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Effect of Increasing Levels of Dietary Zinc (Zn), Manganese (Mn), and Copper (Cu) from Organic and Inorganic Sources on Egg Quality and Egg Zn, Mn, and Cu Content in Laying Hens

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EFFECT OF INCREASING LEVELS OF DIETARY ZINC (ZN), MANGANESE
(MN), AND COPPER (CU) FROM ORGANIC AND INORGANIC SOURCES
ON EGG QUALITY AND EGG ZN, MN, AND CU CONTENT IN LAYING
HENS

A Thesis

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Louisiana State University and
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by
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ABSTRACT

Feeding laying hens or broiler breeder hens organic sources of trace minerals can improve egg quality, especially as related to shell quality. The objectives of this research were to evaluate the effect of increasing laying hen dietary zinc (Zn) levels on the content of Zn and other minerals in eggs (boron (Bo), iron (Fe), and calcium (Ca)), and to evaluate the effect of feeding increasing Zn, manganese (Mn) and copper (Cu) levels from inorganic (ITM) and organic (OTM) sources on egg quality and yolk content of Zn, Mn, and Cu. In the first experiment, a total of 64 (48 weeks of age) commercial hens (Hy-Line W-36) were allotted to one of four treatment diets on day 0. There were four replicates with four hens per replicate. The dietary treatments were: 1) corn-soybean meal (C-SBM) with no added Zn (25 mg/kg total Zn from feed ingredients), 2) C-SBM with 50 mg/kg added Zn, 3) C-SBM with 100 mg/kg added Zn, and 4) C-SBM with 150 added mg/kg Zn. The inorganic source of Zn was ZnSO₄. The trial lasted 30 days. In the second trial, 600 Hy-Line W-36 hens at 26 weeks of age were used. Hens were allotted to one of five treatment diets on day 0 of the trial. A total of 10 replicates with 12 hens per replicate were used. The dietary treatments were: 1) Control - no supplementation of Zn, Mn, or Cu, 2) Zn, Mn, and Cu supplemented at 80-90-8 mg/kg from ITM, 3) Zn, Mn, and Cu supplemented at 80-90-8 mg/kg from ITM+OTM, 4) Zn, Mn, and Cu supplemented at 160-175-16 mg/kg from ITM, and 5) Zn, Mn, and Cu supplemented at 160-175-16 mg/kg from ITM+OTM. The trial lasted 84 days. The response criteria were egg weight, specific gravity, egg shell thickness, egg shell weight, albumen height, yolk height and width, Haugh units, yolk index, feed intake and feed conversion. Data were collected on three consecutive days at the end of each 28 day period. On the second day of each three day collection period, three yolks per replicate were collected and stored for analysis of Zn, Mn, and Cu content. In the first

experiment, hens fed more than 100 mg/kg added Zn had reduced ($P<0.05$) egg production and egg mass. Zinc content in the egg yolk was increased 23% with 150 mg/kg added Zn. In the second experiment there was no effect ($P>0.05$) of dietary treatment on egg production. During the first 28 day collection period, yolk Zn and Mn were highest ($P<0.01$) for hens fed the 160-175-16 mg/kg from ITM+OTM. In the third 28 day collection period, albumen height was highest ($P<0.05$) in eggs laid by hens fed the control diet and similar for hens fed all other treatment diets. Egg weight and egg mass were not affected by increasing dietary inclusion of Zn, Mn, and Cu. Feed intake was improved for the first 28 day collection period. Eggs per kg of feed was increased when hens were fed the 80-90-8 mg/kg from ITM+OTM. Thus, Zn content of the egg yolk may be increased by the inclusion of ITM+OTM at rates above their requirement in layer diets.

CHAPTER 1: INTRODUCTION

A mineral is an inorganic element that is required for normal life processes (Ammerman et al., 1995). The general functions of minerals are structural, physiological, catalytic, and hormonal (McDowell, 2003). Minerals are classified as macro minerals or micro (trace) minerals. Macro minerals are required in relatively large amounts (%) and trace minerals are required in very small amounts (mg/kg). In poultry, mineral deficiencies can be characterized by poor rate of hatch, inadequate skeletal development, reduced embryo mineral content, or increased mortality rate (McDowell, 2003; Angel, 2007; Uni et al., 2012).

Dietary minerals are available in inorganic and organic forms. Inorganic trace mineral sources are the most common sources used in poultry diets. The benefits of inorganic trace minerals are improved growth rate, health, and reproduction (Leeson et al., 2005; Bao et al., 2007; Nollet et al., 2008). Organic trace minerals are more bioavailable in several species of livestock and poultry compared to inorganic trace minerals. Also, organic mineral sources have been reported to enhance intestinal absorption of trace elements as they decrease interference from agents that form insoluble complexes with the ionic micro elements (Abdallah et al., 1994; Nollet et al., 2007; Peric et al., 2007).

Zinc (Zn), manganese (Mn), and copper (Cu) are three of the trace minerals that are required in poultry diets and are available in both inorganic and organic forms. For the laying hen, recent research indicates that dietary supplementation with organic trace mineral sources of Zn, Mn, or Cu results in improved egg quality (Tang et al., 2006; Bao et al., 2007). Zinc is necessary for normal growth, development, and health in humans and animals (Leeson et al.,

2001). Studies show that Zn is essential in laying hens because it is a component of carbonic anhydrase and metalloenzymes (Balnave and Zhang, 1993; Leeson et al., 2001; Mabe et al., 2003; Hudson et al., 2004; Zamani et al., 2005; Favero et al., 2013a).

Thus, the objectives of this research were to evaluate the effect of increasing laying hen dietary Zn levels on the content of Zn and other minerals in eggs, and to evaluate the effect of feeding increasing Zn, Mn and Cu levels from inorganic and organic sources on egg quality and yolk content of Zn, Mn, and Cu.

CHAPTER 2: LITERATURE REVIEW

2.1 Mineral Nutrition in Layers

A mineral is an inorganic element that is essential in the body for different physiological processes. Mineral absorption is realized from the gastrointestinal tract and bioavailability of minerals varies (Ammerman et al., 1995). Macro minerals are required in large amounts that are included as percentages of the diet and are essential for bone, muscle, and nerve function. The macro minerals are calcium, phosphorus, sodium, potassium, chloride, magnesium, and sulfur. Trace minerals, or micro minerals, are required in very small amounts which are included at rates of mg/kg of the diet. They are essential for normal metabolism and reproduction. The micro minerals poultry require are Zn, Mn, iron (Fe), iodine (I), selenium (Se), Cu, and molybdenum (Mo). Dietary trace mineral supplementation is essential for poultry even though feed ingredients contain most of their required minerals (Bell and Weaver, 2002; McNamara, 2006).

Additionally, trace minerals are fundamental because they are co-factors associated with proteins and are needed in metabolic reactions (Larbier and Leclercq, 1992; McNamara, 2006). Iron, Cu, Zn, Mn, I, and Se are the most important trace elements (Bao et al., 2007). Trace minerals cause complex interactions that can affect vitamins, hormones, and neurological functions, both antagonistically and synergistically (Watts, 1990). Thus, the animal has three responses to the concentrations of minerals in their diet. The response to low levels is a deficiency, the response to higher levels is body reserves are kept constant, and the response to amounts over the requirements can be toxicity symptoms such as reduced egg production in laying hens (Larbier and Leclercq, 1992).

In general, the bioavailability of minerals is strongly related to the chemical form, feed composition, age and physiological state of the bird, and mineral interactions (Dobrzański et al., 2003). Furthermore, Uni et al. (2012), while studying the nutritional limitations during poultry embryonic development, stated that the uptake of nutrients by the embryo is fundamental in the fertile egg. A deficiency of dietary minerals can negatively affect incubation, skeletal development, embryo mineral content, and mortality rate (Angel, 2007; Uni et al., 2012).

2.1.1 Inorganic Sources

Inorganic trace minerals are the most common source of trace minerals supplemented in poultry diets. The overall benefits are increased growth rate, health, and reproduction. The rate of dietary trace mineral supplementation is approximately 0.25% of the total diet (Leeson et al., 2005; Bao et al., 2007; Nollet et al., 2008). Inorganic trace minerals are added to feed in the form of inorganic salts, such as sulfates, oxides, and carbonates (Bao et al., 2007; Nollet et al., 2007; Peric et al., 2007; Aksu et al., 2010). Miles et al. (1998) reported that sulfates and chlorides are more soluble than carbonates and oxides. Also, intense usage of inorganic salts may reduce their relative biological availabilities and their excessive use may cause environmental pollution due to increased mineral excretion (Nollet et al., 2008; Aksu et al., 2010).

