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INTERACTION OF DIETARY SUPPLEMENTS OF ORGANIC TRACE MINERALS AND PHYTASE ON GROWTH PERFORMANCE AND MINERAL METABOLISM OF REPLACEMENT PULLETS

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INTERACTION OF DIETARY SUPPLEMENTS OF ORGANIC TRACE MINERALS AND PHYTASE ON GROWTH PERFORMANCE AND MINERAL METABOLISM OF REPLACEMENT PULLETS

THESIS

A thesis submitted in partial fulfillment
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By

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ABSTRACT OF THESIS

INTERACTION OF DIETARY SUPPLEMENTS OF ORGANIC TRACE MINERALS AND PHYTASE ON GROWTH PERFORMANCE AND MINERAL METABOLISM OF REPLACEMENT PULLETS

Effects of dietary supplementation with low levels of organic sources of trace minerals in place of normal levels of their inorganic salts and phytase on growth performance and mineral metabolism were evaluated in two studies using pullets of white and brown shell laying strains. The organic sources were proteinates of copper, iron, manganese and zinc and selenium yeast. A corn-soybean meal diet was fed alone, plus inorganic minerals or plus organic minerals, and with or without phytase in a 3 x 2 factorial arrangement. Twelve groups of 16 pullets, 2 weeks old, were used per treatment. Compared with inorganic minerals, feeding no mineral supplement or organic minerals significantly ($P<0.05$) decreased manure Cu, Fe and Zn for white pullets and Cu, Fe, Mn and Zn for brown pullets. Dietary phytase significantly reduced manure Fe, P and Ca for white pullets and Fe, Mn, Zn, P and Ca for brown pullets. Adding phytase to diets containing inorganic minerals reduced manure Zn concentration for white pullets and manure Fe, Mn, Zn, P and Ca concentrations for brown pullets. These studies indicate manure levels of trace minerals can be decreased by using low levels of organic mineral supplements and phytase in pullet diets.

KEYWORDS: inorganic mineral, organic mineral, laying hen pullets, manure minerals, phytase

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DEDICATION

I will like to dedicate this paper to my beautiful family, my wife Sharon, and my children, Elena, Lucas, Javier, Diego, and Sandra. Thank you for your love and support.

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CHAPTER 1

INTRODUCTION

Pullets require trace minerals to maintain adequate body functions and general life processes. Trace minerals are essential for the formation of the skeleton, for maintaining adequate growth rate, and for keeping the bird's general health (McDowell, 2003). The major sources of trace minerals for pullets are the feed ingredients such as corn, soybean meal, and wheat, which can fulfil the nutritional requirements. Nevertheless, plants vary in their uptake of trace minerals from the soil, which could have an imbalance or depletion of trace minerals. Therefore, mineral supplementation is necessary to meet the nutritional requirements of the bird (Herrick, 1992). Trace minerals are essential minerals which are required at a low concentration (less than 100 ppm) by the pullet's body and in the feed. Nevertheless, twenty-two essential trace minerals have been recognized to be required by some animal species (Lloyd *et al*., 1978). Some of the essential trace minerals required by pullets are: manganese (Mn), iron (Fe), copper (Cu), iodine (I), zinc (Zn), fluorine (F), cobalt (Co), and selenium (Se), these trace minerals account for only 0.3% of the total body mineral concentration (McDowell, 2003).

Trace minerals serve the bird's body in four major functions: as a main component of the skeletal structure, helping to regulate the colloidal state of the body, assisting in the regulation of the body acid-base equilibrium, and as a cofactor or component of an enzyme system. For example, manganese, zinc, and fluorine are present in skeletal structure to contribute to the mechanical stability and rigidity of the bones. Likewise, trace minerals play an important role in controlling the water flow through the intestinal cell wall,

contributing to water and nutrient absorption from the lumen and the exchange of water between the blood and intracellular (and intercellular) fluids. They also are essential for nerve impulse and muscle contraction. Selenium serves as an integral component of the enzyme glutathione peroxidase. Another good example of trace minerals as a component of a biological compound is iron in hemoglobin and cobalt in vitamin B_{12} (Ashmead, 1992). Trace minerals also play a key role in metabolic functions. Underwood and Suttle (1999) described the vital role of Zn in regulating the enzymes retinal reductase and alcohol dehydrogenase, both of which are necessary for the synthesis of retinol to retinene which is essential for normal vision. Likewise, Zn influences in digestion, glycolysis, DNA synthesis, and the integrity of the epithelial tissue. Manganese is part of the metalloenzymes responsible for cartilage development. A deficiency in Mn will result in skeletal defects because of the key role it plays in the activity of glycosyl transferase (Mn dependent). Glycosyl transferase is needed for synthesis of mucopolysaccharides, a vital component of cartilage (Leeson and Summers, 2001). Underwood and Suttle (1999) reported that Mn deficiency (mucopolysaccharide synthesis) in laying hens will have an effect on shell formation and egg production. The liver will serve as the primary storage site for Cu in the body; likewise Cu will be present in most body cells. Leeson and Summers (2001) proposed that Cu deficiency will lead to growth depression, bone disorder, depigmentation of feathers, anemia, and the demyelination of the spinal cord. Lastly, hemoglobin and myoglobin contain 60% of all body iron. Iron is essential for hemoglobin production and oxygen transport in the blood. It also plays a key role in immunity and muscle functions. Trace minerals could be supplied to pullet's diets in two forms: as inorganic salts as organic sources (chelates). Trace minerals salts are most

commonly oxide, chlorides, sulfates and carbonates. Oxide mineral salts are the most utilized in the industry to formulate mineral premixes, for its ability to contain higher percentage of the trace minerals. For example, copper oxide contains 79% CU while copper sulfate with only 21% Cu. One of the issues related to inorganic trace minerals is the lack of current research to determine their actual availability. Most of the availability is determined by reagent grade test. A primary concern from not knowing the actual availability of the trace minerals is that nutritionist over formulate to be sure the requirements are met. This will lead to excess of minerals in manure, used for crop fertilization and results in pollution problems. An advantage of utilizing inorganic sources is they are easily available and inexpensive. Chelated trace minerals, or organic sources, are bound to a carrier such as an amino acids, a partially hydrolyzed protein, small peptide, or to a carbohydrate. The most common organic trace minerals are either a chelation with an amino acid or small peptide. The primary benefit of chelation is the ability to enhance the absorption and utilization by the intestine, and to prevent the minerals from forming insoluble chemical compounds. A mineral chelate can be absorbed by the intestine intact as a chelate or the mineral can be released in ionic form at the intestinal wall. Also, chelates trace minerals possess a high stability and therefore, do not react with single ions, and can be used in a lower concentration (Herrick, 1992). McDowell (2003) reported that chelates trace minerals do not interact with vitamins or other ions in the digestive tract of the bird, nor in the premix. Organic trace mineral sources have been reported to have increased bioavailability compared with their respective inorganic salts.

Cao *et al*. (2000) suggested they have the property of maintaining the structural integrity in the small intestine until they arrive at the absorption site, therefore, improving the

bioavailability of the trace mineral. Most of the studies conducted with organic trace mineral sources have focused on the bioavailability, the concentration in tissues, the improvement of performance, and the reduction of trace minerals in manure. Henry *et al.* (1989), reported Mn-methionine to be 133% more available compared to Mn monoxide. Similarly, Smith *et al.,* (1994), found that Mn proteinate was 120% available compared with Mn sulfate (set at 100%). Aoyagi and Baker (1992) reported that Cu concentration in the bile is a good indicator of Cu absorption by the gut. They found that Cu-Lysine improved availability 32% when compared to Cu sulfate. Also, Aoyagi and Baker (1992) conducted a study to determine the availability of Zn in Zn-Lys by measuring Zn tibia concentration. They reported that it was 11% more available than Zn in Zn sulfate.

Studies on performance in pullets are not abundant, as most of the trials have been conducted in broilers, layers and swine. The primary objective of these trials was to feed equal or lower levels of organic trace minerals and compare then to inorganic sources fed at the NRC (1994) recommended or require level. Cheng *et al.* (1998) fed pigs with Zn-Lys and Zn sulfate at NRC (1998) requirement and reported no significant difference between the treatments, concluding that organic source could be used as a replacement. Likewise, Kienholz *et al.* (1992) reported improvement in egg production when they fed Zn methionine to layers. Mabe *et al.* (2003) reported no significant difference in egg production when comparing organic trace minerals with inorganic minerals.

The ability of monogastric animals, especially poultry and swine, to utilize phytate P has been reported as poor, due to the lack of phytase enzyme to hydrolyze the phytate and release the P. Phytate can also impair the availability of other nutrients such as trace minerals and protein, hindering the performance of animals (Oberleas and Harland, 1996).

Phytase enzyme is used in the industry to improve the use of P from plant sources and reduce the inclusion of phosphates in the diets. Also, studies by Biehl *et al.* (1995) with phytase enzyme reported improvements in P, Zn and Mn utilization by broilers. They found a 64 % improvement in bone ash. Finally, plants can utilize essential mineral elements present in animal manure; therefore, animal waste serves as a resource for crop production. When trace minerals and P are found in high concentration in the soil, they can be transported to rivers or lakes along with nitrogen, resulting in algae formation and pollution. Organic trace minerals fed at a lower concentration of inorganic source will lead to a significant reduction in the manure mineral concentration. In addition, of using phytase may also reduce manure mineral concentrations. Pierce *et al.* (2005) was able to significantly reduce Cu and Zn manure concentration when feeding a combination of phytase and organic minerals when compared to inorganic mineral sources. Likewise, Leeson (2005) reported a reduction of Zn (60%), Mn (70%) and Cu (11%) manure concentration in layers fed organic trace minerals when compare to an inorganic source.

The objectives of these experiments were to determine the effects of: a) using low levels of trace minerals supplied by organic sources (proteinates) in place of normal levels of trace minerals in the form of inorganic salts in pullet diets, b) supplementing the diet with phytase and c) the interaction mineral source X phytase supplementation on growth performance, skeletal development and the concentration of trace minerals in liver and manure of replacement pullets.

CHAPTER 2

LITERATURE REVIEW

Essential minerals

Minerals are naturally occurring, inorganic solid substances having a definite chemical composition and characteristic crystalline structure, color, and hardness. Chickens and other living forms require minerals for maintaining normal life processes. The term mineral can also be used to mean an inorganic element and, in nutritional science, the term mineral is generally used to refer to all inorganic elements. Minerals are inorganic elements that cannot be synthesized or decomposed by simple or ordinary chemical reactions. They constitute the ash remaining when organic matter is ignited. However, not all of the inorganic elements present in nature can be classified as required nutrients from a nutritional basis (McDowell, 2003). Thus, from the elements listed in the periodic table, only a few are present in the body in a measurable amount (Lloyd *et al*., 1978).

Minerals are solid and crystalline elements and are present in nature forming salt complexes with different combinations of inorganic elements or organic compounds (McDowell, 2003). The major sources of minerals for nutrition of animals are feed ingredients (e.g. corn, soybean meal) and mineral supplements. Because of an imbalance of depletion of minerals in the soils and plants, mineral supplementation is required for animals in order to achieve the nutritional requirements (Herrick, 1992).

The most accepted classification scheme for minerals is related to its essentiality for functions such as maintaining adequate growth rate, supporting general health and reproduction throughout the animal life cycle when all the other essential minerals are present in adequate concentrations. However, essential minerals must be present in all animal tissues with a relatively constant physiological concentration and with little variation among animal

species. A deficiency of an essential mineral will result in a reproducible physiological and/or structural abnormality.

A diet lacking enough of one or more essential minerals results in measurable deficiency symptoms. Therefore, the addition of the deficient essential mineral to the diet should reverse or prevent the adverse symptom of the deficiency and prevent the abnormal biochemical changes created by the specific deficiency (Lloyd *et al.,* 1978).

Essential mineral elements can be further classified as macrominerals or trace minerals (also called microminerals) according to the total amount required by the animal. Macrominerals are essential minerals required at concentrations in the diets of approximately 100 mg/kg (ppm) or greater. Of the twenty-nine essential minerals required by at least some animal species, seven are macrominerals (McDowell, 2003).

Microminerals or trace minerals are essential minerals required at lower concentrations in the animal body and in the diets. Their concentrations are typically less than 100 ppm. Twenty-two essential minerals are recognized as trace minerals, which are required by a least some animal species. The terms macrominerals and microminerals do not infer that minerals required in larger amounts are more important for the animal than minerals required in lower concentrations (Lloyd *et al*., 1978). Essential minerals can also be classified based upon the electric charge as anions (ions with an overall negative charge) and cations (ions with an overall positive charge). Also, essential minerals can be classified on the basis of their position on the periodic table and the valence number (McDowell, 2003).

Table 2.1 lists the essential minerals elements required by at least some animal species. It also includes a classification upon electric charge, showing macromineral cations and anions.

Table 2.1 Essential mineral elements

Macrominerals		Microminerals or Trace minerals	
Cations	Anions		
Calcium (Ca)	Phosphorus (P)	manganese (Mn)	Cobalt (Co)
Magnesium (Mg)	Chlorine (Cl)	iron (Fe)	Molybdenum (Mo)
Sodium (Na)	Sulfur (S)	copper (Cu)	Selenium (Se)
Potassium (K)		Iodine (I)	Chromium (Cr)
		zinc (Zn)	Tin (Sn)
		Fluorine (F)	Nickel (Ni)
		Vanadium (V)	Silicon (Si)

(Adapted from McDowell, 2003)

Distribution of essential minerals in the body

The concentration of essential minerals among body tissues is not uniform. Some tissues can store specific concentration of minerals. But the percent or proportion of essential minerals expressed as fat-free dry body substance is similar among poultry and adult mammals. Of the total body essential minerals, calcium (Ca) and phosphorus (P) represent 46 % and 29% respectively. The other macrominerals potassium, sodium, sulfur, chlorine and magnesium represent 25%. Trace minerals account for less than 0.3% of the total body essential minerals.

The primary storage site for essential minerals is the skeleton, accounting for 80% of the total body minerals. Calcium, phosphorus and magnesium are the primary macrominerals in bones. Ninety-nine percent of the total calcium is present in the bones, while 80 to 85% of the total phosphorus and 70 % of the total magnesium are also present in the bones (McDowell, 2003).

General function of essential minerals

Essential minerals can serve in the body for four major functions:

a) Structural, as a component of the skeletal structure, which supports growth and maintenance of hard and soft tissue.

b) Physiological, regulating and maintaining the colloidal state of the body.

c) Regulatory, regulating the body acid-base equilibrium.

d) Catalytic, as a cofactor or a component of an enzyme system.

Some essential minerals serve in only one of the functions while others can serve in all four functions.

