CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND

Poultry diets are usually compounded from cereals, such as wheat, maize and sorghum as the principal source of energy and protein rich ingredients such as oilseed meals, pulses and animal proteins. Conventional feed ingredients are often low in essential micronutrients needed by growing animals. Because of this, micronutrients are supplemented in the feeds as mixes of vitamins, amino acids and trace minerals, or combinations of the three. From the economic point of view, poultry should be supplied with cheap feed to get maximum return with minimum cost. The profit of poultry farming mainly depends on economical feeding of a balanced diet. Researchers are lately trying to find new nutritional solutions for poultry feeding, which will result in improved poultry performance with lower costs. Poultry feeding uses two major types of raw materials; cereal crops and soybeans or soybean by-products. Most diets contain maize for energy, soybean meal for protein and vitamin and mineral supplements. Maize-soybean diets, formulated to satisfy the energy and amino acid needs of the commercial broiler are typically deficient in several vitamins and trace minerals. Consequently such diets are fortified with vitamins and trace mineral premixes to support good broiler performance (Deyhim & Teeter, 1993).

Observations have revealed that growth and performance of many animals improve when diets contain mostly animal proteins such as milk, beef and fish, and growth performance decline when diets include mostly vegetable proteins such as maize, wheat and soybean. Soybean is regarded as the major source of plant protein for poultry diets. Vegetable protein, frequently used in poultry feeding contains a wide spectrum of anti nutritional factors and indigestible

constituents. Soybean meal contains trypsin inhibitors, lectins, saponins and oligosaccharides (raffinose, stachyose).

While some of the antinutritional factors can be eliminated by physical treatments such as the heat treatment that destroys trypsin inhibitors, large amounts of non starch polysaccharides (NSP) from plant protein which have low digestibility are not destroyed. NSP is considered to have minor contribution to poultry nutrition as some of their constituents have antinutritional activity affecting the use of energy and protein particularly in growing poultry. Soybean meal is an excellent source of lysine, tryptophan and threonine, but it is deficient in methionine (Darwin, 1994). The amino acids from soybean meal protein mix properly with maize amino acids yielding a balanced mixture for poultry requirements but needs minimal supplementation with synthetic amino acids to support better broiler performance (Swick *et al.,* 1977).

Some investigations (Donald, 2003) have shown that if vegetable proteins are supplemented with specific amino acids, the overall BV of that protein improves and this adds to the overall diet of the animal resulting in greater performance improvement. The poor biological value of maize-soybean diets (MSD) is usually due to their low levels of amino acids; methionine and lysine. However, it is often considered that maize- soybean diets are first limiting in sulfur containing amino acids and thus require supplementation. The two ingredients, maize and soybean meal, being the major sources of protein in broiler chicken diets, addition of methionine is required to optimize the growth of chickens on the maize-soybean diet (Donald, 2003). Methionine was found to be the first limiting amino acid with threonine, valine and lysine being also marginal when soybean meal was the only protein source fed to poultry (Perilia *et al*., 1997).

Much of the phosphorus and zinc present in soybeans are in bound form, so various methods have been proposed to increase their availability. Microbial

Pyhtase added to the maize-soybean layer diet resulted in increased egg production, improved egg weights and feed consumption (Um & Paik, 1999).

The variability in nutrient composition of most feed ingredients present in the animal rations is an important factor in determining nutritional adequacy. Moreover, an anti-nutrient present in a feed may destroy, or render unavailable to the animal, a particular nutrient present in the diet at adequate levels. The interaction of one nutrient with another and with other dietary constituents also contributes to the development of nutritional deficiencies (Abawi *et al.*, 1989).

Most of the Zambian poultry feeds are based on maize and soybean meal as energy and protein sources respectively. Vitamins A, D, riboflavin and B_{12} are usually low in poultry diets. The vitamins D and B_{12} are almost completely absent on diets based on maize and soybean meal. It should be noted here that vitamins are essential for life and must be provided in proper amounts for the broilers to grow well. Therefore, identification of better nutritional supplements would help poultry farmers not only to cut down on their production costs, but also to improve the efficiency of their production (Teguia *et al.*, 2005). Addition of amino acids, minerals and vitamins to poultry diets is a good insurance to protect birds from deficiency diseases and disorders and ultimately maximize their productivity.

Many nutritional supplements available exhibit variations in their composition but confer the same effect on the performance of broilers on maize-soybean diets. The results from the field have shown inconsistent effect on broiler performance on maize-soybean diets. Some of the reasons attributed to this are; poor storage of feed and feed ingredients by farmers leading to loss of potency of some nutrients and poor efficacy of the available nutritional supplements. Despite the supplements differing in one or more nutrients (table 2), poor performance characterizes some of the commercial supplements leading to low productivity in the small holder broiler production feeding maize-soybean diets.

This problem of poor performance experienced when using some of the commercial supplements can be overcome by providing a balanced supplement with assured efficacy (Teguia *et al.*, 2005).

Commercial supplements are a combination of amino acids, minerals and vitamins added to a formulated diet to boost levels of amino acids, minerals and vitamins that may be deficient in the formulated diet. Inclusion of commercial supplements has become indispensable practice because feed ingredients do not contain all essential amino acids, minerals and vitamins in the right amounts needed for good broiler performance. Variations of requirements may arise out of a number of factors such as climate, life cycle, breed, age and purpose of production hence need for feed supplements inspite of commercial feeds having premixes in their mix.

1.2 Statement of the Problem:

Many nutritional supplements available show variations in their composition but confer the same effect on the performance of broilers on maize-soybean based diets. It is has been speculated that low effectiveness of the available Amino acid-mineral-vitamin supplements has led to low productivity and poor performance in small holder broiler production especially those on maize-soybean diets. The solution to this problem lies in evaluating the nutritional value of available supplements to facilitate effective supplementation.

1.3 Main Objective:

To determine the feeding value of the different selected nutritional supplements in maize-soybean diet fed to broiler chickens under production conditions.

1.4 Specific Objectives:

- To determine the efficacy of different selected nutritional supplements in maize-soybean diet fed to broiler chickens under production conditions.
- To evaluate the growth performance in broilers given different nutritional supplements under production conditions.
- To determine the economic feasibility of using nutritional supplements.
- To evaluate the carcass characteristics and component yields of broilers on different nutritional supplements at slaughter age.

1.5 Research Justification:

Poultry meat contributes 35% of the total animal protein supplied in Zambia (PAZ, 2005). There is great possibility of growth and expansion of the sector at domestic and commercial levels due to increasing demand for animal protein, need for income generation and need for creation of employment opportunities for the people.

In poultry production, feed accounts for 70-75% of the total cost of production. From the economic view point, poultry should be supplied with cheaper and quality feed to get maximum returns with minimum cost. The profit of poultry farming mainly depends on economic feeding of balanced, cheap and quality diets. It is important to pay attention to the formulation of economic poultry diet using local feed ingredients supplemented with cheaper but quality nutritional supplements. It is also worth noting that minerals and vitamins contribute 10% of the total cost of feed (Singh and Panda, 1988a), hence economization on these nutrients or reduction of the safety margins may restrict performance of the broiler chickens. Micro minerals, though required in small amounts, inadequate supply can just be as detrimental to poultry as a lack of one of the macro nutrients (Deyhim *et al.*, 1995). Identification of beneficial nutritional supplements will help poultry farmers not only to cut down on their production

costs, but also improve the efficiency of their production (Teguia *et al*., 2005). Giving supplemental nutrition to poultry on maize-soybean diets is good insurance in protecting them from deficiency diseases and disorders and ultimately maximize their productivity.

The major thrust of broiler nutrition is to maximize the economic production performance of broilers. The main production criteria are body weight, feed conversion, health and body composition. This research therefore has been designed to investigate the efficacy of different nutritional supplements on productive performance of broilers in terms of growth rate, feed consumption, feed efficiency and mortality, and assess the cost per unit live weight of using commercial supplements in maize-soybean diets. It is hoped that this effort will lead to recommending a suitable nutritional supplement to poultry producers among those available on the local market.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Need for nutrient supplements in poultry diets

It is of greatest importance that chickens which are mostly kept intensively with out access to soil, or herbage, and often without any sunlight, receive food which is not deficient in vital elements such as vitamins, minerals and amino acids (Ewer *et al*., 1971).

Deficiency of vitamins and minerals cause various nutritional diseases such as xeropthalmia, cage layer fatigue, rickets, poor growth, reduced egg size, reduced egg production, osteoporosis, low hatchability, depigmentation of feathers, poor feathering, ataxia, progressive incordination, slipped tendons, micromelia, weeping of the skin, exudative diathesis, droopiness, soft beaks, muscular dystrophy decreased feed efficiency, curl toe paralysis and stuntedness.

Singh and Panda (1988a) stated that critical vitamins such as choline, folic acid, pantothenic acid, pyridoxine, riboflavin, Vitamin A, Vitamin D and Vitamin E, trace minerals such as copper, iodine, iron, manganese, and zinc and essential amino acids should be checked carefully in the diets. They further concluded that economization of these nutrients or neglecting or reducing their safety margins may restrict performance of birds with heavy loses.

2.2 Requirements for Amino Acids

Plants and many micro organisms are able to synthesisze proteins from simple nitrogenous compounds such as nitrates. Animals can not synthesize the amino group, and in order to build up body proteins they must have a dietary source of

amino acids. The actual dietary requirements of certain amino acids are dependent on the presence of other amino acids. The requirement for methionine is partially dependent on the cysteine content of the diet. Essential amino acids needed by the chick for proper growth include; Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and valine. The chick requires a dietary supply of 10 amino acids listed above, but in addition needs a dietary source of glycine (McDonald etal., 1995). Because all essential amino acids are from a dietary source, it is therefore sufficient to ensure that diets for poultry contain, first adequate total protein and second, adequate contents of those amino acids most likely to be deficient.

2.3 Vitamins

Vitamins are organic nutrients that are necessary in small amounts for normal metabolism and good health. Vitamins are not sources of energy in the same way as carbohydrates, fats and proteins are but they serve as chemical partners for the enzymes involved in metabolism. They are involved in all biological functions that allow an animal to use energy and protein for maintenance, growth, health, feed conversion and reproduction. Vitamin deficiencies have an adverse effect on metabolic processes and specific diseases are linked to lack of individual vitamins. Deficiencies of various vitamins may cause problems such as; skin lesions, muscle problems, reduced egg production in layers, reduced growth in meat birds and improper chick development in breeding birds (McDonald *et al.*, 1985). The severity of these problems will depend on which vitamin(s) is inadequate and how deficient there is.

It is well established that in commercial poultry operations today there are benefits from routine use of higher levels of vitamins in feed than recommended in reviews such as NRC (1994). Today's elevated levels not only help ensure simple deficiencies are rare, but also help the bird cope with the increased stress and disease challenge of commercial production (Ward, 1996).

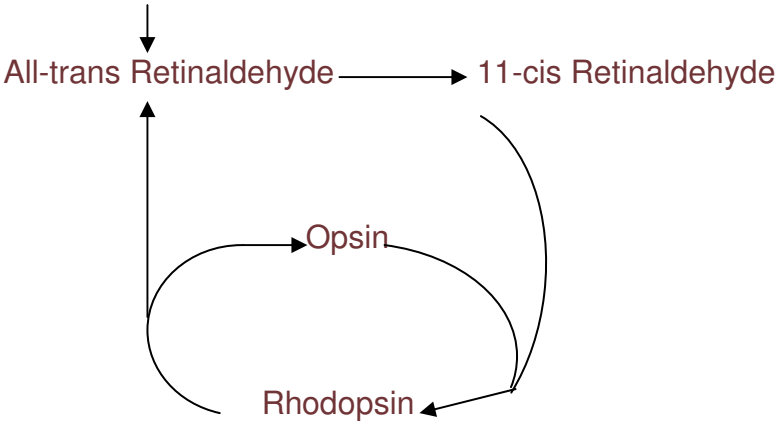
In comparison to other species chickens are more susceptible to vitamin deficiency because gut flora of chickens provide very little vitamin synthesis but compete with the host for dietary vitamins and intensively kept chickens undergo many stresses that precipitate increased metabolic needs or elevate dietary needs due to increased loses. Vitamins A, D, riboflavin and B_{12} are usually low in poultry diets. The Vitamins D and B_{12} are almost completely absent in maize-soybean diet while vitamin K is generally added to poultry diets more than other species diets because birds have less intestinal synthesis due to shorter intestinal tract and faster rate of food passage. Hence, supplementing poultry feeds with vitamins is good insurance to protect chickens from deficiencies and disorders.

2.3.1 Vitamin A

Vitamin A is necessary for growth, reproduction, maintenance of the epithelia tissues, and for normal vision. The chief functions of vitamin A are to ensure adequate growth and help the bird's resistance to disease. The vitamin can be formed naturally in the body from carotene present in vegetable matter or yellow maize, or may be fed either by fortifying the ration with fish liver oil or using synthetic forms of the vitamin (Ewer *et al.*, 1971). Vitamin A and its several precursors (α, β and γ carotene and cryptoxanthin) are relatively unstable therefore, feeds stored for a long time before being fed may lose a large portion of vitamin A activity, especially if the diet contains sources of unstabilised polyunsaturated fats (Fraser *et al.*, 1986). Low and very high Vitamin levels decreases body weight gain in broilers. Low dietary Vitamin A causes depression in invitro T-Lymphocytes responses and in vitro antibody production to defined protein antigens. However, excess Vitamin A intake also decreases immune responses (Abawi and Sullivan, 1989). In mature birds, egg production and hatchability is reduced.

Vitamin A plays two roles in the body according to whether it is acting in the eye or in the general system. In retinal cells of the eye, vitamin A (*all-trans-retinol*) is oxidized to an aldehyde (*all-trans-retinaladehyde*) which is converted to *11-cis isomer*. The latter then combines with the protein opsin to form rhodopsin (visual purple) which is the photo-receptor for vision at low light intensities. When light falls on the retina, the *cis-retinaldehyde* molecule is converted back into the all *trans* form and is released from opsin. This conversion results in the transmission of an impulse up the optic nerve. The *all- trans-retinaldehyde* is isomerised in the dark to *11-cis- retinaldehyde* which is then recaptured by opsin to reconstitute rhodopsin, thus continually renewing the light sensitivity of the retina (McDonald *et al*, 1995).

Fig. 1: Diagramatic presentation of the role of vitamin A (Retinol) in the visual cycle



(Source; Animal nutrition, McDonald et.al., 1995)

In the second role vitamin A is involved in the formation and protection of epithelial tissues and mucous membranes.

Most ingredients present in the diets of poultry are low or lacking in vitamin A or its precursors hence vitamin A deficiency may be a problem unless supplementation is administered (McDonald *et al*, 1995). A deficient diet in

vitamin A results in inapetance and growth failure. The first characteristic signs, other than decline in rate of growth, are very high chick mortality, droopness, ataxia, ruffled feathers, and accumulation of sticky exudates beneath the eyelids leading to blindness and marked nervousness. There is also loss of appetite which has been attributed in part to loss of the sense of taste due to keratinisation of the taste buds and atrophy of accessory glandular tissue. Other deficiencies include constricted optic nerves and increased cerebrospinal fluid (Combs, 1998).

The influence of dietary vitamin A levels on production performance of broilers has been studied by a number of previous researchers, but great differences exist in their results. Aburto and Britton (1998) reported that body weight of broilers decreased when dietary supplemental vitamin A was fed in excess of the requirement i.e. at 80,000IU/kg diet. However, feed efficiency was better when supplemental Vitamin A was fed at 1,000 IU/Kg diet. Song Zhigang and Lin Hai (2004) reported that broilers got better performance when vitamin A supplementation level was 12,000IU/Kg diet. The recommended range for vitamin A is very wide, from 1,500 IU/Kg diet to 2,800IU/Kg diet for some feed producers (NRC, 1998).

2.3.2 Vitamin D

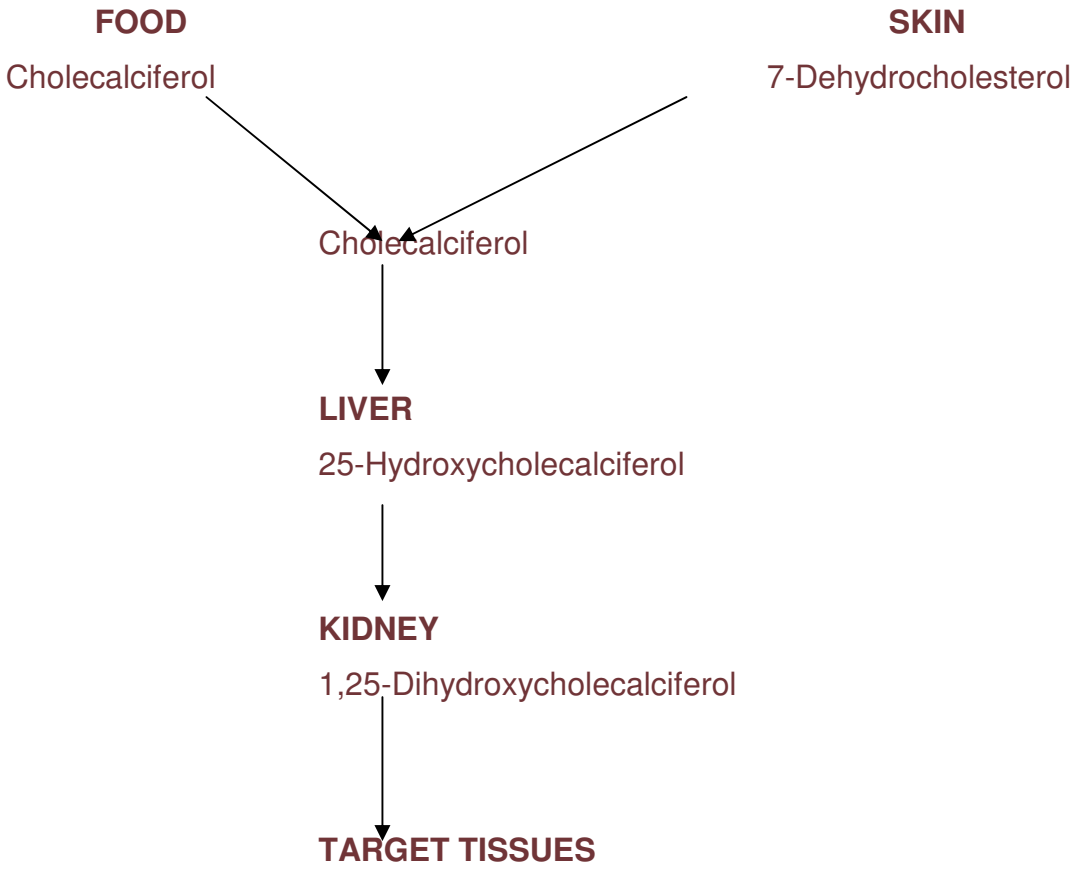
Vitamin D is an essential constituent in both human and animal nutrition. Vitamin D_3, the biologically active form of Vitamin D, plays a critical role in calcium and phosphate homeostasis and is essential for the proper development and maintenance of bone. In poultry, it has been observed that mineralization and body growth is reduced in cases of vitamin D deficiency (Hart, *et al.*, 1930). Little and Bird, 1949 reported that rickets and osteomalacia characterized vitamin D deficiency.