2.1.2 Organic Sources

Over the years, organic trace mineral research has focused on biological value and bioavailability in several species of livestock and poultry. According to AAFCO (2000), organic trace minerals are specific metal amino acid complexes, from a combination of soluble metal salts and amino acids. Similarly, chelates are molecules resulting from a covalent bond between metal ions and a ligand such as proteins or carbohydrates (Vieira, 2008). Organic trace minerals are metal complexes, metal amino acid complexes, metal amino acid chelates, metal proteinates,

metal polysaccharide complexes, metal propionates, and yeast derivate complexes (AAFCO, 1998). Commercially, metal complexes are the form available in the market (Brown and Zeringue, 1994). The use of organic minerals results in improved intestinal absorption. This is related to the small organic molecules and the reduction of the combination of insoluble complexes with ionic trace elements (Abdallah et al., 1994; Nollet et al., 2007; Peric et al., 2007).

Current reports in the literature indicate that feeding laying hens or broiler breeder hens organic sources of trace minerals can improve egg quality, especially as related to shell quality. Also, organic trace minerals can enhance embryonic development, hatchability, hatchling quality, egg production, and growth (Mabe et al., 2003; Tang et al., 2006; Uni et al., 2012). Moreover, several reports indicate that organic sources have greater bioavailability in comparison to inorganic sources (Nollet et al., 2008). Yenice et al. (2015) evaluated the effects of organic and inorganic mixtures of Mn, Zn, Cu and Cr added to the feed of laying hens in the post-peak egg production phase. Barred Rock layers at 50 weeks of age were used. The concentrations of serum Mn, Zn, Cu and Ca; egg Mn, Zn, Cu, and Cr; and eggshell Zn and Cr were increased significantly by feeding the organic trace minerals. They reported that an organic Mn, Zn, Cu, and Cr mixture increased the bioavailability of these elements compared to inorganic sources, and a lower level of trace mineral supplementation resulted in lower mineral excretion, particularly for the organic form. Gheisari et al. (2011) also evaluated the effect of diets supplemented with different levels of Mn, Zn, and Cu from organic or inorganic sources on hen egg production and quality characteristics. Hy-line W-36 layers at 38 weeks of age were used. The results indicated that a corn-soybean meal diet supplemented with the organic form of these elements, with concentrations 50% to 75% lower than the NRC. (1994) recommendation,

was sufficient to maintain laying performance and resulted in improved eggshell and albumen qualities.

Additionally, Mabe et al. (2003) fed aged laying hens organic and inorganic sources of Zn, Mn, and Cu. Three groups of hens, aged 32, 60, and 69 weeks fed C-SBM supplemented with 30-30-5 and 60-60-10 mg/kg of Zn, Mn, and Cu, respectively. They reported that the yolk content of Zn, Mn, and Cu was increased by increasing dietary supplementation of Zn, Mn, and Cu. However, the dietary mineral supplementation did not affect eggshell quality. Stefanello et al. (2014) evaluated the effect of supplementing hens' diets with trace minerals from inorganic and organic sources on productive performance, eggshell quality, and egg shell structure of laying hens. Hy-line W-36 layers between 47 to 62 weeks of age were used. The recommended levels were 65-60-10 mg/kg of Zn, Mn and Cu according to Rostagno et al. (2005). The treatment diets were reduced or increased by the same level to 30-30-5 mg of Zn, Mn and Cu, respectively. The results indicated that increasing dietary trace mineral (Zn, Mn, and Cu) supplementation did not affect egg production or hen feed efficiency, but increasing trace mineral supplementation improved egg shell quality and reduced egg losses. The hens fed organic trace mineral sources had lower egg losses and higher eggshell quality than the hens fed inorganic trace mineral sources. In broiler breeder hens (Cobb 500) Favero et al. (2013a) fed diets supplemented with Zn, Mn and Cu from inorganic and organic sources (amino acid complexes). The treatments contained diets exceeding NRC. (1994) requirements. The results reported increased eggshell weight and thickness when hens were fed organic trace minerals (Zn, Mn, and Cu). Also, these results were improved when hens were fed inorganic trace mineral sources in combination with organic trace minerals.

2.1.3 Zinc

Zinc is essential for humans and animals, and is necessary for normal growth, development, and health. Zinc is a component of carbonic anhydrase, an essential enzyme for eggshell formation and calcification of bone from carbonate ions. Carbonic anhydrase is located in the shell gland of the oviduct in laying hens (Leeson et al., 2001). Additionally, other vital metalloenzymes, which are carboxypeptidases and DNA polymerases, are essential for skin, wound healing, and hormone production (Balnave and Zhang, 1993; Mabe et al., 2003; Hudson et al., 2004; Zamani et al., 2005; Favero et al., 2013a). Deficiency of this enzyme causes a lowered bicarbonate ion secretion that decreases eggshell weight. Zinc plays a major role in erythropoiesis, as it activates the synthesis of alfa-aminolevulinic acid dehydratase (Aksu et al., 2010). Zinc is absorbed in the small intestine of monogastric animals, and a small amount of absorption occurs in the proventriculus of chicks (McDowell, 2003). Moreover, Zn is distributed throughout the body and plays a role in the following functions: reproduction, development of blood cells, immune system function, and bone development (Hudson et al., 2004; Aksu et al., 2010; Hafeez, 2015)

A Zn deficiency can cause adverse effects on erythropoiesis in the marrow, reduction of egg production and eggshell quality, and, consequently, reduced hatchability (Aksu et al., 2010; Favero et al., 2013a). Additionally, Zn deficiency can result in poor growth and abnormal bone development in chicks (Leeson et al., 2001). The chemical form and concentration of Zn are essential for preventing mineral antagonisms. For example, in the case of high calcium intake, intestinal Zn absorption is reduced, and excess Zn intake can reduce Cu absorption (Watts, 1990; Dobrzański et al., 2003). In the same context, declined hatchability and embryonic development (Hudson et al., 2004) can occur.

Hudson et al. (2004) evaluated diets supplemented with either inorganic (ZnSO_4) or organic (amino acid complex) sources of Zn, with 80 ppm fed to layers from hatch to 65 weeks of age. Their results indicated that there is increased absorption in the intestine, increased Zn retention, and enhanced performance when diets are supplemented with both organic and inorganic sources. Commonly, organic Zn sources can be in the form of metal amino acid chelates, metal-proteinates, metal-specific amino acid complexes, and metal-amino acid complexes. In aged hens, Berry and Brake (1987) reported an increase in egg production when Zn was added to layer diets.

2.1.4 Manganese

Manganese is a vital trace mineral, and it plays a significant role in body and bone growth, normal brain function, reproduction, digestion, physiological function, biosynthetic processes within the body, amino acid metabolism, and various enzymatic systems. Manganese is essential for the development of blood cells and immune system function (Bao et al., 2007; Yildiz et al., 2011; Favero et al., 2013a). Additionally, Mn is required in the formation of mucopolysaccharides that are activated for the glycosyltransferases, which are constituents of proteoglycans. These proteins are necessary for bone plate growth and bone cartilage formation of poultry (Leach and Gross, 1983; Zamani et al., 2005; Peric et al., 2007; Yildiz et al., 2011; Favero et al., 2013a). Similarly, keratin and dermatan proteoglycans are involved in eggshell matrix formation (Zamani et al., 2005).

Manganese is an essential nutrient for laying hens. Deficiency of this trace mineral causes reduced egg production, eggs produced with thinner shells, and translucent areas and abnormalities in eggshell ultrastructure (Leach and Gross, 1983; Mabe et al., 2003; Yildiz et al., 2011). Manganese is necessary for the synthesis of polysaccharides, and a deficiency of this

element decreases eggshell content of hexosamine and hexuronic acid (Leeson et al., 2001). Moreover, diets deficient in Mn have a negative effect on hatchability. Manganese deficiency can result in shortened long bones, parrot beak, and wiry down (Favero et al., 2013a). Also, absorption in the intestinal tract is low (Leeson et al., 2001). Manganese is absorbed by binding divalent metal ion transporters (Aksu et al., 2010). Manganese retention in the body of broiler chickens is around of 0.2% (Peric et al., 2007). In poultry feed, organic Mn sources are amino acid complexes, chelates, and proteinates. The bioavailability of chelated and complexed Mn is related to chemical characteristics that are improved by chelation effectiveness and the percentage of organic ligand (Yildiz et al., 2011). Currents research reports that organic Mn sources are more bioavailability than inorganic sources. However, other studies report no difference in bioavailability among organic and inorganic Mn sources (Li et al., 2004; Yildiz et al., 2011).

