavelength of 546 nm (Hg) against the reagent blank within 60 minutes. Using a standard, the concentration of

HDL cholesterol in supernatant was calculated as follows:

$$= \triangle A \text{ sample} / \triangle A \text{ standard} \times \text{Concentration of standard}.$$

In calculating the Low Density Lipoproteins (LDL) cholesterol in mmol/l, the formula below was used:

LDL Cholesterol = Total Cholesterol – (Triglycerides /2.2 + HDL Cholesterol)

The expected values was ranked as to show whether the egg was high or low in cholesterol using the LDL values:

Less than (<) 1.04 mmol/l: Low cholesterol and greater than and equals to (≥ 1.55): high cholesterol.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Commercial Vitamin and Mineral Premixes (CVMP) and Locally Formulated Vitamin and Mineral Premixes (LFVMP 1 and LFVMP 2).

The results of the analyses are presented in Table 4.1. The results obtained shows that CVMPs were high in dry matter, ash and nitrogen-free extract than LFVMP 1 and LFVMP 2,while LFVMP 1 and LFVMP 2 were high in crude protein, crude fibre, ether-extract and nitrogen free extract than CVMPs.

4.2 Vitamins Composition of Commercial Vitamin and Mineral Premixes (CVMPs) and Locally Formulated Vitamins and Minerals Premixes (LFVMP 1 and LFVMP 2).

The results of the vitamins analyzed from different premixes are presented in Table 4.2. The vitamins determined from CVMPs, LFVMP1 and LFVMP2 were vitamin A (retinol), vitamin B_2 (riboflavin), vitamin B_6 (pyridoxine), vitamin B_9 (folic acid) and vitamin E (tocopherol and tocotrienols). CVMP (chick) and CVMP (layer) were numerically high in vitamin A than CVMP (grower) which was similar to that of LFVMP 1 and LFVMP 2. All CVMPs were numerically higher in vitamin B_2 than LFVMP 1 and LFVMP 2, for vitamin B_6CVMP (grower and layer) were relatively high compared to CVMP (chick) and LFVMP 1 and LFVMP 2. The results of vitamin B_9 obtained were relatively the same for CVMP, LFVMP 1 and LFVMP 2. In the case of vitamin E, LFVMP 1 and LFVMP 2 were numerically higher than that of CVMP (chick, grower and layer).

Table 1: Proximate Chemical Composition of Commercial Vitamin - Mineral Premix (CVMP) and Locally Formulated Vitamin - Mineral Premixes (LFVMP).

	CVMP (Chick)	CVMP (Grower)	CVMP (Layer)	LFVMP 1	LFVMP 2
Dry Matter (%)	90.08	91.26	90.43	89.66	87.96
Crude Protein (%)	27.5	5.31	7.31	7.06	21.63
Crude Fibre (%)	1.28	1.01	0.63	0.86	1.10
Ether - Extract (%)	2.11	1.26	1.93	2.01	1.84
Ash (%)	9.28	8.15	6.20	4.66	7.31
Nitrogen Free Extract (%)	59.83	84.27	83.93	85.41	68.12

Table 4.2: Vitamin Composition of Commercial Vitamin - Mineral Premix (CVMP) and Locally Formulated Vitamin - Mineral Premixes (LFVMP 1 and 2).

Vitamin	CVMP (Chick)	CVMP (Grower)	CVMP (Layer)	LFVMP 1	LFVMP 2
A	56.40 mg/kg	21.18 mg/kg	76.75 mg/kg	17.80mg/kg	19.70 mg/kg
B_2	1208.51 mg/kg	1255.67 mg/kg	1255.67 mg/kg	0.04 mg/kg	0.05 mg/kg
B_6	690.08 mg/kg	787.94 mg/kg	746.38 mg/kg	573.39 mg/kg	635.79 mg/kg
B_9	1.90mg/kg	1.97 mg/kg	1.97 mg/kg	1.78 mg/kg	1.78 mg/kg
E	186.13 i.u/kg	53.85 i.u/kg	186.13 i.u/kg	303.91 i.u/kg	329.05 i.u/kg

Vitamins A - Retinol, B_2 - Riboflavin, B_6 - Pyridoxine, B_9 - Folic acid, E - Tocopherol
mg/kg - milligramme per kilogramme, i.u - International unit

4.3 Minerals Composition of Commercial Vitamin and Mineral Premixes (CVMPs) and Locally Formulated Vitamin and Mineral Premixes (LFVMP 1 and LFVMP 2).

The results of the minerals analyzed from different premixes are presented in Table 4.3. The minerals determined from CVMP, LFVMP 1 and LFVMP 2 were calcium, phosphorus, sodium, potassium, copper, zinc, iron and manganese. LFVMP 1 and LFVMP 2 were numerically higher in calcium than CVMPs. LFVMP 2 had numerically higher valueof phosphorus than the remaining premixes. All CVMP's were numerically higher in sodium than LFVMP 1 and LFVMP 2. The potassium content of CVMP (layer) was numerically higher than that of LFVMP 2, CVMP (chick and grower) and LFVMP 1 were similar. All CVMPs had numerically higher copper, zinc, iron and manganese contents than LFVMP 1 and LFVMP 2

Table 4.3: Mineral Composition of Commercial Vitamin - Mineral Premix (CVMP) and Locally Formulated Vitamin - Mineral Premixes (LFVMP 1 and 2).

Minerals (mg/l)	CVMP (Chick)	CVMP (Grower)	CVMP (Layer)	LFVMP 1	LFVMP 2
Calcium	3.18	6.05	10.00	15.04	29.62
Phosphorus	0.00	0.00	0.01	0.01	0.03
Sodium	8.81	4.47	3.28	1.02	1.14
Potassium	0.36	0.49	1.42	0.62	1.06
Copper	2.42	3.42	1.16	0.05	0.03
Zinc	1.68	2.2	1.29	0.11	0.35
Iron	8.32	9.59	9.92	0.61	20.2
Manganese	11.2	14.5	8.46	0.16	0.23

mg/l - milligramme per litre

4.4 Experiment 1: Response of Pullet Chicks to different dietary levels of Commercial Vitamin Mineral Premix, Locally Formulated Vitamin Mineral Premix 1 and Locally Formulated Vitamin Mineral Premix 2 (0 – 8 weeks).

The result of the response of the chicks to the different dietary levels of CVMP (chick), LFVMP 1 and LFVMP 2 are presented in Table 4.4. There was a significant increase in final weight as the levels of the premixes in the diets were increased from half to full dose. The same trend was also observed for weight gain. All the diets with premix gave better performance than the control diet. There was significant ($P < 0.05$) difference in total feed intake (g/bird) and in average daily feed intake (g/bird/day) across the treatments. The levels of CVMP (chick), LFVMP 1 and LFVMP 2 in the diet gave significant ($P < 0.05$) difference on feed to gain ratio. Feed cost expressed as ₦/bird was not significantly ($P > 0.05$) different for all the treatments.

The addition of premixes at half or full doses resulted in better growth performance than the control. Growth performance on full dose was significantly ($P < 0.05$) better than on half dose for all the premixes. Growth performance was best on full dose CVMP (chick) followed by LFVMP 2 and LFVMP 1. The feed costs per kg weight gain were significantly ($P < 0.05$) better on the treatment 3 (CVMP at 0.25%) followed by treatment 7 (LFVMP 2 at 4.60%). All the diets with premixes performed better than the control diet. There was no significant ($P > 0.05$) difference on mortality recorded throughout the chick phase.