Livestock diets deficient in vitamin D have been implicated in a number of growth abnormalities including seizures, bone fracturing, thin or soft egg shells, decreased egg production and hatching, and diseases of the liver and kidneys (Ray *et al*., 1977). This vitamin is also known as a quasi hormone, due the various genes it regulates in living organisms. Evidence of vitamin D receptor presence in brains implies that this vitamin may have some function in this organ. Maternal vitamin D deficiency results in profound brain alterations at birth.

Vitamin D is obtained naturally by animals from irradiated feedstuffs or from synthesis within their own bodies under appropriate environmental conditions. Whilst chickens are able to synthesise vitamin D from sunlight, the amount is often inadequate even under natural conditions. As a result, proper regulation of dietary supplemental vitamin D_3 (active form of Vitamin D in poultry) is critically important.

Dietary ergocalciferol (D_2) and cholecalciferol (D_3) are absorbed through the small intestines and are transported in the blood to the liver where they are converted into 25-hydroxycholecalciferol (McDonald *et al*.,1995). The latter is then transported in the kidney where it is converted into 1, 25-dihydroxycholecalciferol, the most biologically active form of the vitamin. This compound is then transported in the blood to the various target tissues, the intestines, bones and the egg shell gland in laying birds. The compound 1, 25-dihydroxycholecalciferol acts in similar way to a steroid hormone, regulating DNA transcription in the intestinal microvilli, inducing the synthesis of specific messenger RNA which is responsible for the production of calcium binding protein (CBP). This protein is involved in the absorption of calcium from the intestinal lumen. Various pathways involved in these transformations are summarized below;

Fig. 2. Metabolic pathway showing production of hormonally active form of vitamin D



(Source: Animal Nutrition, McDonald *et al*.,1995).

The amount of 1, 25-dihydroxycholecalciferol produced by the kidney is controlled by the parathyroid hormone. When the level of calcium in the blood is low (Hypocalcaemia), the parathyroid gland is stimulated to secrete more parathyroid hormone, which induces the kidney to produce more 1,25-dihydroxycholecalciferol which in turn enhances the intestinal absorption of calcium. In addition to increasing intestinal absorption of calcium, 1, 25-dihydroxycholecalciferol increases the absorption of phosphorus from the intestine and also enhances calcium and phosphorus re-absorption from the kidney and bone.

Maximal growth can only be realized if there is a strong skeletal system to support it. This is especially true with today's rapidly maturing broiler and turkey. The trend towards marketing heavier birds has increased the need for a sound skeletal system (Rennie and Whitehead, 1996).

In poultry, a deficiency of vitamin D causes the bones and beak to become soft and rubbery, growth is usually retarded and the legs may become bowed. Egg production is reduced and egg shell quality deteriorates (McDonald *et al*, 1988). Most foods for poultry, with possible exception of fish meal, contain little or no vitamin D and the vitamin is generally supplied to these animals, if reared indoors, in form of fish liver oils or synthetic preparations. The recommended range for vitamin D is from 200 ICU/Kg diet to 500ICU/Kg diet (NRC, 1998).

2.3.3 Vitamin E

Vitamin E, commonly supplied as ά-tocopherol acetate in feed, is one of the body's main defenses against free radical attacks. It is a dietary essential added in animal diets at levels above minimum requirement due to benefits on tissue integrity, stability against oxidation and normal neurological function. Its main function is to work as a biological antioxidant, but it may also function in membrane structure, prevention of heavy metal toxicity, blood clotting, and biological oxidation-reduction reactions (McDowell, 1992). Oftentimes, excess E is provided in feeds to prevent oxidation and rancidity of added fat.

Vitamin E influences cells of immune system, such as lymphocytes and macrophages (Gebremichael *et al*., 1984). It also has beneficial effects on immunocompetence, limiting the production of prostagladins and leukotriens, which are powerful inducers of inflammation, and alters the release cytokines (Chung & Boren, 1999). Kidd (2004) observed increments in thymus and spleen cell T population for broilers supplemented with this vitamin. This observation agreed with Zhu *et al* (2003) using 200IU / Kg of vitamin E who found out that

there was a significant increment of T lymphocytes in turkeys challenged with Lysteria monocytogenes. Therefore a supplement with adequate levels of vitamin E enhances the immunology by increasing lymphocyte proliferation and has a positive effect on the interaction between lymphocytes B and T and inhibition of substances that prevent proliferation of lymphocytes T resulting in good broiler performance. Supplementing Vitamin E in well balanced diets is in agreement with the observation by Lauzon *et al*. (1983) that there was an increase of humoral immunity for monogastric species treated with vitamin E characterizing good growth.

Vitamin E as earlier indicated functions as a biological antioxidant in the animal body in association with the selenium-containing enzyme glutathione peroxidase, which protects cells against oxidative damage caused by free radicals. Free radicals are formed during cellular metabolism and, as they are capable of damaging cell membranes, enzymes and cell nuclear material, they must be converted into less reactive substances if the animal is to survive.

The animal body has two main methods of protecting itself against oxidative damage. Firstly, radicals are scavenged by vitamin E as the first line of defense and secondly, glutathione peroxidase destroys any peroxides formed before they can damage the cell. These two defense mechanisms complement one another.

Deficiency of the vitamin in chicks may lead to a number of distinct diseases; muscular dystrophy, encephalomalacia, or exudative diathesis. In nutritional myopathy the main muscles affected are the pectorals although the leg muscles also may be involved. Nutritional encephalomalacia or crazy chick disease is a condition in which chicks are unable to walk or stand, and is accompanied by haemorrhages and necrosis of brain cells. Exudative diathesis is a vascular disease of chicks characterized by a generalized oedema of the subcutaneous fatty tissues, associated with an abnormal permeability of capillary walls.

Both selenium and vitamin E appear to be involved in nutritional myopathy and exudative diathesis but selenium does not seem to be important in nutritional encephalomalacia. It should be stressed that selenium itself is a very toxic element and care is required in its use as a dietary additive (McDonald *et al.*, 1995). Whilst there should be a sufficient supply of vitamin E from the cereal fraction of the diet, vitamin E is easily destroyed by bad storage, over heating or rancid oils or fats in the feed hence supplementation becomes critical in poultry feeds (Ewer *et al.*, 1971). In broilers, where meat stability is favored, dosages in excess of 100ppm are recommended (Lauzon *et al.,* 2006).The recommended range for vitamin E in poultry is from 8 IU/Kg diet to 10IU/Kg diet (NRC, 1998).

2.3.4 Vitamin K

Vitamin K regulates the production of certain coagulation factors in the blood plasma e.g. 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. The vitamin is also important in relation to bone formation and bone re-modelling. Synthesis of osteacalcin, one of the main bone proteins, is dependent on vitamin K. Oseteocalcin is found in bone, uterus and egg shell. Low levels of osteocalcin can interfere with bone mineralization during skeletal development and egg shell formation.

Vitamin K absorption is in association with dietary fats, facilitated by the presence of bile salts. Vitamin K_3 (menadione) is the main source in poultry nutrition. The vitamin is metabolized in the liver but very little is stored, hence results in rapid depletion, probably within a week if there is no dietary supplementation (McDonald *et al.*, 1985).

Deficiency of the vitamin increases blood clotting time, resulting in haemorrhagic diseases in most tissues and organs and birds may bleed to death. In young poultry general weakness, rough plumage and paleness as well as discoloration of the comb, wattles and eyelids as a result of anaemia have been described. Subcutaneous and intramuscular haemorrhages and bloody feaces due to bleeding in the crop and in the caeca can occur.

To avoid deficiencies it is recommended that supplementation of 2-3 mg menadione per Kg diet should be routine in poultry feeds (NRC, 1998).

2.3.5 Riboflavin (Vitamin B_2)

Only a few of the feedstuffs fed to poultry contain enough riboflavin to meet the requirements of the growing chick. Hence, if the ingredients of a poultry feed are not carefully selected, or if a special supplement is not included, it may be deficient in riboflavin. Riboflavin occurs in all biological materials. The vitamin can be synthesized by all green plants, yeasts, fungi and most bacteria. The lactobacilli are a notable exception and require an exogenous source. Rich sources are yeast, liver, milk (especially whey), and green leafy crops. Cereal grains are poor sources (McDonald et al., 1995). The vitamin is stored in small quantities in the liver, spleen, kidney and cardiac muscle. These depots are maintained and, even in severe deficiency states, remain at steady level. Riboflavin is eliminated in the urine and daily loses can amount up to 30% of intake.

Riboflavin is of much practical importance for poultry, which have high requirements for it. The Vitamin is an essential part of an enzyme necessary in the oxidation processes of living cells. Riboflavin is essential for growth of animals and for proper nutrition at all ages (Morrison, 1954).

Riboflavin is an important constituent of the flavoproteins. The prosthetic group of these compound proteins contains riboflavin in the form of flavin mononucleotide (FMN) or in a more complex form as flavin adenine dinucleotide (FAD). There are several flavoproteins which function in the animal body and they are all concerned with chemical reactions involving the transport of hydrogen (McDonald *et al*, 1995).

Riboflavin deficiency is potentially fatal in all bird species (Powers, 2003). Chicks reared on a riboflavin deficient diet grow slowly and develop curled toe paralysis, a specific symptom, caused by peripheral nerve degeneration in which chicks walk on their hocks with toes curled inwards (Jylling, 1971). Other signs are stunting, diarrhea after 8-10 days, and high mortality after 3 weeks. There is no apparent impairment of the growth of feathers; on the contrary, the main wing feathers often appear to be disproportionately long (Fraser *et al.*, 1986) In breeding hens, a deficiency results in decreased hatchability (Romanoff and Bauernfeind, 1942). Cereals are poor sources of riboflavin but generally form the major part of the diet of poultry, hence deficiency troubles are likely to occur in practice (McDonald *et al*, 1988). The recommended dietary riboflavin level for broilers is 4.5mg/Kg.

2.3.6 Vitamin B_{12}

Vitamin B_{12} is only found in foods of animal origin (Ewer *et al.*, 1971). The vitamin has limited occurrence in higher plants but this is still controversial, since many think that its presence in trace amounts may result from contamination with bacteria or insect remains (McDonald *et al*, 1988). Vitamin B_{12} is considered to be synthesized exclusively by microorganisms and its presence in foods is thought to be ultimately of microbial origin. The main natural sources of the vitamin are foods of animal origin, liver being a particular rich source. It is therefore recommended to include vitamin B_{12} in most commercial poultry feeds to avoid the likelihood of its deficiency. Vitamin B_{12} is required for the

development of normal red blood cells and also for good hatchability and growth.

Vitamin B_{12} is the antipernicious anaemia factor. This vitamin functions in metabolism in two coenzyme forms, adenosylcobalamin and methyl cobalamin, both of which are related to methionine sythetase, leucine mutase and methyl malonyl-CoA mutase. The role of methionine sythetase, leucine mutase and methyl malonyl-CoA mutase in nutrition, have been widely accepted as that to do with starving off pernicious anaemia (Stabler and Allen, 2004). A possible deficiency of this vitamin affects B_{12} dependent methionine synthetase in folate metabolism, thus directly affecting synthesis of sulfur amino acid methionine from N^5 methyl tetrahydrofolate. Additionally, due to impaired production of transferable methyl groups (from methionine) it reduces synthesis of melatonin, epinephrine and phosphatidylcholine, with a concomitant effect on DNA-histone methylation that selectively modulates gene expression through out embryogenesis.

The cobalt is the active centre of the corrin nucleus which consists of a ring structure comprising five membered rings containing nitrogen. A cyano group is usually attached to the cobalt as an artifact of isolation and, as this is the most stable form of the vitamin, it is the form in which it is commercially produced (McDonald *et al.*, 1995). Before the vitamin can be absorbed from the intestine it must be bound to a highly specific glycoprotein, termed the intrinsic factor, which is secreted by the gastric mucosa. In animals, the cyanide ion is replaced by a variety of ions, hydroxyl (hydroxocobalamin), 5'-deoxyadenosyl (5'-deoxyadenosylcobalamin) and methyl (methylcobalamin), the last two forms acting as coenzymes in animal metabolism (McDonald *et al.*, 1995). The coenzymic forms of the vitamin fuction in several important enzyme systems. These include isomerases, dehydrases and enzymes involved in the biosynthesis of methionine from homocysteine.

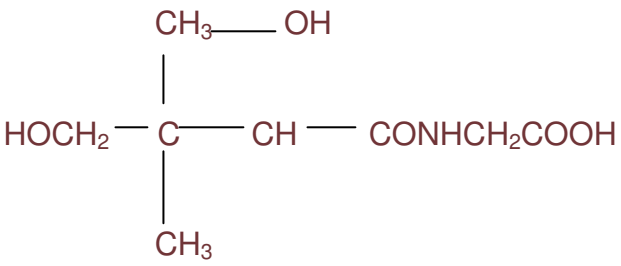
In poultry, in addition to the effect on growth, feathering is poor and kidney damage may occur if the vitamin is deficient. In young chicks, polynurities, curled toe paralysis, perosis and impairment of food utilization is common (Briggs *et al.*, 1942). Hens deprived of the vitamin remain healthy but hatchability is affected. However, if levels of cobalt in the diet are low, a deficiency of the vitamin will arise and cause reduced appetite, emaciation, decreased feed efficiency and anemia. Vitamin B_{12} deficiency is easily prevented and cured by feeding a diet containing feedstuffs of animal origin or a commercial cobalamin supplement.

Marked vitamin B_{12} deficiency is difficult, if not impossible, to produce in birds that have free access to their droppings. However, such birds may not receive optimal vitamin B_{12} levels and may fail to achieve growth at maximal rate (Fraser *et al.*, 1986). Adult animals are generally less affected by vitamin B_{12} deficiency than young growing animals, in which growth is severely retarded and mortality is high (McDonald *et al.*, 1995). The recommended daily range for vitamin B_{12} in poultry is 0.003mgKg diet to 0.009mg/Kg diet (NRC, 1998)

2.3.7 Pantothenic Acid

Pantothenic acid is an amide of pantoic acid and β-alanine and has the following formular;

Figure 3: Pathothenic Acid Chemical Structure (McDonald etal, 1995)



This vitamin is metabolically active as the prosthetic group of coenzyme A (CoA) and the acyl-carrier protein which is involved in the cytoplasmic synthesis of fatty acids (McDonald *et al.*, 1985). Chemically, CoA is a 3-phospho-adenosine-5-diphospho-pantotheine. Pantothenic acid deficiency, although rare, in most species results in reduced growth, decreased efficiency of feed ultilisation, anorexia, changes in hair, feather or skin, locomotor abnormalities, gastrointestinal problems, impaired adrenal functions, altered lipid and carbohydrate metabolism and adverse breeding outcomes (Smith and Song, 1996).

In broilers, deficiency symptoms include myelin degeneration of the spinal column with paralysis, ataxia and lethargy while additionally in mature birds high rates of embryonic and post hatch mortalities are experienced (Watanabe, 1990; Combs, 1998).The sources of the vitamin include liver, groundnuts, peas, yeast and molasses. Cereal grains and potatoes are also good sources o the vitamin (McDonald *et al.*, 1985). The recommended daily requirement for broilers for pantothenic Acid ranges from 10mg/Kg diet to 12mg/Kg diet (NRC, 1998).

2.3.8 Pyridoxine (Vitamin B_6)

The vitamin exists in three forms which are interconvertible in the body tissues. The parent substance is known as pyridoxine, the corresponding aldehyde derivative as pyridoxal and the amine as pyridoxamine. The metabolically active form of this vitamin is pyridoxal phosphate (PP), which serves as a cofactor of numerous enzymes, most of which are in amino acid metabolism as transaminases, decarboxylases and racemases. PP-dependent enzymes function in the biosynthesis of the neurotransimitters, serotonin, epinephrine and norepinephrine, and aminobutyric acid (GABA) an important source of energy for the brain. Lack of any of these PP dependent enzymes results in neurologic impairment (Haenggeli *et al.*, 1991).

Because of the numerous enzymes requiring pyridoxal phosphate, a large variety of biochemical lesions are associated with Vitamin B_6 deficiency. These lesions are concerned primarily with amino acid metabolism and a deficiency affects the animal's growth rate (McDonald *et al.*, 1985). B_6 deficiency affects various enzymes in folate metabolism such as thymidylate synthetase, glycinamide ribonucleotide transferase and N^5 formyl tetrahydrofolate transformylase that invariably results in reduced purine and pyrimidine synthesis, and impaired cell division affecting embryogenesis and brain development. In chickens and turkeys, deficiency of the vitamin exhibits reduced appetite and poor growth, dermatitis, marked anaemia, convulsions, reduced egg production and low fertility. Overall, most species develop paralysis, convulsions and peripheral nueropathies related to reduced neurotransmitter synthesis (Combs, 1998). The recommended range for pyridoxine in poultry is from 2.5mg/Kg diet to 3mgKg diet (NRC, 1998).

2.3.9 Folic Acid

Folic Acid is a B complex vitamin whose chemical name is pteroylmonoglutamic acid and is made up of three moieties; ρ-aminobenzoic acid, glutamic acid and a pteridine nucleus. Several active derivatives of the vitamin are known to occur, some containing up to 11 glutamate residues in the molecule (McDonald *et al.*, 1985). The monoglutamate form is readily absorbed from the digestive tract. The polyglutamates must be degraded by enzymes to the monoglutamate form before they can be absorbed.