Skeletal structure

Minerals form structural constituents in organs and skeletal structures such as bones and teeth, in which calcium, phosphorous, magnesium, fluoride and silicon account for high percentage and contribute to the mechanical stability and rigidity. Bones are formed from the deposition of calcium and phosphorus into the protein matrix in the form of hydroxyapatite (McDowell, 2003). In molecules and membranes, minerals such as zinc and phosphorus contribute structural stability, and in birds, calcium plays a key role in the formation of eggshells (Lloyd *et al.,* 1978).

Colloidal system

Essential minerals control the flow of water through the intestinal cell wall. Because of the effect of minerals on the flow of water and other compounds across membranes, they are important factors in intestinal absorption and the movement of water to and from blood and other body fluids.

Essential minerals also control the osmotic pressure of the cell by regulating the exchange of water and solutes through the cell wall. Transmission of the nerve impulse is regulated by the exchange of sodium and potassium throughout the cell wall. Muscle

contraction is also regulated by the function of essential minerals such as calcium, magnesium, sodium and potassium (Lloyd *et al.,* 1978).

Acid-base equilibrium

Essential minerals regulate the acid-base balance of the body fluid by controlling membrane permeability (McDowell, 2003). The minerals are present in the body fluid as components of the electrolytes. Calcium, magnesium, phosphorus, sodium, potassium and chlorine are the main components of electrolytes.

Acid-base balance is affected by the concentration of minerals in the feed ingested by the animals. Food with a predominance of the cations sodium, potassium, calcium, iron, and magnesium will be base forming. Food with a predominance of chlorine, phosphorus and sulfur will be acid forming (Lloyd *et al.,* 1978).

Enzyme and coenzyme systems

Several essential minerals serve as integral components of enzymes. Examples include selenium in glutathione peroxidase enzyme, iron in cytochrome oxidase, and copper in tyrosinase. Other functions include being part of the structure of biological compounds, such as iron in hemoglobin, cobalt in vitamin B_{12} and iodine in thyroid hormones thyroxine and triiodothyronine (Lloyd *et al*., 1978). As coenzymes, essential minerals participate in enzymatic reactions which are needed for generation of energy. Magnesium, calcium and phosphorus are utilized in the synthesis of ATP (Ashmead, 1992).

Interaction and bioavailability of essential minerals

Uptake of essential minerals in the gastrointestinal tract is affected by numerous antagonistic factors, which can result in ineffective absorption. In general, the chemical interaction between the nutrients present in the diet and the intestinal environment will

determine the degree of mineral absorption. The mechanism by which a mineral reaches the blood stream through the intestinal walls is related to its chemical form when it reaches the mucosal membrane of the intestine cell. In addition, dietary factors, such as vitamins, can affect absorption of minerals positively or negatively. Vitamin D is known to increase calcium absorption. Vitamin C can increase iron absorption. Excess niacin causes hypocalcemia by inactivating vitamin D, thereby influencing calcium absorption (Ashmead, 1992).

Another dietary factor is fat, which affects mineral absorption when added to the feed in large quantities. Insoluble soap formation from fatty acids and calcium has been reported in high fat diets, resulting in steatorrhea and reduction of calcium absorption. This will largely be dependent on the chemical form of the metal and the composition of the fat (Ashmead, 1992).

A third factor is non-digestible fiber, which has been reported to affect mineral utilization. Absorption of calcium, zinc, magnesium and phosphorus is decreased in a high fiber diet. Phytic acid and oxalic acid are also found in fiber sources, reducing the mineral absorption due to binding to these organic acids. Fourthly, the stomach and the intestinal chemical environment affect the percent of mineral absorption.

There are six major mineral interactions in biological systems. The first interaction involves two or more minerals in the intestinal lumen competing for a ligand (with anionic electron bearing), resulting in an insoluble precipitate. An organic compound such as phytic acid or an inorganic compound such as phosphate serves as a ligand. This competition for a ligand will depend on the association constant of the chelates and the solubility of the resulting products (Ashmead, 1992). A physiological description for a competition can be represented by the digestion of the mineral salt. The low pH of the stomach increases the solubility, resulting in an ionization of the salt. Once the mineral reaches the jejunal and ileal portions of the intestine, the higher pH decreases the solubility, and the mineral tends to bind to a ligand,

anion or metal acid radical complexes which are highly insoluble and stable. In order to be absorbed, the mineral must be soluble once it reaches the intestine. The level of phytic acid level directly influences the absorption of certain minerals such as zinc and calcium (Lan *et al.*, 2002). Organic phosphates can form low solubility products with calcium, magnesium, zinc, manganese and iron. Calcium phosphates can interact with iron, forming an insoluble precipitate iron phosphate, resulting in a decrease of absorption of iron. This can lead to iron deficiency anemia by the animal (Ashmead, 1992).

The second type of mineral interaction involves the competition for a carrier between ions for absorption of metals from the lumen to the cytoplasm of the intestinal cell. Carrier molecules are composed of proteins that traverse the intestinal mucosal cell membrane. Ions must bind to the carrier protein in order to be absorbed, and have the capacity to form a chelate or a complex through a functional electron-bearing group. Therefore, a competition for the binding site can occur between trace minerals, macrominerals or both. Iron and copper are good examples of this interaction. Both share the same protein carrier, transferrin, in the intestine cell. If the levels of dietary copper are excessive, iron absorption is inhibited by a higher affinity of copper for transferrin. In normal conditions, the intestine produces sufficient concentration of transferrin to transport both metals throughout the intestine cell. The affinities of essential minerals for electron bearing atoms of the protein carrier depend on their electronic arrangement.

The third mineral interaction group results from the inability of body cells to synthesize the metal binding proteins. This can be a consequence of interference with non-essential heavy metals.

The fourth mineral interaction group is related to the previous group, in which the metal activates an enzyme in this group, forming a metalloenzyme. If the metal is removed, the catalysis can be blocked or accelerated. In the metalloenzyme carboxypeptidase, zinc plays a

key role as integral part. If cobalt replaces Zn for incorporation into carboxypeptidase, the peptidase activity is reduced or when zinc replaces cobalt the peptidase activity of the enzyme is increased (Ashmead, 1992).

The fifth mineral interaction group results from the transport and excretion of minerals from the cell of the intestinal mucosal. Minerals can be returned to the lumen of the intestine without being utilized by the body. This can result in a replacement of metal while at the same time promoting competition of minerals, due to specific interactions among them.

The sixth mineral interaction results as consequence of the previous group, which releases a chain reaction by affecting further enzymatic reaction or production of protein because of a cation precipitated in the intestine. This makes it insoluble and therefore, it will not be absorbed (Ashmead, 1992).

Bioavailability of essential minerals is influenced by intrinsic and extrinsic factors. Kratzer and Vohra (1986) classified these factors as follows:

Intrinsic factors include "Animal species and genetic, age and sex of the animal, monogastric or ruminant species, physiological function, environmental stress and health, food habits and nutritional status, endogenous ligand or chelates". Extrinsic factors are: "Mineral status of the soil in which the plants are grown, transfer of minerals from soil to food supply, bioavailability of mineral elements from food to animal". The bioavailability of minerals from food is affected by: "inorganic salt or chelates, solubility of the mineral complex, absorption on silicates, calcium phosphate, dietary fiber, electronic configuration of the element and competitive antagonism, coordination number, route of administration, presence of complexing agents such as chelates, relative amounts of other mineral elements". The lumen possesses factors which influence bioavailability such as: "interaction with naturally occurring ligands, protein, peptides and amino acids, carbohydrates, lipids, anionic molecules and other metals".

Metabolic functions of trace minerals

Trace minerals play an important role in metabolism. Zinc has been reported to have a vital role in animal metabolism, such as regulating the activity of enzymes retinene reductase and alcohol dehydrogenase. Both are necessary for the conversion of vitamin A alcohol (retinol) to vitamin A aldehyde (retinene), which is essential for normal vision. Zinc is also important in the formation of antibodies and the immune response, and in protein and carbohydrate metabolism, by the regulation of gene expression at the molecular level, in which Zn-finger domains in DNA-binding proteins influence transcription and cell replication. In addition, zinc influences digestion, glycolysis, DNA synthesis, nucleic acid and protein metabolism. Likewise, Zn affects the regulation of growth rate and maintenance of epithelial tissue integrity and animal reproduction (Underwood and Suttle, 1999). Manganese is essential for metalloenzymes which play an important role in cartilage development. Manganese deficiencies cause skeletal defects because the glycosyl transferase enzyme is Mn dependent which increases the synthesis of mucopolysaccharides, a vital component of cartilage. In poultry this causes perosis, a syndrome in which an enlargement and malformation of the tibiometatarsal joint occurs, resulting in the slippage of the gastrocnemius tendon from its condyles (Leeson and Summers, 2001). It has been reported that Mn deficiency in laying hens influences egg production and shell formation by the activity of the mucopolysaccharide synthesis and the reduction of hexosamine content. In addition, the glycosyltransferase is Mn dependent and influences blood clotting, which is involved in the formation of glycoprotein and prothrombin. Similarly, Mn affects lipid and carbohydrate metabolism and reproduction (Underwood and Suttle, 1999).

Copper is essential for reproduction, bone development, growth, connective tissue development, and pigmentation of the skin and feathers. It has been reported that copper is

present in most body cells and stored mainly in the liver. Some of the signs of copper deficiency are anemia, growth depression, bone disorder, depigmentation of feathers, and demyelination of the spinal cord, and fibrosis of the myocardium (Leeson and Summers, 2001).

Iron is required for hemoglobin production in the blood, oxygen transport, muscle function and immunity. Iron is present as hemoglobin and myoglobin (60% of body iron), forming complexes of the protoporphyrin heme and globin (Underwood and Suttle, 1999).

Chelation of Minerals

The Association of American Feed Control Officials Incorporated (AAFCO, 2001) defined several types of mineral chelates a metal amino acid complex, "as the product resulting from a complexion of a soluble metal salt with an amino acid (s)". A metal amino acid chelate is "a product resulting from the reaction of a metal ion from a soluble salt with amino acids with a molar ratio of one mole of metal to one to three (preferably two) moles of amino acids to form coordinate covalent bonds". The average molecular weight of the hydrolyzed amino acids is approximately 150 and the chelate molecular weight must not be higher than 800. A metal proteinate results from "the chelation of a soluble salt with several amino acids and/or partially hydrolyzed protein. The proteinate as an ingredient for feed must be declared as specific metal proteinate", i.e., zinc proteinate or copper proteinate. In addition, AAFCO (2001) stated that the minimum metal content of the chelate must be declared and, when used as an ingredient for commercial feed, stated as a specific metal amino acid chelate such as calcium amino acid chelate or iron amino acid chelate. Mineral chelates are produced by combining hydrolyzed plant, carbohydrates, amino acids or animal proteins with a mineral sulfate salt. Mineral chelates are employed to enhance the absorption and utilization of trace minerals by the intestine and to prevent the minerals from forming insoluble chemical compounds. A mineral chelate can be absorbed by the intestine intact as a chelate or it can be

released in ionic form at the intestinal wall. Chelates are metals complexes in which the metal atom is attached in the complex through several points of attachment to the chelating agent (a ligand); the mineral atom is held in the central position of the complex (McDowell, 2003). Mineral chelates form a ring structure that consists of a metal atom, an active carboxyl oxygen atom, a carbonyl carbon atom, and α-carbon atom or α-nitrogen atom. This results from the attraction between positive charges of a polyvalent cation, in a highly electronegative (two or more sites) chemical compound know as a ligand. A ligand is defined as the organic substance which binds the metal (McDowell, 2003). It requires ionic and covalent bonds to be formed; the covalent bond is known as a coordinate bond. The ligand has the capacity of donating atoms and contributes with two electrons at the same time. Therefore, it facilitates the bonding to the metal of two or three amino acids depending upon its oxidative state and forms bicyclic and or tricyclic ringed molecules (Ashmead, 1992). Chelating agents attach to the metal ion by position two though six and are classified as bidentate, tridentate, quadridentate, quindentate or hexadentate chelating agents. The chelation of minerals generally enhances the absorption of the metal by preventing the interaction of the mineral with other substances in the gastrointestinal tract resulting in no formation of insoluble chemical compounds. Mineral chelates also prevent the mineral from forming an insoluble colloid due to a strong absorption by the intestine cells so that the ion is not released to the lumen. The capacity to carry a mineral into the intestinal mucosal cell without releasing the chelate agent also enhances the absorption of the mineral (Herrick, 1992). Also chelates do not react as singles ions because of their high stability. Due to their high stability, mineral chelates work effectively at low concentrations. They do not interact with vitamins or other ions either in the digestive tract or on premixes. Some examples of chelating agents in living organisms are carbohydrates, lipids, amino acids, phosphates (phytic acid), porphyrins (hemoglobin and chlorophyll) and vitamins (B_{12} and

ascorbic acid). A number of drugs such as penicillin, aspirin and tetracycline also work via chelation. (McDowell 2003).

Herrick (1992) describes the major groups of chelates essential in biological systems. The major function of the first group is to transport and store metal ions. In this class of chelates the proprieties of the ligands are not change. The properties of the ligands aid in the absorption of the metal ion. Transferrin, which transport iron in the blood, is an example as this type of chelate. The second group of chelates participates in the animal's physiological functions, one example of this is hemoglobin which require the binding of iron for it functionality. The third group of chelates are those which can bind nutritionally essential minerals there by interfering with their absorption and utilization. Phytic acid is an example of this group of chelates.

Stability Constant

Stability constant is the quantitative measure of the attraction of a metal to a ligand. Leeson and Summers (2001) describes the stability constant as the log K_f, where K_f is the equilibrium constant for the formation of chelates and is expressed mathematically as:

$$K_f = [MC] / [M^{++}] [L]$$

Where L is the ligand or chelating agent, M^{++} is a cation, and MC is the metal chelate. Therefore, the interaction between L and M^{++} is generally 1:1 and can be expressed as $L + M^{++} \leftrightarrow MC$. The "chelate formation constant" quantifies the stability of metal chelate. Theoretically, a metal ion with a higher stability constant has the ability to form a chelate before a metal ion with lower stability constant, or displace a metal with lower stability constant from the ligand (Kratzer and Vohra, 1986). McDowell (2003) described a proposal in which zinc from soybean protein was chelated to improve the availability. He suggested that in order to have success in chelating zinc, a chelating agent must have a higher stability constant for Zn

than the Zn binding substance present in the feed. Living organisms produce numerous ligands, which form a complex with minerals present in diet and serve as carriers from the lumen of the intestine into the cell of the intestinal wall. The chelating agent will replace the soybean protein and form a zinc-chelate complex in the digestion tract. When the complex is absorbed, a ligand replaces the chelate in the body due to a higher stability constant. This is a result of the property of the chelating agent to serve as an intermediary between the zinc in the soybean protein and the ligand in the body. However, we have to note that using the stability constant to predict improvement on utilization of minerals chelate by the animal is not recommended, because stability constant values are markedly influenced by the pH of the living system (Kratzer and Vohra, 1986). Kratzer and Vohra (1986) describe several factors which influence stability constant when measured under similar conditions of pH. The first factor is the "size of the ring". The most stable complexes are saturated ligands that form five-member rings and unsaturated ligands that form a six-member ring. Secondly, if the "number of rings" of chelated molecules increases, its stability will increase. "Basic strength of the chelating agent" is the third factor. The more basic donor ligands have greater capacity to donate electrons and to form stable complexes. Ligands that are small in size and highly charged yield more stable complexes. Therefore, the size and charge of the ligand is another factor influencing stability constant. The fifth factor is the multidentate ligand, which has the capacity to form more stable complexes than monodentate ligands. Resonance effect is the sixth factor. With an increase in resonance in the ring, the stability of the chelate increases. Therefore, if the resonance is interfered within the chelate ring, the stability decreases.