Table 4.4: Effects of Different Dietary Levels of Commercial Vitamin - Mineral Premix and Locally Formulated Vitamin - Mineral Premixes Diets on the Performance of Chicks (0 - 8 weeks

	Levels of Premixes								
			CVMP		LFVMP1		LFVMP 2		
	1	2	3	4	5	6	7		
Parameters	0.00	0.125	0.25	2.30	4.60	2.30	4.60	**SEM**	**LOS**
Initial wt. g/bird	45.90	46.00	45.90	46.00	46.00	46.00	46.00	0.60	NS
Final wt. g/bird	535.32^{f}	557.35^{e}	610.45^{a}	540.55^{e}	569.30^{c}	540.67^{d}	580.34^{b}	8.20	*
Wt. Gain g/bird	489.42^{f}	511.35^{e}	564.55^{a}	494.55^{e}	523.30^{c}	494.67^{d}	534.34^{d}	8.15	*
Feed intake g/bird	1721.90^{ab}	1706.90^{ab}	1718.80^{ab}	1690.40^{ab}	1733.50^{a}	1720.30^{b}	1719.10^{ab}	21.4	*
Feed intake g/b/day	30.75^{b}	30.48^{ab}	30.69^{ab}	30.19^{a}	30.96^{a}	30.72^{ab}	30.70^{ab}	0.5	*
Feed gain ratio	3.51^{a}	3.34^{b}	3.04^{d}	3.42^{ab}	3.31^{c}	3.48^{b}	3.22^{d}	0.08	*
Feed cost #/bird	125.31^{a}	126.74^{a}	127.83^{c}	127.92^{a}	128.19^{a}	127.69^{a}	127.77^{c}	0.85	*
Mortality (%)	6.67	4.44	4.44	6.67	4.44	6.67	6.67	1.02	NS

Means within the same row with different superscripts are significantly different (P<0.05)
SEM - Standard Error of Means, LOS - Level of Significance
NS - Non significant difference (P > 0.05), * - Significant difference (P < 0.05)

4.5 Experiment 2: Response of growing pullets to different dietary levels of Commercial Vitamin and Mineral Premix (grower) and Locally Formulated Vitamin and Mineral Premixes (LFVMP1 and LFVMP 2).

The results of the response of growing pullets to different dietary levels of CVMP (grower), LFVMP 1 and LFVMP 2 diets are presented in Table 4.5. The final weight, weight gain, total feed intake and average daily feed intake were significantly ($P < 0.05$) better on all levels of premixes than on the control diet. A gradual but insignificant ($P > 0.05$) increase in feed intake was observed as the level of premixes in the diets increased to full doses. Final weight and weight were significantly ($P < 0.05$) better on full doses than half doses for all the premixes.

Feed intake on half doses and full doses of CVMP (grower) and LFVMP 2 were similar but significantly ($P < 0.05$) better than on LFVMP 1. Feed to gain ratio and feed cost (₦/kg gain) were also significantly ($P < 0.05$) better on full doses than half doses for all premixes and also better than the control diet at all levels. There was no significant ($P > 0.05$) difference on mortality recorded throughout the grower phase.

Table 4.5: Response of Growing Pullets to Different Dietary Levels of Commercial Vita
Premix and Locally Formulated Vitamin - Mineral Premixes Diets (9 - 20 weeks)

	Levels of Premixes						
		CVMP		LFVMP 1		LFVMP 2	
	1	2	3	4	5	6	7
Parameters	0.00	0.125	0.25	2.30	4.60	2.30	4.60
Initial wt. g/bird	599.67	599.50	599.50	600.00	599.50	599.67	599.67
Final wt. g/bird	1471.45^{e}	1513.33^{c}	1599.66^{a}	1494.68^{de}	1549.33^{c}	1509.87^{d}	1569.05^{b}
Wt. Gain g/bird	871.78^{e}	913.83^{c}	1000.16^{a}	894.68^{de}	949.83^{e}	910.20^{d}	969.38^{b}
Feed intake g/bird	6700.48^{c}	6822.41^{a}	7094.83^{a}	6644.16^{b}	6800.00^{b}	6864.83^{a}	6900.27^{a}
Feed intake g/b/day	87.02^{d}	88.60^{a}	92.14^{a}	86.29^{c}	88.31^{b}	89.15^{a}	89.61^{a}
Feed gain ratio	7.69^{a}	7.46^{bc}	7.09^{d}	7.43^{a}	7.16^{c}	7.54^{ab}	7.12^{d}
Feed cost #/kg	34.20^{a}	31.49^{bc}	38.10^{d}	35.00^{ab}	36.47^{c}	38.02^{bc}	38.07^{d}
Mortality (%)	5.56	0.00	2.78	5.56	2.78	5.56	2.78

Means within the same row with different superscripts are significantly different (P<0.05)
SEM - Standard Error of Means
LOS - Level of Significance
NS - Non significant difference (P > 0.05), * - Significant difference (P < 0.05)
g/bird - gramme per bird, g/b/day - gramme per bird per day

4.6 Experiment 3: Effects of different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes (LFVMP 1 and LFVMP 2) on the subsequent performance of layers.

Table 4.6 shows the effect of different levels of premixes on the subsequent laying house performance of the growers. In general, final weight and weight gain were significantly ($P < 0.05$) better on full dose than on half doses of premixes. Weight gains for birds on all diets containing premixes were significantly ($P < 0.05$) better than that of the control. Similarly, body weight at first egg was better on full doses than on all half doses for the three premixes and better than on the control diet.

The weight of the first egg was also significantly ($P < 0.05$) higher for hens fed premixes compared control. Age at first egg was significantly ($P < 0.05$) lower on full dose of CVMP (layer), half and full doses of LFVMP 1, which were significantly better than the two doses of LFVMP 2. It took 143 days for hens fed full dose of CVMP (layer0 but 165 day for the control diet, which had the worst performance. For all the premixes, performance for age at peak of production was better ($P < 0.05$) on full dose than on half dose. However, weights of egg at peak production were similar for both levels of premixes except for half dose of LFVMP 1 which was similar to that of control.

Both percent hen – day and hen – housed data show that performance on full dose was better than on half dose for the premixes. The performances on half dose were similar to that of the control. The bottom line of performance assessment which is Income Above Feed Expenses (IAFE) show that CVMP (layer) was significantly (P , 0.05) superior with an IAFE of ₦137.33 per dozen eggs followed by LFVMP 2 with ₦133.33 and ₦131.34 for

LFVMP 1. Performances on half doses although significantly ($P < 0.05$) lower than on full doses were better than that of the control, which was ₦123.28 per dozen eggs. Average daily feed intake (g/bird/day) was not significantly ($P < 0.05$) different across the treatments. There was no record of any mortality throughout the early laying phase.

Table 4.6: Effects of Differrent Dietary Levels of Commercial Vitamin - Mineral Premix and Locally Formulated Vitamin - Mineral Premixes Layer Ration's on the Subsequent Performance of Layer's (20 - 32 weeks)

	Levels of Premixes									
	C V M P				L F V M P 1		L F V M P 2		SEM	LOS
	1	2	3	4	5	6	7			
Initial wt. g/bird	1471.45^{b}	1513.33^{b}	1599.66^{a}	1494.68^{b}	1549.33^{ab}	1509.87^{b}	1569.05^{a}	2.88	*	
Final wt. g/bird	1804.80^{b}	1798.70^{b}	1812.00^{a}	1794.70^{b}	1816.00^{ab}	1799.90^{b}	1829.10^{a}	4.88	*	
Wt. Gain g/bird	333.35^{a}	273.37^{cd}	212.34^{e}	300.02^{b}	266.67^{d}	290.03^{c}	260.05^{d}	0.93	*	
Feed intake g/bird/day	123.42	123.70	125.00	123.63	124.60	124.30	p;m\/	5.32	NS	
Body wt. at first egg (kg)	1.50^{d}	1.63^{b}	1.70^{a}	1.57^{c}	1.65^{b}	1.60^{c}	1.68^{a}	0.03	*	
Wt. of first egg (g)	46.00^{c}	47.00^{bc}	50.00^{a}	48.80^{b}	49.00^{b}	48.00^{b}	51.00^{a}	3.10	*	
Age at first egg (days)	165^{a}	166^{a}	143^{c}	142^{c}	147^{bc}	152^{b}	157^{b}	8.65	*	
Age at 10% prod. (days)	176^{a}	170^{a}	144^{d}	151^{c}	152^{c}	160^{b}	160^{b}	8.58	*	
Age at 50% prod. (days)	198^{a}	185^{b}	152^{e}	196^{a}	165^{d}	176^{bc}	170^{c}	1.92	*	
Age at peak prod. (days)	222^{a}	205^{b}	165^{e}	217^{a}	183^{d}	193^{c}	185^{c}	4.25	*	
Wt. of egg at peak of prod. (g)			59.00^{b}	60.00^{a}	61.20^{a}	59.90^{b}	60.30^{a}	60.00^{a}	60.78^{a}	0.75 *
Feed cost (#/kg)	143.93^{b}	145.49^{a}	146.46^{a}	144.34^{b}	145.57^{a}	145.24^{b}	147.48^{a}	3.27	*	
Feed cost / dozen egg(#)	154.95^{a}	143.80^{b}	136.56^{c}	142.49^{b}	140.29^{b}	143.03^{b}	139.76^{c}	0.67	*	
IAFE at #20.00/egg	123.28^{d}	127.34^{c}	137.33^{a}	125.34^{d}	131.34^{b}	129.34^{c}	133.33^{b}	0.87	*	
% hen day production	73.25^{b}	76.00^{b}	82.20^{a}	74.00^{b}	78.20^{a}	77.06^{b}	78.50^{a}	2.30	*	
% hen housed production	71.80^{c}	76.04^{b}	82.16^{a}	73.49^{b}	78.20^{a}	76.62^{b}	78.50^{a}	2.23	*	
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	NS	