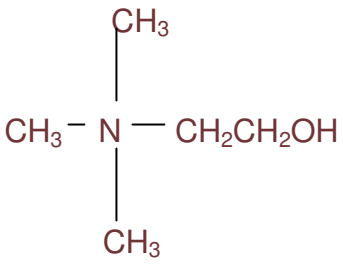
After absorption into the cell, folic acid is converted into tetrahydrofolic acid which functions as a coenzyme in the mobilization and utilization of single carbon groups (e.g. formyl, methyl) that are added to, or removed from such metabolites as histidine, serine, glycine, methionine and purines. The vitamin functions also as an enzyme cosubstrate serving as an acceptor or donor in many single carbon reactions of amino acids and nucleic acids. It is generally

established that the vitamin has an important role in DNA synthesis to support normal cell division (Rosenquist and Finnell, 2001). A deficiency of this vitamin in broilers initially results in severe anaemia, followed by luekopenia (abnormally low numbers of leukocytes), poor growth, poor feathering, perosis (as in niacin), poor growth, poor feed intake and poor bone formation (Singh, 2004). Other highly pigmented feathered birds show achromotrichia and in turkey poults a spatic type of cervical paralysis in which the neck is held rigid is seen (Combs, 1996). In reality Folic Acid deficiency symptoms rarely occur in other species of farm animals because of synthesis by intestinal bacteria. The recommended range for folacin in broiler and turkey production is from 0.25mg/Kg diet to 0.55mg/Kg diet (NRC, 1998).

2.3.10 Choline

Choline is a trimethylated hydroxide compound that occurs in biological tissues in the free form and as a component of lecithin, acetylcholine and certain of the plasmalogens and sphingomyelins. Choline is important as a source of labile methyl groups and is also synthesized by methylation of dimethylaminoethanol, the needed methyl group being supplied by methionine (Pike and Brown, 1975).

Figure 4: Choline Chemical Structure (McDonald *et al*., 1995)



Unlike the other B vitamins, choline is not a metabolic catalyst but forms an essential structural component of body tissues. It plays a vital role in cellular structure and activity (McDonald *et al*., 1985). It also plays an important part in

lipid metabolism in the liver by preventing the accumulation of fat in this organ. This conditional vitamin (known as trimethyl-ethanol amine) is an important methyl donor for the biosynthesis of structural compounds phosphatidylcholine, ceramide and sphingomyelin and neurotransmitter acetylcholine (Zeisel, 1993; 2000). Choline can be synthesized in the liver from methionine and the exogenous requirement for this vitamin is therefore influenced by the level of methionine in the diet (McDonald *et al*., 1985). The vitamin is supplemented to young birds because they do not have fully functional S-methyl transferase enzymes at an early age (Burgos *et al*., 2006). Synthesis of choline by methylation of dimethylaminoethanol, the needed methyl group being supplied by metionine and is catalysed by the S-methyl tranferase enzymes. Absence of the methyl transferase enzymes in poultry chicks inhibits choline synthesis and necessitates dietary supplementation of the vitamin. A deficiency of choline in the diet, even where there are adequate quantities of other nutrients, results in the development of perosis. Perosis is the chief sign of choline deficiency in poultry (Pike and Brown, 1975). Good sources of choline include green leafy materials, yeast, egg yolk and cereal grains. Other good sources of choline are distillers grains, fish meal, liver meal, meat scrap and distillers solubles.

A deficiency of choline expresses as depressed growth, hepatic steatosis, impaired nerve function and defective neurotransmission resulting in limited body movement and consequently infrequent access to food and water (Combs, 1996). The recommended choline range for broilers is from 500 mg/Kg diet to 1500mg/Kg diet (NRC, 1998).

2.3.11 Niacin (Nicotinic Acid)

The vitamin is an amine derivative of nicotinic acid (pyridine 3-carboxylic acid) and is the form in which it functions in the body. The vitamin is stable and is not easily destroyed by heat, acids, alkalis or oxidation (McDonald, *et al*., 1985). It is active in the metabolism as nicotinamide and represents an indispensable

component of hydrogen carrying coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). The vitamin 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 vitamin can be synthesized from tryptophan in the body tissues and since animals can convert the acid to the amide-containing coenzymes, it follows that if the diet is adequately supplied with proteins rich in tryptophan, then the dietary requirement for the vitamin itself should be low. However, the efficiency of conversion of tryptophan into nicotinamide is poor (McDonald *et al.*, 1985). Thus, it is obvious, that a dietary supplementation with niacin is necessary. Rich sources of the vitamins are the liver, yeast, groundnut and sunflower meals.

Deficiency of the vitamin in poultry causes bone disorders, feathering abnormalities and inflammation of the mouth and upper part of the oesophagus. The recommended range for niacin in poultry is very large but for broilers the recommended level is from 11mg/Kg diet to 27mg/Kg diet (NRC, 1998).

2.3.12 Biotin

Biotin is chemically 2-keto-3, 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid. Biotin is absorbed in the small intestine, transported in the blood stream to various tissues, but inadequately metabolized. Excess biotin is excreted in both urine and faeces. Biotin represents an important co-enzyme 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 substrate, and a possible effect on purine and pyrimidine synthesis has been put forward (Pike and Brown, 1975).Three biotin dependent enzymes

of particular importance include; pyruvate carboxylase, acetyl coenzyme A carboxylase and propionyl coenzyme A carboxylase. Biotin is completely available in maize and certain oilseed meals such as soybean meal.

Deficiency in poultry causes reduced growth, dermatitis, leg bone abnormalities, cracked feet, poor feathering and fatty liver and kidney syndrome (FLKS). FLKS mainly affects 2-5 weeks old chicks and is characterized by a lethargic state with death frequently following within a few hours. On autopsy, the liver and kidneys, which are pale and swollen, contain abnormal depositions of lipid (McDonald *et al.*, 1985). The requirement rate for biotin in broilers ranges between 0.10 and 0.15mg./Kg diet (NRC, 1998).

2.4 Trace Minerals

The role of trace minerals in animal production is an area of strong interest for producers, feed manufacturers, veterinarians and scientists. Adequate trace mineral intake and absorption is required for a variety of metabolic functions including immune response to pathogenic challenge, reproduction and growth. Mineral supplementation strategies are critical because trace mineral requirements of all livestock and avian species are important in order to optimize production in modern animal production systems.

Minerals have important biological functions and their requirements for broiler chickens have to be met for optimum growth and performance. NRC (1994) gives the minimum levels that are necessary for optimum productivity. In a review, Pallauf (1979) stated that with exception of trace minerals such as manganese and zinc, requirements are met by raw materials used in poultry rations. However food ingredients grown in some regions have trace element deficiencies due to soil conditions, hence, to ensure the normal levels of such minerals in the diets, trace mineral supplements are added to the rations. Rapid

growth in broilers may increase the need for supplemental minerals hence external source of trace minerals becomes a critical practice.

Sub-clinical or marginal deficiencies may be a larger problem than acute mineral deficiency because specific clinical symptoms are not evident to allow the producer to recognize the deficiency. However, animals continue to grow and reproduce but at reduced rate. As animal trace mineral status declines immunity and enzyme functions are compromised first, followed by a reduction in maximum growth and fertility, and finally normal growth and fertility decreases prior to evidence of clinical deficiency (Fraker, 1983; Wikse, 1992). It is known that particularly mineral and trace mineral deficiencies, caused by insufficient feeding led to growth retardation (Radostits *et al.*, 1995). In order to maintain animals in adequate trace mineral status, balanced intake and absorption are necessary.

Clinical disorders seen in trace element deficiencies include diarrhea, anaemia, loss of hair, depigmentation, bone deformities, parakeratosis, lack of appetite, reduced fertility, retardation in the development of foetus, loss of sperm quality, abortion unrelated to infections and interruptions in the protein synthesis (Chesters and Arthur, 1988; Kozat *et al.*, 2007; Underwood and Suttle, 2001).

To better understand the role of trace minerals in animal production it is important to recognize that trace minerals are functional components of numerous metabolic events. Trace mineral functions can be described by four broad categories (Underwood and Suttle, 1999); structural, physiological, catalytic and regulatory. Structural function refers to minerals forming structural components of the body organs and tissue. Physiological function occurs when minerals in body fluids and tissue act as electrolytes to maintain osmotic pressure, acid base balance, and membrane stability. Catalytic function is probably the largest category for trace mineral as it refers to catalytic role of metalloenzymes in enzyme and hormone systems. Upon removal of the trace

element or lack of adequate trace mineral levels the enzyme activity is compromised followed by loss of performance and reproduction. The importance of enzyme function as it relates to animal performance was illustrated by zinc depletion trials reported by Engle *et al*. (1997). Zinc was shown to have a critical role in proteolytic enzyme systems associated with muscle protein turn over. Muscle protein accretion was shown to decrease when supplemental zinc was removed from the basal forage diet for 21 days , however when zinc was added back to the diet for 14 days, muscle protein accretion returned to normal levels. Regulatory function is exemplified by the role of zinc to influence transcription and iodine serving as a constituent of thyroxine, a hormone associated with thyroid function and energy metabolism. In recent years 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; Fletcher *et al*., 1988). Trace minerals, Iron, iodine, copper, manganese and Zinc were selected for detailed description because they are the ones often considered critical in nutrition and hence supplemented. They play primarily catalytic roles in cellular metabolism and therefore function in a much the same way as vitamins. Some of these elements appear to function entirely as components of larger molecules whose metabolic role, however, also is fundamentally catalytic. A deficiency in one or more of these elements can compromise immunocompetence of an animal (Beisel, 1982; Suttle and Jones, 1989).

2.4.1 Zinc

Zinc is fairly widely distributed. Yeast is a rich source, and zinc is concentrated in the bran and germ of the cereal grains. Animal protein by products such as meat meal and fish meal are usually richer sources of zinc than plant supplements. Zinc has been found in every tissue in the animal body. It tends to accumulate in the bones rather than the liver, which is the main storage organ of

many of the other trace minerals. High concentrations have been found in the skin, hair and wool of animals. Several enzymes in the animal body are known to contain zinc; these include carbonic anhydrogenase, alkaline phosphotase, thymidine kinase, alcohol dehydrogenase, lactatedehydrogenase and pancreatic carboxypeptidedase (Scuttle and Jones, 1989).

It is well documented that Zinc (Zn) is an essential trace element for the growth and development of plants, animals and humans, needed for various physiological functions including bone formation, host defense, sexual maturity, reproduction and tissue growth (McDowell, 1992). In addition Zinc is an activator of several enzyme systems. It is involved in cell replication and differentiation, particularly in nucleic acid metabolism. Among the other physiological functions of zinc are production, storage and secretion of hormones, involvement in the immune system and electrolyte balance (McDonald *et al*, 1995)

Zinc is required in the diet of broilers (NRC, 1994, McDowell, 1992), but it should be noted that Zinc requirements of chicks varies depending upon the nutrient contents as well as protein sources in the diet. Zinc plays a key role in enzyme activity, cell replication, and development of bone and cartilage, and Zn deficiency could cause delayed sexual development (Baker and Ammerman, 1995b). Research has been ongoing for providing Zn in the diet of animals. Often either ZnO or $ZnSO_4$ is added to the diet. If a Zn source that is more available to the animal can be found, it could be fed at lower levels, and thereby decreasing the Zn excreted and reduce costs to producers. Some research has shown that Zn complexed with amino acids, such as methionine or lysine, renders Zn more available to animals (Wedekind *et al*., 1992).

A Zn amino acid complex could be a more available source than $ZnSO_4$ making it a more economical choice for addition to feeds. In many countries zinc requirement is advised to be 40mg/Kg diet throughout the whole period, but some researchers showed that the 40mg zinc/Kg diet was not sufficient for

broilers to reach better performance. Xiuyun Wu (1995) reported that the appropriate zinc level was 83mg/kg diet for weight gain of Arbor Acres broilers, while Dechao Liu (1995) recommended that zinc levels should be 160-172mg/Kg diet for the best weight gain. Dechao Liu (1995) reported that the zinc level of 210mg/Kg diet will give the best feed efficiency but this was contrasted by Mohanna and Nys (1999) who reported that dietary zinc concentration of 45mg/Kg was sufficient to obtain normal broiler performance.

Zinc deficiency has been shown to have an important impact on immunity (Gershwin et al., 1985; Droke and Spears, 1993). Decreased cellular immunity, lowered antibody response and disrupted growth of T-dependent tissue have resulted from inadequate intake of zinc (Fletcher *et al.*, 1988). Zinc deficiency in poultry results in retarded growth in chicks, foot abnormalities, frizzled feathers, parakeratosis and a bone abnormality referred to as swollen hock syndrome. The hock joints may become enlarged and the long bones shortened and thickened. Occasionally, the skin on the foot pads becomes dry and thickened with fissures and hyperkeratosis develops.

In mature hens, zinc deficiency reduces egg production and hatchability. Embryos show a wide range of skeletal abnormalities, including micromelia, curvature of the spine, and shortened, fused thoracic and lumbar vertebrae. Therefore, it is usual practice to include zinc supplement in all practical poultry diets (Fraser *et al.*, 1986). Dewar and Downie (1984) reported that zinc requirements of broilers for maximum performance ranges between 18mg/kg and 50mg/kg diet.

2.4.2 Manganese

Manganese is present in the animal body in extremely small amounts. Most tissues contain traces of the element, the highest concentrations occurring in the bones, liver, kidney, pancreas and pituitary gland. Manganese is important in

the animal body as an activator of many enzymes such as hydrolases and kinases and as a constituent of arginase, pyruvate carboxylase and manganese superoxide dismutase (McDonald *et al.*, 1995).

Many poultry feedstuffs do not contain enough manganese. Yeast and most foods of animal origin are also poor sources of manganese. This is true of most cereals, and diets based on high cereal levels usually are deficient unless they contain a special source, such as manganese sulfate. Manganese deficiency is now much less common because virtually all commercial feeds contain added manganese. Rich sources are rice, bran and wheat offals.

Manganese is required in the diet of broilers. Manganese is essential in the prevention of perosis in broiler chicks. Other processes that Mn is needed for are normal enzyme activity and bone growth. A specific role for manganese in the synthesis of mucopolysaccharrides has also been demonstrated (Leach. and Muester, 1962). Two enzyme systems appear to be the sites of manganese function; the polymerase system which is responsible for polysaccharide chain elongation and the galactosyltransferase system which incorporates galactose into the protein component of the molecule (Leach, 1971).This function has been studied primarily in chicks which develop a syndrome known as slipped tendon or perosis as a result of manganese deficiency. Cartilage from manganese deficient chicks contains less hexuronic acid than from normal chicks and this is the leading cause of perosis where the achilles tendon slips off its groove behind the hock joint, pulling sideways and backwards (Ewer *et al.*, 1971).

Deficiency of Mn could pose abnormal male and female reproductive functions (Henry, 1995). Supplementation of Mn is generally in the form of $MnSO_4$. Manganese requirements of broilers for maximum performance ranges between 50mg/kg and 100mg/kg diet.

2.4.3 Copper

Copper though not actually a constituent of haemoglobin is present in certain other plasma proteins such as ceruloplasmin which are concerned with the release of iron from the cells into plasma. Copper is also a component of other proteins in the blood such as erythrocuperin, which occur in erythrocytes where it plays a vital role in oxygen metabolism. It is also known to play a vital role in many enzyme systems, for example, it is a component of cytochrome oxidase, which is important oxidative phosphorylation. Copper is necessary for the normal pigmentation of feathers, hair, fur and wool. The element is thought to be present in all body cells, being particularly concentrated in the liver, which acts as the main copper storage organ of the body.

Copper (Cu) from various compounds has often been added to poultry diets as an antimicrobial agent at concentrations far in excess of the 8mg/kg requirement established by National Research Council (NRC, 1994). Several authors have observed that the addition of levels ranging from 125 to 250mg/kg as copper sulfate or carbonate, resulted in a positive response in body weight and feed efficiency (Fisher *et al.,* 1973; Hoda & Maha, 1975).

The mechanism by which Cu promotes performance improvements in poultry is not completely clear. One of the mechanisms pointed out as responsible for the effects of Cu in relation to performance improvement would be its action over disease causing microorganisms that, even without displaying clinical signs, might be detrimental to animal growth (Cromwell, 1991). Copper functions in the immune system through the following: energy production, neutrophil production and activity, antioxidant enzyme production, development of antibodies and lymphocyte replication (Niederman *et al*., 1994; Nockels, 1994). Low copper status results in decreased humoral and cell mediated immunity (Jones and Suttle, 1981a and 1981b; Xin et al., 1991, Gengelback *et al*., 1997), as well as decreased neutrophil bactericidal capability in steers.

In this respect, there is evidence that antimicrobial agents are usually more efficient as growth promoters in old facilities or buildings with poor sanitary conditions than in new, isolated environments (Hill *et al.,* 1953; Visek, 1978) Copper is required in broiler diets at 5 ppm of the diet from week 0 to 6, and it decreases to 4 ppm until the market age is reached (NRC, 1994). Copper is essential in many enzyme functions and metabolism. Deficiency of Cu could lead to anemia and could have adverse effects on the cardiovascular system and the central nervous system (Baker and Ammerman, 1995a). Hart *et al.* (1928) first reported that copper is essential for the utilization of iron for hemoglobin formation in rats. Copper in its unbound form is a pro oxidant (Diplock *et al.,* 1998).

Copper has also some catalytic functions in which Copper enzymes catalyze oxidation reactions. Enzymes involved in the oxidation of cytochrome c and mono– and diamines are copper containing proteins. Copper containing enzymes play an important role in connective tissue metabolism, specifically in the oxidation of amino groups of lysine side chains necessary for cross linkage of the polypeptide chains of elastin and collagen.

Since copper has been known to perform many functions in the animal body there are a variety of deficiency symptoms. These include anaemia, poor growth, bone disorders, scouring, infertility, depigmentation of hair, feathers and wool, gastro intestinal disturbances and lesions in the brain stem and spinal cord. The lesions are associated with muscular incoordination, and occur especially in young lambs (McDonald *et al*, 1995). Copper is essential in the synthesis of elastin tissue and a dietary deficiency can result in weak or thin blood vessels causing dissecting aneurysm in poultry, especially in turkeys. Recommended copper requirements of broilers for maximum performance ranges between 3.5mg/kg and 8mg/kg diet (NRC, 1998).