Finally, stearic hindrance can impede chelate formation, thereby decreasing the stability constant.

Bioavailability of chelated minerals

Bioavailability of minerals typically refers to the relative degree of absorption and/or utilization in metabolism of minerals from different sources by a normal animal. Mineral chelates have been reported to have increased bioavailability compared with their respective inorganic salts. Bioavailability of minerals bound to organic compounds, hereafter referred to as organic trace minerals, will be affected by the type of organic ligand, the strength of the bond between organic ligand and the metal, and the percent of ligand which remains bound to the metal under physiological pH conditions (Guo *et al.,* 2001). It has been suggested that organic trace minerals have the property to maintain their structural integrity, arriving at the absorption site in the small intestine as an intact chelate molecule. Therefore, they can be absorbed and metabolized as a chelate mineral, thus improving bioavailability of the metal. This stability of the chelate is influenced by both the metal and the ligand. Thus, chelates with high stability constants will be prone to disassociate in the animal body without the ligand rebinding and creating a loss of the metal (Cao *et al., 2000).* Many studies have been conducted to determine relative bioavailability of organic trace minerals.

Tissue trace mineral concentration has been used to determine bioavailability. Bone and liver trace mineral concentration are most commonly used for minerals such as zinc and manganese. Henry *et al*. (1989) conducted an experiment using day-old broiler chicks to determine the relative bioavailability of manganese (Mn). They used reagent grade manganese monoxide (MnO) and Mn-methionine complex (35% methionine, 15.7 % Mn) and compared them with standard sources of Mn sulfate ($MnSO_4$-H_2O). A basal corn-soybean meal diet containing 93 ppm of Mn (dry matter basis) was supplemented with 0, 700, 1400 and 2100 ppm of Mn from the different sources. The NRC (1994) requirements for Mn for broiler chicks is only 60 mg/kg. Six diets were formulated to contain 700, 1400 and 2100 ppm of Mn sulfate

and Mn monoxide in combination with added DL-methionine (0.16, 0.32 or 0.48 %) to match the concentration of Mn methionine (Mn-met) diets, in order to control for the added methionine provided by Mn-met. Bone Mn increased as Mn concentration in the diet increased with the difference sources of Mn, but was not affected by the source of Mn. Kidney Mn concentration also increased ($P<0.01$) as dietary Mn concentration increased. Kidney Mn concentration was greater in birds fed Mn-met (22.6 ppm) diets than those fed Mn sulfate (19.8 ppm) or monoxide (18.9 ppm) with and without DL-methionine. Availability of Mn from Mn monoxide and Mn-met, compared to sulfate, was 96% and 108% respectively, based on bone Mn concentration, and 86 and 132 %, based on kidney Mn concentration. When they averaged these values, compared with Mn sulfate set at 100% available, relative availability values were 91 and 120% for Mn monoxide and Mn-met, respectively. They concluded that Mn-met was 133% available compared with reagent grades of Mn monoxide. They indicated that high bioavailability of Mn-met may be related partially to its high solubility and small particle size. They also suggested that Mn-met is more available because all or part of the amino acid complex in Mn-met could be protected from binding by a ligand in the diet. Smith *et al.* (1994) studied the bioavailability of different sources of manganese (Mn) in heat distressed broilers. They fed diets containing 0, 1000, 2000 or 3000 mg/kg of supplemental Mn as Mn sulfate, Mn oxide or Mn proteinate. They reported that the biological availability values for Mn was 120 % for proteinate and 91% for oxide, compared with 100% for sulfate, using the slope of multiple linear regression analysis of bone Mn. They also reported Mn availability values for broilers in a thermoneutral environment (18 to 23.9 C) of 125% and 83% from Mn proteinate and Mn oxide, respectively, compared with 100% sulfate, and 145% and 82% for heat distressed broilers. Li *et al.* (2004) investigated the use of chemical characteristics of Mn and predicted Mn bioavailability based on tissue concentration for twelve organic Mn sources and Mn sulfate. They reported that at pH 2, the solubility of organic Mn sources was more than

95%, and at pH 5, it was as low as 24.5% and as high as 99.1%. Using a broiler bioassay they also evaluated three Mn sources in a broiler bioassay: Mn methionine E, with a weak chelation strength (Q_f value of 3.2), Mn amino acid B with a moderate chelation strength (Q_f value = 45.3), and Mn amino acid C with a strong chelation strength (Q_f value = 115.4). They used a basal diet with 60, 120, or 180 mg/kg of Mn added as the sulfate or one of the three organic Mn sources. Using multiple linear regressions for bone Mn concentration, they found that Mn methionine E, B and C were 95, 96, and 93% bioavailable, respectively, when Mn sulfate bioavailability was set at 100%. They concluded that Mn amino acid B (moderate strength) was more available than C or E, and the use of chemical characteristics to predict bioavailability is not a reliable approach. Aoyagi and Baker (1992) investigated the bioavailability of copper (Cu) from inorganic and organic sources in young chicks. They used graded levels of Cu (0, 0.5, and 1.0 mg/kg) from Cu sulfate, Cu oxide, Cu carbonate, Cu chloride and Cu lysine, the NRC (1994) minimum requirement is 8 mg/kg. They found that Cu bile concentration was a linear function of supplemental Cu intake, and indicated that when Cu levels are fed below the requirement, bile Cu concentration was a sensitive indicator of net gut absorption of Cu. Using this measurement, they estimated the bioavailability of the different sources of Cu by multiple linear regressions. When compared with Cu sulfate salt at 100%, they found bioavailability values of 113, 97.3, 105.2 and 132.2 % for Cu lysine, Cu oxide, Cu carbonate and Cu chloride, respectively. Aoyagi and Baker (1993) investigated the bioefficacy of Cu and Zn from lysine complexes in chicks. Copper bioavailability was assessed using Cu bile concentration. Graded levels of Cu (0, 0.5, 1 mg/kg) were fed as Cu sulfate ($CuSO_4$ $5H_2O$) or a Cu lysine complex (10.62 % Cu). Overall growth performance increased linearly as dietary Cu increased. The regression coefficients revealed an efficacy of Cu lysine of 120%, which was not significantly different from Cu sulfate set at 100%. Zinc bioavailability was assessed using Zn tibia concentration. Graded levels of Zn (0, 4 and 8

mg/kg) as Zn sulfate (2.9 and 5.8%) as graded levels of Zn lysine complex (7.21% Zn) were fed. Weight gain, feed intake, and total tibia Zn increased linearly as the concentration of dietary Zn increased, regardless of Zn sources. The regression coefficients showed that Zn lysine bioavailability was 111.1% when Zn sulfate was set at 100%. Guo *et al.* (2001) investigated the bioavailability for poultry of different commercial sources of organic copper and their chemical characteristics in order to predict relative bioavailability of copper. First, they evaluated solubility and bonding structural integrity of five commercial organic copper sources and found Cu solubility at pH 2 buffer was the best fit for a linear model to predict Cu bioavailability. Then two experiments were conducted to determine relative bioavailability of Cu using liver Cu concentration. Diets were formulated with higher Cu concentrations than the NRC (1994) minimum requirement of 8 mg/kg. Experiments 1 and 2 used basal diets containing 16 and 22 mg/kg of Cu respectively. Dietary treatments consisted of feeding the basal diets with 150, 300 and 450 mg/kg of Cu as reagent grade Cu sulfate or 150 or 300 mg Cu/kg as Cu lysine, Cu amino acid and Cu proteinate C (experiment 1) and as Cu lysine, Cu proteinate A and B (experiment 2). Liver Cu concentration increased with increasing dietary Cu. The respective bioavailability values of Cu for Cu lysine, Cu amino acid and Cu proteinates C and B were 124, 122, 111 and 109 %, when Cu sulfate was set at 100%. They concluded that all the sources of organic Cu tested were equally or more bioavailable than Cu sulfate. Cao *et al.* (2000) investigated zinc bioavailability from different organic sources used in poultry diets and a model to predict the degree of binding of the organic ligand to the metal under physiological pH. Eight commercial organic Zn products were evaluated in the laboratory and correlated with bioavailability values based on bone Zn concentration. Reagent grade Zn sulfate, Zn methionine (A and B), Zn polysaccharide, Zn lysine, Zn amino acid chelate, and Zn proteinate (A, B and C), were added to a basal diet containing 59 mg Zn/kg, a Zn concentration higher than the NRC (1994) minimum requirement of 40 mg/kg. Zinc sulfate

were added to the basal diet to provide up to 400 or 600 mg Zn/kg, while the commercial products were used at levels providing 200 or 400 mg Zn/kg. Bioavailability values for a given product varied, depending on the age of the bird at the time of sampling. Most products had bioavailability values equal to or greater than that of zinc sulfate. Of the products tested, the zinc proteinate had the highest availability values. Zinc solubility in a pH5 buffer gave the best estimates of Zn availability of the products based on bone Zn concentration. Pimentel *et al.* (1991) studied the bioavailability of Zn methionine in chicks. They compared the tissue accumulation and overall growth performance of Zn oxide and Zn methionine feeding graded levels of Zn (8, 18, 28, 38, 48 and 58 mg/kg). They found bone Zn concentration was affected by the level of Zn in the diet but not by the source. They also found that pancreatic Zn concentration was affected by the source of the mineral. Overall performance was not affected by mineral source. They concluded that the bioavailability of Zn from methionine and oxide were similar. Wedekind *et al.* (1992) investigated different bioassay procedures to assess Zn bioavailability. They compared feed grade sources of Zn methionine with Zn sulfate using purified crystalline amino acid (AA), semi purified (soy isolate) and complex corn soybean meal basal diets. Zn was supplemented at 0, 7.5 and 15 mg Zn/kg. Bone Zn concentration was used to assess bioavailability. They found Zn methionine bioavailability was 177 % and total tibia concentration when Zn sulfate was set at 100%, using multiple linear regressions. They also used different alternative methods to assess bioavailability such as using crystalline AA purified diet. They reported that Zn methionine was 117% when Zn sulfate was set at 100%, based on multiple linear regressions and also indicated that Zn methionine provided slightly more bioavailable Zn than Zn sulfate.

Absorption of Chelated Minerals

Ashmead (1992) suggested that a mineral chelated with an amino acid is absorbed in the jejunum portion of the large intestine, where it is absorbed intact into the mucosal cell. Then it forms a coordinated link with a tripeptide found in the mucosal membrane cell, a moiety of gamma glutamyl glutathione. This chelate is degraded by gamma glutamyl transpeptidase enzyme resulting in gamma glutamyl amino acid chelate and cysteinyl glycine. This reaction ensues in the transport of the chelate amino acid from the luminal side of mucosal to the cytoplasm within the cell. The next step is an ATP dependent reaction in which gamma glutamyl transferase, cleaves and degrades the gamma glutamyl amino acid resulting in 5-oxyoproline and the original amino acid chelate. This reaction also restores the glutathione-transporting molecules, which transport the next chelate molecule into the cell interior. At the terminal web of the mucosal cell, the entry of the chelate amino acid molecule into the cytoplasm takes place. The chelate amino acid molecule will then traverse the mucosal cell to the basement membrane as an intact molecule. Osmotic pressure and kinetic energy of diffusion facilitate this movement through the cell membrane to the plasma. Once in the plasma it will be transported intact to the site of metabolism. If a metal functions in the body as part of amino acid chelates, hydrolysis of the chelate will not be necessary. When the metal required is unbound from the amino acid, hydrolysis of the coordinating bond occurs. This is due to the enzymatic reaction and pH change in the site of use which lowers the stability constant of the chelate releasing the metal.

Effects of chelated trace minerals on tissue trace mineral concentration

The following studies were conducted to investigate the effect on tissue trace mineral concentration with another type of organic mineral, the use organically bound selenium. Paton *et al.* (2002) reported greater concentration of egg Se concentration when Se yeast (vs. Na selenite) was added to diets of White Leghorns. Yolk and white Se deposition were also increased when increasing levels of organic Se were fed. They also reported an increasing Se embryo concentration (at Days 10, 15 and 20 of development) when organic Se was fed at 0.1 mg/kg vs. 0.3 mg/kg of Se as selenite. Payne *et al.,* (2005) investigated the effects of organic Se on deposition in whole eggs when compared with inorganic sources of Se on laying hens. They supplemented diets with different levels of Se (0.15, 0.30, 0.60 or 3.00 mg Se/kg) from Se enriched yeast (SY) and Na selenite (SS). The laying hen's requirements are 0.05 to 0.08 ppm (NRC, 1994). They reported that the Se egg concentration increased linearly as dietary Se was increased from both sources when compared with the control group ($P<0.01$). They also reported higher Se egg concentration from diets with SY vs. SS diets ($P<0.01$). Whole egg Se concentration was reported to have increased at each collection period with eggs from hens fed SY. They indicated that SY increased deposition of Se in whole eggs, and 3 ppm of Se can be supplemented to laying hens without decreasing egg production. Payne and Southern (2005) compared organic and inorganic sources of Se for broiler diets. They reported an increase in Se concentration in breast muscle and plasma in broilers fed diets containing organic Se as SY when compared to birds fed with Se selenite ($P<0.05$). They indicated that SY as a source of organic Se can increase tissue Se concentration.