The Mn requirement for laying hens is approximately 30 to 200 mg/kg but is dependent on the response criteria, production cycle, hen age, production level, and availability of Mn in the raw material (Yildiz et al., 2011). The toxicity of Mn by interfering with magnesium is related to sodium accumulation and excessive potassium in tissues (Watts, 1990). Consequently, a high level of Mn can interfere with the synthesis of aminolevulinant that averts Fe metabolism (Aksu et al., 2010)

Zamani et al. (2005) evaluated the effects of supplementing the diet of laying hens with a combination of Zn and Mn on performance traits and egg breakage. Hy-line W-36 layers at 28 weeks of age were used. Laying hens were fed with C-SBM containing 50 mg/kg Zn, and 30 mg/kg Mn. The supplemented minerals were Zn oxide and Mn oxide. The results indicate that Mn and Zn alone, and in combination, reduced the number of broken eggs but did not affect the

number of eggs produced, feed consumption, or feed conversion ratio. In laying hens, Yildiz et al. (2011) evaluated the effects of dietary organic (Mn-Bioplex) and inorganic (Mn sulfate) sources at 5 increasing doses (15, 30, 45, 60 and 75 mg/kg) for 12 weeks on performance, egg quality, and bone mineralization in laying hens. The results reported that organic Mn sources improve growth, egg weight, and bone solidity.

2.1.5 Copper

Copper is essential for growth performance, eggshell membrane formation, eggshell structure, shell texture, and egg shape (Favero et al., 2013a; Wang et al., 2014). Copper also is necessary for bone formation, especially for cartilage structure (Leeson et al., 2001). Also, Cu has antimicrobial properties and improves intestinal health, which has a significant influence on intestinal microbiology and pathogenic bacteria growth. Copper is essential for gut health (Ewing et al., 1998; Nollet et al., 2007; Wang et al., 2014). Copper is related to the synthesis of hemoglobin, erythrocyte and other plasma proteins necessary for lipid metabolism, hepatic lipogenic enzyme activities, and the maturation of hematopoietic cells (Ewing et al., 1998; Aksu et al., 2010; Favero et al., 2013a). Moreover, Cu can affect the rate of cholesterol biosynthesis and hepatic glutathione concentrations. Pesti and Bakalli (1996) reported that the addition of 250 mg/kg supplemental Cu from cupric citrate to the diet reduces total plasma cholesterol and increases growth performance. Copper is also a component of blood proteins which is cytochrome oxidase of tissues that are fundamental for metabolic and respiratory functions (Linder, 2013). Likewise, Cu is necessary in certain pigments, such as turacin, a pigment in feathers (Leeson et al., 2001; Linder, 2013).

Anemia is a sign of Cu deficiency in poultry (Leeson et al., 2001; McDowell, 2003). Copper deficiency anemia occurs if supplemental of Cu is not given, that is affected by the

protracted total parental nutrition and chronic diarrhea with severe malnutrition. The origin is the severe neutropenia that precedes the onset of sideroblastic anemia (Linder, 2013). Copper deficiency in laying hens results in abnormal eggshell formation, which includes irregular distribution of the shell membrane. Consequently, the result is increased shell-less eggs and irregular egg size and shape (Mabe et al., 2003; Hafeez, 2015). An excess of Zn intake can result in Cu deficiency. As a result, Cu deficiency results in insufficient Fe utilization in the organism (Watts, 1990; Aksu et al., 2010). The absorption of Cu occurs in the duodenum and is attached to a protein carrier hemocyanin that facilitate diffusion of molecules across the cell membrane (Leeson et al., 2001). Body retention of Cu is approximately 6% in broiler chickens (Peric et al., 2007).

The inorganic Cu sources most commonly used are Cu sulfate pentahydrate and tribasic Cu chloride. Research reports that Cu sulfate pentahydrate is commercially more available and less expensive than tribasic Cu chloride. Also, Cu sulfate pentahydrate is considered to be less reactive and destructive, is less hygroscopic, and is more soluble in water. Other researchers report that tribasic Cu chloride is more bioavailable than Cu sulfate pentahydrate (Ewing et al., 1998; Wang et al., 2014). When Cu sulfate pentahydrate or Cu citrate are added to a diet, lipid metabolism and feed conversion are improved, and total plasma cholesterol is reduced in the young broiler (Pesti and Bakalli, 1996).

Wang et al. (2014) evaluated both Cu and lysine-derived amino acid balance. Their results indicate that the addition of 200 ppm of Cu, in the form of tribasic Cu chloride, enhances growth rate. In laying hens, Pesti and Bakalli (1998) reported that dietary inclusion of 125 mg of Cu decreased egg yolk and plasma cholesterol levels. Old hens fed a Cu deficient diet, 0.7 to 0.9

ppm Cu, had reduced egg production and low levels of plasma, liver, and egg Cu (McDowell, 2003).

2.2 Egg Quality

In the USA, 96.4 billion eggs were produced in 2015, and 561 million were produced in Louisiana (USDA, 2016). Eggshell quality and egg internal quality (Roberts, 2004) are important factors in egg production. The factors that affect egg quality are nutrition, disease, environment, age, and breed of the laying hen (Rabie et al., 1997; Mabe et al., 2003). Additionally, shell breakage and loss of moisture through eggshell pores, which occurs during storage, reduces egg quality. The egg contents are stored in eggshells and the shell is the first barrier against bacteria penetration. Thus, the shell is important in maintaining the nutritional components of the egg for human consumption (Mabe et al., 2003; Zamani et al., 2005). Refrigeration is an effective method of preserving egg quality (Pujols et al., 2014). Currently, egg quality is the most common problem that increases production costs and produces significant losses to the commercial egg industry (Roberts, 2004; Stefanello et al., 2014).

The formation of the egg in laying hens is the result of several steps: ovulation of the yolk, deposition of the albumen, formation of the egg shell (organic matrix), shell calcification, and finally the egg is laid via the cloaca (Larbier and Leclercq, 1992; Roberts, 2004). The three components of the egg are the vitellus or yolk (30-33%), white or albumen (60%), and shell (9-12%) (Larbier and Leclercq, 1992; Stadelman et al., 1995). Quality problems in the egg may arise in any step of the process of formation (Roberts, 2004). For that reason, the diet of laying hens should meet the hen's requirements for energy, amino acids, vitamins, and minerals that are essential for optimum growth, development, egg production, and egg quality (Tang et al., 2006).

Egg quality is measured by physical and chemical properties. These are shell quality, albumen quality, nutritional composition, freedom from defects, yolk pigmentation, and egg size (Larbier and Leclercq, 1992).

2.2.1 Shell Quality

The egg shell is structurally formed by organic and inorganic components which are composed of shell membranes and calcium carbonate crystals, respectively (Stefanello et al., 2014). To measure egg shell quality, there are direct (mechanical properties) and indirect (physical properties) methods. One direct method is a shell breaking strength. Indirect methods are specific gravity, non-destructive deformation, shell thickness, and shell weight (Roberts, 2004).

Egg shell strength is affected by environmental and nutritional factors (Novikoff and Gutteridge, 1949; Larbier and Leclercq, 1992). For the formation of the shells the calcium, vitamin D₃ (aids calcium absorption), and dietary Mn are required to ensure quality of the egg shell. Manganese is necessary for the synthesis of the protein matrix (Larbier and Leclercq, 1992). Shell strength is influenced by the relationship between the amount and thickness of the egg shell. Likewise, egg shell thickness is an essential indicator for shell quality and the major nutrients that are involved are calcium, phosphorus, and vitamin D₃ (Leeson et al., 2001). The shell breaking strength is determined by breaking open an egg, drying it, and then measuring shell weight. In addition, shell thickness is measured using a digital caliper (Roberts, 2004). Specific gravity is an indirect method that obtains the correlation between the amount of shell present and the size of the egg. It is measured by the immersion of the whole egg in salt solutions, of different specific gravity, to determine at what concentration of solution the egg floats (Novikoff and Gutteridge, 1949; Roberts, 2004).