2.4.4 Iodine

Iodine is one of the most important microelement in animal and human health. As far as is known the role of iodine in the animal body is related solely to the function as a constituent of thyroxine and other related compounds synthesized by the thyroid gland (Pike & Brown, 1975). Symptoms of iodine deficiency may develop in humans and animals (Grossman, 1994), therefore improvement of iodine supply is still a great challenge for nutritionists. Although it is known to be distributed through out the tissues and secretions, it's only known role is in the synthesis of two hormones, triiodothyronine and tetraiodothyronine (thyroxine) produced in the thyroid gland. Iodine also occurs in the gland as monoiodotyrosine and diiodotyrosine which are intermediates in the biosynthesis of the hormones from the amino acid tyrosine.The two hormones are stored in the thyroid gland as components of the protein thyroglobulin which releases the hormones into the blood capillaries when required. The thyroid hormones accelerate reactions in most organs and tissues in the body, thus increasing the basal metabolic rate, accelerates growth and increases the oxygen consumption of the whole organism.

Investigations on supplementing poultry and other livestock feed with different forms and dosages of iodine have been carried out in different research units seeking to improve the quality of poultry products. Biologically active substances, normally contained in poultry meat and eggs in variable quantities, can be increased by supplementing poultry feed with vitamins, minerals and specific microelements such as iodine.

At the Texas Agricultural Research Center, Stanely and Bailey (1998) studied the effect of iodine enriched drinking water on broilers grown at different densities. They found that adding 2ppm iodine to the drinking water significantly improved broiler growth. But it was also found that excess iodine in feed of

growing chickens may delay sexual maturity and in layer feeds it may lead to gradual decrease in rate of production until at 2500mg iodine /Kg feed ovulation stops completely.

When a diet deficient in iodine is fed, production of thyroxine is decreased. The main indication of such a deficiency is an enlargement of the thyroid gland, termed endemic goiter, and is caused by compensatory hypertrophy of the gland. In breeding hens, reproductive abnormalities are one of the most outstanding consequences of reduced thyroid function and results in poor egg hatchability. The iodine requirements of broilers for maximum performance ranges between 0.3mg/kg and 0.5mg/kg diet and is often added as a supplement (NRC, 1998).

2.4.5 Iron

Iron functions in living systems in the respiratory process. Inorganic iron compounds possess some oxidative activity and ability to transport electrons, and this property is enhanced when iron is present in combination with protein. Iron also occurs in blood serum in a protein called transferin which is concerned with the transport of iron from one part of the body to another. Ferritin, a protein containing up to 200g/Kg of the element is present in the liver, spleen, kidney and bone marrow and provides a form of storage for iron. Haemosiderin is a similar storage compound which may contain up to 350g/Kg of the element. Iron has a major role in a host of biochemical reactions, particularly in connection with enzymes of the electron transport chain (cytochromes). Electrons are transported by the oxidation and reduction activity of bound iron. Among the enzymes containing or activated by iron are catalase, peroxidases, phenylalanine hydroxylase and many others including all the tricarboxylic acid cycle enzymes (Morrison, 1954; McDonald *et al*, 1995).

Oxygen is transported in the blood by hemoglobin, which is an iron containing compound in the red blood cells. Iron has other vital functions in the body. It is necessary for the functioning of all body organs and tissues, and is present in the nuclei, or life centers, of the body cells.

Iron being part of the haemoglobin molecule can induce an anaemic condition in an animal in a case of deficiency. A deficiency of iron, due to its role in the red blood cells (RBC) synthesis, has a direct causative effect on aneamia. A microcytic, hypochromic anaemia with no change in the number of RBC can be produced by iron deficiency. Though it may not be expressed in broiler chicks, it does however, sometimes occur in laying hens, since egg production represents a considerable drain on the body reserves. From the above, it is clear that adequate supply of iron is essential for normal metabolism and growth in animals. Iron requirements of broilers for maximum performance ranges between 60mg/kg and 80mg/kg diet (NRC, 1998).

2.5 Protein

Proteins are very critical in normal growth of a broiler. The nutritional value of protein depends on the relative abundance of certain amino acids in the protein source. However, not all protein is the same as the components (amino acids) that make up crude protein can differ in type and concentration from one source to the next. The challenge is determining what levels of amino acid density are cost effective for specific market weights and products. The amount of protein needed to provide the required amino acid content and balance will depend upon the amino acid composition of the feed ingredients. Protein is a relatively expensive component of a diet and it seems that the requirement for the first limiting amino acid increases nearly in direct proportion to the CP content of the diet (Morris *et al.*, 1999). It is therefore a desirable practice to formulate diets to meet the individual amino acid requirements at the lowest economical CP content. Failure to meet the requirement for an amino acid can result in

depressed growth but without the appearance of any specific lesions. Broilers have an appetite for both protein and energy and will regulate their intake to meet their needs for both of these nutrients. Thus a bird can overconsume a diet marginally deficient in protein or amino acid in order to optimize its intake of the limiting nutrient. Over consumption of energy will be consequence of this adaptation with excess energy being deposited as fat. Conversely, a bird will consume less of a diet containing high protein content and will have an improved feed conversion and leaner.

In poultry ration, protein costs involve 45% of the total feed cost (Lester, 1989). Among the vegetable protein sources, soybean meal is comparatively cheaper and readily available. The nutrients are readily digestible and the protein has a high biological value placing it on top of vegetable concentrate feeds (Popa *et al.,* 1980, Burlacu, 1983). Its nutritive value is quite fine when compared with other plant protein sources. But this mostly available and cheaper soybean meal, if not treated, is not suitable for using in higher amounts in poultry diets because of some anti nutritive factors like phytate phosphorus, trypsin inhibitors, non starch polysaccharides, oligosaccharides and lectins (NRC, 1994) which decreases feed consumption, growth rate and feed ultilisation.

Protein can be found in most feedstuffs, but concentrated protein supplements are often used to meet amino acid requirements. Natural feedstuffs alone can not provide enough of the essential amino acids or balanced amino acid profile hence essential amino acids must be provided in the diets. The dietary balance of amino acids is very critical and satisfactory growth would not result without sufficient quantities and proper balance of all indispensable amino acids (Fraser *et al*., 1986)

In practical poultry diets, methionine is the first limiting amino acid followed by lysine. Therefore supplementation of methionine and lysine to practical diets provides a means for increasing the efficiency of protein utilization. The lysine

requirement of broilers ranges between 10g and 12g/Kg of the diet while that for methionine + cystine ranges between 8g and 9.2g/Kg of the diet. If cystine, or its metabolically active form cysteine, is deficient in the diet, it is synthesized by the animal from methionine. The requirement for methionine is therefore partially dependent on cystine (or cysteine) content of the diet, and the two amino acids are usually considered together (i.e. the requirement is stated for methionine plus cystine) (McDonald *et al.*,1985). It should be noted however that the two amino acids are not mutually interconvertible, methionine is not synthesized from cystine and therefore a part of the total requirement must always be met by methionine (McDonald *et al.*, 1985). Poultry amino acid requirements are not easily assessed, as they are influenced by several factors such as metabolizable energy of the diet, age, gender, feed intake and environmental conditions (Rostagno *et al.*, 1995).

The optimal level of balanced protein intake for the young growing chick appears to be 21-23% of the diet. When protein content is reduced below the optimal level, the birds tend to grow more slowly (Fraser *et al.*, 1986). Even though a diet contains the above specified quantities of protein, satisfactory growth will not result without sufficient quantities and the proper balance of all the indispensable amino acids. A deficiency of protein results in lowering of the available protein in the diet; thus calorie: protein ratio usually increases, which in turn can result in a reduction of growth and an increase in fat deposition in the bird (Fraser *et al.*, 1986). A deficiency of various amino acids, with exception of loss of pigment in some of the wing feathers, may result in growth retardation, reduced egg size and reduced egg production depending on the degree of deficiency and the particular amino acid.

The health and well being of chickens depends upon the interaction between their genetic potential and exogenous factors like adequate nutrition, proper growth environment, reduced exposure to stressors and appropriate managerial practices (Burgos etal.,2006). Nutrition has a prominent role in promoting

growth, development, immunity and reproduction. Micro nutrients (vitamins and minerals) coupled with essential amino acids are required for the integrity and optimal function of living systems. There are 13 accepted vitamins 4 of which are lipid soluble (Vitamins A, D, E, K) and 9 are water soluble (B_1, B_2, Nicotinamide, B_6, Pantothenic Acid, Folic Acid, Biotin, Choline, B_{12},).

Table 1. NRC Nutrient Requirements of Poultry: Ninth Revised Edition, 1994.

Nutrient	Weeks 0-3[a]	Weeks 3-6[a]	Weeks 6-8[a]
ME/kg diet	3,200[b]	3,200[b]	3,200[b]
Protein[c] (%)	23.0	20.0	18.0
Arginine (%)	1.25	1.10	1.00
Glycine + Serine (%)	1.25	1.14	0.97
Histidine (%)	0.35	0.32	0.27
Isoleucine (%)	0.80	0.73	0.62
Leucine (%)	1.20	1.09	0.93
Lysine (%)	1.10	1.00	0.85
Methionine + Cystine (%)	0.90	0.72	0.60
Methionine (%)	0.50	0.38	0.32
Phenylalanine + Tyrosine (%)	1.34	1.22	1.04
Phenylalanine (%)	0.72	0.65	0.56
Proline (%)	0.60	0.55	0.46
Threonine (%)	0.80	0.74	0.68
Tryptophan (%)	0.20	0.18	0.16
Valine (%)	0.90	0.82	0.70
Linoleic acid (%)	1.00	1.00	1.00
Calcium[d] (%)	1.00	0.90	0.80
Phosphorus, available (%)	0.45	0.35	0.30
Potassium (%)	0.30	0.30	0.30
Sodium (%)	0.20	0.15	0.12
Chlorine (%)	0.20	0.15	0.12
Magnesium (mg)	600	600	600
Manganese (mg)	60.0	60.0	60.0
Zinc (mg)	40.0	40.0	40.0
Iron (mg)	80.0	80.0	80.0
Copper (mg)	8.0	8.0	8.0
Iodine (mg)	0.35	0.35	0.35
Selenium (mg)	0.15	0.15	0.15
Vitamin A (IU)	1,500	1,500	1,500
Vitamin D_3 (ICU)	200	200	200

Vitamin E (IU)	10	10	10
Vitamin K (mg)	0.50	0.50	0.50
Riboflavin (mg)	3.60	3.60	3.00
Pantothenic acid (mg)	10.0	10.0	10.0
Niacin (mg)	35.0	30.0	25.0
Vitamin B12 (mg)	0.01	0.01	0.007
Choline (mg)	1,300	850	500
Biotin (mg)	0.15	0.15	0.12
Folacin (mg)	0.55	0.55	0.50
Thiamin (mg)	1.80	1.80	1.80
Pyridoxine (mg)	3.50	3.50	3.00

Note: Where experimental data are lacking, values typeset in bold italics represent an estimate based on values obtained for the other ages or related species.

[a] The 0-3, 3-6 and 6-8 week intervals for nutrient requirements are based on chronology for which research data were available, however, these nutrient requirements are often implemented at younger age intervals or on a weight of feed consumed basis.

[b] These are typical dietary energy concentrations, expressed in kcal ME/kg diet. Different energy values may be appropriate depending on local ingredient prices and availability.

[c] Broiler chickens do not have a requirement for crude protein perse. There, however, should be sufficient crude protein to ensure an adequate nitrogen supply for synthesis of non essential amino acids. Suggested requirements for CP are typical of those derived with maize-soy diets, and levels can be reduced when synthetic amino acids are used.

[d] The calcium requirement may be increased when diets contain high levels of phytate phosphorus.

The scientific feeding of farm animals is based on standards expressed in terms of either nutrient requirements or nutrient allowances. These figures in the tables are mainly nutrient requirement as they do not include safety margins. Only the figures for vitamins have safety margins (NRC, 1984).

CHAPTER 3

3.0 MATERIALS AND METHODS

Commercial nutritional supplements are a combination of amino acids, vitamins and minerals which are added to the formulated diet to meet the requirements of at least the amino acids, vitamins and minerals deficient in the formulated diet.

Critical vitamins (choline, folic acid, pantothenic acid, pyridoxine, riboflavin, Vit A, VitD and VitE), Minerals (calcium, phosphorus, copper, iodine, iron, manganese, sodium and zinc) and essential amino acids are usually supplied by inclusion of nutritional supplements in broiler diets and this has become an indispensable practice because feed ingredients do not contain all the essential nutrients in the right amounts needed for good broiler performance. It is now not unusual to add all vitamins, minerals and amino acids in poultry diets in form of supplements.

The selected supplements (Vita Flash Amino, Vitamino Trace Oral and Amino Vitasol and Chick-A-min) were purchased from a local market within Lusaka district.

3.1 Vitamino Trace Oral (VTO)

Vitamino Trace Oral is a commercial supplement that contains amino acids, minerals and vitamins. The supplement is a liquid, amino acid, trace element and vitamin preparation for administration via drinking water. It is especially recommended during periods of peak production, vaccinations and recovery from infections. As indicated on the label by the manufacturer, the supplement is used for treatment and prevention of vitamin deficiencies in animals, weakness of new born, growth disturbances, anorexia, osteoporosis, disturbances of

intestinal flora, poor condition stress, vaccinations, and excessive changes in temperature, movement of animals, change of hen house and poor diet conditions. It does not contain any antibiotics and can be used to treat post treatment after coccidiosis, worm infestation, bacterial and viral infections. It is used as a supplement to the feed and is particularly recommended for use in day old chicks.

3.2 Amino Vitasol (AV)

Amino Vitasol is a water soluble commercial powder for oral treatment of deficiencies of vitamins and amino acids. The label on the product, from the manufacturer, indicates that it is also used for the treatment of stress, growth disturbances and post treatment after treatment against coccidiosis, worm infestation, bacterial and viral infections in poultry. The supplement is used for prevention, treatment completion and deficiency of vitamins and amino acids. It is useful in the state of threat of infectious stress, convalescence after disease and parasitic infections, metastasis groups and changes in feeding and maintenance regimes. It is an antibiotic free supplement administered orally via water and used to supplement the daily diet for routine feeding of poultry.

3.3 Chick-A-min (CM)

Chick-A-min is a food grade water soluble powder containing vitamins, amino acid and minerals. As per label from the manufacturer of the product, Chick-A-Min is 100% soluble in water, non toxic, and antibiotic and hormonal free. It is used to treat and prevent vitamin, mineral and amino acids deficiencies in day old chicks. It is also effectively used for treating various forms of stress such as, nutritional, vaccinations, severe temperature changes, growth disturbances, and as post treatment of coccidiosis, and worm infestation. It is suitable for all liquid and dry feed systems.

3.4 Vita Flash Amino (VFA)

Vita Flash Amino is a commercial watable soluble powder (WSP) made up of vitamins and amino acids. Indications, as prescribed on the manufacturer's label, are that it is recommended for use in stressful periods especially during the first days of life, vaccinations, and excessive changes in temperature and change of hen house effects. It can also be used for post treatment after a cocciodiosis treatment, worm, bacterial and viral infections. It gives better results if used during off feed periods and during noted periods of deficiency or nutritional inadequacy.

Table 2. Comparative composition of different supplements (per Kg)

Content	Amino Vitasol	VitaFlash Amino	Vitamino Trace Oral	Chick-A-min
Vitamin A (IU)	500 000	10, 000, 000	15,000,000	550, 000
Vitamin D (IU)	50 000	2,000,000	4,000,000	360, 000
Vitamin E (mg)	500	15, 000	3,500	66, 000
Vitamin K_3 (mg)	150	2, 500	1,000	300
Vitamin B_1 (mg)	2 500	1, 000	2,000	250
Vitamin B_2 (mg)	200	2, 000	-	380
Nicotinic acid (mg)	1 000	20 000	-	2,500
Pantothenic acid (mg)	800	7, 500	4,000	800
Vitamin B_6 (mg)	50	2, 000	1,250	2,000
Vitamin B_{12} (mg)	1	10, 000	-	20
Vitamin B_9 (mg)	-	1, 000	-	-
Folic acid (mg)	10	300	-	500
Ascorbic acid (mg)	-	150 000	3,500	250
Inositol (mg)	200	-	-	200
Biotin (mg)	2	-	-	40
Choline Chloride (mg)	-	15,000	-	250
Zinc (mg)	-	-	100	60
Potassium (mg)	-	-	830	-
Calcium(mg)	-	-	220	-
Copper (mg)	-	-	35	80
Cobalt (mg)	-	-	-	-
Iodine (mg)	-	-	-	50
Iron (mg)	-	-	250	800
Sodium (mg)	-	-	-	152

Selenium (mg)	-	-	-	30
Manganese (mg)	-	-	-	140
Methionine (mg)	1 450	40, 000	80	1 500
Lysine (mg)	3 000	50, 000	440	1 200
Cystine (mg)	220	-	-	300
Tryptophan (mg)	200	-	220	260
Arginine (mg)	1 630	-	480	1 800
Threonine (mg)	1 000	-	80	1 000
Isoluecine (mg)	1 000	-	100	1 200
Luecine (mg)	1 700	-	300	1 800
Valine (mg)	1 200	-	280	1 600
Histidine (mg)	460	-	100	500
Phenylalanine(mg)	700	-	200	1 200
Tyrosine (mg)	800	-	50	1 000
Glycine(mg)	2 000	-	2,600	2 000
Glutamic Acid (mg)	5 400	-	1,000	6 000
Aspartic acid (mg)	2 200	-	500	3,500
Proline (mg)	1 200	-	1,700	1,800
Serine (mg)	1 100	-	100	1,200
Alanine (mg)	1 700	-	1,000	2,000

Most studies have revealed that not all nutrients are supplied in the right amounts by the maize-soybean meal diet hence need for supplementation with the vital nutrients earlier outlined. In order to meet short falls vital in nutrition of poultry, supplements; Amino Vitasol (AV), Vita flash amino (VFA), Vitamino Trace Oral (VTO) and Chick-A-min (CM) were used. The compositions of the commercial supplements, AV, VFA and VTO and CM are as outlined in table 2.

3.5 Location of Experiments

The study was carried out at the University of Zambia, School of Agricultural Sciences Field Station.

3.5.1 Experiment 1

3.5.2 Birds

Two hundred (200) day old Cobb broiler chicks from Hybrid Poultry Farm were reared under the same environment and management. The day old broilers were mixed sex with an initial mean live weight of 41.88 gms.

3.5.3 Housing

The day old Cobb broilers were housed in a poultry pen at the field station measuring 10 x 70 m^2.The house was made of concrete blocks and a concrete floor for easy cleaning and disinfecting. It was an open sided poultry house with a good site orientation (east – west) that assured good ventilation. The roof of the house was made of galvanized aluminum sheets. The pens measuring $4m^2$ were divided four times into $1m^2$ sub pens. The experimental pens were partitioned using timber and wire mesh.