Effects of chelated trace minerals on animal performance

Several research studies have been conducted to evaluate the effect of trace minerals on animal performance and the differences that exist among trace minerals sources. Cheng *et al.* (1998) conducted two pig trials to compare Zn lysine and Zn sulfate as sources of Zn. Diets were formulated to meet the NRC (1998) minimum requirement of 100 mg Zn/kg for starter pigs utilizing both sources of Zn. They reported that overall performance, average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio was similar for pigs fed diets containing Zn sulfate or Zn lysine in both trials. Also, tissue Zn concentrations in liver, kidney, rib and in serum did not show significant differences between sources. Based on the similarity in responses in performance and serum and tissue concentrations, concluded that both sources were equally effective. Van Heugten *et al.* (2003) compared the effects of dietary of Zn methionine, Zn lysine and Zn sulfate diets on performance, tissue Zn concentration, cellular Zn distribution and immune response of weanling pigs. Experimental diets were formulated to meet or exceed the NRC (1998) minimum requirement of 100 mg Zn/kg with the different sources of Zn. Growth performance was not improved by the addition of diets with Zn methionine or lysine compared with Zn sulfate. In addition, weight and Zn concentration in the liver, pancreas, and spleen were not significantly different among treatments. Intracellular distribution of Zn was not different among Zn sources and levels. They concluded that Zn from organic sources at NRC (1998) levels is sufficient to maintain growth performance and immune response. Coffey *et al.* (1994) evaluated the efficacy of Cu lysine, as a growth promoter in weaning pigs. They found that a Cu lysine complex was more effective than Cu sulfate as a growth promoter. They reported that, overall, the dietary inclusion of Cu lysine (100 and 200 ppm of Cu in diet) improved daily gain, daily feed intake and feed: gain ratio by 16.8, 14.1 and 2.2 % respectively compared with the basal diet and improved growth rate and feed intake compared with Cu sulfate. They also reported that liver Cu concentration was lower

in pigs fed Cu lysine than those fed Cu sulfate. They suggested that Cu lysine complex had 50% of Cu lysine as a source of Cu and 50% of Cu sulfate as the remaining source of Cu. They concluded that Cu lysine complex was as effective as Cu sulfate as a growth promoter. Kienholz *et al.* (1992) reported improvement in egg production in low calcium diets of stressed birds when fed with 1 g/kg of Zn methionine. They also reported that birds fed 2 g/kg of Zn methionine during the recovery time from the stress caused by the low calcium diet produced more eggs than those on control diets. Mabe *et al.* (2003) investigated the effect of supplementing inorganic and organic sources of Zn, Mn and Cu with high (60-60-10 mg/kg respectively) and low (30-30-5 mg/kg) levels of inclusion in layer diets. They reported increased concentration of Zn (5 to 10 %) and Mn (20 to 47 %) in the egg yolk when hens were fed with low or high levels as compared with the control diet (0-0-0 mg/kg). They also reported lower percentage of shell and eggshell index values in birds fed with inorganic sources of minerals. Bone breaking strength was increased by 8.5 % and 9.1% with the supplementation of both organic and inorganic sources of minerals respectively when compared with the control at high levels of inclusion. Fremaut (2003) reported a 12% reduction of weight loss during lactation of sows when organic Zn was fed, compared in inorganic Zn. Also, there was a shortened weaning to estrus interval (7%) in sows fed organic Zn. In suckling pigs an 8% improvement of daily weight gain was reported when organic Zn was fed.

Interaction of Phytic acid and trace minerals

Phytic acid (myo-inositol-1, 2, 3, 4, 5, 6,-hexakis-dihydrogenphosphate) serves as the vegetative phosphorous (P) storage form for seeds. Phytic acid contains six phosphate molecules combined with one molecule of inositol; therefore, its chemical composition is $C_6H_{24}O_{27}P_6$ with 18 tritrable hydrogens. Between contiguous phosphates there are three P-O-P linkages present.

In mature seeds phytic acid forms a complex salt with Ca, Mg and K designated as phytin (Kratzer and Vohra, 1986). Phytic acid can be designated as a chelating agent because it possesses six reactive phosphates with the ability to form a complex with cations such as calcium, zinc, iron, magnesium, manganese and copper (Ravindran *et al.*, 1996). These cations can form a complex within one or two phosphate molecules of the phytic acid and between two phytic acid molecules (Kratzer and Vohra, 1986). Phytic acid has a phosphorus content of 28 % and accumulates in seeds during the ripening period (Ravindran, 1996). During dormancy and germination of the seeds, phytic acid serves important physiological functions which include antioxidant protection, storage of P that will be used during the germination period, and initiation of dormancy. It has been reported that 60 to 90% of the P content in cereals and oilseeds are in the form of phytic acid, as shown in Table 2.2. In corn 90% of phytic acid is found in the germ (Table 2.2) (Ravindran *et al.,* 1996). The concentration of phytate P in feedstuff depends on the plant part from which they are derived. Cereals and legumes hold moderate amounts of phytate P, whereas oilseed meals and cereal byproducts contain high concentrations. A typical corn-soybean diet for poultry contains 0.89 to 0.90% of phytic acid or 0.22 to 0.25% phytate P concentrations (Harland and Oberleas, 1996).

Table 2.2 shows distribution of phytic acid for the two varieties.

Table 2.2. Distribution of phytic acid in normal and high Lysine corn (%)

	Corn	
	Normal	**High Lysine**
Germ	88.0	88.9
Endosperm	3.2	3.0
Hull	0.4	1.5

(Adapted from Kratzer and Vohra, 1986)

The distribution of phytate P in common feed ingredients is shown in table 2.3.

Table 2.3 Concentration of total and phytate P in some common feed ingredients

Ingredients	Total P (%)	Phytate P, % of total P
Corn	0.26	66
Grain sorghum	0.31	68
Barley	0.34	56
Wheat	0.30	67
Oats	0.34	56
Soybean meal	0.61	61
Cottonseed meal	1.07	70
Sesame meal	1.27	81
Wheat bran	1.37	70
Wheat middlings	0.47	74
Alfalfa meal	0.30	0

(Adapted from Ravindran *et al.* 1996)

The ability of monogastric animals, especially poultry and swine, to utilize phytate P has been reported as poor, due to the lack of phytase enzyme to hydrolyze the phytate and release the P. Phytate can also impair the availability of other nutrients such as trace minerals and protein, hindering the performance of animals (Oberleas and Harland, 1996).

Several research publications report the influence of dietary phytic acid on performance of animals and their response to the addition of phytase enzyme. Ravindran *et al.* (1996) reported improvement of feed intake in broiler chicks when phytase enzyme was supplemented to a diet containing high levels of phytic acid (1.57%). They also studied the effects of three levels of dietary phytic acid (0.46, 0.82, 1.18%) on weaned pigs. They reported that increasing levels of dietary phytic acid depressed daily gain ($P< 0.06$) and increased feed/gain ($P< 0.01$). However, the addition of phytase enzyme overcame this effect.

Biehl *et al.* (1995) studied the effect of adding high levels of phytase to broiler diets on P, Zn and Mn utilization. They reported that supplementation of 1200 units /kg of phytase increased bone ash by 64%. Also, the combination of phytase and 1, 25-$(OH)_2$ D_3 increased tibia Zn concentration by 86% compared with the basal diet (0 units/kg phytase) and tibia Mn concentration by 123 %. Sebastian *et al.* (1996) reported that adding phytase to low-P diets fed to male and female broiler chicks increased body weight. They also reported that phytase supplementation to the low-P diet increased ($P<0.05$) Ca retention by 9.1 % and Cu retention by 19.3% in male chicks. It also increased Zn retention by 62% in male and by 44% in female chicks. Lan *et al.* (2002) studied the effect of *Mitsoukella jalaludinii* (AMJC), a phytase producing bacterial species, on nutrient utilization in broiler chicks. They used 250, 500, 750 and 1000 U phytase/kg of feed combined with a low nonphytate P diet. They reported that body weight gain was significantly increased by 14 to 18% when AMJC (250 to 1000 U phytase/kg) was supplemented to low nonphytate diets. They also reported that P retention increased by 19%, Ca retention by 16%, when 500, 750 or 1000 U phytase/kg was fed, zinc retention was also increased when 1,000 U phytase was fed. Viveros *et al.* (2002) studied the effect of dietary phytase on utilization of minerals (Ca, P, Mg and Zn) in broiler chicks fed low-P diets. They reported an improvement in weight gain by 6% when there was an inclusion of 500 U phytase/kg diet. They also reported an increase of P, Mg, Zn and Ca retention when phytase was added to the diets. Likewise, they reported that phytase supplementation to a low-non phytate P diet significantly increased tibia ash by 5%. Ahmad *et al.* (2000) studied the effects of phytase (synthesized using *Aspergillus niger*) on P and Ca bioavailability and performance of broiler chicks fed corn and soybean meal diets. They fed three diets with normal P content, normal P + phytase and low P content + phytase. They reported an increase in P retention in birds fed low P + phytase diets ($P<0.05$) by 20 and 12 % when compared to normal P and normal P + phytase diets respectively. Likewise, they reported a significant

increase of ash content of tibia and toe by 62 and 12 g/100g respectively in birds fed microbial phytase. Phytase also increased tibia P and Ca concentration by 1 and 2 g/100g, respectively. Brenes *et al.* (2003) studied the effect of feeding graded levels of phytase in combination with citric acid on mineral utilization (Ca, P, Mg and Zn) in broiler chicks. They reported an increase of 7% in weight gain and 5% feed consumption ($P<0.001$) when phytase (200 U/kg) was added to diets. Likewise, Ca, P and Zn retention was increased by 9, 10 and 16 % respectively when dietary phytase was fed. They also reported that Ca, P and Zn concentration in tibia ash increased ($P<0.001$) by 2, 1 and 4%, respectively, when phytase was fed to low available P diets. Peter *et al.* (2001) evaluated the effect of microbial phytase (two levels) on late finishing pigs fed diets with no supplementation of P, Zn, Cu and Mn. They reported that addition of microbial phytase (300 and 500 U/kg) significantly increased ($P<0.05$) bone weight and bone ash. However, removal of supplemental P, Zn, Cu and Mn for the diets did not deleteriously affect growth performance or carcass characteristics. Mohanna and Nys, (1999) studied the effect of microbial phytase on Zn and Mn availability in broiler chicks. They reported that the addition of microbial phytase (800 U/kg) significantly increased tibia Zn concentration and plasma Zn concentration by 30 and 20% respectively. Microbial phytase supplementation also enhanced Zn concentration in feathers when added to low Zn diets. Therefore, they concluded that microbial phytase reduced the antagonistic effects of phytate on Zn bioavailability, and allowed lowering dietary Zn supplementation. Adeola *et al.* (1995) studied the influence of phytase on utilization of Ca, P, Zn, Mn, Mg and Cu in pigs' diets. They reported that body weight gain and feed efficiency were improved by addition of phytase to diets. Plasma Mg concentration and Zn, Ca, P and Cu retention increased by dietary phytase supplementation. Revy *et al.* (2003) studied the interaction between Zn from inorganic and organic sources and phytase on Zn bioavailability in weanling pigs. They reported that Zn concentration in bone ash was increased by 75 % with phytase supplementation. Likewise Zn

liver concentration was increased by phytase supplementation. They concluded that dietary phytase (1200 U phytase/kg) supplementation improved Zn bioavailability in piglets in contrast to the substitution of inorganic by organic (20 ppm) sources of Zn. Jongbloed *et al.* (2004) studied the effect of microbial phytase on mineral digestibility and sow performance. They reported that Ca, P, Mg, Cu and Zn digestibility was significantly increased by phytase supplementation in lactating sows. Phytase increased Ca, P, Cu and Zn digestibility at 70 days gestating sows and Cu digestibility at 100 days of gestation.

Organic trace minerals in waste management

Plants can utilize essential mineral elements present in animal manure; therefore, animal waste serves as a resource for crop production. Ruminants, poultry and swine are the largest contributors of animal manure for crop application (Coelho, 1996). Therefore, the majority of manure produced by these species is land applied, representing more than 140 million dry tons of land manure application per year. Since this agricultural practice is utilized by crop producers all around the world, a satisfactory waste management plan must be followed to prevent potential negative environmental effects of excess nutrients and to improve the potential use of the waste (Williams, 1996). Therefore, when animal waste is utilized, the primary goal of a waste management plan is to prevent the accumulation of nutrients in the soil to the point where they can cause environmental pollution. Waste application should not surpass the capacity of the soil to contain it and the crop to utilize it, or else environmental pollution problems could potentially develop, such as contamination of ground water and surface water with nutrients (Mikkelsen, 1996). Applying animal manure to crop land to meet N requirements can result in a large over application of P and trace minerals. Phosphorus is present in animal manure as organic and inorganic compounds. Depending on the animal and

feed sources, phosphorus present in organic compounds can account for 2/3 of the total phosphorus present in animal waste. Animal waste will contain significant amounts of phosphorus, because 70-90% of the total phosphorus ingested by the animal will be excreted. Several research studies have investigated the ability of organic phosphorus to utilize by crops. Some of them have reported that organic phosphorus is not effective as phosphorus sources for crops. Phosphorus can be tightly bound by soil and will not be considered a leaching risk in most soils. However, when phosphorus is present in high concentrations, it can be transported to rivers or lakes and, along with nitrogen, and can result in a growth of algae and aquatic vegetation, which in turn will lead to foul odors, insect problems, and harm to the fish population. Likewise, when copper is found in high concentrations in animal waste, it can lead to soil accumulation. This results from the affinity of copper to complex with organic matter, clays and oxide minerals. This affinity to form a complex makes copper the least mobile plant micronutrient in the soil (Williams, 1996). Zinc is another nutrient of concern in waste material. When its concentration is high, toxicity may result. Zinc toxicity will be determined by the pH of the soil. A high soil pH can reduce the risk of toxicity (Mikkelsen, 1996). A number of research studies have been conducted to reduce phosphorus and trace mineral excretion in animal production by dietary manipulation. Pierce *et al.* (2005) studied the effects of reducing the dietary trace mineral levels in mineral excretion of broilers, utilizing organic mineral sources. Copper, iron, and zinc as organic sources at 100, 50 and 25% of the NRC (1994) requirements were used to supplement the diets. They reported a linear decrease ($P<0.01$) of Cu and Zn manure concentration as the dietary supplementation decreased. They concluded that 25 % of the NRC (1994) was needed to meet the minimum requirements of broilers to maintain normal performance and reduce mineral excreta. Leeson (2005) reported reduction of trace mineral output in broiler chicks fed diets with organic minerals compared whit inorganic mineral salts. They also reported reduction in manure trace mineral

concentration in layer hens fed diets with organic minerals. Zinc and Mn were reduced by 60 to 70% while Cu was reduced by 11%. Pierce *et al.* (2005) reported a linear increase in Cu retention by 35%, when increased levels of organic Cu were used to feed birds as compared with inorganic Cu sources. They also reported that organic Cu increased Zn retention by the birds. They concluded that organic Cu increased Cu and Zn retention. Nollet *et al.* (2008) studied the effects of organic trace mineral supplementation on trace mineral concentration of broilers. They fed diets with organic sources of Cu, Fe, Mn and Zn and compared them with inorganic sources of the same trace minerals. They reported a reduction in manure concentration of Cu by 41 %, of Mn by 49%, of Zn by 36% and of Fe by 50% for organic trace mineral sources. Burkett *et al.* (2005) studied the effects of organic trace mineral sources on reducing manure trace mineral concentration in pigs. They reported that organic sources of Cu reduced ($P<0.05$) concentrations of Cu, Zn and Fe in manure by 75%, 50%, and 14% respectively without reducing animal performance, compared with using inorganic salts of these minerals. Leeson (2005) studied the effects of organic minerals on reducing trace mineral manure concentration of laying hens. They fed organic sources of Cu, Mn, Fe and Zn levels providing 20% of the minerals provided of inorganic sources. They reported a reduction of manure concentration of Mn and Zn by 60 and 70%, respectively, and 11% reduction of Cu when feeding organic mineral sources.