Factors affecting egg shell quality are age and genetics of the hen, induced molt, nutrition, water quality, stress, disease, or type of production system. The diet of laying hens must contain adequate nutrients for optimal shell quality to be achieved (Roberts, 2004). Some minerals are necessary in small quantities to improve egg shell. These include Zn and Mn are essential in hen diets due to their function as cofactors of enzymes in egg shell formation. Proteoglycans and carbonic anhydrase enzyme are the enzymes of Mn and Zn respectively. (Mabe et al., 2003; Roberts, 2004). Also, they are involved in the interaction with the crystals of calcium during shell formation (Mabe et al., 2003; Yildiz et al., 2011; Stefanello et al., 2014). The trace elements Zn, Mn, and Cu, are necessary for the organic matrix of the eggshell and, subsequently, the mechanical properties (Mabe et al., 2003; Stefanello et al., 2014).

2.2.2 Internal Quality

Internally, the egg is a compound of the yolk and the white or albumen. Egg quality is good when it is free from internal blemishes, such as blood spots, pigment spots, or meat spots. Internal egg quality is measured by two methods, yolk quality and albumen quality (Roberts, 2004). The composition of the yolk consists of protein, lipids, water, minerals, vitamins, and small amounts of glucose and amino acids (Larbier and Leclercq, 1992). The majority of minerals are located within the yolk (Larbier and Leclercq, 1992; Angel, 2007; Uni et al., 2012). Yolk quality is determined by two methods, color of the yolk and strength of the perivitelline membrane (Roberts, 2004).

The albumen consists of water and protein, which contains small amounts of minerals, water soluble vitamins, and free glucose (Larbier and Leclercq, 1992). Albumen quality depends on the gel structure of the albumen which is formed from proteins (Leeson et al., 2001). The quality of albumen is calculated from the height of the albumen which is converted into Haugh

units. This is a measure of the viscosity of the thick albumen (Rabie et al., 1997; Scott and Silversides, 2000; Roberts, 2004). Egg storage time and conditions are factors that affect albumen height. Additionally, another method for measuring the effects of storage on egg quality is the rise in albumen pH (Scott and Silversides, 2000). Haugh units are affected by several factors, such as storage time and temperature, hen age, strain of bird, nutrition, disease, dietary supplements, artificial exposure to ammonia, induced molt, and medications (Roberts, 2004).

Sahin et al. (2002) evaluated the effect of chromium (chromium picolinate) and zinc sulfate on egg production and egg quality. The results reported that dietary inclusion of 400 µg of Cr more 30 mg of Zn was higher for Haugh unit.

CHAPTER 3:

EFFECT OF INCREASING LEVELS OF INORGANIC ZINC (ZN) FED TO COMMERCIAL LAYERS ON EGG QUALITY AND ZINC CONTENT

3.1 Introduction

Zinc is essential because it is required for normal growth, development, and health (Leeson et al., 2001). This element is important in cell replication and it is a component of different metalloenzymes (Balnave and Zhang, 1993; Mabe et al., 2003; Hudson et al., 2004; Zamani et al., 2005; Favero et al., 2013a). A Zn deficiency in hens may produce fetal abnormalities or decrease the rate of hatchability of fertile eggs (Aksu et al., 2010; Favero et al., 2013a). Thus, Zn supplementation is needed in poultry diets. The bioavailability of Zn in corn is about 45%, while the bioavailability of Zn in inorganic sources is 100% (Ammerman et al., 1995). There are two inorganic sources of Zn used by the poultry industry: Zn oxide and Zn sulfate (ZnSO_4) monohydrate (Idowu et al., 2011).

A Zn deficiency can cause adverse effects on erythropoiesis in the marrow, reduction of egg production and eggshell quality, and, consequently, reduced hatchability (Aksu et al., 2010; Favero et al., 2013a). Additionally, Zn deficiency can result in poor growth and abnormal bone development in chicks (Leeson et al., 2001).

Therefore, the objective of this research was to evaluate the effect of increasing dietary Zn levels on egg quality and egg Zn content.

3.2 Materials and Methods

All methods used in this investigation were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

3.2.1 Experimental design

An experiment was conducted with 64 Hy-Line W-36 hens that were 48 weeks of age. The trial lasted 30 days. Layers were housed in a tunnel-ventilated house at the Louisiana State University AgCenter Central Research Station Poultry Farm. The cages were metal wire (52x34x30 cm) in double-decker rows providing 520 cm² per hen. Each cage had two nipple waterers. Metal feed troughs were divided by replicate to ensure that the hens were not able to consume feed assigned to adjoining replicates. A divider was inserted into the egg collection area to prevent mixing of eggs from separate replicates. Hens were provided mash form feed and water ad libitum.

3.2.1.1 Treatments

Hens were allotted to one of four treatment diets on day 0 of the experiment. A total of four replicates with four hens per replicate were used. Diets were corn-soybean meal based and formulated to meet the nutrient requirements suggested in the Hy-Line W-36 management guide (Hy-Line, 2014) except for Zn. Diets were formulated to contain 2800 kcal/kg metabolizable energy, 0.82% total lysine, 4.80% Ca and 0.43% non-phytate phosphorus (nPP). The dietary treatments were: 1) corn-soybean meal (C-SBM) with no added Zn (25 mg/kg total Zn from feed ingredients), 2) C-SBM with 50 mg/kg added Zn, 3) C-SBM with 100 mg/kg added Zn, and 4) C-SBM with 150 mg/kg added Zn. Analyzed total Zn was 25, 75, 125, 175 mg/kg of Zn for diets 1,2,3, and 4 respectively. Zinc sulfate (40.5% Zn) was the source of added Zn. The diet compositions and calculated nutrient contents of the treatment diets are in Table 3.1.

Table 3.1 Percentage composition of diets fed to laying hens, as fed basis.

Ingredient, %	Added Zinc treatment (mg/kg)			
	0	50	100	150
Corn	60.68	60.68	60.68	60.68
Soybean meal, 48%	22.92	22.92	22.92	22.92
Soy oil	2.08	2.08	2.08	2.08
DL-Methionine	0.29	0.29	0.29	0.29
Salt	0.50	0.50	0.50	0.50
Mineral premix ¹	0.10	0.10	0.10	0.10
Vitamin premix ²	0.25	0.25	0.25	0.25
Choline chloride ³	0.13	0.13	0.13	0.13
Ethoxyquin	0.10	0.10	0.10	0.10
Monocalcium phosphate	1.58	1.58	1.58	1.58
Limestone	11.58	11.58	11.58	11.58
Zinc sulfate (g)	0	9.3	18.6	27.9
Calculated values				
Non-phytate P, %	0.43	0.43	0.43	0.43
Total P, %	0.66	0.66	0.66	0.66
Ca, %	4.8	4.8	4.8	4.8
Lysine, %	0.82	0.82	0.82	0.82
ME (kcal/kg)	2800	2800	2800	2800

Analyzed values

Total Zinc (mg/kg)	25	75	125	175
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¹ Provided per kilogram of diet: Cu (copper sulfate), 7 mg; I (calcium iodate), 1 mg; Fe (ferrous sulfate•H₂O), 50 mg; manganese (manganese sulfate), 100 mg; Se (sodium selenite), 0.15mg.

² Provided per kilogram of diet: vitamin A, 8,002.78 IU; vitamin D3, 3003.8 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg.

³ Contains 750,000 mg/kg of choline.

3.2.2 Data Collection

3.2.2.1 Performance

Number of eggs produced was recorded during the 3 day collection period. Feed consumption was measured to determine average daily feed intake (ADFI).

3.2.2.2 Egg Quality

Specific gravity, egg weight, albumen height, albumen weight, yolk height, and yolk weight were determined on three consecutive days at the end of the 30 day period. Specific gravity was measured using saline solutions with varying concentrations ranging from 1.060 to 1.095 in intervals of 0.005. Albumen and yolk height were determined using a tripod micrometer (Baxlo Precision, Barcelona, Spain). Egg weight was measured using a balance (Mettler-Toledo GmbH, Greifensee, Switzerland).