3.5.4 Diets

Diet (table 3) was formulated based on NRC (1994) tables of feedstuffs to meet nutrient requirements recommended for broilers

Table 3: Composition and nutrient content of the trace mineral and vitamin deficient diet.

Ingredient	Quantity (Kg)
Maize	60.00
Soybean Meal	36.00
DCP	1.50
LSF	2.00
Methionine	0.46
Lysine	1.30
Salt	0.25
Total	101.51

Calculated nutrient content	
ME (Kcal/Kg)	2903
Crude protein%	22.5
Lysine, %	1.41
Methionine + Cystine %	0.85
Calcium %	1.52
Phosphorus %	0.70
Analysed Values	
Crude protein, %	22.90
Calcium, %	1.42
Total phosphorus %	0.63

To achieve uniformity in the basal diet (deficient diet i.e. without vitamin mineral supplement), one mixture was made first. The mixed diet was then divided into five equal quantities, four of which were supplemented with the commercial supplements (Amino Vitasol, Vita Flash Amino and Vitamino Trace Oral and

Chick-A-min). The basal diet was used as control. These five different portions represented the treatments. Hence all the treatment diets were isocaloric and isonitrogenous in nature.

3.5.5 Allotment of Treatments and Experimental Design

Chicks were randomly assigned into the experimental groups of 40 chicks each keeping approximately similar initial live body weight. Each experimental group was replicated four times; each replicate with 10 chicks reared in $1m^2$ partition. A Completely Randomized Design (CRD) with five treatments involving 40 chickens per treatment was used. CRD was used to study the responses of the broilers to different nutritional supplements on a deficient diet.

3.5.6 Feeding

The five treatment diets were fed to chicks in different experimental pens. Feeds were weighed daily to ensure equal quantities are fed to the birds on different treatments. The treatment diets were fed to the birds *ad libitum* using feeding trays from day 1 to day seven and tubular feeders up to the end of the experiment.

3.5.7 General Management

The chicks were raised on concrete floor pens covered with wood shavings under similar managerial and hygienic conditions. During the first 2weeks, supplemental heat was provided by an infra red bulb per pen and a 24 hour lighting program was maintained. Temperature was kept at 32° C for the first week, 28°C in the second week and 21° C thereafter. Chicks were weighed weekly and so was the feed consumed with FCR calculated at 28 days of age. Water was offered *ad libtum* in chick founts at early age up to 14 days and later 15 litre bucket drinkers were used.

All the birds were monitored for health clinically and by way of pathological examinations on the dead birds. Prior to placement the chicks were vaccinated against Newcastle Disease post hatch via a coarse spray at the hatchery.

The birds were vaccinated against Gumboro and Newcastle on the 10th and 18th Day, and 12th and 21st day, respectively. These two diseases are endemic to the area and hence the need for the vaccinations. At 28 days of age 20 birds were slaughtered, 4 birds from each treatment to determine carcass body weight and yields and weights of the internal organs. The selected birds had weights nearest to the average of the group, weighed, slaughtered, bled and defeathered. Before slaughter the birds were starved for 12hrs to ensure that the intestines were free of any feed and feacal materials inside them. The offals were weighed as a whole as giblets.

Mortality or number of dead birds was recorded through out the experiment period from first day to 28 days of age.

3.5.8 Chemical analysis

The feed ingredients were analyzed in the food science laboratory for Crude protein (CP), and Ca and P according to AOAC (1990) procedures of proximate analysis.

3.5.9 Data Collection

Data on weekly live weights of chicks, mortality, feed consumption, carcass and organ yields at slaughter were collected for statistical analysis.

3.5.10 Statistical Analysis

Data collected were analyzed using GLM procedure of SAS® (SAS institute, 1985) statistical program using the Analysis of variance (ANOVA). Significant differences among treatment means were determined using the Duncan's Multiple Range test (Duncan, 1955) at P≤ 0.05.

3.6 Experiment 2

3.6.1 Birds

A total of 200 unsexed day old Cobb broiler chicks sourced from Hybrid Poultry Farm Hatchery were used for experiment 2. The chicks' initial average day old live weight was 53.7gms

3.6.2 Housing

The day old Cobb broilers were housed in a poultry pen at the field station measuring 10 x 70 m^2.The house was made of concrete blocks and a concrete floor for easy cleaning and disinfecting. It was an open sided poultry house with a good site orientation (east – west) with assured good ventilation. The roof of the house was made of galvanized aluminum sheets. The experimental pens measuring 4m^2 and divided four times into 1m^2 sub pens were constructed from wood and wire mesh partitions.

3.6.3 Diets

The standard starter basal diet (SBD) with a vitamin mineral premix added to it was mixed first and then divided into 5 equal portions to ensure uniformity among the treatment diets. The standard finisher mash basal diet (FBD) (table

4) with a mineral-vitamin premix added was mixed and was also divided into five equal portions to ensure uniformity among the treatment diets.

Table 4: Composition and nutrient content of the standard diets

Ingredient	Quantity (Kg)	
	Starter	Finisher
Maize	60.00	64.00
Soybean Meal	36.00	31.00
Fish Meal	-	-
DCP	1.50	1.50
LSF	2.00	2.00
Methionine	0.46	0.41
Lysine	1.30	1.00
Vitamin mineral premix	0.25	0.25
Salt	0.25	0.25
Total	101.76	100.41
Calculated nutrient content		
ME (Kcal/Kg)	2903	3140
Crude protein %	22.6	18.4
Lysine, %	1.42	0.94
Methioninine + Cystine %	0.85	0.41
Calcium %	1.51	0.88
Total Phosphorus %	0.70	0.40
Analysed Values		
Crude protein, %	22.80	18.9
Calcium, %	1.43	0.80
Total phosphorus %	0.66	0.37

Premix provided the following per kilogram diet: vitamin A, 9,000IU; vitamin D_3, 1,500IU; vitamin K, 0.5mg; vitamin B_{12}, 0.007mg; thiamine, 0.4mg; riboflavin, 6mg; folic acid, 1mg; biotin, 0.15mg; pantothenic acid, 12mg; niacin, 35mg; pyridoxine, 4mg; choline, 1000mg; iodised salt, 2g; manganese, 60mg; copper, 5mg; zinc, 50mg and selenium, 0.1mg.

The standard starter and finisher basal diets were used as control and while the other four portions of starter and finisher diets were supplemented with the three commercial supplements and an experimental supplement as in experiment one. The selected supplements (Amino Vitasol, Vita Flash Amino, Vita Trace Oral and Chick-A-min) were purchased from the local market within Lusaka district.

3.6.4 Allotment of Treatments and Experimental Design

Chicks were randomly assigned into five experimental groups of 40 chicks each keeping approximately similar initial live body weight in each group. Each experimental pen had a holding space of $4m^2$ replicated four times and each replicate had 10 chicks reared in $1m^2$ partition. A Completely Randomized Design (CRD) with five treatments involving 40 chickens per treatment was used. Each treatment was replicated four times, a replicate being composed of 10 chickens.

3.6.5 Feeding

In experiment 2, chicks in the experimental groups were fed five treatment diets; four of them fortified with commercial supplements (CM, VFA, VTO and AV) while the standard basal diets were fed as control. The feeding of the birds was divided into two phases; the starter and finisher phases. In the starter phase, birds were fed the standard starter diet from day one to day 28. In the finisher phase, the birds were fed a standard finisher diet from day 28 to 42 days of age.

In both the starter and finisher phases, four treatment diets were supplemented with the commercial supplements; Chick-A-min (CM), VitaFlash Amino (VFA), Vitamino Trace Oral (VTO) and Amino Vitasol (AV) while the basal diet or control remained unsupplemented.

In the starter phase (0-28 days), the standard starter diet was fed to the birds *ad libitum* using feeding trays from day 1 to day seven. After seven days, the experimental pens were fitted with tubular feeders for the supply of the starter diet *adlibitum* up to the 28th day. In the finisher phase, from 28-42 days, the chickens were fed a standard finisher diet *adlibitum.*

3.6.6 General Management

The birds were kept on deep litter of wood shavings spread on the concrete floors with each pen supplemented with heat from one infra red bulb up to 14 days. The temperature was maintained at 32℃, 28℃ and 21℃ in the first, second and third weeks respectively. Water and feed were offered *ad libitum.* In the initial stage, water and feed were offered in chick founts and feeder trays respectively. After 14 days water and feed were offered in 15 litre bucket drinkers and tubular feeders respectively. The birds were provided with a 24 hours lighting program.

Through out the experiment, weekly live weights and mortality of the chickens were monitored. The health of the chicks was assessed clinically by gross pathological examinations on the dead birds. Prior to placement the chicks were vaccinated against Newcastle Disease post hatch via a coarse spray at the hatchery. All the birds were vaccinated against Gumboro on the 10th and 18th day and New Castle Disease on the 12th and 21st day. This was done because the two diseases are endemic in the area. All the birds were exposed to similar routine care and management through out the experimental period.

On the last day of the experiment (42 days), a total of 20 birds were selected 4 from each experimental group and slaughtered to determine carcass yield (both hot and cold), with regards to share of carcass percentage and yields and the weight of the internal organs. The selected birds had weights nearest to the average of the group, weighed, slaughtered, bled and defeathered. Before slaughter the birds were starved for 12hrs to ensure that the intestines were free of any feed and feacal materials which may add weight. The offals were weighed as a whole as giblets.

3.6.7 Chemical analysis

The feed ingredients were analyzed in the food science laboratory for Crude protein (CP), Ca and P according to AOAC (1990) procedures of proximate analysis.

3.6.8 Data Collection

Data on weekly live weights of chicks, mortality, feed consumption, carcass quality and organ yields on slaughter for each treatment were collected for statistical analysis.

3.6.9 Statistical Analysis

Data collected were analyzed using General Linear Model (GLM) procedure of SAS® (SAS institute, 1985) statistical program using the Analysis of variance (ANOVA). Significant differences among treatment means were determined using the Duncan's Multiple Range test (Duncan, 1955) at $P \leq 0.05$.

3.6.10 Cost Analysis

Feed cost per kilogram diet was calculated using the prevailing market price of feed ingredients. The unit cost of each supplement and the cost of supplement per kilogram of feed were calculated. Feed cost per kilogram live weight was derived by multiplying the feed to gain ratio i.e. (efficiency of feed utilization) with the unit feed cost of respective diets.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Experiment 1

The results of broiler performance with regards to feed intake, live weights, feed conversion ratio and mortality are presented in Table 5.

4.1.1 Feed Intake

Results in table 5 show that there were significant (P≤0.05) differences in feed intake at 28 days of age among the treatments. There were no significant differences among the treatments CM, AV and VFA but all differed significantly with the control. (P≤0.05). No significant differences were recorded among the treatments VTO, AV and VFA. Birds on VTO had a higher feed intake than CM while the control had lower feed intake than the rest. The results indicate that birds fed supplements had significantly higher intake than the control (P≤0.05). VTO was significantly different from CM and the control but not significantly different with VFA and AV. VFA and AV had similar effect on feed intake but not significantly different with CM. The result did show significant reduction in feed intake in birds on control diet. This implies that adding supplements had significant effect on feed intake. The result of this experiment also suggests that the supplements could have influenced appetite of the birds as the intake on all treatments was significantly different from the control.

4.1.2 Live weights

The average live weights at 28 days were not significantly different among CM, AV and VFA but were significantly different from VTO and the Control (P≤0.05). Treatments CM, AV and VFA had equal effect on the live weight. The live

weights for the birds on CM, AV and VFA were higher than those of birds on VTO and the control (P≤0.05). Birds fed VTO had significantly higher liveweight than the control (P≤0.05). This shows that supplementation can cause appreciable induction of improvement in growth as the case is with CM, AV, VTO and VFA compared to the control.

The performance of CM, AV and VFA noted at 28 days proved that the nutrient composition in them increased the rate of body weight due to their influence on feed utilization. The increased live weights in the present findings resembles the findings of Deyhim *et al.*(1995) and Gavrilona *et al.*(1989) who reported that vitamin-mineral supplement added to basal low vitamin-mineral diet increased the growth of broiler chickens by 5 to 12 percentage.

The poor performance of VTO compared to the other supplements agrees with Whitehead (2002) who observed that vitamins and trace minerals are involved in all biological functions that allow an animal to use energy and protein for maintenance, growth, health, feed conversion and reproduction. If one or more are deficient, no increase in other nutrients will overcome the deficiency and permit these functions to occur. VTO in its nutrition composition (table2) exhibits clear absence of some essential vitamins and trace minerals such as Vitamin B_{12}, Nicotic Acid, Riboflavin, Folic Acid, Biotin, Choline, Iodine, manganese, selenium and sodium. Most of these nutrients are involved in supporting good growth and formation of the skeletal and nerve systems. The majority is critical in enzymic and metabolic functions and their inadequacy in the diet could be one reason why VTO's performance was affected. The other reason could be possible low stability in its liquid form, arising from the fact that synthetic vitamins are highly stable but possible losses may occur prior to consumption by the bird. He further noted that liquid vitamin mineral supplements would also lack the specific protection used for solid products as such might be less stable during storage. These observations coupled with the inadequate nutrition

composition of the supplement VTO could be the best explanation for poor performance of VTO.

The findings of Wang et al. (2008) that total removal of trace mineral vitamin supplements resulted in a significant reduction in body weight of all ages compared to birds fed with the standard level of supplementation and Radostits *et al.*(1995) observation that particularly mineral and trace element deficiencies, caused by insufficient feeding led to growth retardation, agrees with the findings of this study in the control of the experiment were the live weights were significantly lower from those on supplements ($P<0.05$). This finding indicates proof of the efficacy of the supplements to support good growth on a basal low nutrient diet.

Table 5: Feed intake, Live weight, Feed Conversion Ratio and Mortality of broilers fed a trace mineral and vitamin deficient diet to 28 days of age.

	Treatments				
Parameters	**CM**	**VFA**	**VTO**	**AV**	**CONTROL**
Feed Intake (g)	1381.75^{b}	1500.00^{ab}	1533.00^{a}	1416.25^{ab}	1084.50^{c}
Live weight (g)	755.75^{a}	698.50^{a}	507.50^{b}	715.25^{a}	316.50^{c}
FCR (Kg feed/kg wt)	1.83^{b}	2.17^{b}	3.08^{a}	1.98^{b}	3.44^{a}
Mortality (%)	0.25^{c}	1.25^{bc}	2.25^{b}	2.00^{b}	8.00^{a}

^{abc} *Means within the row with the same superscript do not differ significantly ($P\leq0.05$).*

4.1.3 Feed Conversion Ratio

The FCR values were low in the diets supplemented with CM, AV, and VFA and significantly different from the Control and VTO ($P\leq0.05$). The higher feed intake on VTO did not impact positively on the live weight hence higher FCR. This finding agrees with the findings of Deyhim et al. (1995) and Gavrilona et al. (1989) who reported that the increased rate of body weight may only be possible on supplementation with quality vitamin mineral supplement to increase feed

efficiency, enhance digestion, absorption and metabolism of supplied nutrients especially protein which is essential for the health and higher live weights. They also observed that a low quality supplement enhanced feed intake but did not impact positively on the live weight. Wang *et al.*, (2008) observed significant reduction in feed intake in birds fed a poor diet with no supplementation and attributed this finding to the absence of the trace mineral vitamin supplement which he thought affected the appetite of the birds resulting in poor live weights. Wang findings agrees totally with the findings of this study as the case has been with the control which was a diet with out trace mineral vitamin supplementation.

4.1.4 Mortality

The obtained results on mortality are shown in table 5. Birds consuming CM and VFA supplements had significantly lower mortality (P≤0.05) compared to the Control. No significant differences were recorded among the treatments, VFA, VTO and AV but all were significantly different from the control (P≤0.05). Overall, all the supplements CM, VFA, VTO and AV had significantly lower mortality than the control (P≤0.05). With these findings, it is clear that mortality was significantly influenced by dietary supplementation. The pathoanatomical findings of the dead birds on the control did indicate the connection between diet and the cause of death.

According to the findings, causes of death were leg deformities, inapetence and stunted growth. Maiorka *et al.* (2002) reported that absence of trace minerals and vitamins supplementation in the diet had a negative effect on bone strength and resulted in more deleterious growth and deaths. This is in agreement with the findings of Zapata *et al.* (1998) that vitamins and minerals are important ingredients used in poultry diets and are included as supplements in appropriate amounts to satisfy the nutrient requirements for a healthy and productive performance of birds. Possibilities of the trace minerals and vitamins in the modification of the immunological functions can not be ruled out as their

deficiency can affect in a negative manner the immune response of the birds, decrease the hormonal synthesis and attenuate the cellular immunity leading to eventual death (Macdonald, 1995; Cordain *et al.*,2005; Lowenthal *et al.*, 2005).

The findings in this experiment correlates well with the findings of Bowman *et al.*,1990; Friedman, *et al.*, 1991 that a deficiency in the diet is associated with immunosuppression and reduced resistance to infection leading to uncontrolled deaths. Ram Rao *et al.* (2004) observed that nutrients have influence on the maturity of the immune system and magnitude of the antibody. They indicated that the process requires more energy and amino acids than are normally needed for responding leucocytes with the imbalance leading to disturbances in normal physiology of the bird, with consequent immunosuppression in chickens. Supplementation of the deficient diet with commercial supplements significantly increased the livability of the birds compared to the control which had no supplementation.

4.1.5 Carcass and Organ weights

Carcass and organ weights of birds fed dietary treatments are shown in table 6.

4.1.5.1 Carcass weight

Carcass weight and organ or offal weights (weight of heart, spleen, gizzard, liver and intestines) are shown in table 6. All the treatments had significantly higher carcass weights than the control ($P \leq 0.05$). Birds fed CM, VFA and AV had higher carcass weights after slaughter than those fed VTO and the control ($P \leq 0.05$). The carcass weight on VTO significantly differed from the control ($P \leq 0.05$).

The carcass weights expressed as percentages of live weight were not significantly different among CM, VFA and AV but significantly higher than for

VTO and the control (P≤0.05). This suggests that the treatments resulted in significant positive metabolic reactions that improved muscle growth, lipogenesis and osteoblastic activity. Carcass weight expressed as % of live weight for the birds fed VTO did not differ significantly from the control. This is reflected by the low live weight of the birds on VTO compared to the other treatments. The poor performance of VTO compared to the other supplements could also be attributed to inability of the supplement nutrient composition to sustain higher live weights on a marginally nutrient deficient diet. This result is in agreement with the observation made by Bamgbose and Niba (1998) that live weight determines the carcass yield and it is an indication of the quality and ultilisation of the ration.