Means within the same row with different superscripts are significantly different (P<0.05)

SEM - Standard Error of Means, LOS - Level of Significance

NS - Non significant difference (P > 0.05) * - Significant difference (P < 0.05

, IAFE - Income Above Feed Expenses g - gramme, Kg - Kilogramme, #/kg - Naira per Kilogramme

4.7 Experiment 4: Response of Laying Hens to different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes (LFVMP1 and LFVMP 2).

The responses of the laying hens to the different dietary levels of premixes are presented in Table 4.7. The finalweights of birdswere significantly ($P < 0.05$) higherin CVMP (0.25%) and LFVMP 2 (4.60%) treatments.The same pattern was observed for the percentage change in body weight. Similarly, half doses of all the premixes followed the same pattern with CVMP (0.25%) and LFVMP 2 (4.60%) performing better than other treatments and resulting into better egg production, higher weights of eggs produced for the laying period. Feed intake (g/bird/day) was significantly ($P < 0.05$) different across the treatments, but the control diet which had no premix was similar with half – dose of premixes diets.

Feed cost per dozen eggs, hen – day and hen – housed egg production, percent production at peak and average egg weight were found to be significantly ($P < 0.05$) higher than the control diet, which contained no premix. All the three premixes performed better than the control diet at half or full doses. Except for CVMP (layer), hen day egg production performance at half dose was less than on full dose premix diets. Performance on half dose of CVMP was similar to full dose of CVMP and full doses of LFVMP 1 and LFVMP 2. Performance was same on full dose for all the three premixes, which means that for laying hens any of the premixes can be used in the absence of CVMP (layer). Income above feed expenses (IAFE) was found to be significantly ($P < 0.05$) higher for the birds on the premix diets than birds on the control diet. As the level of premixes increased in the diet to full doses, there was a corresponding increase in the profit margin. IAFE was best for full dose of CVMP (layer) at ₦404.42 per dozen eggs followed by full dose of LFVMP 2 with

₦389.88 and that of LFVMP 1 with ₦383.09. The least was on the control diet with ₦350.84 per dozen eggs. There was no significant ($P > 0.05$) difference on mortality recorded across the treatments throughout the laying phase.

Table 4.7: Response of Laying Hens to Different Dietary Levels of Commercial Vitamin - Mineral Premix and Locally Formulated Vitamin - Mineral Premixes Diets

	Levels of Premixes							
		C V M P		LFVMP 1		L FVMP 2		
	1	2	3	4	5	6	7	
Parameters	0.00	0.125	0.25	2.30	4.60	2.30	4.60	**SEM**
Initial wt. g/bird	1508.00	1506.00	1506.00	1508.00	1506.00	1508.00	1506.00	0.02
Final wt. g/bird	1870.83^{c}	1985.67^{b}	2088.00^{a}	1895.00^{c}	2010.50^{b}	1957.67^{b}	2058.83^{a}	1.33
Change in body wt (%)	24.06^{ab}	31.85^{b}	38.65^{a}	25.66^{ab}	33.50^{b}	29.83^{b}	36.71^{a}	1.45
Feed intake g/bird/day	134.25^{d}	134.87^{bc}	136.51^{a}	134.50^{cd}	135.00^{bc}	134.85^{b}	135.97^{a}	0.77
Feed cost (#/kg)	171.91^{c}	173.73^{b}	177.96^{a}	170.68^{c}	173.37^{b}	174.39^{b}	177.25^{a}	13.3
Feed cost/ dozen egg (#)	180.09^{b}	182.72^{b}	183.29^{b}	195.78^{a}	190.45^{a}	190.85^{a}	189.35^{ab}	25.7
IAFE at #23.00 / egg	350.84^{c}	383.96^{a}	404.42^{a}	381.39^{b}	383.09^{a}	379.97^{b}	389.88^{a}	89.3
% hen day production	69.11^{c}	76.19^{a}	77.38^{a}	75.60^{b}	76.00^{a}	75.38^{b}	76.79^{a}	1.60
% hen housed production	71.43^{c}	76.19^{a}	77.38^{a}	75.60^{b}	78.57^{b}	77.98^{a}	79.37^{a}	1.62
% hen day at peak	75.85^{c}	76.65^{b}	78.30^{a}	76.00^{b}	78.00^{a}	76.10^{b}	78.10^{a}	1.64
Average egg weight (g)	58.85^{c}	61.42^{b}	62.30^{a}	61.38^{b}	62.13^{a}	61.43^{b}	62.20^{a}	0.06
Mortality (%)	3.33	0.00	0.00	0.00	3.33	3.33	3.33	1.47

Means within the same row with different superscripts are significantly different ($P<0.05$)
SEM - Standard Error of Means
g - Gramme, Kg - Kilogramme
#/kg - Naira per kilogramme
IAEF - Income Above Feed Expenses
g/bird - gramme per bird, g/b/day - gramme per bird per day

4.8 The Economic benefits of feeding Commercial Vitamin Mineral Premix (layer), Locally Formulated Vitamin Mineral Premix 1 and Locally Formulated Vitamin Mineral Premix 2 at the early and late laying phases of hens (20 – 32 and 32 – 56 weeks)

The results shown in Table 4.8 indicate that the inclusion of CVMP (layer) and LFVMP1 and 2 at full doses in the diets of layers significantly ($P < 0.05$) increased the cost of kg feed. Feed costs per kg weight gain were significantly ($P < 0.05$) higher for all the premix diets compared to the control diet. Feed consumed per dozen eggs was significantly ($P < 0.05$) different across the treatments. At the early laying phase, it was highest and poorest ₦154.95 for the control diet and lowest and better with the CVMP (layer) diet at full dose ₦136.56. At the laying phase, it was highest ₦195.78 at the LFVMP 1 half dose and lowest ₦180.09 at the control diet.

4.9 Effects of different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes (LFVMP 1 and LFVMP 2) on Egg Quality Parameters.

The egg quality characteristics of eggs laid by hens fed the different types of premixes are shown on Table 4.9. Egg quality parameters showed significant ($P < 0.05$) differences for all parameters analyzed for both external and internal qualities across the treatments. Full doses diets of CVMP (layer) did better than the LFVMP 2 and LFVMP 2 performed better than LFVMP 1. Half doses of CVMP (layer), LFVMP1 and LFVMP 2 followed the same trend as infull doses. All premixes resulted in eggs that had better quality than the control diet even at half dose. All premixes at full dose gave rise to eggs of higher quality than half dose. Egg weight, shell thickness and Haugh unit were best for eggs from hens fed CVMP (layer) followed by LFVMP 2 and LFVMP 1.