3.2.2.3 Chemical Analysis

On day 30, three eggs per replicate were selected for further analysis. From those eggs, one egg (yolk and albumen) was stored. The other two eggs were separated into yolk and albumen, placed in 50 ml tubes, and frozen until analysis. To determine mineral and Zn content, the eggs were defrosted, mixed, and 0.5 g was placed in a plastic high-pressure tube for digestion using 7 ml of nitric acid and 2 ml of hydrochloric acid. The sample digestion was accelerated in a microwave digester using an organic program. After cooling, the samples were transferred to 100 ml flasks and brought to 100 ml volume with deionized water for the homogenization. Then, samples were homogenized. Approximately 2 ml was transferred to disposable vials and the inductively coupled plasma (ICP1) analysis was performed using mass spectrophotometry (Powell et al., 2011).

3.2.3 Statistical Analysis

Data analysis was conducted using the SAS software (SAS-Institute, 2014). All data were analyzed with the MIXED procedure of SAS with treatment as the fixed effect in a completely

randomized design. Linear and quadratic contrasts were determined for the effect of Zn.

Treatments means were separated by the PDIFF option of SAS at α level of $P < 0.05$.

3.3 Results

The effects of increasing levels of dietary Zn on hen performance and egg mineral content are in Table 3.2. Average daily feed intake decreased ($P < 0.05$) with increasing Zn supplementation in hen diets. There was no effect ($P > 0.05$) of dietary treatment on specific gravity, albumen height, albumen weight, or yolk weight. The number of eggs produced and total egg weight decreased linearly ($P < 0.05$) with increasing dietary Zn. Albumen weight increased quadratically ($P < 0.05$) with increasing dietary Zn. Yolk Zn was increased ($P < 0.05$) by increased dietary Zn supplementation. Zinc content in the egg yolk was increased 23% with 150 mg/kg dietary Zn. The Zn content in albumen and the whole egg was not affected ($P > 0.05$) by dietary treatment. Yolk Fe content increased quadratically ($P < 0.05$), and yolk Ca increased linearly with increasing levels of dietary Zn. Yolk sulfur (S) was increased ($P < 0.05$) by increased dietary Zn supplementation. Yolk Cu and Bo content were decreased ($P < 0.05$) with increasing levels of dietary Zn.

3.4 Discussion

Hens fed 0, 50, or 100 mg/kg added Zn had similar ADFI, while hens fed 150 mg/kg added Zn had lower ADFI. Idowu et al. (2011) also reported approximately 5-11% reduction in feed intake of hens fed diets supplemented with Zn compared to the control group that was fed basal diet without Zn supplement.

Idowu et al. (2011) reported that hen day egg production was similar for hens fed ZnSO₄ supplemented diets compared to hens fed Zn proteinate supplemented diets. Similarly, Sahin et

al. (2002) reported that dietary supplementation of 30 mg of ZnSO₄ increases egg weight and specific gravity of laying hens when they are housed in low ambient temperatures. Our results agree with these data for egg weight, but not for specific gravity. Albumen weight was increased quadratically by Zn supplementation. The findings in this study are in agreement with Klecker et al. (2002) who reported that using 40 % of Mn and Zn in chelated form increases albumen weight.

Yolk Ca content linearly increased by increased dietary Zn. This also is in agreement with Klecker et al. (2002) who reported that the addition of dietary Zn increases the utilization of Ca in hens. Additionally, a Zn and Cu antagonism was observed in this study. Our results indicated that yolk Cu content was linearly decreased with increasing levels of dietary Zn. Similarly, Skřivan et al. (2005) reported a Zn-Cu antagonism where the Cu content on the yolk decreased by supplementation with 80 mg/kg dietary Zn. In addition, Zn content of the yolk was decreased by increased dietary Cu (25 mg/kg).

Thus, hens fed more than 100 mg/kg added Zn had reduced egg production, egg mass, and ADFI. Hens can be fed diets supplemented with up to 100 mg/kg added Zn without negatively affecting egg quality or yolk mineral content.

Table 3.2 The effect of increasing levels on dietary Zinc (Zn) on hen performance and egg quality¹

Variable	Added Zinc treatment (mg/kg)				SEM ²	P- Values		
	0	50	100	150		Trt	Linear	Quadratic
ADFI ³ , g/d	71.74 ^a	72.10 ^a	70.99 ^a	68.21 ^b	0.88	0.01	0.01	0.03
Number of Eggs	10.5 ^{ab}	10.8 ^a	9 ^{ab}	8 ^b	0.8	0.12	0.03	0.6
Avg egg weight, g	66.06	66.14	67.67	66.12	1.04	0.65	0.63	0.46
Total egg weight,g	693.2 ^{ab}	709.9 ^a	609.3 ^{ab}	559.8 ^b	50.72	0.09	0.02	0.45
Specific gravity	1.086	1.086	1.085	1.084	0.0012	0.65	0.25	0.78
Albumen height, mm	8.84	8.31	7.89	8.64	0.54	0.54	0.3	0.21

Table 3.2 (continued)

Albumen weight, g	33.58	26.99	30.63	34.58	2.43	0.14	0.44	0.04
Albumen Zn, ppm	9.67	8.23	9.89	11.04	2.14	0.64	0.85	0.74
Yolk weight, g	19.2	18.3	20.2	19.8	0.8	0.42	0.26	0.76
Yolk Zn, ppm	47.82 ^b	48.25 ^b	59.47 ^a	49.05 ^b	3.48	0.03	0.14	0.06
Yolk boron, ppm	41.6 ^a	21.0 ^b	18.9 ^b	11.4 ^b	4.57	0.006	0.002	0.18
Yolk calcium, %	0.15 ^b	0.15 ^b	0.20 ^a	0.16 ^b	0.009	0.01	0.04	0.10
Yolk copper, ppm	6.38 ^a	1.72 ^b	1.59 ^b	ND ⁴	1.28	0.001	0.0007	0.08
Yolk iron, ppm	61.9 ^b	71.2 ^a	73.4 ^a	68.6 ^{ab}	3.07	0.05	0.09	0.02
Yolk magnesium, %	0.013	0.013	0.019	0.013	0.001	0.12	0.37	0.15
Yolk phosphorus, %	0.57	0.55	0.54	0.54	0.01	0.24	0.16	0.39
Yolk potassium, %	0.11	0.11	0.11	0.10	0.003	0.15	0.61	0.53
Yolk sodium, %	0.05	0.05	0.05	0.05	0.002	0.98	0.90	0.92
Yolk sulfur %	0.070 ^b	0.073 ^b	0.079 ^a	0.071 ^b	0.002	0.04	0.23	0.02
Egg Weight	49.97	51.3	50.59	50.45	2.7	0.99	0.98	0.79
Whole egg Zn, ppm	28.95	33.5	26.57	24.85	2.72	0.20	0.11	0.28

¹ Data are means of four replicates per treatment.

² SEM = Standard error of mean.

³ ADFI = Average Daily Feed Intake.

⁴ ND = Not detected.

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

CHAPTER 4:

EFFECT OF INCREASING LEVELS OF DIETARY ZINC (ZN), MANGANESE (MN), AND COPPER (CU) FROM ORGANIC AND INORGANIC SOURCES ON EGG QUALITY AND EGG ZN, MN, AND CU CONTENT IN LAYING HENS

4.1 Introduction

Current reports in the literature indicate that feeding laying hens and broiler breeder hens organic sources of trace minerals can improve egg quality, especially as related to shell quality (Fernandes et al., 2008; Yildiz et al., 2011; Stefanello et al., 2014). Yenice et al. (2015) reported that dietary supplementation of organic Zn, Mn, Cu and Cr for laying hens increases the concentrations of egg Mn, Zn, Cu, and Cr, as well as eggshell Zn and Cr. They reported that an organic Mn, Zn, Cu, and Cr mixture increases the bioavailability of these elements compared to inorganic sources. Hudson et al. (2004) reported that dietary supplementation with both organic and inorganic trace mineral sources, especially Zn, increases absorption sites or transporters in the intestine, increases Zn retention, and improves performance. Favero et al. (2013b) demonstrated that the source of trace minerals affects egg mineral content. This occurs because the egg mineral content was affected by period with an initial increase from 35 to 55 weeks and decrease between 55 to 65 weeks of age.