The reports above describe benefits from the use of organic minerals as well as phytase for poultry and swine production. Therefore, it was of interest to investigate the effects of using both organic minerals and phytase, alone or in combination, on the production performance and mineral metabolism of replacement pullets.

CHAPTER 3

INTERACTION OF ORGANIC MINERALS AND PHYTASE ON REPLACEMENT PULLETS

Objectives

The objectives of these experiments were to determine the effects a) using low levels of trace minerals supplied by organic sources (proteinates) in place of normal levels of trace mineral in the form of inorganic salts in pullet diets, b) supplementing the diet with phytase and c) the interaction mineral source X phytase supplementation on growth performance, skeletal development and the concentration of trace minerals in liver and manure of replacement pullets.

Materials and Methods

This project involved two similar trials, one with replacement pullets of a white shell laying strain (Hy-Line W-36) and one with a brown shell laying strain (Hy-Line Brown), hereafter referred to as white and brown pullets, respectively. The same procedures described below were used for both trials, unless otherwise noted.

Dietary treatments

Dietary treatments consisted of feeding corn-soybean meal starter, grower and developer pullet diets without supplemental trace minerals (basal diet) or supplemented with inorganic sources of Cu, Fe, Mn and Zn or organic source of minerals Cu, Fe, Mn and Zn and without or with phytase either 0 or 0.025% (250 phytase units/kg) in a 3 x 2 factorial arrangement (mineral source x phytase). The starter and growers diets were fed from weeks 3 through 7 and weeks 8 through 12, respectively. Then developer diets were fed through weeks 17 and 18 for brown and white birds, respectively. The experimental dietary

treatments are presented in Table 3.1. The ingredients and calculated composition of nutrients are presented in Table 3.2 and Table 3.3, respectively. The levels of Ca and available P in diets supplemented with phytase were both reduced by 0.1% by slightly decreasing the percentages of dicalcium phosphate and limestone and increasing the percentage of corn. The inorganic trace mineral supplement provided levels of Cu, Fe, Mn and Zn that met or exceeded the NRC (1994) minimum requirement for pullets. The organic trace mineral supplement levels of Cu, Fe, Mn, and Zn equivalent to providing 40 % of the NRC (1994) minimum requirements for pullets. Levels and sources of trace minerals in these supplements are presented in Table 3.4 and Table 3.5, respectively. Table 3.6 shows the analyzed concentration of minerals in the feed and major ingredients (corn and soybean meal). Starter diet PS2 and PS5 were inadvertently not supplemented with ZnO (Table 3.5). Therefore, the Zn level in these starter diets originated from the feed ingredients (Table 3.6). The analyzed value of all trace minerals in the feeds were in line with the expected concentration based on the dose levels supply by the mineral premix (Table 3.6). Likewise, the analysis of the starter basal diet showed it contained Cu, Fe, Mn and Zn at 100, 145, 30 and 55% of NRC (1994) requirements respectively. Also, similar results were obtained from the analysis of the grower and developer basal diets, where they contain Cu, Fe, Mn, and Zn at 100, 135, 40 and 50 % of NRC (1994) requirements respectively.

Table 3.1. Dietary treatments

Treatments	Diet
1	Basal (no trace mineral supplement)
2	Basal + inorganic trace minerals
3	Basal + organic trace minerals*
4	Basal (no trace mineral supplement) + phytase**
5	Basal + inorganic trace minerals + phytase
6	Basal + organic trace minerals+ phytase

* As Bioplex® mineral source of Cu, Fe, Mn and Zn.

** As Allzyme SSF®, equivalent to 250 phytase units/kg diet.

Table 3.2 Ingredients composition of pullet basal diets

Ingredients	Starter	Grower	Developer
	(%)		
Corn	62.00	67.86	73.10
Soybean meal (48% CP)	30.00	24.00	18.80
Dehydrated alfalfa meal(17% CP)	2.86	3.40	3.40
Corn oil	1.20	0.80	0.80
Iodized salt	0.42	0.42	0.42
Limestone	1.10	1.20	1.27
Dicalcium phosphate	2.04	1.92	1.84
DL-Methionine	0.13	0.15	0.12
Vitamin mix[1]	0.25	0.25	0.25

[1]Supplied per kg diet: 9,920 I.U. Vitamin A, 2,755 I.U. Vitamin D_3, 33 I.U. Vitamin E, 1.9 mg Vitamin K, 1.9 mg thiamin, 6.6 mg riboflavin, 44 mg niacin, 11 mg Ca pantothenate, 3.9 mg pyridoxine HCl, 0.11 mg biotin, 1.3 mg folic acid, 496 mg choline, 19µg Vitamin B-12.

Table 3.3 Calculated nutrient composition of pullet basal diets[1]

Nutrient	Starter	Grower	Developer
AME_n [2], Mcal/kg	2.95	2.97	3.02
CP, %	20.38	18.07	15.98
Fat, %	3.68	3.43	3.56
Fiber, %	3.16	3.18	3.09
Ca, %	1.00	1.01	1.00
Available P, %	0.50	0.47	0.45
Methionine, %	0.46	0.45	0.39
Methionine + cysteine, %	0.78	0.74	0.66
Lysine, %	1.12	0.95	0.80
Sodium, %	0.19	0.19	0.19

[1] Values reported are calculated on an "as-is" basis.
[2] Apparent metabolizable energy, nitrogen corrected.

Table 3.4 Sources and levels of minerals in organic trace mineral mix

Mineral[1]	Source	Starter	Grower and Developer
		(mg/kg diet)	
Cu	Cu proteinate (Bioplex Cu*)	1.8	0.9
Fe	Fe proteinate (Bioplex Fe*)	9.6	4.8
Mn	Mn proteinate (Bioplex Mn*)	16	8.0
Zn	Zn proteinate (Bioplex Zn*)	16	8.0
Se	Selenium yeast (Sel-Plex *)	0.2	0.2

[1]Values are amount of mineral, not compound

*Alltech Biotechnology Center, Nicholasville, KY 40356

Table 3.5 Sources and levels of minerals in inorganic trace mineral mix

		Starter	Grower and Developer
Minerals	Provided by	mg/kg diet	mg/kg diet
Cu	$CuSO_4 \bullet 5H_2O$	5	4
Fe	$FeSO_4 7H_2O$	80	60
Mn	$MnSO_4 \bullet H_2O$	60	30
Zn	ZnO	0	35
Se	Na_2SeO_3	0.3	0.3

Table 3.6. Mineral concentration of feeds and ingredients (Experiments C04-03 & C05-03)[1]

Diets and Ingredients[2,3]	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	P (g/kg)	Ca (g/kg)
PS1	4.74 ± 0.29	175 ± 34.2	17.69 ± 2.01	21.93 ± 2.34	9.01 ± 0.90	12.15 ± 1.30
PS2	8.66 ± 0.87	290 ± 20.5	63.53 ± 11.8	21.73 ± 1.30	8.89 ± 0.21	12.42 ± 0.53
PS3	5.73 ± 0.03	158 ± 10.7	29.12 ± 0.07	33.88 ± 0.54	8.45 ± 0.15	11.91 ± 0.48
PS4	4.62 ± 0.53	95.0 ± 12.2	15.12 ± 0.02	20.27 ± 1.05	7.25 ± 0.05	10.80 ± 1.68
PS5	8.28 ± 0.17	269 ±12.0	65.40 ± 11.3	20.65 ± 0.02	7.76 ± 0.27	12.20 ± 0.82
PS6	5.51 ± 0.18	107 ± 9.46	25.68 ± 0.01	31.25 ± 0.43	7.36 ± 0.25	10.25 ± 0.17
PG1	3.51 ± 0.19	168 ± 3.65	16.06 ± 0.38	20.61 ± 0.06	8.87 ± 0.05	12.04 ± 1.16
PG2	6.92 ± 0.83	266 ± 27.0	33.52 ± 3.56	40.50 ± 2.27	8.42 ± 0.00	12.68 ± 0.19
PG3	4.88 ± 0.29	201 ± 4.44	24.24 ± 5.01	27.12 ± 0.12	8.40 ± 0.30	12.94 ± 0.41
PG4	3.83 ± 0.17	108 ± 4.24	14.88 ± 0.34	19.64 ± 0.21	7.03 ± 0.15	10.81 ± 0.59
PG5	6.98 ± 1.11	239 ± 9.11	36.59 ± 5.92	41.31 ± 1.04	7.08 ± 0.03	10.57 ± 0.30
PG6	4.73 ± 0.23	126 ± 1.52	19.42 ± 0.09	25.29 ± 0.54	7.31 ± 0.08	10.73 ± 0.15
PD1	3.14 ± 0.19	187 ± 29.7	12.35 ± 0.69	17.66 ± 0.34	7.88 ± 0.15	12.57 ± 0.04
PD2	9.70 ± 3.22	285 ± 11.0	40.64 ± 1.94	52.20 ± 3.14	8.26 ± 0.36	11.61 ± 0.45
PD3	4.26 ± 0.09	150 ± 5.90	16.68 ± 0.80	21.87 ± 1.70	7.42 ± 0.13	11.72 ± 0.11
PD4	3.39 ± 0.01	89.0 ± 4.37	11.39 ± 0.35	17.36 ± 0.77	6.48 ± 0.08	10.07 ± 0.45
PD5	8.39 ± 4.45	191 ± 3.45	35.02 ± 1.70	46.88 ± 2.75	6.29 ± 0.06	11.80 ± 0.17
PD6	3.97 ± 0.02	114 ± 7.63	17.23 ± 0.10	23.67 ± 0.21	6.52 ± 0.30	10.95 ± 0.27
Corn	2.46 ± 1.20	60.0 ± 5.88	4.54 ± 3.90	14.09 ± 2.00	2.62 ± 0.17	0.05 ± 0.06
SBM (CP 48%)	12.6 ± 0.80	101 ± 3.55	30.3 ± 5.40	45.18 ± 1.12	6.51 ± 0.07	5.18 ± 0.25

[1]Means ± SD of 3 samples.
[2]PS = Pullets Starter Diet; PG = Pullets Grower Diet; PD = Pullet Developer Diet; SBM = soybean meal (CP 48%)
[3] 1= basal diet (no trace mineral supplement), 2= basal + inorganic trace mineral, 3= basal + organic trace mineral, 4= as 1 + phytase, 5= as 2 + phytase and 6= as 3 + phytase

Animals and housing

The experiments were conducted at the University of Kentucky Poultry Research Facility. Each experiment was conducted with 1,152 pullets. Pullets were 2 weeks of age at the start of the experiments. The pullets were housed in mesh wire-floored pullet starter-grower cages (61 cm wide x 51 cm deep x 36 cm high) in environmentally controlled rooms. All pullets were initially housed in the same room from the start of the experiments at 16 birds/cage. At 37 days of age, the brown pullets were moved to a similar environmentally controlled room with the same size of cages. At that time the birds were split into two cages per replication, cage density was reduced to eight birds per cage in both rooms. During the pre-trial period low intensity light was provided at 22 hours per day (22 L: 2 D) during Days 1 to 7 and 16 L: 8 D during Days 8 to 14. From Day 15 (start of the trial) until photo stimulation at 18 weeks of age light was provided at 8 L: 16 D. Each cage had one feeder that was removable for weighing and two adjustable nipple drinkers. Feed and water were supplied on an *ad libitum* basis. The temperature in the room was 31° C for the first week and then lowered approximately 3° C per week until it reached 21° C. Textured papers were placed on the floor of the cages for the first week.

Daily animal care sheets were filled out, providing a record of mortality, temperature, and feed and water availability. Birds and feed were weighed initially and then every three or four weeks for weight and feed every two weeks. After every 2 weeks, mortality, average body weight gain and average daily feed intake were calculated. The study protocol was approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC).

Materials

A commercial organic mineral mix of mineral proteinates was supplied by Alltech Inc., Nicholasville, KY. The minerals supplemented in the mix were Cu, Fe, I, Mn, and Zn. In addition it contained Se in the form of selenium yeast. The commercial fungal source of phytase was supplied by Alltech Inc., Nicholasville, KY, as Allzyme SSF to provide a measured activity of 250 phytase units/kg diet.

Experimental Design

Each experimental unit consisted of one group of 16 birds with eight birds in one cage on the top tier of cages and eight birds in another cage on the bottom tier of cages. The experiments used a randomized complete block design, with blocks based on physical location of the cages within the room. There were four replicates for each of the six dietary treatments within each of three blocks.

Excreta and tissue samples

The experiments started when the birds were 14 days of age and lasted until the pullets were 17 and 18 weeks of age (brown and white respectively). At Week 14, excreta samples were collected for 48 hours from the bottom cage of eight replicate groups per treatment in Blocks 1 and 2, by hanging a collection board underneath the bottom cage. Samples were weighed and dried in an oven at 60° C for 72 hours. At Week 16, one bird from each replicate group (a total of 72 birds) was randomly selected and killed by asphyxiation with argon gas followed by cervical dislocation. Liver and left tibia samples were collected from each bird. The liver samples were weighed and then dried in an oven at 60° C for 48 hours.

Laboratory Analyses

Samples of feed, oven-dried liver, and oven-dried excreta were ground in a coffee grinder to a fine texture. Feed, liver, and manure samples were further digested using a microwave digester (Microwave Sample Preparation System MDS-2000, CEM Corporation, Matthews, NC 28106), according to the method described by the CEM Corporation, Operation Manual, General Guidelines for Microwave Sample Preparation, 1994 (Appendix 1, 2 and 3). Trace mineral determination of feed, liver, and manure were determined by inductively coupled plasma-atomic emission spectrometry (ICP, Basta-Pro., Varian, Australia), according to the method 6010B, (Appendix 4, 5 and 6). Bone breaking strength was determining using an Instron Testing Machine (Instron Model 4301, Instron Ltd. Coronation Rd., High Wycombe Buckinghamshire HP123SY England), according to the method described by Cantor *et al.* (1980). Bones were placed across two fulcra with the long axis in the horizontal position and were broken at the mid-point using a probe speed of 25 mm per minute and a 1000 N load cell. Percent ash was analyzed using the method of AOAC (1995). Bones were dried, fat extracted with petroleum ether, and ashed in an oven at 600° C, overnight (Appendix 7).