Table 4.8 Economic Benefits of Using Different Dietary Levels of CVMP and LFVMP'S Diets at the Early Laying and Laying Phases

	Levels of Premixes								
	CVMP			LFVMP 1		LFVMP 2			
	1	2	3	4	5	6	7		
Parameters	0.00	0.125	0.25	2.30	4.60	2.30	4.60	**SEM**	**LOS**
Feed Cost (#/kg)	143.93[a]	145.49[a]	146.46[a]	144.34[b]	145.57[a]	145.24[b]	147.48[a]	3.27	*
Feed Cost / Dozen egg (#)	154.95[a]	143.80[b]	136.56[c]	142.49[b]	140.29[b]	143.03[b]	139.76[c]	0.67	*
IAFE at #20.00/ egg	123.28[d]	127.34[c]	137.33[a]	125.34[d]	131.34[b]	129.34[c]	133.33[b]	0.87	*
Feed cost (#/kg)	171.91[c]	173.73[b]	177.96[a]	170.68[c]	173.37[b]	174.39[b]	177.25[a]	13.3	NS
Feed cost/ dozen egg (#)	180.09[b]	182.72[b]	183.29[b]	195.78[a]	190.45[a]	190.85[a]	189.35[ab]	25.7	NS
IAFE at #23.00 / egg	350.84[c]	383.96[a]	404.42[a]	381.39[b]	383.09[a]	379.97[b]	389.88[a]	89.3	NS

Means within the same row with different superscripts are significantly different (P<0.05)
SEM - Standard Error of Means
LOS - Level of Significance
NS - Non significant difference (P > 0.05)
* - Significant Difference (P < 0.05)
#/KG - Naira per kilogramme
IAEF - Income Above Feed Expenses

Table 4.9: Effect of Different Dietary Levels of Commercial Vitamin - Mineral P
Vitamin - Mineral Premixes Diets on Egg Quality Paran

	Levels of Premixes						
		C V M P		L FVMP 1		LFVM	
	1	2	3	4	5	6	
Parameters	0.00	0.125	0.25	2.30	4.60	2.30	4
	External Quality						
Egg weight (g)	58.12^{e}	601.0^{c}	61.51^{a}	59.12^{d}	60.08^{c}	59.48^{d}	60.
Egg height (mm)	54.01^{d}	55.86^{c}	58.00^{a}	55.57^{c}	56.32^{b}	55.58^{c}	56.
Shell weight (g)	5.62^{e}	6.00^{cd}	6.31^{a}	5.92^{d}	6.11^{bc}	6.04^{cd}	6.2
Shell thickness (mm)	0.47^{d}	0.48^{c}	0.51^{a}	0.48^{c}	0.49^{c}	0.48c	0.5
Egg diameter (mm)	43.48^{e}	44.91^{c}	46.23^{a}	44.39^{d}	45.23^{c}	44.89^{c}	45.
	Internal Quality						
Albumen height (mm)	8.00^{d}	8.14^{c}	8.46^{a}	8.12^{cd}	8.22^{bc}	8.15^{c}	8.3
Albumen diameter (mm)	60.33^{e}	62.49^{d}	65.51^{a}	57.48^{d}	64.30^{c}	63.66^{d}	64.
Albumen weight (g)	33.88^{f}	35.80^{d}	38.93^{a}	35.38^{e}	36.91^{c}	35.81^{d}	37.
Yolk height (mm)	16.54^{d}	17.20^{bc}	18.20^{a}	17.04^{c}	17.57^{bc}	17.49^{b}	17.
Yolk diameter (mm)	36.36^{d}	37.50^{c}	38.81^{a}	37.31^{c}	37.90^{b}	37.73^{b}	38.
Yolk weight (g)	15.02^{e}	15.93^{d}	18.66^{a}	15.42^{e}	16.79^{c}	15.77^{d}	17.
Yolk index	0.44^{e}	0.45^{d}	0.47^{a}	0.45^{d}	0.46^{c}	0.45^{d}	0.
Haugh unit	90.30^{d}	90.70^{cd}	91.83^{a}	90.73^{cd}	91.03^{bc}	90.85^{cd}	91.3

abcdef - Means within the same row with different superscripts are significantly different

SEM - Standard Error of Means

g - Gramme

mm - Millimeter

4.10 Determination of Cholesterol contents of yolk of eggs laid by hens fed different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes on subsequent performance of layers.

The results of cholesterol determination in egg yolk of eggs laid by birds on different treatments are presented in Table 4.10. There were significant ($P < 0.05$) differences on the total cholesterol (T.C), triglycerides (TRIG) and high density lipoprotein (HDL) analyzed from the yolk of eggs laid by the hens on different premix diets. The low density lipoprotein (LDL) content of the yolk was calculated. CVMP (layer) fed hens laid eggs with higher total cholesterol than LFVMP. CVMP (layer) and LFVMP 1 fed hens tended to have higher triglycerides. However, eggs from CVMP (layer) fed hens had higher HDL cholesterol but less LDL cholesterol than eggs from LFVMP fed hens which are desirable.

Table 4.10: Cholesterol Content of Yolk of Eggs laid by hens fed Different Dietary Levels of CVMP and LFVMP 1 and 2 on Subsequent Performance of Layers.

	Levels of Premixes							
	CVMP			LFVMP 1		LFVMP 2		
	1	2	3	4	5	6	7	
Parameters	0.00	0.125	0.25	2.30	4.60	2.30	4.60	**SEM**
TC (mmol/l)	2.77^{c}	3.10^{b}	3.37^{a}	2.70^{c}	3.03^{b}	2.47^{d}	2.63^{cd}	0.021
Trig (mmol/l)	0.83^{b}	1.00^{a}	1.07^{a}	1.00^{a}	1.13^{a}	0.47^{c}	0.60^{c}	0.019
HDL (mmol/l)	1.00^{b}	1.43^{a}	1.50^{a}	0.63^{e}	0.87^{c}	0.63^{e}	0.77^{d}	0.01
LDL (mmol/l)	1.39^{b}	1.21^{c}	1.38^{b}	1.61^{a}	1.65^{a}	1.62^{a}	1.59^{a}	0.013

abcd - Means within the same row with different superscripts are significantly different ($P < 0.05$). SEM - Standard Error of Means
TC - Total Cholesterol, Trig - Triglycerides, HDL - High Density Lipoprotein
LDL - Low Density Lipoprotein, Mmol/l - Millimole per litre
CVMP - Commercial Vitamin - Mineral Premix
LFVMP - Locally Formulated Vitamin - Mineral Premix

4.11 Determination of Cholesterol contents of yolk of eggs laid by hens fed different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes at Laying Phase.

The results of cholesterol determination in egg yolk of eggs laid by birds on different treatments are presented in Table 4.11. There were significant ($P < 0.05$) differences on the total cholesterol (TC), triglycerides (TRIG) and high density lipoprotein (HDL) analyzed from the yolk of eggs laid by hens on different premix diets. The low density lipoprotein (LDL) content of the yolk was calculated. The results followed the same trend as that of the early laying phase.

Table 4.11 Cholesterol Content of Yolk of Eggs Laid by Hens Fed Different Dietary Levels of CVMP and LFVMP 1 and 2 at the Laying Phase.

