ABSTRACT

MALHEIROS, DIMITRI M. Effect of Microencapsulated Trace Minerals and Dietary Supplementation Level on Broiler Breeder Egg Production, Egg Quality, and Progeny Performance (Under the direction of Dr. Ferket).

The aim of this thesis is to assess the effects of dietary supplementation of lipid microencapsulated inorganic trace minerals (TM) at 100% and 200% of commercial recommendations on broiler breeder production, egg quality, carry over effects on the progeny. At 27 weeks of age, 108 broiler breeder hens Ross 708, and 24 roosters Ross HY were distributed among 12 pens with 9 females and 2 males per floor pen in a temperature and humidity-controlled room. Each pen was assigned to one of 4 treatments consisting of a factorial arrangement of 2 mineral forms (free (FR) and microencapsulated (MI)) and 2 mineral supplement dosages (100% and 200%). Broiler breeder performance was evaluated from 27 to 55 weeks of age (1-27 weeks of lay). At 55 weeks of age, 15 hens per treatment were taken randomly to access jejunum histomorphology, and tibia bone strength and mineral composition. The breeders that were fed the MI TM diets produced 11 more eggs and 15 more chicks/hen than those fed the FR TM diets ($P<.005$) but produced 1.2g lighter chicks at hatch ($P<.05$). There were no differences in the histomorphology parameters with regards to form of TM supplemented, but the 100% dose resulted in greater villus height, crypt depth, and villi area than TM 200% ($P<0.5$). There were no effects on bone weight, length, and bone mass density (BMD), but MITM increased tibia bone thickness, and FRTM supplementation increased tibia ash and Cu contents. Hens fed 200% TM had higher bone Mn content than 100% TM, whereas hens fed MI100% had the highest Fe content. Evidently, feeding MITM to broiler breeders positively impacts egg production rate and feed efficiency, but does not affect their jejunum histomorphology or tibia bone parameters.

At 1, 6, 10, 13, 21, and 27 weeks of lay (WOL), 18 eggs per treatment were collected to evaluate the internal and external egg quality, and mineral composition of eggshell and whole egg. Hens fed the MI TM diets had lower yolk color score at 1 WOL, improved Haugh unit scores at 10 WOL, and lower egg weights at 21 WOL. The higher dietary TM dose increased egg weights at 1 WOL, yolk color at 6 and 2 WOL, and shell strength at 21WOL. Treatment interaction effects were only observed intermittently. The MI100% treatment increasing egg weights, vitelline membrane strength and bending moment at 13 WOL, reduced shell strength 6 WOL. Lastly, FR200% resulted in the highest shell elasticity at 13 WOL. In conclusion, TM premix form or dietary dose had marginally intermittent effects on external or internal egg quality, or mineral composition of hatching eggs of broiler breeders.

To evaluate the progeny performance sixty chicks from each of these 4 broiler breeder treatments were assigned to 10 replicates in alternative design cages, and fed one of 2 dietary levels of MITM (100% or 200%). Broiler performance was accessed at 7, 14, and 21 days. At 21 days jejunum histomorphology, bone characteristics, and bone mineral composition were all assessed and statistically analyzed as a 4x2 factorial. No treatment effects were observed on growth performance. Progeny fed MI100% had higher villi height and greater bone bending moment and lower bone ash than those fed MI200%. Progeny from broiler breeders fed free TM at 200% level had the greatest tibia diaphysis diameter, weight, and ash content. Carry-over effects for tibia ash% and BMD were observed, where progeny from broiler breeders fed free minerals at 100% and 200% and then fed 200% MITM had higher values. There were no treatment effects on tibia bone mineral composition. This study demonstrated broiler breeders fed diets supplemented with microencapsulated trace mineral premix may improve skeletal and

enteric development of progeny, especially when progeny is also fed microencapsulated TM premixes, regardless of supplementation level.

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Effect of Microencapsulated Trace Minerals and Dietary Supplementation Level on Broiler Breeder egg production, egg quality, and progeny performance

by
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APPROVED BY:

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Dr. Kenneth E. Anderson

DEDICATION

I would like to dedicate this work to my family and friends who are always there for me whenever I need a helping hand. My life is nothing without family and friends.

BIOGRAPHY

Dimitri Moraes Malheiros was born in Ribeirao Preto SP, Brazil on December 26th, 1996. He is the son of Ramon Malheiros and Vera Moraes who are both in the poultry research world and have instilled from an early age the principles of hard work, kindness, and good nature onto Dimitri. Through the opportunities provided by the poultry science research field, Dimitri was able to follow his parents to many countries and experience many different aspects of life from the time he was born to the present.