Table 6: Carcass and organ weights of broilers fed a trace mineral and vitamin deficient diet to 28 days of age.

	Treatments				
Parameters	**CM**	**VFA**	**VTO**	**AV**	**CONTROL**
Carcass Analysis					
Carcass Wt (g)	675.75[a]	633.50[a]	446.50[b]	630.00[a]	277.75[c]
Carcass wt (as % LWt)	89.42[a]	90.69[a]	87.98[b]	88.08[a]	87.76[b]
Organ weight (g)	67.50[a]	63.25[a]	44.50[b]	63.00[a]	27.75[c]
Organ wt (as % LWt)	8.93[a]	9.06[a]	8.76[a]	8.81[a]	8.76[a]

[abc] *Means within the row with the same superscript do not differ significantly (P≤0.05).*

4.1.5.2 Organ weights

No significant differences were observed in organ weights for birds fed CM, VFA and AV but were significantly different from VTO and the control (P≤0.05). The organ weights of birds fed VTO differed significantly with the control (P≤0.05). Birds fed CM, VFA, VTO, AV and the control did not differ significantly in organ weights expressed as percentage of live weight. Two reasons can be adduced

for this observation. The nutrient composition of the supplements CM, VFA, VTO and AV were so close that the effect on organ development was not significantly different statistically.

The lack of tangible difference in supplements CM, VFA, VTO and AV on the organ weight expressed as a percentage of live weight might also be due to the ability of all the supplements to supply essential nutrients for maximum organ growth. Generally birds fed treatment diets improved their body weight compared to the birds on control but did not differ significantly from the control on organs expressed as a % of live weight. This can be explained as a result of the beneficial influence of the nutritional value of the supplements and the minimum provision from the composition of the control diet.

It can also be argued that nutrients being supplied by the basic diets (raw materials) may, due to their form, be poorly available or in such low concentrations that the animals benefit and the organ growth is induced. This could explain why there were no significant differences among the treatments and with the control. The carcass weights recorded for all treatments were significantly higher ($P \leq 0.05$) than the control. However, the proportion of organs was not significantly different for birds fed treatment diets and the control. It is worthy to note that in deficient diet situation the supplements (CM, VFA and AV) induced higher weights but did not influence organ weights thus in contrast with earlier observation made by Atteh (2004) that the weight of organs in broilers reflects the anatomical response of birds to the type of nutrition consumed, balanced or unbalanced and that poor nutrition has been known to affect organ development and weight.

4.2 Experiment 2

The results of the broiler performance with regards to feed intake, live weights, feed conversion ratio and mortality are presented in Tables 7 and 8.

Table 7: Feed intake, Live weight, Feed Conversion Ratio and Mortality of broilers fed standard diets supplemented with trace minerals and vitamins to 28 days of age.

	Treatments				
Parameters	**CM**	**VFA**	**VTO**	**AV**	**CONTROL**
Feed Intake(g)	1445.25^{b}	1542.50^{ab}	1480.00^{b}	1461.50^{b}	1631.00^{a}
Live weight (g)	728.25^{a}	626.50^{b}	603.75^{b}	697.75^{ab}	626.50^{b}
FCR (Kg feed/kg wt)	1.99^{b}	2.48^{ab}	2.46^{ab}	2.10^{ab}	2.66^{a}
Mortality (%)	0.50^{a}	1.00^{a}	1.50^{a}	2.00^{a}	1.75^{a}

^{ab} *Means within the row with the same superscript do not differ significantly (P≤0.05).*

Table 8: Feed intake, Live weight, Feed Conversion Ratio and Mortality of broilers fed standard diets supplemented with trace minerals and vitamins to 42 days of age.

	Treatments				
Parameters	**CM**	**VFA**	**VTO**	**AV**	**CONTROL**
Feed Intake (g)	3501.00^{b}	4046.00^{a}	3943.00^{a}	3537.00^{b}	4107.50^{a}
Live weight (g)	2141.25^{a}	1826.75^{b}	1869.75^{b}	1948.25^{ab}	1797.50^{b}
FCR (Kg feed/kg wt)	1.64^{b}	2.24^{a}	2.11^{a}	1.82^{b}	2.29^{a}
Mortality (%)	1.25^{a}	1.25^{a}	2.25^{a}	3.00^{a}	3.25^{a}

^{ab} *Means within the row with the same superscript do not differ significantly (P≤0.05)*

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4.2.1 Feed Intake

The effect of commercial supplements on feed intake of broilers fed a standard diet is shown in tables 7 and 8 at 28 days and 42 days of age, respectively. Broilers on VFA and the control diet had the highest feed intake at 28 days ($P \leq 0.05$) compared to the intake of broilers on VTO, CM and AV supplemented diets. The intake among broilers fed supplemented diets VFA, VTO, CM and AV was statistically not different. VFA was statistically not different from the control. However, differences in intake emerged at 42 days with broilers on supplements VFA and VTO being statistically not different to those on control. Broilers on VTO, VFA and the control had significantly higher intake than CM and AV ($P \leq 0.05$). On complete removal of a premix and without supplementation as in Experiment 1, feed intake was significantly depressed up to age 28 on broilers fed the control diet. In Experiment 2 at the same age there was no significant difference in intake in broilers among supplemented diets. CM, VTO and AV were significantly different from the control at 28 days ($P \leq 0.05$). VFA was not significantly different from the control. The inconsistence in results at 28 days and 42 days however are not supported by the findings of Macdonald F. (1995) who found significant increase on feed intake by broiler chicks on supplemented experimental diets against the control.

This variation in results could be from the effect of the premix in the control diet. On the other hand in experiment 1 no varied effects of diets with supplements were noted but all were significantly different from the control at 28 days of age ($P \leq 0.05$). This lower intake on the control was due low levels of available nutrition in the control diet in experiment 1. This effect was also reported by Weber (2009) whose findings agrees with the results of this study that as intake and utilization of vitamins and minerals from natural sources is unpredictable due to differing content of vitamins and minerals in feed stuffs and variable composition, it is safer to cover the entire vitamin and mineral requirement of poultry through dietary supplementation. It is clear from Experiment 1 that the

minimum dietary vitamin and mineral levels required to prevent clinical deficiencies may not support the health, performance and welfare of the broilers on the deficient diet without supplementation. The result also signals the importance of adequate nutrition at early age as all the birds on diets supplemented with CM, VFA, VTO and AV showed good health and growth. This observation concurs with that of Skinner (1992) who observed that the need for vitamins and minerals, however, may be more crucial at the early age of body development than any other time.

At 42 days feed intake of broilers fed diets with supplements CM and AV did not differ significantly.There were no statistically significant differences among birds fed diets supplemented with VFA, VTO and the Control. However, feed intake differed significantly between birds fed diets supplemented with CM and AV and those on diets with VFA, VTO and the control ($P \leq 0.05$). Result for feed intake for the birds on CM and AV could not significantly increase the feed intake as compared to VFA, VTO and the Control meaning that good supplementation of a standard diet with quality and efficient supplements entails efficient usage of nutrients with less feed eaten. Efficacy of CM and AV could be described as better to the rest in the context of the composition of the nutrients as in table 2. Composition influences efficacy and is an important factor in this respect especially that most trace elements are low in maize–soybean meal diet.

4.2.2 Live weights

Live weights of birds on different experimental treatments are presented in tables 7 and 8. Birds on the diet supplemented with CM, showed a significantly higher live weight than the supplements VFA and VTO ($P \leq 0.05$) but was not significant when compared to AV at 28 days and 42 days. No significant differences were recorded among birds fed diets supplemented with VFA, VTO, AV and the control. The higher live weights at 28 days and 42 days of age for birds CM might be due to increased supply of nutrients (table 2) to support the

birds' health and live weight increases. At 42 days of age birds fed a diet supplemented with CM had significantly higher final live weights (P≤0.05) than those fed VFA, VTO and the control but not significantly different from AV (table 8). The differences in the effects of the supplement CM to the other commercial supplements could mainly be influenced by its composition. The composition exhibits a complete and balanced amino acid, vitamin and trace mineral profile as compared to VFA, VTO and AV (table 2).

The higher live weights in the present findings on CM resembles the findings of Oduguwa *et al*.(1996) that a balanced vitamin and mineral profile can ensure an efficient utilization of various feed components better than unbalanced one. In broiler production, body weight is a primary consideration for farmers. This is due to the fact that higher body weight gives high returns on investment. A large part of this body weight depends on muscular tissue of substantial protein content. This is supported by the fact that for optimum health and performance, the animal diet must contain adequate quantities of all nutrients required including amino acids.

A shortage of the limiting amino acid will constrain animal growth and reduce feed efficiency (D'Mello, 1994). In terms of trace minerals, Cunxia Sun (1996) reported that the zinc level of 230mg/diet got the best live weights. Aburto and Britton (1998) reported that the body weight of broilers decreased when supplementation of vitamin A was less than recommended but Abawi and Sullivan (1989) showed that higher body weights in broilers were significantly affected by dietary supplementation of vitamin A in excess. From the findings of this research, there was a real advantage gained by supplementing the broiler diet with quality commercial supplements as it resulted in statistically significant higher live weights (P≤0.05).This is what is relevant to a modern farmer as high live weights improves the profitability of the broiler meat production.

4.2.3 Feed Conversion Ratio (FCR)

Feed conversion ratios among supplements showed no significance difference at 28 days of age. No significant differences in FCR on birds fed VFA, VTO, AV and the control were observed at the same age. The good performance of the control diet could be attributed to the premix in the diet. CM was significantly different from the control (P≤0.05) but not significantly different from VFA, VTO and AV at 28 days. FCR for birds on VFA, AV and VTO were not significantly different from the control. At 42 days, no significant differences were observed among VTO, VFA and the control. FCR for birds on CM and AV differed significantly (P≤0.05) with VFA, VTO and the control. FCR is a measure of the bird's efficiency in converting feed mass into increased body mass. Birds with low FCR are considered efficient users of feed (Brown *et al*., 2001). This result is supported by Kassim and Suwanpradit (1996) who observed that supplements containing more nutrients resulted in higher feed efficiency than the control and the result was similar with the comment downed by Nigra and Sethi (1993) who observed that feed efficiency was best with the birds that were provided with a supplement with better nutrient composition. Donmez *et al.*(2002) reported significantly higher body weight and lower FCR with birds on diet supplemented with trace minerals. Similarly, the influence of dietary vitamins on performance of broilers has been studied by a number of previous researchers. This explains the influence of the trace mineral and vitamin supplementation on nutrient utilization efficiency in diets fed to poultry and broilers in particular.

4.2.4 Mortality

There were no significant differences among the treatments CM, VFA, VTO, AV and the control for mortality at both starter (28 days of age) and finisher stages (42 days of age). This implies that the supplements CM, VFA, VTO and AV had no influence on livability. No overall effects of dietary treatments were noticed,

including any visible disorders in the surviving birds. The presence of a broiler premix in the control diet must have helped in the control of mortality and hence did not differ significantly from the diets supplemented with CM, VFA, VTO and AV (tables 7 and 8).This helps us understand the relationship between livability and nutrient intake. In experiment 1, it was apparent that mortality was influenced by the supply of extra nutrition to the deficient diet from the supplements. Deficiency of vitamins and minerals is responsible for various diseases. Like vitamins, the importance of certain trace minerals in immune function has been established (Suttle and Jones, 1989). It is known that particularly mineral and trace mineral deficiencies, caused by insufficient feeding led to growth retardation and deaths (Radostits *et al*., 1995).

Increasing the dietary levels of amino acids as observed by Valfre et al.(1988) improved the agglutinin titers leading to improved immunity and livability in birds. Antibodies are proteins, therefore any deficiency of essential amino acids, particularly during the growing chicken, must have resulted in poor immunocompetence in experiment 1 at 28 days. This agrees with findings of this study that amino acids, vitamins and trace minerals are important for the normal physiological functions of broilers and hence could have played a leading role in keeping mortality under check. With regards to mortality the findings of this study agree with those of Jahan (2000) and Khatun (2000) who reported that there were no significant differences in the survivability of the broiler birds when different dietary supplements were used to support a standard diet. This finding is critical to a farmer as more of the birds are able to reach the market and enhance profitability. If more poultry is sold from a single batch, it will allow continued expansion and competitiveness in small scale poultry production as success of the sector depends on low mortality, higher weights and low cost feed.

4.2.5 Carcass and Organ weights

Carcass and organ weights of birds fed dietary treatments are shown in tables 9 and 10.

Table 9: Carcass weight, organ weight and cost analysis of broilers fed standard diets supplemented with trace minerals and vitamins to 28 days of age.

	Treatments				
Parameters	**CM**	**VFA**	**VTO**	**AV**	**CONTROL**
Carcass Analysis					
Carcass Wt (g)	653.75^{a}	552.25^{bc}	530.75^{c}	622.50^{ab}	553.50^{bc}
Carcass wt (as % LWT)	89.80^{a}	88.20^{b}	87.90^{b}	89.22^{a}	88.34^{b}
Organ weight (g)	65.75^{a}	55.25^{bc}	53.00^{c}	62.25^{ab}	55.25^{bc}
Organ wt (as % LWT)	9.02^{a}	8.82^{b}	8.78^{b}	8.92^{a}	8.81^{b}
Cost Analysis					
Feed cost ZMK /Kg Wt gain	4287.70^{b}	5355.70^{ab}	5289.10^{ab}	4524.00^{ab}	5746.70^{a}

^{abc} *Means within the row with the same superscript do not differ significantly (P≤0.05).*

Table 10: Carcass weight, organ weight and cost analysis of broilers fed standard diets supplemented with trace minerals and vitamins to 42 days of age.

	Treatments				
Parameters	**CM**	**VFA**	**VTO**	**AV**	**CONTROL**
Carcass Analysis					
Carcass Wt (g)	1914.75^{a}	1532.25^{c}	1589.75^{b}	1736.00^{ab}	1522.50^{bc}
Carcass wt (as % LWT)	89.40^{a}	83.80^{bc}	85.00^{b}	89.10^{a}	84.70^{b}
Organ weight (g)	191.75^{a}	165.75^{bc}	168.75^{b}	173.50^{b}	162.25^{bc}

Organ wt (as % LWT)	8.95[a]	9.07[b]	9.03[b]	8.90[a]	9.02[b]
Cost Analysis					
Feed cost ZMK /Kg Wt gain	3522.50[b]	4836.30[a]	4562.80[a]	3915.80[b]	4939.50[a]

[abc] *Means within the row with the same superscript do not differ significantly (P≤0.05).*

4.2.5.1 Carcass weights

Birds fed a diet supplemented with CM had higher carcass weight than those fed VFA, VTO and Control at 28 days of age but was not significantly different from AV (table 9). Carcass weight for the birds on VFA and VTO were not statistically different with the control at 28 days. Contrary to the expectation, supplementation of the starter diet composition with VFA and VTO did not result in positive carcass weights compared to the control. Some studies have emphasized the influence of external factors such as supplementation on the dynamics of growth cells during the starter stage leading to better carcass weights (Halevy *et al.*,2000). However, the results observed in this study indicate that VFA and VTO supplementation were not sufficient in quality to influence the expected carcass weights. At 42 days CM had significantly higher final carcass weight than VTO, VFA and the control (P≤0.05) but was not significantly different to AV (table 10).

The higher live weight for birds on CM and AV resulted in higher carcass weights since the surface area and the weight determine the amount of final carcass weight. This result agrees with reports of Tuleun and Igba (2007) that lower carcass weights of broilers results from smaller live weights. The higher live weights and subsequently significantly higher carcass weights for birds on the diet with CM and AV could be attributed to good balance of all amino acids in their composition as earlier alluded to on live weights. Protein forms the structure of organs and tissues and an adequate protein in the diet if properly utilized will more likely lead to more deposition of protein in such tissues that

increased weight. This indicates that the vitamin mineral composition and a balanced amino acid composition of CM and AV had higher capabilities of inducing more carcass weights than those of VTO, VFA and the control.

4.2.5.2 Organ weights

The organ weights and the organ weights expressed as a percentage of live weights are as presented in tables 9 and 10. The internal organ weights expressed as percentages of live weight increased significantly (P≤0.05) on birds fed CM and AV at 28 days and 42 days of age (Tables 9 and 10). However, internal organ weights of VFA, AV and VTO were not significantly different from the control at 28 days of age. This suggests that the dietary supplements VFA, AV and VTO did not result in any significant metabolic reactions that could affect the growth and characteristics of the internal organs. The results obtained from the study showed that heavy birds had high carcass weights and subsequently heavy organs. Two reasons can be adduced from this observation. The nutrient composition and in particular the amino acid content of each supplement were so variant that the effect of the difference was felt and expressed in the organ weight differences (table2). Secondly the effect of the premix in the control diet was a source of extra nutrition and this could explain why there were no differences between the control and supplements VFA, AV and VTO. This emphasizes the importance of good nutrition and in particular the protein in organ development.

The result of this study is in agreement with Featherston and Scholz (1968) who reported that the organ weights expressed on body weight basis significantly increased in chicks fed high protein diet for the first two days. Other studies, found out that the effect of amino acids on immune organs were crucial for optimum immune responses. At present limited information is available on how excess essential amino acids influence the development of organs or immune function. However, in some studies, researchers (De Jonge *et al.*, 2002., Kwak

et al., 1999) have found that deficiency of essential amino acids can impair lymphoid organ weight and functions. The weight of organs in broilers is known to indicate the response of birds to feed intake in relation to their growth or age of the birds.

The weight of the organs in broilers also reflects the anatomical response of birds to the type of diets consumed, such as texture of feed or fibre content (Atteh, 2004). This is in contradiction with the findings of this study were high organ weight were recorded on low intake of feed but with very high final weights (table 10). This explains the fact that organ development requires balanced nutrition to evoke dramatic physical organ development and health which has a major influence on growth performance of commercial broilers as it affects feed digestion, nutrient absorption and mortality.

4.2.6 Cost Analysis

The cost analysis was calculated on the basis of total feed consumed to the Kilogram live weight. The impact of the supplements CM, VFA, VTO and AV on the overall feed cost was as shown in tables 9 and 10. The data on the feed cost per Kg live weight showed that at 28 days treatment diets VFA, VTO and AV were not significantly different from the control as their feed cost per Kg live weight ratio were statistically not different. Diet supplemented with CM was significantly different from the control ($P \leq 0.05$) but not significantly different from VTO, VFA and AV.