	Levels of Premixes							
	CV M P			LFVMP 1		LFVMP2		
	1	2	3	4	5	6	7	
Parameters	0.00	0.125	0.25	2.30	4.60	2.30	4.60	**SEM**
TC (mmol/l)	2.80^{d}	3.07^{b}	3.38^{a}	2.78^{d}	3.07^{c}	2.47^{c}	2.72^{d}	0.02
Trig (mmol/l)	0.83^{c}	0.95^{b}	1.13^{a}	1.03^{ab}	1.10^{ab}	0.53^{c}	0.65^{d}	0.01
HDL (mmol/l)	1.03^{c}	1.32^{b}	1.55^{a}	0.67^{e}	0.83^{d}	0.65^{e}	0.78^{d}	0.01
LDL (mmol/l)	1.37^{c}	1.32^{d}	1.32^{d}	1.65^{ab}	1.72^{a}	1.58^{b}	1.64^{ab}	0.01

abcd - Means within the same row with different superscripts are significantly different ($P<0.05$)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition of Commercial Vitamin and Mineral Premix (CVMP) and Locally Formulated Vitamin and Mineral Premixes (LFVMP1 and LFVMP2)

The results obtained for the proximate analyses of the various premixes showed that commercial vitamin and mineral premixes (chick, grower and layer) were higher in dry matter, ash and nitrogen free extract compared to locally formulated vitamin and mineral premixes 1 and 2. LFVMP1 and LFVMP2 were higher in crude protein, crude fibre and ether extract due to the presence of blood meal, egg and fishmeal. These results agreed with the findings of Malik *et al*. (2010) but disagreed with the findings of Anonymous[2] (2011). They reported that locally formulated vitamin and mineral premix was higher in nutrient contents than the commercial vitamin and mineral premix. Asaduzzaman *et al*. (2005) reported that different commercial vitamin and mineral premixes have varying degree of efficacy in cage laying pullets, which was directly related to the nutrient composition of the premixes.

5.2 Vitamins Composition of Commercial Vitamin and Mineral Premix (chick, grower, layer) and Locally Formulated Vitamin and Mineral Premixes (LFVMP1 and LFVMP2)

The results obtained from the analyses, indicated that commercial vitamin and mineral premixes for chicks, growers and layers were richer in all the five vitamins analyzed except vitamin E when compared to locally formulated vitamin and mineral premixes 1 and 2. These results agreed with that of Malik *et al*. (2010) who reported that commercial vitamin mineral premix was richer in terms of vitamins than the locally formulated vitamin mineral premix. But disagreed with the results obtained by Anonymous[2] (2011), they reported that locally formulated

vitamin mineral premix was richer in terms of vitamins than the commercial vitamin mineral premix. Jakic – Dimic *et al*. (1997) and Asaduzzaman *et al*. (2005) evaluated the quality of vitamin and mineral premixes obtained locally and from abroad and reported that there were noticeable discrepancies between the declared and the actual contents of premixes. Research study of Ogunwole *et al*. (2008) highlighted the varying potency of different commercial premixes in broiler diets.

5.3Minerals Composition of Commercial Vitamin and Mineral Premixes (chicks, growers, layers) and Locally Formulated Vitamin and Mineral Premixes (LFVMP1 and LFVMP2).

The results obtained in this study showed that, all commercial vitamin and mineral premixes (chicks, growers and layers) were richer in all the eight minerals analyzed except for calcium when compared to locally formulated vitamin and mineral premixes 1 and 2. These results disagreed with the report of Trouw Nutrition Approach to poultry feeding (2011), they reported that locally formulated vitamin and mineral premixes were richer in mineral contents than the commercial vitamin and mineral premixes. Jakic – Dimic *et al*. (1997) and Asaduzzaman *et al*. (2005) observed different growth performances in feeding trials on poultry with various premixes, attributed the reason to differences in mineral contents of the premixes.

5.4Experiment 1: Response of Pullet Chicks to different dietary levels of Commercial Vitamin and Mineral Premix (CVMP) and Locally Formulated Vitamin and Mineral Premixes 1 and 2 (0 – 8 weeks)

The results of the final weight and weight gain obtained in this study compares favourably with the results reported by Malik *et al.* (2010) who fed diets containing commercial vitamin premix and locally formulated vitamin premix and observed that the final weight and weight gain of

commercial vitamin premix (CVP) diets were better than the locally formulated vitamin premix (LFVP) diets. Daily feed intake was insignificant (P > 0.05) tochick commercial vitamin premix diets than for those obtained from the locally formulated vitamin premix diets.

Locally formulated vitamin and mineral premix 1 and locally formulated vitamin and mineral premix 2 were richer in vitamin E than commercial vitamins and minerals premix. Surai (1991) reported that vitamin E has a positive effect on growth performance of poultry by increasing resistance to disease and stress. Surai (1999) and Biswas*et al*., (2010) reported that vitamin E supplementation have been widely used in poultry diets and the levels for enhancing productive and reproductive performances.

Most of the comparative studies using CVMP and LFVMP were done with broiler chicks, for the simple reason that they respond faster to different premixes than pullet chicks. Bolu and Balogun (2001) studied the comparative performance and carcass evaluation of broilers fed locally formulated vitamin premix (LFVP) and two CVMP (starter and finisher) and reported that the LFVP compete favourably with the CVMP (starter and finisher) in terms of final weight and weight gain and that it was cheaper to use LFVP than CVMP as a source of vitamins and minerals for broilers. This report contrasts the findings of this study in the case of feed cost ₦ / bird. Bolu and Balogun (2003) studied the effect of graded levels of iron – fortified locally formulated vitamin premix on the performance and carcass characteristics of broilers and reported that feed utilization efficiency and weight gain were inversely related to feed intake for birds fed LFVP – based diets and these measurements were lower than values observed for birds fed CVMP based diets.Bolu and Balogun (2004) studied the effects of improved (addition of anti – microbial and anti – oxidant) LFVP on the performance of broilers and reported that additives

to the premix enhanced weight gain and carcass characteristics than the ordinary LFVP.

Ogunbawo*et al.* (2010)reported that broilersfed CVMP (finisher) had the higher growth rate, weight gain, feed intake and feed conversion ratio than birds fed locally prepared premix. Oyewole*et al.* (2013) fed diets containing CVMP (starter and finisher) and LFVMP to broilers; they reported that birds on CVMP full dose performed better than those on LFVMP full dose in terms of body weight, weight gain and feed to gain ratio.

Feed cost per bird in grammes was lower for birds on CVMP when compared with birds on LFVMP. Cost per kg gain was not significantly ($P > 0.05$) influenced but numerically the controlled (CVMP diets) was the least economical in terms of economy of utilization. Mortality recorded during the experimental period was insignificant ($P > 0.05$) across the treatments. This could be attributed to good management and to the safety of the test ingredients.

5.5Experiment 2: Response of growing pullets to different dietary levels of Commercial Vitamin and Mineral Premix (grower) and Locally Formulated Vitamin and Mineral Premixes 1 and 2 (9 – 20 weeks)

Results obtained in terms final weight, weight gain, feed intake and feed to gain ratio in this study agreed with the results of Malik *et al.* (2010). They fed diets containing commercial vitamin premixes (grower) and locally formulated vitamin premixes (LFVP) to growing pullets and observed that birds on CVP diets consumed more feeds and expressed it positively in terms of final body weight and weight gain. Trouw Nutrition Approach to poultry feeding (2011) reported that whenbroilers at finishing stage were fed diets containing locally formulated vitamin and mineral premixes called Greenline^{TN} broiler 2,birds on LFVMP diets performed better than

those on the control diets in terms of weight gain, feed conversion ratio and low mortality.These results disagreed with that of Malik *et al.* (2010) and the present study which showed that addition of premixes at half and full doses resulted in better growth performance than the control. Furthermore, growth performance on full dose was significantly better than on half dose for all the three premixes. While all the diets with premixes performed better than the control diet.

5.6 Experiment 3: Effects of different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes (LFVMP 1 and LFVMP 2) layer diets on the subsequent performance of layers (20 – 32 weeks)

The results obtained in this study, shows that performance was significantly ($P < 0.05$) higher with respect to final weight and weight gain in birds fed commercial vitamin and mineral premix (layer) and locally formulated vitamin and mineral premix 2. This is an indication that the more qualitative the premixes were the better the productive performance of birds on those diets. These results obtained was similar to that of Jakic – Dimic *et al*. (1997) and Asaduzzaman *et al*. (2005) who compared the efficacy of different commercial vitamin – mineral premixes on productive performance of caged laying pullets. They concluded that better quality vitamin – mineral premixes had better effects on feed quality and consequently the performance of the hens.