According to previous research, trace minerals are transferred into the egg from the ovary to the yolk, and from the oviduct to the albumen, shell membrane, and egg shell (Richards, 1997). Additionally, because the yolk is the major storage area of most minerals for the embryo, it is important to study the deposition of minerals into the egg. Uni et al. (2012) reported that the yolk contains the majority of the Mn and P in eggs.

Inorganic trace minerals are the most common source of trace minerals supplemented in poultry diets. There is growing interest in the evaluation of the combination of inorganic and organic trace mineral sources in laying hen diets because organic trace mineral supplementation has been reported to improve egg quality, especially shell quality. Therefore, the objectives of this study were to evaluate the effect of feeding increasing Zn, Mn and Cu levels from inorganic and organic sources on egg quality and egg content of Zn, Mn, and Cu.

4.2 Materials and Methods

All methods used in this investigation were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

4.2.1 Experimental design

An experiment was conducted with 600 Hy-Line W-36 hens that were 26 weeks of age. The trial was conducted for 84 days. Layers were housed in a tunnel-ventilated house at the Louisiana State University AgCenter Central Research Station Poultry Farm. The cages were metal wire (60x50x35 cm) in double-decker rows providing 516 cm² per hen. Each cage had two nipple waterers. Metal feed troughs were divided by replicate to ensure that the hens were not able to consume feed assigned to adjoining replicates. A divider was inserted into the egg collection area to prevent mixing of eggs from separate replicates. Hens were provided mash form feed and water ad libitum.

4.2.1.1 Treatments

Hens were allotted to one of five treatment diets on day 0 of the experiment. A total of ten replicates with 12 hens per replicate were used. Each replicate consisted of two adjoining cages with six hens per cage for a total of 12 hens per replicate. Diets were corn-soybean meal

based and formulated to meet the dietary nutrient requirements suggested in the Hy-Line W-36 management guide (Hy-Line, 2015) except for Zn, Mn, and Cu. Diets were fed in two phases during the experiment: Peaking (weeks 26-37 of age) and Layer 2 (weeks 38-41 of age). Diets were formulated to contain 2,900 kcal/kg and 2,894 kcal/kg metabolizable energy in each phase, respectively (Table 4.1). The dietary treatments were: 1) Control (C) with no supplementation of Zn, Mn, or Cu, 2) Zn, Mn, and Cu supplemented at 80-90-8 mg/kg from inorganic sources (80-90-8 ITM), 3) Zn, Mn, and Cu supplemented at 80-90-8 mg/kg from inorganic and organic sources (40-45-4 from inorganic and 40-45-4 from organic sources; 80-90-8 ITM+OTM), 4) Zn, Mn, and Cu supplemented at 160-175-16 mg/kg from inorganic sources (160-175-16 ITM), and 5) Zn, Mn, and Cu supplemented at 160-175-16 mg/kg from inorganic and organic sources (80-87.5-8 from inorganic and 80-87.5-8 from organic sources; 160-175-16 ITM+OTM). The inorganic sources of Zn, Mn, and Cu were Zn sulfate, Mn sulfate and Cu sulfate. The organic sources of Zn, Mn, and Cu were Availa-Zn, Availa-Mn, and Availa-Cu (Zinpro Corporation®, Eden Prairie, MN). The supplemental mineral content of the dietary treatments is in Table 4.2.

Table 4.1 Percentage composition of control diets fed to laying hens, as fed basis.

Feeding Phase	PEAKING First egg until production drops 2% below peak	LAYER 2 2% below peak to 90% of production
Weeks of age	26 - 37	37 - 41
Ingredient%	%	%
Corn	46.29	57.48
Soybean meal, 48%	32.44	24.87
Limestone	11.39	10.46
Poultry fat	5.99	3.89
Monocalcium phosphate	2.21	1.83
Salt	0.49	0.43
Corn starch ¹	0.40	0.40
Vitamin premix ²	0.25	0.25
Mineral premix ³	0.10	0.10
DL-Methionine	0.33	0.22
Choline chloride ⁴	0.05	0.05

Table 4.1 (continued)

L-Threonine	0.06	0.02
Calculated composition		
ME (kcal/kg)	2900	2894
Ca, %	4.94	4.48
P, %	0.58	0.49
Non-phytate P, %	0.6	0.6
Total amino acids, %		
Lysine	1.05	0.86
Methionine	0.60	0.41
Methionine + Cysteine	0.91	0.74
Threonine	0.79	0.64
Tryptophan	0.24	0.20
Arginine	1.07	0.87
Isoleucine	0.80	0.66
Valine	0.93	0.76

¹ Organic trace minerals were provided at the expense of corn starch.

² Provided per kilogram of diet: vitamin A, 8,002.78 IU; vitamin D3, 3003.8 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg.

³ Provided per kilogram of diet: I (calcium iodate), 1.25 mg; Fe (ferrous sulfate•H₂O), 50 mg; Se (sodium selenite), 0.3 mg.

⁴ Contains 750,000 mg/kg choline.

Table 4.2 Supplemental mineral content of dietary treatments.

Item, g	Treatments				
	CONTROL	80-90-8 ITM	80-90-8 ITM+OTM	160-175-16 ITM	160-175-16 ITM+OTM
ITM					
ZnSO ₄	-	102.31	51.15	204.62	102.31
MnSO ₄	-	127.69	63.84	248.28	124.14
CuSO ₄	-	14.53	7.26	29.06	14.53
OTM					
Availa Zn	-	-	151.33	-	302.67
Availa Mn	-	-	255.38	-	496.56
Availa Cu	-	-	18.16	-	36.32

4.2.2 Data Collection

4.2.2.1 Performance

Egg production was recorded daily. Feed consumption was recorded for each 28 day period to determine average daily feed intake (ADFI) and eggs/kg of feed.

4.2.2.2 Egg Quality

Specific gravity, egg weight, egg shell thickness, shell weight, albumen height, yolk height, and yolk width were determined on three consecutive days at the end of each 28 day period. All eggs from each pen were collected on the three consecutive days at the end of each 28 day period.

Specific gravity was measured using saline solutions with varying concentrations ranging from 1.060 to 1.095 in intervals of 0.005. Eggshell thickness (mm) was measured at one point on the egg's equatorial zone using a digital caliper (General Tools & Instruments, NY, USA).

Haugh units were calculated by the following formula: $\text{Haugh unit} = 100 \log (H + 5.57 - 1.37 W^{0.37})$. Where: H is albumen height (mm) and W is egg weight (g). Albumen height was measured with a tripod micrometer (Baxlo Precision, Barcelona, Spain) and egg weight was measured using a balance (Mettler-Toledo GmbH, Greifensee, Switzerland).

Yolk index was calculated by the following formula: $\text{Yolk Index} = (\text{Yolk Height (mm)} / \text{Yolk width (mm)})$. Yolk height was measured with a tripod micrometer (Baxlo Precision, Barcelona, Spain) and yolk width was measured using a digital caliper (General Tools & Instruments, NY, USA).

4.2.2.3 Chemical Analysis

On the second day of each three-day collection period, three eggs per replicate were selected for further analysis. The three yolks were separated from the albumen, placed in 50 ml

tubes, and frozen until analysis. Prior to analysis, the egg yolks were defrosted, mixed, and 0.5 g was placed in a plastic high-pressure tube for digestion using 7 ml of nitric acid and 2 ml of hydrochloric acid. The sample digestion was accelerated in a microwave digester using an organic program. After cooling, the samples were transferred to 100 ml flasks and brought to 100 ml volume with deionized water for the homogenization. Approximately 2 ml was transferred to disposable vials and the inductively coupled plasma (ICP1) analysis was performed using mass spectrophotometry (Powell et al., 2011).

4.2.3 Statistical Analysis

Data analysis was conducted using the SAS software (SAS-Institute, 2015). All data were analyzed by ANOVA as a completely randomized design using the GLM procedure in SAS. The two adjoining cages, containing twelve layers, was the experimental unit. Treatment means were separated by the LSD option of SAS at α of $P < 0.05$.