The impact of the type of supplement used on the overall feed cost per unit live weight was more pronounced at 42 days where CM and AV were not significantly different but were significantly different from VFA, VTO and the control ($P \leq 0.05$). The feed cost per Kg live weight value on VFA, VTO and the control were significantly not different at 42 days. The lower the value the better and the more cost effective is the diet and vice versa. The data on feed cost per

Kg live weight showed that the diet supplemented with VFA and VTO were most inefficient and expensive. From 28 days CM and AV showed consistence in their influence and improved the ratio of feed cost to that of live weight by 42 days (table10). A major concern of modern poultry enterprise is to reduce the feed cost for optimal economic returns because feed constitutes 70% of the total cost of production. One way of reducing the feed cost is through improvement in the feed efficiency of the birds. The better performance and net return for birds on CM and AV emphasizes the importance of the use of excellent quality nutritional supplements to obtain maximum growth and feed conversion and therefore maximum income from the broilers.

The major thrust of field investigations among small scale farmers in the country through the Poultry Association of Zambia (PAZ) Annual General Meeting (2005) reviewed the situation as critical where the cost of feed was getting beyond them with commercial supplements not performing to their expectations. In the PAZ AGM report (2005) the farmers from all over the country submitted that some of the commercial supplements were not cost effective as they encouraged more feed intake with low live weights while others encouraged moderate intake and high live weights on the same feeds. The farmers reported that about 75% of their cost of doing poultry business in this country is attributed to feed. This is supported by Nyoupayou (1990) who confirmed that about 75% of the costs of production in raising or maintaining broilers are attributed to feed costs with three fourths of this cost being allotted to maintenance needs and the remainder for productive purposes. He further argued that the high cost of feeds is an important problem affecting smallholder broiler production in Africa because of inadequate financial resources.

The protein component is the biggest cost in feed making and to reduce costs, amino acid supplements which allows for meeting the needs for essential amino acids at lower protein content can be used. In addition, the use of synthetic amino acids to meet the amino acid needs of broilers can reduce feed costs,

increase flexibility in raw material selection and can be used to balance amino acids of the diet thus minimizing excess crude protein and/or amino acids (Bercovici and Fuller, 1995). Given the importance that feeds play in intensive broiler systems, it becomes imperative to identify least cost feed options for the farmer. This option agrees with the observation made in this study that the well balanced supplements (CM and AV) enhanced the biological parameters such as growth, mortality, health and feed conversion efficiency resulting in a most cost effective combination compared to the control, VFA and VTO.

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATIONS.

Amino acid-mineral-vitamin supplements have a definite effect on broiler performance and feed cost per unit live weight as shown in this study when fed to the deficient and standard maize-soybean poultry diets. The study demonstrated that the profit of poultry farming depends on economic feeding of balanced rations.

From this study, it is obvious that the supplements (CM, VFA, VTO and AV) have differing capabilities for supporting growth of broilers up to market weight as seen in experiment 1 and 2. This has gone to prove right the complaints of the farmers on the performance of some commercial supplements in broiler production and that care must be taken to feed a proven supplement of good quality at the starter phase in order to maximize growth and improve feed efficiency.

The results of this study indicate that CM and AV were found to give the best results as determined through their ability to sustain good performance and / or maintain better performance at marginally deficient nutrition levels. The results showed that the two supplements had positive effect on live weight, FCR, organ development and mortality in comparison with a standard ration.

Feed cost per unit live weight values on the use of the nutritional supplements as methods to improve feed efficiency should further be conducted to determine the cost effectiveness of the use of the supplements recommended in this study.

The results of this study would therefore be useful to the farmers looking for tools to select the best supplements to improve the effects of maize-soybean diets on performance of broilers. As the results of this study revealed,

supplements of amino acids, minerals and vitamins had a positive effect on the live weight and feed ultilisation efficiency in comparison to standard diets therefore the use of quality commercial amino acid-mineral-vitamin supplements on maize soybean diets should be encouraged to become a phenomenon for maintenance of normal growth and health in broiler chickens and ultimately improved profitability of the broiler business.

In conclusion, it is therefore suggested that supplementation of amino acids, mineral and vitamins on standard maize-soybean poultry diets is essential for proper growth, disease resistance and decreased mortality rate of poultry especially in the growing chicks. This study safely concludes that as intake and utilization of nutrients from natural sources is unpredictable due to differing content of nutrients in the feedstuffs and variable composition, it is safer to cover the entire nutrient requirement of poultry through quality dietary supplementation.

CHAPTER 6

6.0 REFERENCES

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APPENDIX

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: CW1

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 1859.433

Number of Means	2	3	4	5
Critical Range	64.99	68.13	70.08	71.41

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	675.75	4	CM
A			
A	633.50	4	VFA
A			
A	630.00	4	AV
B	446.50	4	VTO
C	277.75	4	CONTROL

Analysis Variable: CW 1

TREAT	N Obs	Mean	Std Error
AV	4	630.0000000	12.7279221
CM	4	675.7500000	4.7675116
CONTROL	4	277.7500000	5.2021630
VFA	4	633.5000000	25.9566947
VTO	4	446.5000000	37.9308582

Duncan's Multiple Range Test for variable: cW1 % LWT

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 1500.633

Number of Means	2	3	4	5
Critical Range	58.38	61.20	62.95	64.15

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	89.42	4	CM
A			
A	90.69	4	VFA
A			
A	88.08	4	AV
B	87.98	4	VTO

B 87.76 4 CONTROL

Analysis Variable : cw1 % Lwt

TREAT	N Obs	Mean	Std Error
AV	4	88.0800000	11.3137085
CM	4	89.4200000	4.2890364
CONTROL	4	87.7600000	4.7258156
VFA	4	90.6900000	23.3358630
VTO	4	87.9800000	34.0954542

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: OW1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4499.70000000	1124.92500000	58.29	0.0001
Error	15	289.50000000	19.30000000		
Corrected Total	19	4789.20000000			

R-Square	C.V.	Root MSE	OW Mean
0.939551	8.257851	4.39317653	53.20000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	4499.70000000	1124.92500000	58.29	0.0001

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: OW1

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 19.3

Number of Means	2	3	4	5
Critical Range	6.621	6.941	7.139	7.275

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	67.500	4	CM
A			
A	63.250	4	VFA
A			
A	63.000	4	AV
B	44.500	4	VTO
C	27.750	4	CONTROL

Analysis Variable: OW1

TREAT	N Obs	Mean	Std Error
AV	4	63.0000000	1.4142136
CM	4	67.5000000	0.5000000
CONTROL	4	27.7500000	0.4787136
VFA	4	63.2500000	2.6259919
VTO	4	44.5000000	3.8405729

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: OW1 %Lwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4499.70000000	1124.92500000	58.29	0.0001
Error	15	289.50000000	19.30000000		
Corrected Total	19	4789.20000000			

R-Square	C.V.	Root MSE	OW Mean

0.939551	8.257851	4.39317653	53.20000000	

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	4499.70000000	1124.92500000	58.29	0.0001

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: OW1 % Lwt

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 19.3

Number of Means	2	3	4	5
Critical Range	6.621	6.941	7.139	7.275

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	8.930	4	CM
A			
A	9.060	4	VFA
A			
A	8.810	4	AV
A	8.760	4	VTO
A	8.760	4	CONTROL

Analysis Variable: OW1 % Lwt

TREAT	N Obs	Mean	Std Error
AV	4	8.8100000	1.4142136
CM	4	8.9300000	0.5000000
CONTROL	4	8.7600000	0.4787136
VFA	4	9.0600000	2.6259919
VTO	4	8.7600000	3.8405729

Experiment No 2

OBS	TREAT	BW4	BW6	CW4	CW6	EW4	EW6	OW4	OW6
1	CM	755	2180	698	1962	628	1766	70	196
2	VFA	720	1660	648	1494	583	1334	65	150
3	VTO	590	2000	531	1800	478	1620	53	180
4	AV	760	2005	684	1800	616	1620	68	180
5	CONTROL	700	1910	630	1720	820	1548	63	172
6	CM	730	2100	657	1890	591	1701	66	190
7	VFA	570	1675	513	1508	462	1357	51	151
8	VTO	555	1890	499	1701	449	1531	50	170
9	AV	685	1860	617	1674	555	1507	62	167
10	CONTROL	520	1725	468	1552	422	1397	46	156
11	CM	750	2120	675	1908	607	1717	68	191
12	VFA	565	1875	508	1687	457	1519	51	168

13	VTO	590	1750	531	1575	478	1418	53	157
14	AV	692	1995	622	1796	560	1616	62	180
15	CONTROL	600	1875	558	1688	502	1520	56	168
16	CM	650	2110	585	1899	526	1709	59	190
17	VFA	600	1600	540	1440	500	1296	54	144
18	VTO	624	1870	562	1683	506	1515	56	168
19	AV	630	1860	567	1674	510	1507	57	167
20	CONTROL	620	1700	558	1530	502	1377	56	153

Experiment No 1

OBS	TREAT	BW	CW	EW	OW
1	CM	755	679	611	68
2	VFA	760	684	616	68
3	VTO	545	490	441	49
4	AV	700	630	567	63
5	CONTROL	275	292	263	29
6	CM	760	684	616	68
7	VFA	730	657	591	66
8	VTO	430	387	349	38
9	AV	720	648	583	65
10	CONTROL	325	270	243	27
11	CM	755	678	610	68
12	VFA	700	630	567	63
13	VTO	420	378	340	38
14	AV	720	648	583	65
15	CONTROL	310	279	251	28
16	CM	735	662	596	66
17	VFA	570	563	507	56
18	VTO	590	531	478	53
19	AV	660	594	535	59
20	CONTROL	300	270	243	27

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: OW 21

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 28.46667

Number of Means	2	3	4	5
Critical Range	8.041	8.429	8.671	8.835

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	65.750	4	CM
A			
B A	62.250	4	AV
B			
B C	55.250	4	CONTROL
B C			
B C	55.250	4	VFA
C			
C	53.000	4	VTO

Analysis Variable : OW21

TREAT	N Obs	Mean	Std Error

AV	4	62.2500000	2.2500000
CM	4	65.7500000	2.3935678
CONTROL	4	55.2500000	3.4970225
VFA	4	55.2500000	3.3260337
VTO	4	53.0000000	1.2247449

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: OW22

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3309.80000000	827.45000000	12.03	0.0001
Error	15	1032.00000000	68.80000000		
Corrected Total	19	4341.80000000			

R-Square	C.V.	Root MSE	OW6 Mean
0.762311	4.882034	8.29457654	169.90000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	3309.80000000	827.45000000	12.03	0.0001

Duncan's Multiple Range Test for variable: OW22

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 68.8

Number of Means	2	3	4	5
Critical Range	12.50	13.10	13.48	13.74

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TREAT
	A	191.750	4	CM
	B	173.500	4	AV
	B			
	B	168.750	4	VTO
	B			
C	B	162.250	4	CONTROL
C				
C	B	165.750	4	VFA

Analysis Variable: OW22

TREAT	N Obs	Mean	Std Error
AV	4	173.5000000	3.7527767
CM	4	191.7500000	1.4361407
CONTROL	4	162.2500000	4.5893899
VFA	4	153.2500000	5.1538820
VTO	4	168.7500000	4.7147817

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: CW21 % Lwt

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 2802.95

Number of Means	2	3	4	5
Critical Range	79.79	83.65	86.04	87.67

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	89.80	4	CM
A			
A	89.22	4	AV
B	88.34	4	CONTROL
B	88.20	4	VFA
B	87.90	4	VTO

Analysis Variable: CW21 % Lwt

TREAT	N Obs	Mean	Std Error
AV	4	89.2200000	2.9669912
CM	4	89.8000000	2.4041492
CONTROL	4	88.3400000	3.1700166

```
VFA           4   88.2000000    3.6812255

VTO           4   87.9000000    1.8606311
-------------------------------------------


 Duncan's Multiple Range Test for variable: CW22 %Lwt

NOTE: This test controls the type I comparisonwise error rate, not the
      experimentwise error rate

            Alpha= 0.05  df= 15  MSE= 7776.233

         Number of Means     2     3     4     5
         Critical Range  132.9 139.3 143.3 146.0

Means with the same letter are not significantly different.

      Duncan Grouping              Mean      N  TREAT

                  A            89.40      4  CM
                  A
                  B            84.70      4  CONTROL
                  B
                  A            89.10      4  AV
                  A
            C     B            83.80      4  VFA
            C
                  B            85.00      4  VTO


       Analysis Variable : CW22 % Lwt

       TREAT     N Obs          Mean     Std Error
       -------------------------------------------
       AV            4   89.1000000    1.0193270

       CM            4   89.4000000    1.0113607

       CONTROL       4   84.7000000    2.2057254

       VFA           4   83.8000000    2.1275929

       VTO           4   85.0000000    1.6359715
       -------------------------------------------


 Duncan's Multiple Range Test for variable: OW21 % LWT

NOTE: This test controls the type I comparisonwise error rate, not the
      experimentwise error rate

            Alpha= 0.05  df= 15  MSE= 5774.25

         Number of Means     2     3     4     5
         Critical Range  114.5 120.1 123.5 125.8

Means with the same letter are not significantly different.

      Duncan Grouping              Mean      N  TREAT

                  A           9.02      4  CM

                  A           8.92      4  AV
                  B
                  B           8.78      4  VTO
                  B
```

B	8.81	4	CONTROL
B	8.82	4	VFA

Analysis Variable: OW21 %Lwt

TREAT	N Obs	Mean	Std Error
AV	4	8.92	1.0533410
CM	4	9.02	1.1194790
CONTROL	4	8.81	1.0125951
VFA	4	8.82	1.1367140
VTO	4	8.78	1.3783357

Duncan's Multiple Range Test for variable: OW22 %Lwt

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 5882.833

Number of Means	2	3	4	5
Critical Range	115.6	121.2	124.6	127.0

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
B	9.03	4	VTO
B			
B	9.07	4	VFA
A	8.90	4	AV
A	8.95	4	CM
B	9.02	4	CONTROL

Analysis Variable :OW22 % LWt

TREAT	N Obs	Mean	Std Error
AV	4	8.90	1.8232644
CM	4	8.95	1.8885465
CONTROL	4	9.02	2.0159449
VFA	4	9.07	2.0166428
VTO	4	9.03	2.7753683

Cumulative Mortality

OBS	TREAT	MORT14	MORT24	MORT26
1	CM	0	0	2
2	VFA	2	2	2
3	VTO	3	1	1
4	AV	2	4	4
5	CONTROL	8	0	1
6	CM	0	1	2
7	VFA	1	1	1
8	VTO	2	2	3
9	AV	1	3	5
10	CONTROL	9	3	4
11	CM	0	1	1
12	VFA	2	1	1
13	VTO	2	1	1
14	AV	3	1	1
15	CONTROL	7	4	6
16	CM	1	0	0
17	VFA	0	0	1
18	VTO	2	2	4
19	AV	2	0	2
20	CONTROL	8	0	2

Duncan's Multiple Range Test for variable: MORT1

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 0.55

Number of Means	2	3	4	5
Critical Range	1.118	1.172	1.205	1.228

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	8.0000	4	CONTROL
B	2.2500	4	VTO
B			
B	2.0000	4	AV
B			
C B	1.2500	4	VFA
C			
C	0.2500	4	CM

Analysis Variable: MORT1

TREAT	N Obs	Mean	Std Error
AV	4	2.0000000	0.4082483
CM	4	0.2500000	0.2500000
CONTROL	4	8.0000000	0.4082483
VFA	4	1.2500000	0.4787136
VTO	4	2.2500000	0.2500000

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: MORT21

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.80000000	1.45000000	0.81	0.5362
Error	15	26.75000000	1.78333333		
Corrected Total	19	32.55000000			

R-Square	C.V.	Root MSE	MORT24 Mean
0.178187	98.91963	1.33541504	1.35000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	5.80000000	1.45000000	0.81	0.5362

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: MORT21

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 1.783333

Number of Means	2	3	4	5
Critical Range	2.013	2.110	2.170	2.211

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	2.0000	4	AV
A			
A	1.7500	4	CONTROL
A			
A	1.5000	4	VTO
A			
A	1.0000	4	VFA
A			
A	0.5000	4	CM

Analysis Variable : MORT21

TREAT	N Obs	Mean	Std Error
AV	4	2.0000000	0.9128709
CM	4	0.5000000	0.2886751
CONTROL	4	1.7500000	1.0307764
VFA	4	1.0000000	0.4082483
VTO	4	1.5000000	0.2886751

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: MORT22

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.20000000	3.55000000	1.52	0.2461
Error	15	35.00000000	2.33333333		
Corrected Total	19	49.20000000			

R-Square	C.V.	Root MSE	MORT26 Mean
0.288618	69.43297	1.52752523	2.20000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	14.20000000	3.55000000	1.52	0.2461

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: MORT22

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 2.333333

Number of Means	2	3	4	5
Critical Range	2.302	2.413	2.482	2.529

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	3.250	4	CONTROL

```
A
A            3.000      4  AV
A
A            2.250      4  VTO
A
A            1.250      4  CM
A
A            1.250      4  VFA
```

Analysis Variable: MORT22

TREAT	N Obs	Mean	Std Error
AV	4	3.0000000	0.9128709
CM	4	1.2500000	0.4787136
CONTROL	4	3.2500000	1.1086779
VFA	4	1.2500000	0.2500000
VTO	4	2.2500000	0.7500000

OBS	TREAT	MORT14	MORT24	MORT26	CFC14	FCR14	CFC24	FCR24	CFC26	FCR26
1	CM	0	0	2	1370	1.81	1459	1.88	3499	1.61
2	VFA	2	2	2	1508	1.98	1562	2.16	4034	2.42
3	VTO	3	1	1	1501	2.61	1494	2.50	3875	1.93
4	AV	2	4	4	1450	2.05	1484	1.93	3554	1.75
5	CONTROL	8	0	1	980	3.32	1497	2.08	3977	2.09
6	CM	0	1	2	1375	1.79	1453	1.97	3503	1.66
7	VFA	1	1	1	1495	2.03	1575	2.76	4090	1.94
8	VTO	2	2	3	1544	3.55	1465	2.46	3980	2.09
9	AV	1	3	5	1395	1.89	1463	2.15	3492	1.89
10	CONTROL	9	3	4	1070	3.23	1922	3.68	4442	2.55
11	CM	0	1	1	1377	1.81	1459	1.96	3499	1.63
12	VFA	2	1	1	1511	2.08	1562	2.72	4034	2.05
13	VTO	2	1	1	1497	3.52	1494	2.58	3875	2.07
14	AV	3	1	1	1388	1.89	1484	2.17	3554	1.76
15	CONTROL	7	4	6	970	2.94	1497	2.43	3977	2.13
16	CM	1	0	0	1405	1.91	1410	2.15	3503	1.64
17	VFA	0	0	1	1486	2.60	1471	2.31	4026	2.57
18	VTO	2	2	4	1590	2.67	1467	2.29	4042	2.38
19	AV	2	0	2	1432	2.11	1415	2.15	3548	1.87
20	CONTROL	8	0	2	1318	4.25	1608	2.47	4034	2.40