The results obtained in this study was similar to that reported by Asaduzzaman*et al.* (2005), that the egg weight produced by birds in the different premixes diets differed significantly($P<0.05$).Saly*et al*., (1996) observed that vitamin E increased egg weight. Michael and Edward (1992) reported that vitamin B_{12} increased egg weight when the diet contained 8.0

μ/kg of vitamin B_{12}. In this study, most of the parameters measured were significantly ($P < 0.05$) different for most of the treatments which included ages at first egg, 10% lay, 50% lay and peak lay. The range of values obtained in this work as shown in Table 4.6 are comparable to values obtained by Abeke(2005), Vantsawa (2007) and Onimisi (2010). There was a gradual increase in percent hen – day and hen – housed egg production as the premixes in the layer rations increased to full doses.

Kilogramme feed consumed per dozen eggs produced did not showed any significant ($P > 0.05$) effect among the treatments, although there was a gradual decrease in these values as the levels of premixes in the layer rations increased. However, feed cost per dozen eggs showed a significant ($P < 0.05$) difference across the treatments. These costs decreased as the level of premixes in the diets increased to full doses. It means therefore, that any cost savings made as a result of feeding poor diets to growing pullets may not necessarily mean better profit for the egg production period.

Ogundipe *et al.* (1992) and Abeke (1997) had earlier reported that unnecessary tendency to save cost by feeding poor quality diets as opposed to proper feeding of growing pullets may result in loss of profit during the laying phase. Aduku(1992) also reported that unnecessary delay in sexual maturity of growing pullets might also mean a delay in their productive time and consequent reduction of their productive life. This is coupled with high cost of feeding them for a longer period of time, which will definitely result in an increase in the cost of production. Income above feed expenses showed a significant ($P < 0.05$) increase as the birds were fed diets with full doses of premixes. There was no mortality recorded throughout the early laying phase..

5.7 Experiment 4: Response of Laying Hens to different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes (LFVMP1 and LFVMP 2)

The responses of the laying hens to the different dietary levels of premixes shows that the finalweights of birdswere significantly ($P < 0.05$) higherin CVMP (0.25%) and LFVMP 2 (4.60%) treatments.The same trend was observed for the percentage change in body weight. Similarly, half doses of all the premixes followed the same trend with CVMP (0.25%) and LFVMP 2 (4.60%) performing better than other treatments and resulting into better egg production, higher weights of eggs produced for the laying period.This agrees with the result of Malik *et al*. (2010), who reported that laying birds fed diet premixed with CVMP performed better than the ones fed with LFVMP.

Bolu and Balogun (1998) reported that laying hens fed graded levels of LFVMP did well in terms of final weights, change in body weights, better egg production. LFVMP can be a favourable substitute for CVMP. Feed intake (g/bird/day) was significantly ($P < 0.05$) different across the treatments.

Feed cost per dozen eggs, hen – day and hen – housed egg production, percent production at peak and average egg weight were found to be significantly ($P < 0.05$) higher than the control diet. Income above feed expenses was found to be significantly ($P < 0.05$) higher for the birds on the premix diets than birds on the control diet. As the level of premixes increased in the diet to full doses, there was a corresponding increase in the profit margin. There was no significant ($P > 0.05$) difference on mortality recorded across the treatments throughout the laying phase.

Final weights of birds on premixed diets were significantly ($P < 0.05$) better than those birds on the control diet.The ones on commercial vitamin-mineral premix did better and followed by the ones on locally formulated vitamin-mineral premix diet 2. These results agreed with that of Malik *et al.* (2010), who reported that there were significant differences between the final weights of birds on commercial vitamins premixes diets and locally formulated vitamins premixes diets. Weight changes were however significantly ($P < 0.05$) different with treatments 3 and 7 having higher weight gains than the remaining treatments. Full doses of premixes diets were generally better in maintaining body weight during the laying period. Similarly, full doses of commercial vitamin and mineral premixes and locally formulated vitamin and mineral premix 2 resulted into better egg production and higher weight of eggs produced for the laying period.

This agreed with the results of Asaduzzaman*et al.* (2005) that qualitative premixes significantly ($P < 0.01$) enhanced egg production. Treatments without premix caused the poorest egg production, these results might occur due to the continuous absence of essential vitamins and minerals in the control group. Banerjee (1988) was of the opinion that diets continuously deficient in any one of the required vitamins will seriously tell initially upon the egg production of chickens.

Sato*et al*. (1994) observed that vitamin A deficiency lowered the egg production but recommended levels of vitamin A improved egg production rapidly. Bartov*et al*. (1990) reported that recommended doses of vitamin E improved egg production. Panda and Reddy (1976) reported that a marked deficiency of any one or more of calcium, manganese,vitamin A, vitamin D, riboflavin and choline causes a reduction or even a cessation of egg production.

Feed consumption was similar (P > 0.05) for all the treatments. Asaduzzaman*et al.* (2005) reported efficient feed utilization for treatments with qualitative premixes and poorer feed utilization occurred in the control diets, which lack premix. Reza*et al*.(1983) also observed such a low pattern of egg production in an experiment using diets with and without premixes.

Hen – day egg production was significantly (P < 0.05) higher in CVMP (0.125%), CVMP (0.25%) and full doses of LFVMP 1 (4.60%) and LFVMP 2 (4.60%) compared to other treatments. Hen – housed egg production was significantly (P < 0.05) higher in CVMP (0.25%), LFVMP 1 (4.60%) and LFVMP 2 (2.30 and 4.60%) than the other treatments. Percent at peak production and average egg weight were significantly (P < 0.05) higher with birds fed full doses of CVMP (0.25%), LFVMP 1(4.60%) and LFVMP 2 (4.60%) compared to other treatments. Aduku (2004), Asaduzzaman *et al*.(2005) and Avitech (2007) earlier reported that qualitative premix had profound effects on productive performance of poultry. All the three premixes in this study performed better than the control diets at half and full doses. Performance on half dose of CVMP was similar to full doses of CVMP, LFVMP 1 and LFVMP 2. Performance was same on full dose for all the three premixes, which means that for laying hens any of the premixes can be used in the absence of CVMP. There was no significant (P > 0.05) difference in mortality across the treatments. Most of the mortality occurred due to fighting and aggression between birds shortly after randomization into treatments at the end of the grower phase.

5.8 The economic benefits of feeding Commercial Vitamin Mineral Premix, Locally Formulated Vitamin Mineral Premix 1 and Locally Formulated Vitamin Mineral Premix 2 at the early and late laying phases of hens (20 -32 and 32 – 56 weeks)

Inclusion of CVMP and LFVMP at full doses in the diets of layers significantly ($P < 0.05$) increased the cost of kg feed in CVMP (0.25%) and LFVMP2 (4.60%) above the other treatments. Feed cost was similar for all the LFVMP1 diets at half and full doses and for CVMP (0.25%), LFVMP2 (2.30% and 4.60%). This may be attributed to the cost of CVMP and the ingredients for premix formulation. Feed cost ₦/kg weight gain was significantly ($P < 0.05$) better for premix diets when compared with the control diets. This is an indication that the inclusion of premix in egg type chicken diets is economical. This is in agreement with the findings of Afolayan*et al*. (2009) who reported that feed cost / kg gain is a determinant of how much profit accrues to the farmer after sales.

However, feed consumed per dozen eggs was significantly ($P < 0.05$) different across treatments. Hens on diets containing locally formulated vitamin – mineral premixes had significantly ($P < 0.05$) higher values for feed cost per dozen eggs compared to those on CVMP and the control. This also had similar value with LFVMP 2 (4.60%). The income above feed was significantly ($P < 0.05$) higher in the CVMP groups (T_2 and T_3) and full doses of LFVMP (T_5) and LFVMP 2 (T_7) compared to other treatments. Hens on CVMP and LFVMP 2 (4.60%) diets have therefore used similar cost of feed to produce significantly ($P < 0.05$) better quality eggs than those on LFVMP 1 and the control diets. Commercial vitamin and mineral premix diets performed better than locally formulated vitamin and mineral premixes diets at half doses, consequently the significantly higher income above cost of feed. The results obtained in this study is in contrast

to that of Malik *et al.* (2010) where they reported that birds fed LFVP diets had better gross profit values than birds fed the CVP diets.