4.3 Results

The effects of increasing levels of dietary inorganic and organic Zn, Mn, and Cu supplementation on performance parameters of laying hens are presented in Table 4.3. During the first 28 d collection period, ADFI was improved ($P < 0.05$) for hens fed treatment diets containing a combination of ITM+OTM or ITM 160-175-16 mg/kg of Zn, Mn, and Cu. During the third 28 d collection period, ADFI decreased ($P < 0.05$) in hens fed ITM+OTM 80-90-8 mg/kg of Zn, Mn, and Cu. During the first 28 d collection period, eggs/kg of feed was greater ($P < 0.05$) for hens fed control diet or ITM 80-90-8 mg/kg of Zn, Mn, and Cu. During the third 28 d collection period, eggs/kg of feed was improved ($P < 0.05$) for layers fed ITM+OTM 80-90-8 mg/kg of Zn, Mn, and Cu. The differing dietary Zn, Mn, and Cu did not affect ($P > 0.05$) the

number of eggs, egg weight, and hen day production (expressed in %) during any of the collection periods during the trial.

Table 4.3 Effect of dietary supplementation of inorganic (ITM) and organic (OTM) Zn, Mn and Cu on the performance of laying hens¹.

Supplement (mg/kg)					Response Criteria ²				
Zn	Mn	Cu	Sources	Month	Number of Eggs	ADFI (g)	Eggs / kg of feed	Egg wt (g)	HDP %
0	0	0	-	1	318.90	110.55 ^c	8.59 ^a	60.34	94.91
80	90	8	ITM		321.40	110.58 ^c	8.67 ^a	59.86	95.65
80	90	8	ITM+OTM		317.00	115.20 ^b	8.19 ^b	58.21	94.35
160	175	16	ITM		321.70	117.95 ^a	8.12 ^b	59.68	95.74
160	175	16	ITM+OTM		317.20	117.89 ^a	8.01 ^b	60.35	94.40
SEM ³					3.63	0.91	0.12	1.02	1.08
P-Value					0.82	<0.001	0.00	0.57	0.82
0	0	0	-	2	309.40	111.68	8.25	61.55	92.08
80	90	8	ITM		310.00	109.78	8.42	61.31	92.26
80	90	8	ITM+OTM		309.40	107.49	8.58	61.35	92.08
160	175	16	ITM		310.10	111.63	8.27	60.46	92.29
160	175	16	ITM+OTM		314.40	109.25	8.58	61.57	93.57
SEM					2.27	1.30	0.12	0.34	0.68
P-Value					0.49	0.14	0.16	0.15	0.49
0	0	0	-	3	322.40	112.52 ^a	8.53 ^b	60.86	95.95
80	90	8	ITM		326.10	110.61 ^a	8.80 ^b	61.31	97.05
80	90	8	ITM+OTM		327.30	104.78 ^b	9.30 ^a	61.12	97.41
160	175	16	ITM		326.20	111.55 ^a	8.71 ^b	60.75	91.08
160	175	16	ITM+OTM		327.40	110.07 ^a	8.87 ^b	61.00	97.44
SEM					3.07	1.31	0.14	0.34	0.91
P-Value					0.78	0.00	0.00	0.80	0.78

¹ Data are means of 10 replicates with 12 hens per replicate.

² Response criteria: Number of eggs, Average Daily Feed Intake (ADFI), Eggs produced per kilogram of feed, Average egg weight (Egg wt), Hen day production (HDP).

³ SEM = Standard error of mean.

^{abc} Means within a column with different superscripts are different (P<0.05).

The egg quality parameters are presented in Table 4.4. Egg shell thickness, shell weight, yolk height, and yolk width were not affected (P>0.05) by increasing levels of Zn, Mn, and Cu

supplementation from organic and inorganic sources. In the third 28 day collection period, albumen height was higher ($P<0.05$) in eggs laid by hens fed the Control diet than eggs from hens fed a combination of ITM+OTM or ITM 160-175-16 mg/kg of Zn, Mn, and Cu. In the same period, specific gravity was greater ($P<0.05$) for hens fed 160-175-16 mg/kg of Zn, Mn, and Cu from ITM than from hens fed 80-90-8 mg/kg from ITM or a combination of ITM+OTM 160-175-16 mg/kg of Zn, Mn, and Cu. Yolk index, Haugh units, and egg shell (expressed in %) were not affected ($P>0.05$) by increasing levels of Zn, Mn, and Cu supplementation (Table 4.5).

Table 4.4 Effect of dietary supplementation of inorganic (ITM) and organic (OTM) Zn, Mn and Cu on egg quality of laying hens¹

Supplement (mg/kg)					Response Criteria ²					
Zn	Mn	Cu	Sources	Month	SG	EST (mm)	SW (g)	AH (mm)	YH (mm)	YW (mm)
0	0	0	-	1	1.0875	0.37	8.36	10.10	19.15	40.30
80	90	8	ITM		1.0874	0.37	8.22	10.11	19.11	40.19
80	90	8	ITM+OTM		1.0874	0.37	8.27	10.10	19.20	40.22
160	175	16	ITM		1.0872	0.37	8.23	9.90	19.02	40.27
160	175	16	ITM+OTM		1.0868	0.37	8.33	9.94	19.13	40.31
SEM ³					0.00	0.03	0.31	0.64	0.57	2.83
P-Value					0.58	0.99	0.31	0.58	0.79	1.00
0	0	0	-	2	1.0852	0.33	8.42	9.37	18.86	42.19
80	90	8	ITM		1.0961	0.32	8.33	8.94	18.94	42.30
80	90	8	ITM+OTM		1.1316	0.33	8.30	9.19	18.91	42.35
160	175	16	ITM		1.1221	0.45	8.26	9.14	23.18	42.39
160	175	16	ITM+OTM		1.0851	0.33	8.38	8.94	19.38	42.48
MSE					0.09	0.31	0.53	1.00	10.93	0.94
P-Value					0.12	0.41	0.80	0.41	0.48	0.81
0	0	0	-	3	1.0853 ^{ab}	0.26	8.11	8.53 ^a	18.08	41.70
80	90	8	ITM		1.0851 ^b	0.26	8.08	8.27 ^{ab}	18.05	41.48
80	90	8	ITM+OTM		1.0853 ^{ab}	0.33	8.16	8.32 ^{ab}	18.09	41.70
160	175	16	ITM		1.0859 ^a	0.34	8.08	8.17 ^b	17.97	41.58
160	175	16	ITM+OTM		1.0847 ^b	0.25	8.11	8.19 ^b	17.95	41.37
MSE					0.00	0.29	0.29	0.53	0.41	1.41
P-Value					0.05	0.64	0.81	0.05	0.51	0.92

¹ Data are means of 10 replicates with 12 hens per replicate.

² Response criteria: Specific gravity (SG), Egg shell thickness (EST), Shell weight (SW), Albumen height (AH), Yolk height (YH), Yolk width (YW).

³ SEM = Standard error of mean.

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

Table 4.5 Effect of dietary supplementation of inorganic (ITM) and organic (OTM) Zn, Mn and Cu on internal egg quality of laying hens¹

Supplement (mg/kg)					Response Criteria		
Zn	Mn	Cu	Sources	Month	Yolk Index	Haugh Unit ²	Egg Shell %
0	0	0	-	1	0.48	99.53	13.87
80	90	8	ITM		0.48	99.70	13.74
80	90	8	ITM+OTM		0.48	99.59	13.73
160	175	16	ITM		0.47	98.85	13.79
160	175	16	ITM+OTM		0.48	98.89	13.80
SEM ²					0.04	2.87	0.46
P-Value					0.96	0.66	0.79
0	0	0	-	2	0.38	95.89	13.67
80	90	8	ITM		0.37	93.83	13.58
80	90	8	ITM+OTM		0.38	95.03	13.53
160	175	16	ITM		0.48	94.85	13.66
160	175	16	ITM+OTM		0.39	93.82	13.61
SEM					0.29	4.49	0.68
P-Value					0.56	0.34	0.92
0	0	0	-	3	0.44	91.93	13.33
80	90	8	ITM		0.44	90.43	13.19
80	90	8	ITM+OTM		0.43	90.84	13.36
160	175	16	ITM		0.43	90.12	13.31
160	175	16	ITM+OTM		0.43	90.09	13.31
SEM					0.02	2.80	0.42
P-Value					0.99	0.07	0.59

¹ Data are means of 10 replicates with 12 hens per replicate.

² The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: $100 \log (H + 5.57 - 1.37 W^{0.37})$. Where: H is albumen height (mm) and W is egg weight (g).

² SEM = Standard error of mean.