Statistical Analyses

Data were subjected to ANOVA for a randomized complete block design for a 3 x 2 factorial arrangement of treatments using linear models of Statistix V.9 (2003) (Analytical Software, Tallahassee, FL). Differences among means were determined using Fisher's least significant difference test. Significance was declared when probability was less than 5%.

Results & Discussion

Effect of trace mineral source and phytase supplementation on growth performance

The effects of trace mineral source and phytase supplementation on average feed intake (AFI) of white pullets measured during weeks 3 to 7, weeks 8 to 12, weeks 13 to 18, and during Weeks 3 to 18 are summarized in Table 3.7. The only significant effect observed was that organic trace minerals reduced AFI ($P<0.05$) by 2 % (50 g/bird), when compared to no mineral supplementation during Weeks 8 to 12. The AFI during 3 to 7 weeks, 13 to 18 weeks of age, and for the entire growing period (weeks 3 to 18), was not significantly affected by treatments ($P<0.05$). The inclusion of 0.025% phytase enzyme to diets had no significant effect ($P<0.05$) on AFI for the entire growing period (3 to 18 weeks) nor for any to the phases of the growing period (weeks 3 to 7, 8 to 12, and 13 to 18). There were no significant interactive effects of mineral sources X phytase on AFI during the growing period (18 weeks). Average feed intake for the entire growing period was 5,573 g per bird.

The effects of treatments of brown pullets during Weeks 3 to 18 are shown in Table 3.8. During Weeks 3-18, AFI was not significant affected by mineral sources, phytase, nor their interaction. Average feed intake for the entire growing period was 6,416 g per bird.

The effects of organic trace minerals and phytase supplementation on average body weight (ABW) of white measured at 2, 6, 8, 13 and 19 weeks of age are summarized in Table 3.9. Adding either organic or inorganic mineral supplements to the diet improved ABW ($P<0.05$) by 3% at 6 and 8 weeks of age, but not at 13 or 19 weeks of age. Phytase supplementation increased ABW ($P<0.05$) by 10 g (3%) at 8 weeks of age. However,

ABW was not significantly affected by phytase supplementation at other times. The interaction of mineral source X phytase did not have a significant effect on ABW at any time during the entire growing period. Average body weight was 1.21 kg at 19 weeks of age. The same significant effect of mineral supplementation (improved ABW at 6 and 8 weeks of age) and phytase supplementation (improved ABW at 8 weeks of age) was observed with the brown pullets (Table 3.10). Average body weight was 1.52 kg at 18 weeks of age.

The overall performance was not affected by the dietary treatment. The unsupplemented basal diet, which was the most likely to results in low concentration of trace minerals did not results in a loss of performance. Leeson and Caston (2007) reported no significant difference in performance in layers when feeding none, inorganic and organic source of trace minerals. They concluded the diets had sufficient levels of trace minerals coming from the major ingredients in the diets. Likewise, they made the similar observation, ingredients such corn and soybean meal are capable to supply significant amount of trace minerals to the bird to meet the requirements, but the bioavailability is unknown and the concentration on the ingredients is variable depending the soil were they growth. Therefore, the author suggested a revision of the layer's requirements. Star *et al.* (2012) made similar observation, when conducting a 21 day study in broilers to compare organic Zn vs zinc sulfate, in which the basal diet with no Zn supplementation showed not significant reduction in performance when compare to the other two source of Zn. They concluded Zn (31.7 ppm) was not limiting in the basal diet. Likewise, a broiler study conducted by Nollet *et al*. (2008), were they fed a basal diet with no trace mineral supplementation, organic and an inorganic trace minerals diets with or without phytase

indicated overall performance was not significant affected. The inorganic source contained 15 ppm of Cu and Fe, Mn and Zn were added at 45 ppm each. The organic source was added to supply five levels of trace minerals, 100%, 67%, 50%, 30% and 17% of the inorganic source concentration. Similar to our study, for the entire trials (42 days) performance was not affected by dietary treatments or trace mineral concentration. The basal diet had a FCR equal to the organic and inorganic trace mineral source. Only BW was significant affected ($P<0.05$) by the minerals source when compare to the basal diet for the overall period. The performance results on this study were similar to performance found by Ao *et al*. (2008) which concluded that feeding organic sources of minerals Cu, Mn, Fe and Zn had no significant effect on AFI, BW or uniformity when fed to brown pullets. Their results showed that feeding lower levels of trace minerals at 25% of NRC (1994) recommendations had no negative effect on performance parameters. Also, they found no significant difference in AFI and BW for pullets fed the basal diet (no added trace minerals) and those fed the supplemented diets. Ao *et al.* (2009) had similar findings with white layer pullets. They showed no significant differences on overall performance during a 17 week study among the treatments. The basal diet (no added trace minerals) showed no detrimental effect on growth performance during the study. The findings of Ao *et al.* (2008 and 2009) are in agreement with the results of the present studies. An experiment conducted by Nollet *et al.* (2007) with broilers fed a wheat-corn-SBM diet examined substituting inorganic minerals with organic minerals. No significant differences in feed conversion rate (FCR) nor BW were noted. They concluded that overall productive performance was not affected by feeding a lower level of organic mineral at 10 mg/kg for Zn, Mn, and Fe and 2.5 mg/kg for Cu vs 12, 37, 70 and 45 mg/kg for Cu, Zn, Mn and Fe

respectively. Shelton and Southern (2006) conducted a study to investigate the effect of completely removing a trace mineral premix from a corn-SBM broiler diet on growth performance. They reported that growth performance was not affected by removing the trace mineral premix, but bone strength was decreased. The study also reported a significant increase in gain to feed ratio when phytase was added. These findings agree with our study in which the addition of phytase had a positive effect on BW at week 8 for the white and brown pullets. Similar results were observed by Creech *et al.* (2004) when studying the effect of feeding reduced levels (an average of 15% reduction) of organic and inorganic trace minerals on swine performance. In nursery pigs they reported no adverse effect on gain and average daily feed intake (ADFI). Also, there were no significant effects on performance during the growing phase. Likewise, Leeson and Caston (2007) conducted a study in broilers to measure the effect of feeding lower levels of trace minerals as an organic source when compare to an inorganic trace minerals. The organic trace mineral were formulated to supply 80%, 60%, 40% and 20% of the level of Inorganic source. They found no significant difference in performance in 42 day trial with broilers. They concluded that organic trace minerals are capable to sustain performance due to a higher bioavailability and a good supply of trace minerals by the major ingredients use in the diets. They did not use phytase, which is different from our study. Other studies in poultry were designed to compare inorganic and organic trace minerals, without a basal diet with none trace mineral supplementation on performance. Zhao *et al.* (2010) reported no difference in performance in broilers when evaluation trace minerals sources (inorganic and organic). They conducted two commercial trials, where they fed inorganic trace minerals which provided 80, 120, 8 and 30 ppm of Zn, Mn, Cu and Fe respectively, as well as 40, 60, 8,

and 30 ppm of organic source (Cu was fed ad same level). Both trials showed similar results on feed efficiency (adjusted feed conversion rate) and BW gain. They concluded that feeding reduce levels of organic trace minerals to broilers had no negative effect on overall performance. Manangi *et al.* (2012) found that performance in broilers fed reduced levels of chelates trace minerals was not affected. They were able to feed chelate Zn 30% of the concentration of inorganic source and not find any negative effect on performance in a 54 day study. Similarly, they conducted a second broiler study in commercial facilities, where for 54 day broilers were the fed organic Zn, Cu and Mn at a reduce concentration at 30, 6.4 and 35 % respectively of the inorganic source which resulted in no difference in BW, FI, FCR and mortality. They concluded that chelated trace minerals can be fed to broilers for the entire growing period at a reduced level without sacrificing performance. In a study conducted by Stefanello *et al* (2014), layers (Hy-Line W36) were fed increasing concentration levels of either inorganic or organic trace minerals for 20 weeks. Similar to our study the fed a basal diet with no trace mineral supplementation. They reported no significant response to either mineral source or level of supplementation. They made the observation that several trials conducted in layers had similar findings. Analyzed levels of trace minerals in the final feed or ingredients were not reported.

Table 3.7. Average feed intake (AFI) of Hy-Line W-36 pullets (Experiment C04-03)[1]

Treatments		AFI, g/bird weeks 3 to 7	AFI, g/bird weeks 8 to 12	AFI, g/bird weeks 13 to 18	AFI, g/bird weeks 3 to 18
Mineral supplement	Phytase				
None		1163	2166[a]	2255	5585
Inorganic		1157	2132[ab]	2304	5593
Organic		1154	2116[b]	2273	5542
	0	1151	2151	2283	5586
	+	1165	2123	2271	5559
None	0	1167	2144	2241	5552
Inorganic	0	1150	2167	2341	5658
Organic	0	1137	2143	2268	5548
None	+	1159	2190	2271	5620
Inorganic	+	1165	2093	2265	5522
Organic	+	1171	2089	2278	5537
SEM[2]		11	20	10	46
Sources of variation		Significance of treatment effects[3]			
Mineral source		ns	*	ns	ns
Phytase		ns	ns	ns	ns
Mineral source x phytase		ns	ns	ns	ns

[1]Twelve replicate groups of 16 birds were assigned to each of the six treatments
[2]Standard error of mean for interactive (mineral x phytase) effects
[3] ns = not significant; * = P<0.05
[a, b] Means with no common superscript are significantly different (P <0.05)

Table 3.8. Average feed intake (AFI) of Hy-Line Brown pullets (Experiment C05-03)[1]

Treatments		AFI, g/bird weeks 3 to 7	AFI, g/bird weeks 8 to 12	AFI, g/bird weeks 13 to 17	AFI, g/bird weeks 3 to 17
Mineral supplement	Phytase				
None		1355	2237	2810	6402
Inorganic		1374	2237	2820	6431
Organic		1368	2235	2811	6415
	0	1371	2241	2834	6447
	+	1360	2232	2793	6385
None	0	1370	2270	2869	6508
Inorganic	0	1379	2230	2821	6430
Organic	0	1366	2223	2813	6402
None	+	1340	2205	2751	6296
Inorganic	+	1370	2244	2819	6432
Organic	+	1371	2247	2810	6428
SEM[2]		14	24	32	59
Sources of variation		Significance of treatment effects[3]			
Mineral source		ns	ns	ns	ns
Phytase		ns	ns	ns	ns
Mineral source x phytase		ns	ns	ns	ns

[1]Twelve replicate groups of 16 birds were assigned to each of the six treatments
[2]Standard error of mean for interactive (mineral x phytase) effects
[3] ns = not significant; * = P<0.05

Table 3.9. Average body weight (ABW) of Hy-Line W-36 pullets at various ages (Experiment C04-03)[1]

Treatments		ABW, kg (2 wk)	ABW, kg (6 wk)	ABW, kg (8 wk)	ABW, kg (13 wk)	ABW, kg (18 wk)
Mineral supplement	Phytase					
None		0.128	0.381[b]	0.553[b]	0.993	1.209
Inorganic		0.129	0.389[a]	0.560[a]	0.999	1.216
Organic		0.128	0.391[a]	0.564[a]	1.006	1.219
	0	0.129	0.385	0.554[b]	0.993	1.209
	+	0.128	0.389	0.564[a]	1.006	1.222
None	0	0.129	0.380	0.545	0.984	1.203
Inorganic	0	0.129	0.387	0.557	0.998	1.214
Organic	0	0.128	0.390	0.562	0.998	1.211
None	+	0.127	0.383	0.561	1.002	1.216
Inorganic	+	0.129	0.391	0.564	1.001	1.219
Organic	+	0.129	0.392	0.567	1.014	1.226
SEM[2]		2.4E-04	7.3E-04	9.5E-04	1.7E-03	2.3E-03
Sources of variation		Significance of treatment effect[3]				
Mineral source		ns	*	*	ns	ns
Phytase		ns	ns	*	ns	ns
Mineral source x phytase		ns	ns	ns	ns	ns

[1] Twelve replicate groups of 16 birds were assigned to each of the six treatments.
[2]Standard error of mean for interactive (mineral x phytase) effects
[3]ns = not significant; * = P< 0.05
[a, b] Means with no common superscript are significantly different (P <0.05)

Table 3.10. Average body weight (ABW) of Hy-Line Brown pullets at various ages (Experiment C05-03)[1]

Treatments		ABW, kg (2 wk)	ABW, kg (6 wk)	ABW, kg (8 wk)	ABW, kg (13 wk)	ABW, kg (17 wk)
Mineral supplement	Phytase					
None		0.136	0.447[b]	0.674[b]	1.236	1.513
Inorganic		0.139	0.466[a]	0.694[a]	1.262	1.532
Organic		0.137	0.463[a]	0.796[a]	1.257	1.524
	0	0.137	0.457	0.683[b]	1.246	1.518
	+	0.137	0.461	0.693[a]	1.258	1.529
None	0	0.137	0.441	0.663	1.233	1.512
Inorganic	0	0.138	0.464	0.688	1.255	1.520
Organic	0	0.138	0.466	0.699	1.251	1.521
None	+	0.136	0.454	0.685	1.240	1.515
Inorganic	+	0.140	0.468	0.700	1.269	1.544
Organic	+	0.136	0.460	0.693	1.264	1.528
SEM[2]		4.9E-04	1.5E-03	1.6E-03	2.0E-03	3.1E-03
Sources of variation		Significance of treatment effect[3]				
Mineral source		ns	*	*	ns	ns
Phytase		ns	ns	*	ns	ns
Mineral source x phytase		ns	ns	ns	ns	ns

[1] Twelve replicate groups of 16 birds were assigned to each of the six treatments.
[2]Standard error of mean for interactive (mineral x phytase) effects
[3]ns = not significant; * = P< 0.05
[a, b] Means with no common superscript are significantly different (P <0.05)

Effect of trace mineral source and phytase supplementation on liver mineral concentration

The effects of trace mineral and phytase supplementation on liver mineral concentrations of white pullets are summarized in Table 3.11. Liver Mn concentration was significantly increased by organic trace mineral supplementation, compared with no supplementation. A further significant increase liver Mn was obtained by inorganic trace mineral supplementation. However, trace mineral supplementation did not significantly affect Cu, Fe, Zn, P and Ca liver concentrations. Phytase supplementation did not have a significant effect on liver Cu, Fe, Mn, Zn, P and Ca concentrations. The interaction of mineral sources and phytase supplementation did not significantly affect liver mineral concentrations.