5.9 Effect of different dietary levels of Commercial Vitamin and Mineral Premix (layer) and Locally Formulated Vitamin and Mineral Premixes (LFVMP 1 and LFVMP 2) on Egg Quality Parameters.

In this study, egg quality parameters showed significant ($P < 0.05$) differences for all parameters analyzed for both external and internal qualities across the treatments. Full doses diets of CVMP did better than the LFVMP 2 and LFVMP 2 performed better than LFVMP 1 (Malik *et al*., 2010). Half doses of CVMP, LFVMP1 and LFVMP 2 followed the same trend as infull doses. The weight of the egg shell followed the same trend as the egg weight. There was increase in weight of the egg shell to the diets containing full doses of CVMP, LFVMP 1and LFVMP 2. The reason for this is glaring, the bigger the egg, there is great need to secrete more calcium and phosphorus for the shell to accommodate the egg.

The performances of birds on diets containing CVMP and LFVMP 2 were similar. All premixes resulted in eggs that had better quality than the control diet even at half dose. Egg weight, shell thickness and Haugh unit were best for eggs from hens fed CVMP, followed by LFVMP 2 and LFVMP 1.

5.9.1 Egg external quality

Egg weight, egg height, shell weight, shell thickness and egg diameter were significantly ($P < 0.05$) different across the treatments. Hens on CVMP (0.25%) diets produced significantly ($P < 0.05$) better qualities, followed by those on LFVMP 2 (4.60%). This report disagrees with that of Malik *et al.*(2010), who reported that there were no significant ($P > 0.05$) difference in the egg weight and shell weight of birds fed commercial vitamin premixes and locally formulated vitamin premixes diets across the treatments.

Full doses of premix diets in treatments 3, 5 and 7 resulted into higher values for shell weight and shell thickness than the other treatments. Sultana*et al*.(2007) reported that different dietary calcium sources did not significantly ($P > 0.05$) influence egg weight, shell weight and shell percentage but had significantly ($P < 0.05$) positive effect on shell thickness of Japanese quail.

5.9.2 Egg internal quality

Albumen height, yolk diameter and Haugh unit were significantly ($P < 0.05$) higher in eggs from hens on full doses of CVMP (0.25%) and LFVMP 2 (4.60%) compared to other treatments. Albumen diameter, albumen weight, yolk weight and yolk index were significantly ($P < 0.05$) higher in eggs from hens on full doses of CVMP (0.25%) compared to other treatments. This was closely followed by eggs from hens on full doses of LFVMP 2 (4.60%) which have values that were significantly ($P < 0.05$) higher than the other treatments. Malik *et al.* (2010), reported no significant differences among the dietary treatments in egg specific gravity, egg shell thickness, yolk colour score, yolk index and Haugh unit. Haugh unit indicates the freshness or age of eggs. The unit ranges from 0 – 130 and eggs are ranked as shown below:

AA 72 – 130 Haugh unit (H.U.)

A 71 – 60 H.U

B 59 – 31 H.U

C 30 or less H.U

Scores of 90 H.U and above are considered excellent, 70 H.U is acceptable;All the eggs analyzed in this work were of the excellent (AA) quality. Commercial vitamin and mineral premix improved egg production and egg weight in laying hens. It also improved feed conversion and increased income accruable from the egg production enterprise. Hens on locally formulated vitamins and minerals premix 2 produced eggs with similar qualities to hens fed commercial vitamin and mineral premix diets.

5.10 Determination of Cholesterol contents of yolk of eggs laid by hens fed different dietary levels of Commercial Vitamin and Mineral Premix and Locally Formulated Vitamin and Mineral Premixes on subsequent performance of layers.

In this study, the yolk of eggs analyzed for the different cholesterol contents shows that, the total cholesterol was significantly ($P < 0.05$) higher in CVMP (0.25%) compared to the other treatments and that of half dose LFVMP (2.30%) and full dose of LFVMP (4.60%) had the lowest values. Eggs from hens fed the control diet also had high levels of cholesterol; this is because of the importance of cholesterol which is use in synthesizing steroid hormones, cell membranes and other body structures.

The animal body produces cholesterol daily to meet its minimum requirement. United State Department of Agriculture (USDA) 2002 reported that the total cholesterol in an egg fluctuates from 140 – 270 milligrammes (mg) depending on size, brand and how farmers raise their

chicken. The bigger the egg, the more cholesterol it will have and the more a chicken roams around on a pasture the less cholesterol will be in the eggs. Behrenbeck (2012) reported that one large conventional egg has about 186 mg of cholesterol, all of which is found in the yolk. A healthy person is recommended to limit his dietary cholesterol to less than 300 mg a day while unhealthy person with cardiovascular disease, diabetes or high low density lipoprotein blood cholesterol level should limit his dietary cholesterol to less than 200 mg a day. The standard blood cholesterol level in humans is 2.2 millimole per litre (mmol/l) or 200 milligrammes per deciliter (mg/dl). Therefore, eggs laid by hens on CVMP had higher total cholesterol, followed by LFVMP 1, control and LFVMP 2. They cholesterol level in all the eggs laid by the hens were above the desired level for human consumption.

Triglyceride values were significantly ($P < 0.05$) higher in CVMP and LFVMP 1 supplemented diets compared to the control. LFVMP 2 supplemented diets had the least value of triglycerides. A simple blood test revealed the different categories of triglycerides: normal level is less than 1.7 mmol / l; borderline high level is between 1.8 – 2-2 mmol/l; high level is between 2.3 – 5.6 mmol/l and very high level is 5.7 mmol/ l and above. The American Heart Association (A.H.A) recommends that a triglyceride level of 1.1 mmol/ l or lower is considered optimal. The optimal level improves your heart health. In this study, the triglycerides in eggs laid by hens on CVMP and LFVMP 1 diets were higher than that of the control and LFVMP 2 but within the desirable levels.

CVMP diets also had significantly ($P < 0.05$) higher values of high density lipoproteins (HDL), while LFVMP 2 (4.60%) had the least value. HDL or “good” cholesterol act as cholesterol

scavengers, picking up excess cholesterol in your blood and taking it back to your liver where it is broken down. In men, HDL cholesterol level that is less than 1.0 mmol/l is below the body requirement therefore the person is at risk while 1.6 mmol/ l or above is the desirable level. In women, HDL cholesterol that is less than 1.3 mmol/ l the person is at risk while 1.6 mmol/ l or above is the desirable level. In this study, eggs laid by hens on CVMP diets had higher values for HDL cholesterol, followed by the control then the LFVMP's comparable to the standard of 1.6 mmol/ l.

All eggs laid by hens fed on LFVMP 1 and LFVMP 2 diets had significantly ($P < 0.05$) higher values for low density lipoprotein (LDL) compared to the other treatments. LDL cholesterol is the lipoprotein particle that is mostly involved in atherosclerosis. LDL cholesterol is usually calculated using the Friedewald equation, based on that we have different categories: below 1.8 mmol/l is ideal for people at very high risk of heart disease; below 2.6 mmol/l is ideal for people at risk of heart diseases; 2.6 – 3.3 mmol/l is the level near ideal; 3.4 – 4.1 mmol/l is level at borderline high; 4.1 – 4.9 mmol/l is high level and above 4,9 mmol/l is very high level.

In this study, eggs laid by hens fed LFVMP 1 and 2 diets had higher values compared to eggs laid by hens fed CVMP and control diets. Despite that the values are not as high as 2.6 – 3.3 mmol/l for the level near ideal. Therefore, all the eggs are safe for human consumption. In general, the lower your LDL cholesterol level is the better. There is no evidence that really low LDL cholesterol levels are harmful (Behrenbeck, 2012). Total cholesterol (TC), triglycerides (Trig), HDL and LDL values in Table 4.11 followed the same pattern as those presented in Table 4.10.

5.11 Determination of Cholesterol contents of yolk of eggs laid by hens fed different dietary levels of Commercial Vitamin and Mineral Premix and Locally Formulated Vitamin and Mineral Premixes at Laying Phase.