With an interest in poultry from a young age, Dimitri has officially and unofficially worked with his parents during trials throughout high school. Needless to say, that when time to apply for college came knocking, Dimitri applied for the poultry science degree at NC State.

Dimitri finished his bachelor's degree and wanted to pursue higher education in poultry nutrition. Under the direction of Dr. Peter Ferket, Dimitri continued his education in graduate school, where he studied the Effect of Lipid Microencapsulated Trace Minerals on Broiler Breeder Feed and its Effect on Progeny Production and Egg Quality. Dimitri plans to continue his education as a PhD student under the direction of Dr. Kenneth Anderson.

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To my girlfriend Paige, thank you so much for being a part of my life. You are a beautiful ray of sunshine in my life, you are my best friend in this world.

To my parents, who I would not even be in this world without. A huge thank you from the bottom of my heart for always being there for me. With the uncommon mixture of personal and professional life we have created, I am truly happy to call myself your son. I owe everything that I am to my parents and I would be nothing without their support. Words cannot describe how much I love them.

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Chapter 2: Production of Broiler Breeders Fed Diets Supplemented with Conventional Free or Lipid Microencapsulated Premix Forms of Trace Minerals at Standard or High Levels

CHAPTER 1

Literature review

The goal of these studies was to evaluate the effect of Microencapsulated inorganic trace minerals (MITM) on the broiler breeder performance, jejunum histomorphology, tibia bone physical characteristics, and tibia bone composition. In addition to the effects on the broiler breeders, the carry-over effects on the progeny will also be evaluated by looking at performance, jejunum histomorphology, tibia bone characteristics, tibia bone composition. This current body of work also has the objective of evaluating the internal and external egg quality, whole egg mineral composition, and shell mineral composition.

Poultry nutrition is focused on the main dietary components that supply protein, energy, vitamins, and minerals that are formulated into concentrate feed to satisfy animal nutritional requirements (Lopez-Alonzo, 2012; Switkiewicz et al., 2014). Indeed, all of the ingredients in poultry feed are important, but trace minerals (TM) like zinc, copper, manganese, iodine, and selenium hold key roles in the biological functions of animals. These trace minerals are so called due to their small inclusion levels in the feed, which does not diminish their nutritional importance for the animal. Although these minerals are included in the feed in small quantities, it is very important that they are added in the correct quantities and chemical forms so as to meet minimum nutritional requirements for the animal yet not so much as to catalyze oxidative reactions with other dietary components, or adverse toxic effects or excessive environmental emissions. The industry has innovated the mode by which trace minerals are delivered, for example, new products like (MITM) have been hypothesized to diminish losses due to oxidative stress, allowing for better absorption during digestion. As MITM are released slower in the gut they are not contributing to formation of free radicals, which promote oxidative stress, but are

instead released in a more controlled and prolonged mode by which they can be absorbed and can take part as cofactors in antioxidants which help mitigate oxidative stress (Goff, 2018).

Trace minerals (TM) supplementation to poultry feed has been studied for the last several decades as a means to improve the poultry production of eggs, meat, and fertile eggs. The requirements of TM for poultry are not totally defined, and there is considerable variation in research results and recommended levels for all poultry sectors and feeding phases. Almost all research used to estimate TM requirements for poultry was done between the 1950's and 1980's, and very little has been done since that time (NRC, 1994; Oviedo-Rondon, 2012).

The aim of this review is to assess dietary TM supplementation on the performance and health of broiler breeders, their egg quality, and health and growth performance of their progeny. Trace mineral supplementation is important to the broiler breeder because it affects their feathering and skin integrity, their immune function, hormone levels that affect metabolic homeostasis, and reproduction. Broiler breeders have a significant impact on the number and productivity of their progeny as commercial broilers for meat. The egg is the vehicle by which the broiler breeder supplies all of the nutrients necessary for the developing embryo and hatchling chick. The mineral composition and profile of the egg can be modified by TM supplementation of the breeder's diet, which ultimately may induce carry-over effects that produce a more robust chick (Latshaw, 1991).

Dietary TM supplementation can positively affect several economically important production traits for broiler breeders and broilers. TM supplementation has been shown to improve eggshell strength, internal egg quality, deposition of TM in yolk, bone strength, reduce leg problems and foot pad dermatitis, enhance biomechanical properties of bone and skin associated with collagen formation, and modulate immune function and resistance to infectious

diseases. All of these biological functions should be considered as parameters to estimate requirements and bioavailability values rather than just mineral accumulation in bone or tissues (Oviedo-Rondon, 2012). Broiler breeder diets with insufficient TM levels may reduce egg production, impair eggshell quality, and increase embryonic mortality and the production of unviable chicks. In addition, TM nutrition of broiler breeders is important because many of the ingested TM are deposited in the egg yolk (Kidd, 2003; Dibner et al., 2007), and are utilized by embryos for their development, ensuring the hatch of high-quality chicks, which are essential for good broiler production (Araujo et al., 2019).