The Zn, Mn, and Cu concentrations in the egg yolk are presented in Table 4.6. During the first 28 day collection period, yolk Zn was highest (P<0.01) for hens fed 160-175-16 mg/kg of

Zn, Mn, and Cu from ITM+OTM. In the same period, yolk Mn was higher ($P<0.05$) for hens fed 160-175-16 mg/kg of Zn, Mn, and Cu from ITM+OTM, 80-90-8 ITM, or 160-175-16 ITM than for hens fed Control. Also, yolk Mn increased with increasing levels of ITM+OTM. Yolk Cu was greater ($P<0.05$) for yolks from hens fed 80-90-8 ITM than for yolks from hens fed Control. During the second and third 28 day collection periods, no differences were observed in yolk Zn, Mn, or Cu as dietary inclusion of Zn, Mn, and Cu increased ($P>0.10$).

Table 4.6 Yolk content of copper (Cu), manganese (Mn), and Zinc (Zn) of eggs from hens fed increasing inorganic (ITM) and organic (OTM) Zn, Mn, and Cu¹.

Supplement (mg/kg)			Sources	Month	Response Criteria		
Zn	Mn	Cu			Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
0	0	0	-	1	30.76 ^b	0.63 ^c	1.42 ^b
80	90	8	ITM		32.97 ^b	1.66 ^{ab}	3.36 ^a
80	90	8	ITM+OTM		32.76 ^b	1.18 ^{bc}	2.17 ^{ab}
160	175	16	ITM		29.52 ^b	1.35 ^{ab}	2.21 ^{ab}
160	175	16	ITM+OTM		47.58 ^a	1.94 ^a	2.64 ^{ab}
SEM ²					7.78	0.77	1.39
P-Value					<0.001	0.01	0.05
0	0	0	-	2	34.46	1.01	2.78
80	90	8	ITM		37.11	1.41	2.85
80	90	8	ITM+OTM		34.85	1.01	2.52
160	175	16	ITM		37.27	1.15	3.62
160	175	16	ITM+OTM		37.40	1.07	4.16
SEM					7.15	0.41	1.94
P-Value					0.81	0.17	0.31
0	0	0	-	3	47.78	1.58	3.15
80	90	8	ITM		48.80	2.07	3.26
80	90	8	ITM+OTM		45.66	2.21	3.87
160	175	16	ITM		44.29	1.34	4.92
160	175	16	ITM+OTM		48.90	1.69	4.50
SEM					7.00	0.78	2.72
P-Value					0.50	0.10	0.53

¹ Data are means of 10 replicates with 12 hens per replicate.

² SEM = Standard error of mean.

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

4.4 Discussion

The objective of this research was to evaluate the effect of increasing levels of dietary Zn, Mn, and Cu from organic and inorganic sources on egg quality and egg Zn, Mn, and Cu content in laying hens. Hens fed Zn, Mn, and Cu from inorganic and organic trace mineral sources had increased ADFI and lower eggs/kg of feed. Zamani et al. (2005) who reported that feed intake was increased by increased Mn and Zn added to the diet in different periods of age. The supplementation minerals were Zn oxide and Mn oxide in levels from 0-0 to 150-90 mg/kg of Zn and Mn. In contrast, Stefanello et al. (2014) reported dietary supplementation of Mn, Zn, and Cu does not affect laying hen production, feed intake, or feed conversion (kg/dz and kg/kg). The supplementation levels for Mn, Zn, and Cu, were, respectively, from 35-30-5 to 125-120-20 mg/kg. Fernandes et al. (2008) reported that feed intake and feed conversion ratio were not affected by supplementation level of organic trace mineral blend per kg of product (Se, 300 mg; Zn, 30 g; Mn, 30 g) compared to inorganic sources.

The egg quality parameters were not affected by the supplementation of Zn, Mn, and Cu from organic and inorganic sources used in the present study. Specific gravity and albumen height, for the third 28 d collection period, were significantly affected. Hens supplemented with 160-175-16 mg/kg of Zn, Mn, and Cu from ITM, 80-90-8 mg/kg of Zn, Mn and Cu from ITM+OTM and control diet had improved egg specific gravity. In agreement, Hudson et al. (2004) reported that providing hens with Zn from a mixture of a Zn amino acid complex and ZnSO₄ (80 ppm zinc from each) optimized egg specific gravity. In contrast, Lim and Paik (2003) reported no effect of supplementary methionine chelates 15% Mn and 17% Zn on egg specific gravity. Also, Fernandes et al. (2008) reported no effect of organic trace mineral blend

supplementation on egg specific gravity. In the current study, albumen height was improved in hens fed the control diet and similar for hens fed the diets with organic and inorganic sources.

Yolk index, Haugh Units, and egg shell percentage were not affected by the addition of Zn, Mn, and Cu to hen diets at any inclusion level or source. Yildiz et al. (2011) also reported no difference in the egg shell weight of hens supplemented with 5 increasing levels of Mn (15, 30, 45, 60 and 75 mg/kg) with organic (Mn-Bioplex) or inorganic (Mn-sulfate) sources. Fernandes et al. (2008) reported no differences in Haugh Units of eggs from hens fed diets supplemented with organic trace mineral blend, and Mabe et al. (2003) observed no beneficial effects of dietary supplementation of C-SBM diets with 30-30-5 and 60-60-10 mg/kg of Zn, Mn, and Cu, respectively, from inorganic or organic sources on egg shell percentage. By contrast, Stefanello et al. (2014) reported a linear increase in the percentage of eggshell in hens fed increasing levels of Mn, Zn, and Cu, were, respectively, from 35-30-5 to 125-120-20 mg/kg. Zamani et al. (2005) suggest that the addition of combined Zn oxide and Mn oxide at levels from 0-0 to 150-90 mg/kg of Zn and Mn in hen diets increases egg shell percentage.

These results indicated that Zn, Mn, and Cu content in the egg yolk during the first 28 day collection period were increased by dietary Zn, Mn and Cu from ITM+OTM 160-175-16 mg/kg. However, ADFI was increased when the laying hens were supplemented with a combination of ITM+OTM or ITM 160-175-16 mg/kg of Zn, Mn, and Cu. Thus, the increased deposition in the yolk may be due to the increased ADFI.

During incubation, the minerals for the embryo, which are phosphorus, Zn, Cu, Mn, and Fe, are located in the yolk (Uni et al., 2012). According to previous research, as well as our results, Zn content in the egg yolk may be increased by the inclusion of a combination of ITM+OTM in layer diets. Favero et al. (2013b) observed that hens fed inorganic trace mineral

sources in combination with organic trace minerals have increased Zn, but not Mn and Cu content in the egg yolk. Hudson et al. (2004) reported that breeder hens fed organic Zn have increased egg Zn content. Thus, feeding hens diets supplemented with inorganic and organic sources of Zn, Mn, and Cu may increase yolk content of Zn, Mn, and Cu.

CHAPTER 5: SUMMARY AND CONCLUSIONS

The objectives of this research were to evaluate the effect of feeding increasing levels of dietary Zn, Mn and Cu from inorganic and organic sources on egg quality and egg content of Zn, Mn, and Cu in laying hens.

In evaluating the effect of feeding increasing Zn levels on the content of Zn and other minerals in eggs, the results indicate that feeding hens more than 150 mg/kg dietary Zn reduced egg production and egg mass. Additionally, ADFI decreased with increasing Zn supplementation. Yolk Zn increased 23% when hens were fed 150 mg/kg dietary Zn. Iron and calcium content of the egg increased with increasing levels of Zn, and egg Cu and Bo decreased.

Zinc and Mn content in the egg yolk increased when hens were fed ITM+OTM 160-175-16 mg/kg of Zn, Mn, and Cu from ITM+OTM. But, supplemental Zn, Mn, and Cu did not affect internal egg quality (Haugh units, yolk index, or eggshell percentage), egg shell thickness, egg weight, or egg mass. Feed intake and eggs/kg of feed were improved for the first and third 28 day collection period.

Based on the results of this trial, Zn content in the egg yolk may be increased by the inclusion of a combination of ITM+OTM in layer diets. For humans, eggs are a great source of energy, protein and other essential dietary nutrients (vitamins, carotenoids, minerals and certain fatty acids). Furthermore, eggs are an economical ingredient in the human diet. Consequently, further research is needed to determine the potential benefit of consuming eggs with increased Zn content produced from hens fed a combination of inorganic and organic trace minerals.

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