In this study, the yolk of eggs analyzed for the different cholesterol contents shows that, the total cholesterol was significantly ($P < 0.05$) higher in CVMP (0.25%) compared to the other treatments, while half dose of LFVMP (2.30%) and full dose of LFVMP (4.60%) had the lowest values. Triglycerides values were significantly ($P < 0.05$) higher in CVMP and LFVMP 1 compared to other treatments. LFVMP 2 had the least value. CVMP had significantly ($P < 0.05$) higher values of high density lipoproteins (HDL) compared to the other treatments, while LFVMP 2 (4.60%) had the least value. All groups on LFVMP 1 and LFVMP 2 had significantly ($P < 0.05$) higher values for low density lipoprotein (LDL) compared to the other treatments. Total cholesterol (TC), triglycerides (Trig), HDL and LDL values in Table 4.11 followed the same pattern as those presented in Table 4.10.

In cholesterol test, fat has four major components namely total cholesterol, low – density lipoprotein, high – density lipoprotein and triglyceride. High – density lipoprotein is considered to be the "good cholesterol" because HDL's role in the body is to take cholesterol to the liver for processing. LDL is considered to be "bad cholesterol" because this type of lipoprotein is circulated from the liver to other organs and tissues in the body carrying cholesterol to where it is needed. Jones *et al.* (2009) reported that, if the cholesterol level is < 1.04 millimole per litre (mmol/l) and ≥ 1.55mmol/l; then the cholesterol level in the egg yolk is low and high respectively. These values are based on the low density lipoprotein (LDL), from this work high cholesterol levels were reported in treatments 4, 5, 6 and 7. This may be due to the utilization of

blood meal and whole hard egg in formulating locally produced vitamin and mineral premixes 1 and 2. Blood meal as an ingredient is known to be high in cholesterol (Armstrong, 2002) and one large egg has about 186 milligrams of cholesterol, all of which is found in the yolk (Behrenbeck, 2012).The results obtained for the different components of cholesterol in an egg at the laying phase of the birds followed the same trend as in the early laying phase of the birds across the treatments. The value of low density lipoprotein (LDL) was used in the ranking to evaluate whether the egg is low or high in cholesterol.

CHAPTER SIX

6.0 GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 General summary

Studies were conducted to evaluate the efficacy of commercial and locally formulated vitamin and mineral premixes in the diets of egg – type chickens. In 3.1, the proximate compositions of the premixes were determined. The commercial vitamin and mineral premix was found to be higher in dry matter, crude fibre, nitrogenfree extract and ash. The locally formulated vitamin and mineral premix 2 was higher in etherextract, ash, nitrogen free extract. Locally formulated vitamin and mineral premix 1 was higher in crude protein, crude fibre, nitrogen and moisture than locally formulated vitamin and mineral premix 2. Over all, locally formulated vitamin and mineral premix 1 was richer in nitrogen and moisture than commercial vitamin and mineral premix and locally formulated vitamin and mineral premix 2.

In 3.2, chemical compositions of the premixes were determined. The commercial vitamin and mineral premixes was higher in vitamins and minerals, followed by locally formulated vitamin and mineral premix 2 and lastly locally formulated vitamin and mineral premix 1. These reflected in the growth performance of the birds in all the parameters studied.

In the first feeding trial that is experiment 1, the effects of the three premixes on the performance of pullet chicks from day old to eight weeks were assessed. Itwas observed that performance of birds was better on the commercial premix but locally formulated vitamin and mineral premix 2 compared favourably; however, performances on full doses were better than on half doses. For other parameters studied, full doses of commercial and locally formulated vitamin and mineral

premixes 1 and 2 did better than half doses of these premixes. All the diets with premixes performed better than the control diet.

In the second feeding trial, which is experiment 2 compared the performance of growing pullets (9 – 20 weeks)fed the three levels of commercial and locally formulated vitamin and mineral premixes 1 and 2 was conducted. It was observed that the performance of the birds in terms of final weight, weight gain and total feed intake was significantly ($P < 0.05$) lower on the locally formulated premixes but LFVMP 2 was better than LFVMP 1. The addition of premixes at half and full dose resulted in better growth performance than the control. There was no significant ($P > 0.05$) difference on mortality recorded throughout the experiment, a proof that the premixes are safe for bird's consumption.

In the third feeding trial, which was experiment 3 a study was conducted to determine the effect of the three premixes layer diets on the subsequent performance of layers. The results showed that, for all the premixes tested full doses produced better results than half doses of premixes across the treatments. The results showed no carry over effects from the growing phase to early laying phase in terms of hen – day and hen – housed egg production. No mortality was recorded throughout the early laying period.

The fourth study, which wasexperiment 4was carried out to determine the response of laying hens to the three different types of premixes. Results obtained indicate there was significant ($P < 0.05$) difference in final weight, change in body weight, and feed intake per bird among other parameters. Commercial vitamin and mineral premixes at full doses out performed locally

formulated vitamin and mineral premixes 1 and 2, the same trend followed for half doses of premixes. Performance on half dose of commercial vitamin and mineral premix was similar to full dose of CVMP and full doses of LFVMP 1 and LFVMP 2. Therefore, performance was same on full dose for all the three premixes which means that for laying hens any of the premixes can be used in the absence of CVMP.

The fifth study, which was experiment 5, was on the analyses of eggs laid by birds in various treatments on the external and internal egg quality parameters. Results obtained shows that, eggs laid by birds on commercial vitamin and mineral premixes diets possessed better external and internal qualities than eggs laid by birds on locally formulated vitamin and mineral premixes 2. Followed by locally formulated vitamin and mineral premixes 1and least were eggs laid by birds on the control diets.

The sixth and seventh studies, which were on the determination of cholesterol content in egg yolk of eggs laid based on the three different levels of commercial and locally formulated vitamin and mineral] premixes 1 and 2 at subsequent performance of layers and at laying phases. Results obtained shows that at the early laying and laying phases, eggs laid by hens fed locally formulated vitamin-mineral premixes were higher in cholesterol.

6.2 Conclusion

The following conclusions were made from these studies:

1. Vitamins and minerals premixes represent only 0.05% of the diets and 10% of cost of feed production but have profound effects on bird's performance.
2. The commercial and locally formulated vitamin and mineral premixes 1 and 2 utilized in different diets formulation in this study proved that the efficacy of commercial vitamin and mineral premixes, locally formulated vitamin and mineral premixes 1 and 2 was not the same.
3. Commercial vitamin and mineral premixes were higher in vitamins and minerals than the locally formulated; because the non- vitamin and mineral contents in the locally and naturally sourced feed stuffs were included.
4. Locally formulated vitamin and mineral premixes 2was better than locally formulated vitamin and mineral premixes 1, these types of premixes reflected in the productive performance of the pullet birds.
5. All the three premixes performed better than the control diets at half or full doses.
6. Performance on half dose of CVMP was similar to full dose of CVMP and full doses of LFVMP 1 and 2 in all studies.
7. Performance was same on full dose for all the three premixes, which means that for laying hens any of the premixes can be used in the absence of CVMP.
8. Using blood meal, whole hard egg or any related feed stuff as an ingredient in producing locally formulated vitamin and mineral premixes, the quantity to be use need to be taken into cognizance because of its influence on the low density cholesterol level of egg yolk.

9. Result of this work showed that hens fed locally formulated vitamin and mineral premixes 2 diets, their growth and egg production performance was comparable to that of the hens fed commercial vitamin and mineral premixes diets.

6.3 Recommendations

The following recommendations were made from the study:

1. CVMP at 0.25% and LFVMP 2 at 4.60% are levels recommended for farmers and researchers.
2. It is suggested that further studies should be carried out on varieties of locally and naturallysourced feedstuffs in premix formulation, this will provide more information on how to assemble premixes.
3. Locally Formulated Vitamin and Mineral premixes should be graded into chick, grower and layer premixes. In other words, further research is needed to develop LFVMP formulae for the use of small scale rural poultry and piggery farmers who may not have easy access to quality premixes nor afford whatever is available.

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