The absorption of most minerals follows two possible pathways. When fed in high concentrations, many minerals can utilize paracellular absorption, where the mineral diffuses across the tight junction or moves with the bulk flow of water between intestinal epithelial cells to enter the blood. At lower dietary concentrations, the body relies on transcellular absorption to meet its mineral needs. Transcellular absorption requires special transporters to move the mineral from the chyme across the apical membrane of the enterocyte, a method to move the mineral across the cell, and another transporter that will move the mineral across the basolateral membrane of the enterocyte

The absorption of most minerals follows two possible pathways, paracellular absorption and transcellular absorption. When minerals are fed in higher concentrations, many minerals go through paracellular absorption where the minerals diffuse across the tight junction or moves with the flow of the water between intestinal epithelial cells to enter the blood. When minerals are present in lower concentrations, minerals go through transcellular absorption. Transcellular absorption requires specialized transporters to move minerals from the chyme across the apical

membrane of the enterocyte, in order to move the mineral across the cell, and another transporter will move the mineral across the basolateral membrane of the enterocyte (Goff, 2018).

One mineral that plays an integral part in the activation of hundreds of enzymes and plays a major role in egg production in hens is zinc (Zn) (Bhoyar, 2018). Zinc plays a very important role in biological functions like tissue growth and repair, skeletal development, and immune competence (Salim et al., 2008; Abdallah et al., 2009; Richards et al., 2010; Gheisari et al., 2011; Burns, 1983). Because broiler breeders must be maintained for a longer period than commercial meat broilers, life-long fitness is an economically important trait and they are needed to have a competent immune system to resist disease challenges over time and pass that immunocompetence *via* in ovo antibodies to their progeny. Dietary trace minerals like Zn are vital in maintaining a robust immune system throughout production, provided bioavailability is not compromised as is more likely with MITM. Moreover, animals fed Zn deficient diets have a lower humoral response (Burns, 1983). This becomes an issue when broiler breeders that feed-restricted to control body weight consume enough bioavailable Zn to deposit into the egg (Richards and Steele, 1987), predisposing the progeny to a poor immune response (Kidd et al., 1993). Along with zinc, copper, manganese and selenium play important roles in broiler breeder performance and embryo development (Favero et al., 2013). Deficiencies in these minerals will result in low egg production, poor fertility, poor hatchability, reduction in eggshell strength, and bone strength (Favero et al., 2013).

Copper (Cu) is another essential trace mineral for poultry that has many roles in metabolism, most of them related to enzyme function (Richard et al., 2010: Karimi et al., 2011). A primary function of Cu is related to its role in iron (Fe) oxidation, as part of ceruloplasmin, an essential step in Fe absorption and hemoglobin synthesis (Chen et al., 1994; Reeves et al., 2005;

Chen et al., 2006). Needed for the adequate reproductive functions, Cu is a precursor of β-monooxygenase, which catalyzes the hydroxylation of dopamine to norepinephrine needed for production of gonadotropin-releasing hormone. Cu deficiency negatively affects embryo development, resulting in gross structural and biochemical abnormalities (Roychoudhury et al., 2016). Because of the role of Cu in collagen synthesis, an adequate supply of this mineral is also essential for embryo bone development, which will allow for good post-hatch mobility and feeding behavior (Berwanger et al., 2018).

Manganese (Mn) is another an essential nutritional trace mineral for poultry. Mn is essential for embryonic development, body weight gain, bone growth, and reproduction (Olgun, 2017). It is involved in the metabolism of carbohydrates and lipids in which activates enzymes catalyzing biochemical transformations, such as pyruvate carboxylase, glycoside transferase, agmatinase, arginase, glutamine synthetase, and superoxide dismutase (Suttle, 2010). Mn deficiency may result in growth inhibition, reduced blood cholesterol, anemia, thyroid enlargement, and hypogonadism (Costanzoa et al., 2016). In addition, Mn has a very important role in preventing perosis in birds and maintaining egg shell quality (Lu et al., 2007; Subtle, 2010; Bai et al. 2014; Xiao et al., 2014; Xie et al., 2014; Xiao et al., 2015).