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: FI 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	505593.30000000	126398.32500000	21.49	0.0001

Error	15	88242.50000000	5882.83333333		
Corrected Total	19	593835.80000000			

R-Square	C.V.	Root MSE	CFC14 Mean
0.851403	5.545487	76.69963059	1383.10000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	505593.30000000	126398.32500000	21.49	0.0001

Duncan's Multiple Range Test for variable: FI 1

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 5882.833

Number of Means	2	3	4	5
Critical Range	115.6	121.2	124.6	127.0

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	1533.00	4	VTO
A			
B A	1500.00	4	VFA
B A			
B A	1416.25	4	AV
B			
B	1381.75	4	CM
C	1084.50	4	CONTROL

Analysis Variable : FI 1

TREAT	N Obs	Mean	Std Error
AV	4	1416.25	14.8232644
CM	4	1381.75	7.8885465
CONTROL	4	1084.50	81.0159449
VFA	4	1500.00	5.8166428
VTO	4	1533.00	21.7753683

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: FI 21

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	4	92484.20000000	23121.05000000	2.59	0.0788
Error	15	133694.75000000	8912.98333333		
Corrected Total	19	226178.95000000			

R-Square	C.V.	Root MSE	CFC24 Mean
0.408898	6.243748	94.40859777	1512.05000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	92484.20000000	23121.05000000	2.59	0.0788

Duncan's Multiple Range Test for variable: FI 21

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 8912.983

Number of Means	2	3	4	5
Critical Range	142.3	149.2	153.4	156.3

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	1631.00	4	CONTROL
A			
B A	1542.50	4	VFA
B			
B	1480.00	4	VTO
B			
B	1461.50	4	AV
B			
B	1445.25	4	CM

Analysis Variable : FI 21

TREAT	N Obs	Mean	Std Error
AV	4	1461.50	16.2711401
CM	4	1445.25	11.8348004
CONTROL	4	1631.00	100.4664123
VFA	4	1542.50	24.0294958
VTO	4	1480.00	8.0932070

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: FI 22

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1321892.80000000	330473.20000000	27.98	0.0001
Error	15	177135.00000000	11809.00000000		
Corrected Total	19	1499027.80000000			

R-Square	C.V.	Root MSE	CFC26 Mean
0.881833	2.839615	108.66922287	3826.90000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	1321892.80000001	330473.20000000	27.98	0.0001

Duncan's Multiple Range Test for variable: CFC26

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 11809

Number of Means	2	3	4	5
Critical Range	163.8	171.7	176.6	179.9

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	4107.50	4	CONTROL
A			
A	4046.00	4	VFA
A			

```
                A           3943.00      4  VTO

                B           3537.00      4  AV
                B
                B           3501.00      4  CM


                          Analysis Variable : FI 22

                          TREAT     N Obs          Mean     Std Error
                          ------------------------------------------
                          AV            4       3537.00    15.0665192

                          CM            4       3501.00     1.1547005

                          CONTROL       4       4107.50   112.3065003

                          VFA           4       4046.00    14.7873820

                          VTO           4       3943.00    41.2492424
                          ------------------------------------------
                              Analysis of Variance Procedure
                                   Class Level Information

                            Class    Levels    Values

                            TREAT         5    AV CM CONTROL VFA VTO

                            Number of observations in data set = 20

                                 Analysis of Variance Procedure
Dependent Variable: FCR 1

Source            DF             Sum of Squares               Mean Square    F Value      Pr > F

Model              4                 8.16297000                 2.04074250      14.84      0.0001

Error             15                 2.06315000                 0.13754333

Corrected Total   19                10.22612000

           R-Square                     C.V.                   Root MSE             FCR14 Mean

            0.798247                    14.82288                  0.37086835             2.50200000

Source            DF                   Anova SS               Mean Square    F Value      Pr > F

TREAT             4                  8.16297000               2.04074250      14.84      0.0001


                        Duncan's Multiple Range Test for variable: FCR 1

                  NOTE: This test controls the type I comparisonwise error rate, not the
                        experimentwise error rate

                               Alpha= 0.05  df= 15  MSE= 0.137543

                            Number of Means     2     3     4     5
                            Critical Range  .5590 .5859 .6027 .6141

                  Means with the same letter are not significantly different.

                        Duncan Grouping              Mean      N  TREAT
```

A	3.4350	4	CONTROL
A			
A	3.0875	4	VTO
B	2.1725	4	VFA
B			
B	1.9850	4	AV
B			
B	1.8300	4	CM

Analysis Variable : FCR 1

TREAT	N Obs	Mean	Std Error
AV	4	1.9850000	0.0561991
CM	4	1.8300000	0.0270801
CONTROL	4	3.4350000	0.2835049
VFA	4	2.1725000	0.1439546
VTO	4	3.0875000	0.2587269

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: FCR 21

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.28515000	0.32128750	2.60	0.0786
Error	15	1.85545000	0.12369667		
Corrected Total	19	3.14060000			

R-Square	C.V.	Root MSE	FCR24 Mean
0.409205	15.03014	0.35170537	2.34000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	1.28515000	0.32128750	2.60	0.0786

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: FCR 21

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 0.123697

Number of Means	2	3	4	5
Critical Range	.5301	.5557	.5716	.5824

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TREAT
	A	2.6650	4	CONTROL
	A			
B	A	2.4875	4	VFA
B	A			
B	A	2.4575	4	VTO
B	A			
B	A	2.1000	4	AV
B				
B		1.9900	4	CM

Analysis Variable : FCR 21

TREAT	N Obs	Mean	Std Error
AV	4	2.1000000	0.0568624
CM	4	1.9900000	0.0570088
CONTROL	4	2.6650000	0.3494877
VFA	4	2.4875000	0.1491853
VTO	4	2.4575000	0.0611521

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: FCR 22

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.29443000	0.32360750	9.04	0.0006
Error	15	0.53682500	0.03578833		
Corrected Total	19	1.83125500			

R-Square	C.V.	Root MSE	FCR26 Mean

0.706854	9.358301	0.18917805	2.02150000	

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	1.29443000	0.32360750	9.04	0.0006

Duncan's Multiple Range Test for variable: FCR 22

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 0.035788

Number of Means	2	3	4	5
Critical Range	.2851	.2989	.3074	.3133

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	2.2925	4	CONTROL
A			
A	2.2450	4	VFA
A			
A	2.1175	4	VTO
B	1.8175	4	AV
B			
B	1.6350	4	CM

Analysis Variable : FCR 22

TREAT	N Obs	Mean	Std Error
AV	4	1.8175000	0.0363719
CM	4	1.6350000	0.0104083
CONTROL	4	2.2925000	0.1100284
VFA	4	2.2450000	0.1492481
VTO	4	2.1175000	0.0944612

Live Weights of Birds

OBS	TREAT	LW11	LW12	LW13	LW14	LW21	LW22	LW23	LW24	LW25	LW26
1	CM	117	314	496	757	115	311	541	775	1023	2175
2	VFA	114	289	501	763	137	358	502	724	1069	1670
3	VTO	112	256	406	574	124	275	499	597	1033	2004
4	AV	123	315	478	706	101	309	424	768	1115	2030
5	CONTROL	114	211	276	295	108	326	497	718	1071	1900
6	CM	121	341	483	769	106	282	526	737	1165	2105
7	VFA	131	312	447	735	101	247	411	571	1005	2105
8	VTO	119	275	420	435	104	280	470	596	942	1900
9	AV	121	309	518	740	123	350	485	680	985	1850
10	CONTROL	121	255	308	331	106	253	492	522	1142	1745
11	CM	122	348	490	760	104	290	542	745	1145	2145
12	VFA	128	315	400	725	103	242	420	575	1035	1965
13	VTO	120	260	405	425	102	258	425	580	965	1875
14	AV	124	310	520	735	125	340	475	685	1005	2015

15	CONTROL	122	260	315	330	102	280	494	615	1013	1865
16	CM	106	282	526	737	126	336	449	656	1390	2140
17	VFA	101	247	411	571	118	298	426	636	823	1567
18	VTO	104	280	470	596	120	328	425	642	899	1700
19	AV	123	350	485	680	136	319	442	658	916	1898
20	CONTROL	106	240	305	310	124	298	450	651	764	1680

Analysis of Variance Procedure

Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: LW1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	544651.70000000	136162.92500000	40.41	0.0001
Error	15	50542.50000000	3369.50000000		
Corrected Total	19	595194.20000000			

R-Square	C.V.	Root MSE	LW14 Mean
0.915082	9.695573	58.04739443	598.70000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	544651.70000000	136162.92500000	40.41	0.0001

Duncan's Multiple Range Test for variable: LW1

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 3369.5

Number of Means	2	3	4	5
Critical Range	87.49	91.71	94.33	96.12

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	755.75	4	CM
A			
A	715.25	4	AV
A			
A	698.50	4	VFA
B	507.50	4	VTO
C	316.50	4	CONTROL

Analysis Variable: LW1

TREAT	N Obs	Mean	Std Error
AV	4	715.2500000	13.9366125
CM	4	755.7500000	6.7500000
CONTROL	4	316.5000000	8.6458082
VFA	4	698.5000000	43.2540942
VTO	4	507.5000000	45.0157380

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: LW21

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	45728.70000000	11432.17500000	3.28	0.0403
Error	15	52242.25000000	3482.81666667		
Corrected Total	19	97970.95000000			

R-Square	C.V.	Root MSE	LW24 Mean
0.466758	8.988713	59.01539347	656.55000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	45728.70000000	11432.17500000	3.28	0.0403

Duncan's Multiple Range Test for variable: LW21

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 3482.817

Number of Means	2	3	4	5
Critical Range	88.95	93.24	95.91	97.73

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	728.25	4	CM
A			
B A	697.75	4	AV
B			

```
                    B                   626.50      4  CONTROL
                    B
                    B                   626.50      4  VFA
                    B
                    B                   603.75      4  VTO

                         Analysis Variable: LW21

                  TREAT     N Obs          Mean     Std Error
                  ------------------------------------------
                  AV            4   697.7500000    24.1397839

                  CM            4   728.2500000    25.4341470

                  CONTROL       4   626.5000000    40.8503366

                  VFA           4   626.5000000    35.7409662

                  VTO           4   603.7500000    13.3315103
                  ------------------------------------------
                      Analysis of Variance Procedure
                           Class Level Information

                    Class    Levels    Values

                    TREAT         5    AV CM CONTROL VFA VTO

                    Number of observations in data set = 20

                         Analysis of Variance Procedure

Dependent Variable: LW22

Source             DF             Sum of Squares                Mean Square    F Value      Pr > F

Model               4            303688.20000000            75922.05000000       3.88      0.0234

Error              15            293768.00000000            19584.53333333

Corrected Total    19            597456.20000000

            R-Square                      C.V.                  Root MSE              LW26 Mean

            0.508302                  7.301338             139.94475100          1916.70000000

Source             DF                   Anova SS               Mean Square    F Value      Pr > F

TREAT               4            303688.20000000            75922.05000000       3.88      0.0234


                        Duncan's Multiple Range Test for variable: LW22

                  NOTE: This test controls the type I comparisonwise error rate, not the
                        experimentwise error rate

                              Alpha= 0.05  df= 15  MSE= 19584.53

                           Number of Means     2     3     4     5
                           Critical Range  210.9 221.1 227.4 231.7

                  Means with the same letter are not significantly different.

                        Duncan Grouping              Mean      N  TREAT

                                    A           2141.25      4  CM
                                    A
                              B     A           1948.25      4  AV
                              B
                              B                 1869.75      4  VTO
```

	B			
	B	1826.75	4	VFA
	B			
	B	1797.50	4	CONTROL

Analysis Variable : LW22

TREAT	N Obs	Mean	Std Error
AV	4	1948.25	44.0801826
CM	4	2141.25	14.3432621
CONTROL	4	1797.50	51.3363094
VFA	4	1826.75	125.3584029
VTO	4	1869.75	63.1009443

Cost Analysis

OBS	TREAT	LW14	LW24	LW26	FCKGWT14	FCKGWT24	FCKGWT26
1	CM	757	775	2175	3898.44	4055.27	3465.38
2	VFA	763	724	1670	4257.38	4647.38	5203.38
3	VTO	574	597	2004	5632.93	5390.66	4165.24
4	AV	706	768	2030	4424.14	4162.35	3771.27
5	CONTROL	295	718	1900	7155.99	4491.21	4508.87
6	CM	769	737	2105	3851.61	4246.82	3584.71
7	VFA	735	571	2105	4381.47	5941.69	4185.40
8	VTO	435	596	1900	7645.82	5294.89	4512.27
9	AV	740	680	1850	4060.77	4634.48	4066.01
10	CONTROL	331	522	1745	6963.40	7931.38	5483.39
11	CM	760	745	2145	3902.89	4218.57	3513.84
12	VFA	725	575	1965	4489.44	5851.66	4422.21
13	VTO	425	580	1875	7587.50	5548.66	4451.81
14	AV	735	685	2015	4067.88	4666.69	3799.34
15	CONTROL	330	615	1865	6331.75	5243.39	4593.49
16	CM	737	656	2140	4106.53	4630.00	3526.08
17	VFA	571	636	1567	5605.94	4982.20	5534.40
18	VTO	596	642	1700	5746.68	4922.22	5121.69
19	AV	680	658	1898	4536.28	4632.30	4026.74
20	CONTROL	310	651	1680	9158.40	5320.73	5172.40

Duncan's Multiple Range Test for variable: FCKGWT21

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 571966.1

Number of Means	2	3	4	5
Critical Range	1140	1195	1229	1252

Means with the same letter are not significantly different.

	Duncan Grouping		Mean	N	TREAT
		A	5746.7	4	CONTROL
		A			
	B	A	5355.7	4	VFA
	B	A			
	B	A	5289.1	4	VTO
	B	A			
	B	A	4524.0	4	AV

B
B 4287.7 4 CM

Analysis of Variance Procedure
Class Level Information

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: FCKGWT22

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6009816.22818218	1502454.05704555	9.26	0.0006
Error	15	2434284.41650170	162285.62776678		
Corrected Total	19	8444100.64468389			

R-Square	C.V.	Root MSE	FCKGWT26 Mean
0.711718	9.249376	402.84690363	4355.39556952

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	6009816.22818214	1502454.05704553	9.26	0.0006

Duncan's Multiple Range Test for variable: FCKGWT22

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 162285.6

Number of Means	2	3	4	5
Critical Range	607.2	636.5	654.7	667.1

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	4939.5	4	CONTROL
A			
A	4836.3	4	VFA
A			
A	4562.8	4	VTO
B	3915.8	4	AV
B			
B	3522.5	4	CM

Duncan's Multiple Range Test for variable: CW22

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 7056.617

Number of Means 2 3 4 5
Critical Range 126.6 132.7 136.5 139.1

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	1914.75	4	CM
B	1736.00	4	AV
B			
B	1689.75	4	VTO
B			
C B	1622.50	4	CONTROL
C			
C	1532.25	4	VFA

Analysis Variable : CW22

TREAT	N Obs	Mean	Std Error
AV	4	1736.00	35.8050276
CM	4	1914.75	16.1728940
CONTROL	4	1622.50	47.7170480
VFA	4	1532.25	53.6258256
VTO	4	1689.75	46.0929767

Class	Levels	Values
TREAT	5	AV CM CONTROL VFA VTO

Number of observations in data set = 20

Analysis of Variance Procedure

Dependent Variable: CW21

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	44442.70000000	11110.67500000	3.96	0.0217
Error	15	42044.25000000	2802.95000000		
Corrected Total	19	86486.95000000			

R-Square	C.V.	Root MSE	CW4 Mean
0.513866	9.088129	52.94289376	582.55000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	4	44442.70000000	11110.67500000	3.96	0.0217

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: CW21

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 2802.95

Number of Means 2 3 4 5
Critical Range 79.79 83.65 86.04 87.67

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TREAT
	A	653.75	4	CM
	A			
B	A	622.50	4	AV
B				
B	C	553.50	4	CONTROL
B	C			
B	C	552.25	4	VFA
	C			
	C	530.75	4	VTO

Analysis Variable: CW21

TREAT	N Obs	Mean	Std Error
AV	4	622.5000000	23.9669912
CM	4	653.7500000	24.4041492
CONTROL	4	553.5000000	33.1700166
VFA	4	552.2500000	32.6812255
VTO	4	530.7500000	12.8606311

Analysis of Variance Procedure

Duncan's Multiple Range Test for variable: CW21 %Lwt

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 19.3

Number of Means 2 3 4 5
Critical Range 6.621 6.941 7.139 7.275

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	TREAT
	A	89.800	4	CM
	A			
	B	88.200	4	VFA
	B			
	A	89.220	4	AV
	B	87.900	4	VTO
	B	88.340	4	CONTROL

Analysis Variable: cW 21 % Lwt

TREAT	N Obs	Mean	Std Error
AV	4	89.2200000	1.4142136
CM	4	89.8000000	0.5000000
CONTROL	4	88.3400000	0.4787136
VFA	4	88.2000000	2.6259919
VTO	4	87.9000000	3.8405729

Duncan's Multiple Range Test for variable: cW 22 %Lwt

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 15 MSE= 7776.233

Number of Means	2	3	4	5
Critical Range	132.9	139.3	143.3	146.0

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TREAT
A	89.40	4	CM
A			
B	84.70	4	CONTROL
B			
A	89.10	4	AV
A			
C B	83.80	4	VFA
B	85.00	4	VTO

Analysis Variable : CW 22 % Lwt

TREAT	N Obs	Mean	Std Error
AV	4	89.1000000	1.7193270
CM	4	89.4000000	2.0113607
CONTROL	4	84.7000000	2.2057254
VFA	4	83.8000000	2.1275929
VTO	4	85.0000000	1.6359715