The effects trace mineral and phytase supplementation on liver mineral concentration of brown replacement pullets are summarized in Table 3.12. Liver Mn concentration for birds given the inorganic mineral supplementation was significantly higher by 2 ppm than for those fed no trace mineral supplementation. However, liver Mn concentration for birds fed the organic trace mineral supplement was not different from that of birds given the other mineral treatments. Liver Ca concentration was significantly higher ($P<0.05$) for birds fed inorganic trace mineral supplements than for those given the organic mineral source, but not different from that of birds given no mineral supplementation. Mineral supplementation did not significantly affect Cu, Fe, Zn and P liver concentration. Likewise phytase supplementation did not have a significant effect on liver Cu, Fe, Mn, Zn, P and Ca concentrations. The interaction of mineral source X phytase supplementation did not significantly affect liver mineral concentration.

Mineral deficiency will result in a depletion of tissue concentration and storage, leading to reduction or change in metabolism, transport rate and sign of deficiency in the bird. Therefore, trace minerals concentration in tissue have been used to measure the status of body storage and bioavailability (Zhao *et al*. 2010). The concentration of Cu in the liver is a good indicator of bioavailability and normal status of the mineral. Cu liver concentration is sensitive to a change in mineral source and dietary supplementation.

Results from a trial with brown pullets conducted by Ao *et al.* (2008) concur with results shown in Tables 3.11 and 3.12 on liver mineral concentrations of white and brown pullets. They concluded that supplementation of reduced levels of trace minerals (25% of NRC [1994] recommendations) as proteinates did not increase mineral concentrations in liver compared with using a basal diet with no added trace minerals or with an inorganic trace mineral supplement. Similarly, Zhao *et al.* (2010) reported no reduce liver concentration of Cu in broilers fed either organic or inorganic trace mineral source. The organic Cu was fed at same concentration (8 ppm) as inorganic source. Likewise, Manangi *et al.* (2012) conducted two broilers trials (54 days each) to evaluate feeding reduce level of chelates trace minerals and compare to inorganic trace mineral source. They reported no significant difference in liver minerals concentration. Cu liver concentration was not difference among treatments. A study in pigs by Coffey *et al.* (1994) resulted in a reduction of Cu liver concentration of 68% when fed with Cu-Lys complex vs Cu sulfate ($CuSO_4$). Both sources were fed at the same level. Similarly, Wang *et al.* (2007) was able to show a significant increase in liver Cu concentration when feeding broilers with organic Cu. They reported interaction with source (organic vs inorganic) and level of dietary Cu and a relative bioavailability of 111 % of organic Cu, when compare to sulfate Cu.

Table 3.11. Concentration of minerals in liver of Hy-Line W-36 pullets at 16 weeks of age (Experiment C04-03)[1,2]

Treatments		Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	P (g/kg)	Ca (g/kg)
Mineral supplement	Phytase						
None		14	726	7[c]	85	24	5
Inorganic		15	845	11[a]	88	24	5
Organic		15	781	9[b]	84	24	5
	0	15	821	9	86	24	5
	+	14	746	9	85	23	4
None	0	14	758	8	84	25	5
Inorganic	0	16	925	12	92	25	5
Organic	0	14	781	9	82	24	5
None	+	14	693	6	86	23	4
Inorganic	+	15	764	11	85	23	5
Organic	+	15	781	9	85	24	5
SEM[3]		0.40	41	0.40	2	0.40	0.40
Sources of variation		Significance of treatment effects[4]					
Mineral source		ns	ns	*	ns	ns	ns
Phytase		ns	ns	ns	ns	ns	ns
Mineral source x Phytase		ns	ns	ns	ns	ns	ns

[1]Twelve replicate groups of 16 birds were assigned to each of the six treatments
[2] All values are on a dry matter basis
[3]Standard error of mean for interactive (mineral x phytase) effects
[4]ns = not significant; * = P< 0.05
[a, b, c] Means with no common superscript are significantly different (P <0.05)

Table 3.12. Concentration of minerals in liver of Hy-Line Brown pullets at 16 week of age (Experiment C05-03)[1,2]

Treatments		Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	P (g/kg)	Ca (g/kg)
Mineral supplement	Phytase						
None		12	596	8[b]	93	12	28[ab]
Inorganic		12	547	10[a]	95	12	30[a]
Organic		12	519	9[ab]	96	11	26[b]
	0	12	542	9	95	12	29
	+	12	566	9	94	11	27
None	0	12	604	8	93	12	30
Inorganic	0	11	498	9	95	11	31
Organic	0	12	522	9	98	12	25
None	+	12	588	7	93	11	27
Inorganic	+	13	596	10	95	12	28
Organic	+	12	515	9	94	11	26
SEM[3]		0.50	44	0.50	4	0.29	1
Sources of variation		Significance of treatment effects[4]					
Mineral source		ns	ns	*	ns	ns	*
Phytase		ns	ns	ns	ns	ns	ns
Mineral source x Phytase		ns	ns	ns	ns	ns	ns

[1]Twelve replicate groups of 16 birds were assigned to each of the six treatments

[2] All values are on a dry matter basis

[3]Standard error of mean for interactive (mineral x phytase) effects

[4]ns = not significant; * = P< 0.05; [a, b] Means with no common superscript are significantly different (P <0.05)

Effect of trace mineral source and phytase supplementation on tibia breaking strength

The effects of trace mineral source and phytase supplementation on tibia breaking strength and bone ash of white pullets are summarized in Table 3.13. Both parameters were unaffected by treatments. The average bone breaking strength was 26 kg. Likewise, phytase supplementation and the interaction of mineral sources and phytase did not have a significant effect on breaking strength. Percent bone ash was not significantly affected by mineral supplementation, nor by phytase supplementation. Likewise, the interaction of mineral source X phytase supplementation had no effect on bone ash. The average bone ash was 60 %. Similar results were observed in the trial with the brown shell laying replacement pullets (Table 3.14), in which the average bone ash was 60 %. Star *et al* (2012) conducted a 21 day broiler trial to evaluate the effect of Zn mineral source (none, inorganic and organic) on tibia ash and Zn content. They reported dietary treatment did not affect tibia ash content (g/kg). Nevertheless, they found significantly ($P<0.05$) increased levels of Zn in the tibia for the inorganic and organic source when compare to the basal treatment (none trace mineral source). Zhao *et al.* (2010), also reported no significant difference in tibia ash (41%) and strength (25 kg/m^2), when evaluation an inorganic source of trace minerals with an organic source in broilers in a 52 days study. A study conducted by Manangi *et al.* (2012), found no significant difference in bone breaking strength in broilers fed chelates trace minerals at a reduced level. The inorganic trace minerals source were supplemented at a concentration of 100, 125 and 90 ppm of Zn, Cu and Mn respectively and the chelated trace minerals at 32, 8 and 32 ppm of Zn, Cu and Mn respectively.

Table 3.13. Tibia breaking strength and tibia ash of Hy-Line W-36 pullets at 16 weeks of age (Experiment C04-03)[1]

Treatments		Breaking strength, kg[2]	Bone ash (%)
Mineral supplement	Phytase		
None		26	60
Inorganic		26	60
Organic		26	60
	0	26	60
	+	26	60
None		27	60
Inorganic		25	60
Organic		26	61
None		26	58
Inorganic		26	60
Organic		26	59
SEM[3]		0.82	0.50

Sources of variation	Significance of treatment effects[4]	
Mineral source	ns	ns
Phytase	ns	ns
Mineral source x phytase	ns	ns

[1] Twelve replicate of group of 12 birds were assigned to each of the six treatments; bones were sampled from one hen per replicate group
[2] The force of weight, in kg, required to break a bone, when the bone is placed with its long axis perpendicular to the movement of the force
[3] Standard error of mean for interactive (mineral x phytase) effects
[4] ns= not significant; * = $P < 0.05$

Table 3.14. Tibia breaking strength and tibia ash of Hy-Line Brown pullets at 16 weeks of age (Experiment C05-03)[1]

Treatments		Breaking strength, kg[2]	Bone ash (%)
Mineral supplement	Phytase		
None		24	60
Inorganic		23	59
Organic		22	59
	0	23	60
	+	24	59
None		24	60
Inorganic		22	60
Organic		22	58
None		25	60
Inorganic		24	58
Organic		22	60
SEM[3]		1.41	0.82

Sources of variation	Significance of treatment effects[4]	
Mineral source	ns	ns
Phytase	ns	ns
Mineral source x phytase	ns	ns

[1] Twelve replicate of group of 12 birds were assigned to each of the six treatments; bones were sampled from one hen per replicate group
[2] The force of weight, in kg, required to break a bone, when the bone is placed with its long axis perpendicular to the movement of the force
[3] Standard error of mean for interactive (mineral x phytase) effects
[4] ns= not significant; * = $P < 0.05$

Effect of trace mineral source and phytase supplementation on manure mineral concentration

The effects of trace mineral source and phytase supplementation on manure mineral concentrations of white pullets are summarized in Table 3.15. The results indicated that manure levels of Cu, Fe and Zn were lower for pullets supplemented with organic minerals, compared with values for birds supplemented inorganic minerals. However, organic trace mineral supplementation did not decrease Mn and P manure concentrations. Phytase supplementation significantly reduced the concentrations of Fe, P and Ca but did not significantly affect Cu, Mn and Zn concentrations. The interaction of mineral source X phytase showed a significant ($P<0.05$) effect on manure Zn and Ca concentrations. However, the interaction was not significant for Cu, Fe, Mn, and P concentrations.

The effects of trace mineral and phytase supplementation of brown pullets on manure mineral concentration are summarized in Table 3.16. The results mainly indicated that organic trace minerals supplementation at 40% the inclusion rate significantly reduced concentrations of Cu, Fe, Mn, and Zn but did not have a significant effect on manure P and Ca concentrations. Although phytase supplementation significantly reduced ($P<0.05$) Fe, Mn, Zn, P and Ca manure concentration, it did not significantly affect manure Cu concentration. The interaction of mineral source and phytase showed a significant effect on Cu, Fe, Mn and Zn manure concentrations. However, the interaction was not significant for P and Ca concentrations. Nollet *et al*. (2007) studied the effect of replacing inorganic with organic trace mineral source in broiler on the reduction of trace mineral in manure. They reported a significant reduction ($P<0.05$) of Mn, Zn, Fe and Cu concentration in manure by 40, 69, 73 and 55% respectively from birds fed organic source. They concluded that the lower concentration in the manure was due to feeding lower levels of trace minerals

in organic source (by 20% in Cu, 27% in Zn, 14% in Mn and 22% in Fe) and to the higher bioavailability of the same source of mineral. Leeson and Caston (2007) reported a significant ($P<0.01$) reduction in total Zn, Mn and Cu manure concentration when feeding broilers with organic trace mineral source. They fed diets that supply 80%, 60%, 40% and 20% of the level of Inorganic source. They were able to reduce Zn, Mn and Cu by 38%, 52% and 21% when comparing the inorganic trace mineral with the lover level of organic trace mineral supplementation. They concluded, that trace mineral can be reduce in manure by feeding organic trace mineral source due to the higher bioavailability, which allow to feed lower levels. A similar study in broilers was conducted by Nollet *et al*. (2008), were they compared feeding a basal diet (no trace mineral supplementation), an inorganic (15 ppm of Cu and Fe, Mn and Zn were added at 45 ppm each) and an organic trace mineral source (100%, 67%, 50%, 30% and 17% of the inorganic source's concentration). They reported a significant ($P<0.05$) reduction of manure Cu, Fe, Mn and Zn concentration in birds fed the lower concentration of organic source (67%, 50%, 30% and 17% of the inorganic). This treatments had similar concentration of trace minerals in manure than the basal diets. The birds fed the inorganic and organic trace minerals source (same level as the inorganic) showed no reduction in trace mineral excretion. They discussed that feeding higher level of the organic trace mineral did not resulted in a higher retention or bioavailability due to the inability of the gut to absorb the trace mineral at faster rate.

Leeson and Caston (2007) conducted a study in layers were they fed none, inorganic or organic trace mineral source for 32 weeks. Birds fed the organic source significant ($P<0.01$) reduced the level of Zn and Mn by 67% and 81% respectively when compare to inorganic trace mineral source.

The result from Manangi *et al.* (2012), on manure liter trace minerals concentration showed similar finding as ours. They were able to reduce trace minerals in manure by 40, 74 and 35 % for Zn, Cu and Mn respectively. They attribute this reduction on the ability of chelated source to increase the bioavailability of the trace minerals, therefore, a reduced concentration of organic minerals can be fed to broilers to decrease trace mineral output.

Table 3.15. Concentration of minerals in manure of Hy-Line W-36 pullets (Experiment C04-03)[1,2]

Treatments		Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	P (g/kg)	Ca (g/kg)
Mineral supplement	Phytase						
None		25[b]	651[b]	220	112[c]	31	47[a]
Inorganic		35[a]	1068[a]	318	279[a]	30	42[b]
Organic		27[b]	694[b]	192	154[b]	30	46[ab]
	0	31	912[a]	289	187	33[a]	47[a]
	+	27	697[b]	198	176	28[b]	43[b]
None	0	26	757	215	108[d]	34	51
Inorganic	0	38	1195	385	300[a]	32	42
Organic	0	29	785	268	151[c]	33	48
None	+	24	546	225	115[d]	28	43
Inorganic	+	32	942	252	258[b]	28	41
Organic	+	26	602	115	157[c]	28	44
SEM[3]		2	33	54	9	1	2
Sources of variation		Significance of treatment effect[4]					
Mineral source		*	*	ns	*	ns	*
Phytase		ns	*	ns	ns	*	*
Mineral source x phytase		ns	ns	ns	*	ns	*

[1]Twelve replicate groups of 16 birds were assigned to each of the six treatments
[2] All values are on a dry matter basis
[3]Standard error of mean for interactive (mineral x phytase) effects [4]ns = not significant; * = P< 0.05
[a, b, c, d] Means with no common superscript are significantly different (P <0.05)

Table 3.16. Concentration of minerals in manure of Hy-Line Brown pullets (Experiment C05-03)[1,2]

Treatments		Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	P (g/kg)	Ca (g/kg)
Mineral supplement	Phytase						
None		7[b]	1019[b]	38[c]	31[c]	38	57
Inorganic		32[a]	1552[a]	246[a]	284[a]	38	53
Organic		8[b]	981[b]	80[b]	72[b]	37	57
	0	17	1357[a]	132[a]	137[a]	40[a]	57[a]
	+	14	1003[b]	105[b]	115[b]	35[b]	54[b]
None	0	8[c]	1174[c]	40[d]	31[d]	41	59
Inorganic	0	38[a]	1863[a]	295[a]	338[a]	40	52
Organic	0	7[c]	1098[c]	83[c]	67[c]	41	59
None	+	6[c]	865[d]	35[d]	30[d]	35	54
Inorganic	+	27[b]	1280[b]	202[b]	238[b]	36	54
Organic	+	9[c]	864[d]	77[c]	78[c]	34	54
SEM[3]		2	14	4	6	0.82	1
Sources of variation		Significance of treatment effect[4]					
Mineral source		*	*	*	*	ns	ns
Phytase		ns	*	*	*	*	*
Mineral source x phytase		*	*	*	*	ns	ns

[1]Twelve replicate groups of 16 birds were assigned to each of the six treatments
[2] All values are on a dry matter basis
[3]Standard error of mean for interactive (mineral x phytase) effects.
[4]ns = not significant; * = P< 0.05;
[a, b, c, d] Means with no common superscript are significantly different (P <0.05)

CHAPTER 4

CONCLUSIONS

The overall pullet performance, AFI and BW, was not affected by the dietary treatments. Basal diet with no trace mineral supplementation resulted in similar performance as inorganic and organic source, suggesting that trace minerals were not limiting in the diets. Nevertheless, pullets fed organic trace mineral source formulated to provide 40% of the NRC (1994) minimum requirement, performed as well as birds supplemented with inorganic trace mineral or not supplemented with trace minerals. Likewise, tibia breaking strength and tibia ash, showed no significant response to dietary treatments. Tibia Zn concentration is a good indicator of mineral availability, therefore, this measurement should be included in future studies. Liver Mn concentration showed a significant ($P<0.05$) reduction in pullets fed organic minerals. Manure mineral concentration showed the bigger effect in pullets fed the basal and the organic treatment. The pullets fed the organic minerals were able to reduce the concentration to same levels of the basal diet with no trace mineral supplementation. The W36 pullets were able to reduce Cu, Fe and Zn when fed organic mineral. Likewise, the addition of phytase had an effect on P and Ca manure concentration. Brown pullets fed with organic mineral were able to reduce Cu, Fe, Mn, Zn and Ca. Also Phytase supplementation was able to significantly reduce Fe in addition to P and Ca. This finding suggest that feeding organic minerals at a low dosage will lead to reduction of trace mineral in manure compared to inorganic mineral at a higher dosage.

APPENDIX 1: FEED GRAIN DIGESTION

Reference:

CEM Corporation, Operation Manual, General Guidelines for Microwave Sample Preparation, 1994.

Apparatus:

Microwave Sample Preparation System MDS-2000.

Reagents:

Nitric acid (HNO_3)

Procedure:

1. Weigh 1.0 gram of ground sample into each of 12 vessels.
2. Add 10 mL of HNO_3 to each vessel.
3. Seal all vessels and place on the turntable.
4. Connect the vent tubes from the vessels to the collection vessel.
5. Place the turntable into the system.
6. Program as follows:

Procedure to Program Microwave for Feed Digestion

Stage	1	2	3	4	5
% POWER	46	46	46	46	0
PSI	20	40	80	150	175
TIME	15:00	15:00	15:00	15:00	0
TAP	3:00	3:00	3:00	3:00	0
FAN SPEED	100	100	100	100	100

7. Run heating program to completion.
8. Cool the samples for 5 min., and remove the turntable from the system.
9. Open the vessels and place the liquid content in a 25 mL volumetric flask.
10. Fill to the 25 mL mark with nano-pure water.
11. Place the final volume (25 mL) in a plastic sample cup.

APPENDIX 2: PULLET LIVER DIGESTION

Reference:

CEM Corporation, Operation Manual, General Guidelines for Microwave Sample Preparation, 1994.

Apparatus:

Microwave Sample Preparation System MDS-2000.

Reagents:

Nitric acid (HNO_3), and nano-pure water.

Procedure:

1. Weigh 0.5 gram of ground sample into each of 12 vessels.
2. Add 5 mL of HNO_3 and 2 mL of Nano-pure water to each vessel.
3. Seal all vessels and place on the turntable.
4. Connect the vent tubes from the vessels to the collection vessel.
5. Place the turntable into the system.
6. Program as follows:

Procedure to Program Microwave for Liver Digestion

Stage	1	2	3	4	5
% POWER	74	74	74	0	0
PSI	50	100	150	0	0
TIME	10:00	10:00	10:00	0	0
TAP	5:00	5:00	5:00	0	0
FAN SPEED	100	100	100	100	100

12. Run heating program to completion.

13. Cool the samples for 5 min., and remove the turntable from the system.

14. Open the vessels and place the liquid content in a 25 mL volumetric flask.

15. Fill to the 25 mL mark with nano-pure water.

16. Place the final volume (25 mL) in a plastic sample cup.

APPENDIX 3: PULLET MANURE DIGESTION

Reference:

CEM Corporation, Operation Manual, General Guidelines for Microwave Sample Preparation, 1994.

Apparatus:

Microwave Sample Preparation System MDS-2000.

Reagents:

Nitric acid (HNO_3)

Procedure:

1. Weigh 0.5 gram of ground sample into each of 12 vessels.
2. Add 10 mL of HNO_3 to each vessel.
3. Seal all vessels and place on the turntable.
4. Connect the vent tubes from the vessels to the collection vessel.
5. Place the turntable into the system.
6. Program as follows:

Procedure to Program Microwave for Feed Digestion

Stage	1	2	3	4	5
% POWER	46	46	46	46	0
PSI	20	40	80	150	175
TIME	15:00	15:00	15:00	15:00	0
TAP	3:00	3:00	3:00	3:00	0
FAN SPEED	100	100	100	100	100

7. Run heating program to completion.
8. Cool the samples for 5 min., and remove the turntable from the system.
9. Open the vessels and place the liquid content in a 50 mL volumetric flask.
10. Fill to the 50 mL mark with nano-pure water.
11. Place the final volume (50 mL) in a plastic sample cup.

APPENDIX 4: PULLET FEED TRACE MINERAL DETERMINATION

Reference:

Inductively coupled plasma atomic emission spectrometry, Method 6010B.

Apparatus and Materials:

1. Inductively coupled argon plasma-atomic emission spectrometry (ICP, Basta-Pro., Varian, Australia).
2. Computer-controlled emission spectrometer with background correction.
3. Radio frequency generator compliant with FCC regulations.
4. Mass flow controller for argon nebulizer gas supply.
5. Peristaltic pump.
6. Autosampler.
7. Argon gas supply high purity.

Reagent

Nitric acid 10 %, (HNO_3). Add 10 mL concentrated HNO_3 to 90 mL nano-pure water in an appropriately sized beaker.

copper reference solution, as copper nitrate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

manganese reference solution, as manganese nitrate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

zinc reference solution, as zinc oxide, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

Calcium reference solution, as calcium carbonate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

iron, ICP reference solution, certified 1000 ppm ± 1%, 500 mL. Spexcertiprep, 203 Norcross Ave. Metuchen N.J. 08840.

Phosphorus ICP reference solution, certified 1000 ppm ± 1%, 250 mL. Spexcertiprep, 203 Norcross Ave. Metuchen N.J. 08840.

Procedure:

1. Open the argon gas tank, turn on the ICP machine, and ignite the plasma.
2. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration). Operating conditions: the analyst should follow the instructions provided by the instrument manufacturer.
3. Calibrate the ICP machine, following the instructions provided by the instrument manufacturer.
4. Select specific wavelengths for each of the trace minerals base on the best percent recovery of the spiked sample. Wavelength use were: Cu 223.009; Fe 239.563, Mn 293.931, Zn 206.200, Ca 445.478 and P 213.618.
5. The operation conditions for determination of trace minerals usually vary from 110 to 1200 watts forward power, 14 to 18 mm viewing height, 15 to 19 liter/minutes argon coolant flow, 0.6 to 1.5 L/min argon nebulizer flow, 1 to 1.8 mL/min sample pumping rate with a 1 minute preflush time and measurement time near 1 second per wavelength peak for sequential instruments.
6. Place the sample and standards solutions on the autosampler.

7. Input data in the program with the parameters of standards solutions.
8. Start reading the sample.

Quality Control:

1. Run sample in duplicate for precision, and coefficient of variation must be calculated.
2. Run a spiked matrix sample every 12 feed samples for accuracy. The samples to be spiked must be run in triplicate, and a percent of recovery must be calculated.
3. Run a sample blank for every batch.
4. Run a one laboratory control sample (LCS) for every batch.

APPENDIX 5: PULLET LIVER TRACE MINERAL DETERMINATION

Reference:

Inductively coupled plasma atomic emission spectrometry, Method 6010B.

Apparatus and Materials:

1. Inductively coupled argon plasma-atomic emission spectrometry (ICP, Basta-Pro., Varian, Australia).
2. Computer-controlled emission spectrometer with background correction.
3. Radio frequency generator compliant with FCC regulations.
4. Mass flow controller for argon nebulizer gas supply.
5. Peristaltic pump.
6. Autosampler.
7. Argon gas supply high purity.

Reagent

Nitric acid 10 %, (HNO_3). Add 10 mL concentrated HNO_3 to 90 mL nano pure water in an appropriately sized beaker.

copper reference solution, as copper nitrate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

manganese reference solution, as manganese nitrate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

zinc reference solution, as zinc oxide, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

Calcium reference solution, as calcium carbonate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

iron, ICP reference solution, certified 1000 ppm ± 1%, 500 mL. Spexcertiprep, 203 Norcross Ave. Metuchen N.J. 08840.

Phosphorus ICP reference solution, certified 1000 ppm ± 1%, 250 mL. Spexcertiprep, 203 Norcross Ave. Metuchen N.J. 08840.

Procedure:

1. Open the argon gas tank, turn on the ICP machine, and ignite the plasma.
2. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration). Operating conditions: the analyst should follow the instructions provided by the instrument manufacturer.
3. Calibrate the ICP machine, following the instruction provided by the instrument manufacturer.
4. Select specific wavelengths for each of the trace minerals base on the best percent recovery of the spiked sample. Wavelength use were: Cu 327.395, Fe 234.350, Mn 293.931, Zn 334.502, Ca 616.217 and P 213.618.
5. The operation conditions for determination of trace minerals usually vary from 110 to 1200 watts forward power, 14 to 18 mm viewing height, 15 to 19 L/minutes argon coolant flow, 0.6 to 1.5 L/min argon nebulizer flow, 1 to 1.8 mL/min sample pumping rate with a 1 minute preflush time and measurement time near 1 second per wavelength peak for sequential instruments.

6. Place the sample and standards solutions on the autosampler.
7. Input data in the program with the parameters of standards solutions.
8. Start reading the sample.

Quality Control:

1. Run sample in duplicate for precision, and coefficient of variation must be calculated.
2. Run a spiked matrix sample every 20 samples for accuracy. The samples to be spiked must be run in triplicate; a percent of recovery must be calculated. Run a sample blank for every batch.
3. Run a one laboratory control sample (LCS) for every batch.

APPENDIX 6: PULLET MANURE TRACE MINERAL DETERMINATION

Reference:

Inductively coupled plasma atomic emission spectrometry, Method 6010B.

Apparatus and Materials:

1. Inductively coupled argon plasma-atomic emission spectrometry (ICP, Basta-Pro., Varian, Australia).
2. Computer-controlled emission spectrometer with background correction.
3. Radio frequency generator compliant with FCC regulations.
4. Mass flow controller for argon nebulizer gas supply.
5. Peristaltic pump.
6. Autosampler.
7. Argon gas supply high purity.

Reagent

Nitric acid 10 %, (HNO_3). Add 10 mL concentrated HNO_3 to 90 mL nano pure water in an appropriately sized beaker.

copper reference solution, as copper nitrate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

manganese reference solution, as manganese nitrate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

zinc reference solution, as zinc oxide, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

Calcium reference solution, as calcium carbonate, certified 1000 ppm ± 1%, 100 mL. Fisher scientific, Fisher chemicals, Fair Lawn, N.J. 07410.

iron, ICP reference solution, certified 1000 ppm ± 1%, 500 mL. Spexcertiprep, 203 Norcross Ave. Metuchen N.J. 08840.

Phosphorus ICP reference solution, certified 1000 ppm ± 1%, 250 mL. Spexcertiprep, 203 Norcross Ave. Metuchen N.J. 08840.

Procedure:

1. Open the argon gas tank, turn on the ICP machine, and ignite the plasma.
2. The instrument must be allowed to become thermally stable before beginning (usually requiring at least 30 minutes of operation prior to calibration). Operating conditions: the analyst should follow the instructions provided by the instrument manufacturer.
3. Calibrate the ICP machine, following the instructions provided by the instrument manufacturer.
4. Select specific wavelengths for each of the trace minerals base on the best percent recovery of the spiked sample. Wavelength use were: Cu 324.754 Fe 234.563, Mn 293.305, Zn 334.502, Ca 616.217 and P 213.618.
5. The operation conditions for determination of trace minerals usually vary from 110 to 1200 watts forward power, 14 to 18 mm viewing height, 15 to 19 liter/minutes argon coolant flow, 0.6 to 1.5 L/min argon nebulizer flow, 1 to 1.8 mL/min sample pumping rate with a 1 minute preflush time and measurement time near 1 second per wavelength peak for sequential instruments.
6. Place the sample and standards solutions on the autosampler.

7. Input data in the program with the parameters of standards solutions.
8. Start reading the sample.

Quality Control:

1. Run sample in duplicate for precision, and coefficient of variation must be calculated.
2. Run a spiked matrix sample every 20 samples, for accuracy. The samples to be spiked must be run in triplicate; a percent of recovery must be calculated. Run a sample blank for every batch.
3. Run a one laboratory control sample (LCS) for every batch.

APPENDIX 7: BONE ASH (%) DETERMINATION

Reference:

AOAC: Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, 1995.

Apparatus:

Soxhlet apparatus.

Isotemp®, Muffle Furnace. Fisher Scientific, Fair Lawn, N.J. 07410.

Reagent:

Petroleum ether, 100%, Z139- 20 L. Fisher Scientific, Fair Lawn, NJ 07410.

Procedure:

1. Boil the bone samples for approximately 2 min.
2. Deflesh and clean the bone samples.
3. Label the samples with wing bands and wrap in cheesecloth net.
4. Place on the Soxhlet apparatus and fill with petroleum ether for 48 hours for fat extraction.
5. Dry for 24 hours at room temperature to let the petroleum ether evaporate.
6. Dry bones at 100° C for 24 hours with the crucible and store on desiccators until cool.
7. Weigh samples and record weight.
8. Weigh crucible, record the weight, and place the bones inside.

9. Place them in muffle furnace oven and ash at 600° C overnight.

10. Store them on desiccators and cool. Weigh them and record.

11. Calculate ash % as: $\frac{\text{wt of ash}}{\text{dried defatted sample wt}} \times 100$

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