Trace minerals also play a very important role in the fertility of broiler breeders, as they are involved in the regulation of many hormone excretions (Peters and Mahan, 2008). Luteinizing hormone (LH) is a key hormone of the female reproductive system. It is produced in the interior hypophysis gland and triggers ovulation. Copper is of major importance in the synthesis of LH by prostaglandin E2 (PGE2), and it also plays an important role in maintaining the concentration of LH and follicle-stimulating hormone (FSH) in serum (Rajeswari and Swaminathan, 2014). FSH is the key hormone in the development and growth of the egg yolks in

the ovaries, so without its proper function infertility would become prevalent. Gonadal hormone receptors (GHR) cannot be properly expressed if zinc is deficient in the diet (Tapiero and Tew, 2003). Cholesterol metabolism is also regulated by manganese as a co-factor; cholesterol is the precursor of many steroid hormones (Xie et al., 2014). Studies with laying hens demonstrated the negative effects of Mn deficiency, as it is associated with the decrease of circulating hormones like progesterone, estradiol, LH, and FSH (Feng and Feng, 1998; Yang, 2008).

Furthermore, on the topic of hormones, iodine has a vital function as a key component in the thyroid hormones. Thyroxine molecule studies have shown that four iodine atoms are attached in the inner and outer tyrosine rings (Suttle, 2010). Thyroid hormones have a role in thermoregulation, increasing cellular respiration and energy production, effect on metabolism, growth, immune defense, and circulation (Hopkins, 1975; Goff, 2018). Thyroid hormones have interactions with insulin, growth hormones, and corticosterone which in turn have effects in the performance of poultry (Menedez et al., 2003). Like all anionic elements, iodine is readily absorbed in the GI tract and allows any iodine secreted prior to absorptive sites to be extensively recycled. Extra thyroidal iodine circulates in small portions in free ionic form which accumulates in soft tissues such as muscle and liver when in excess availability (Downer et al., 1981). In leghorn male studies where iodine was deficient in the diet the testes remained small and without spermatozoa, whereas in hens with their thyroids removed had reduced egg production (Suttle, 2010).

The Nutritional Research Council (NRC, 1994) contains the nutritional guidelines for poultry production, the most current NRC is about two decades old (Wang et al., 2018). A cause for concern in the poultry industry is that the NRC is very outdated and could potentially not reflect the modern-day broilers' nutritional requirements (Wang et al., 2018; Savage, 1968).

There is limited knowledge on the TM requirements of modern-day broilers, unlike other nutrients (Wang et al., 2018). With the rapid genetic selection for meat production, the genetic potential of these broilers is not the same and casts doubt on their modern broiler nutritional requirements (Savage, 1968).

Protection of Chemically Active Trace Minerals

Bioavailability can be defined as "the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal" (Ammerman et al., 1995). If the animals are fed a more bioavailable source of minerals, they will be able to utilize more of that mineral before it is excreted. These free mineral cations are vulnerable to antagonistic complexing with other feed ingredients and nutrients, thereby reducing their bioavailability for utilization by the animal that ultimately increases mineral emissions that may adversely affect environmental sustainability (Underwood and Suttle, 1999).

Trace minerals (TM) in feed are generally supplemented as the inorganic salts. Cation metal trace minerals are electrovalently bound to anions of oxide, sulfate, and in some cases carbonate. In contrast, the iodine anion is electrovalently bound to cations, such as calcium. These inorganic salts can easily dissociate into the free ions in aqueous solutions such as digesta, where they can react with other dietary covalently charged molecules, like other metals, phytate, phosphate, polyphenols or ascorbic acids. These reactions can form complexed compounds that are resistant to dissociation and difficult to absorb or they can change the form of the ion to a non-absorbable state. Additionally, free ions can affect enzymatic activities in the intestinal tract (Oviedo-Rondon, 2012). Inorganic trace minerals are not bound to any matrices and can react with materials in the gut. Feedstuffs and forages can bind to minerals by electrostatic binding or

by trapping the minerals within fiber particles, forming stable mineral complexes that prevent the mineral from being absorbed (Kabaija and Smith, 1988; Laszlo, 1989).

Organic trace minerals

The word chelate is derived from the Greek “chele”, which is synonymous to claw. It is name in such a way due to the structure of chelates. Chelates are the result of electron sharing between a metal and a ligand. A ligand is usually an anion or a molecule that has an atom or pair of electrons with available valences. Chelated minerals have nonmetallic ligands and are considered organic. Atoms that are able to donate electrons are called donor atoms. Ligands with one donor atom are called monodented, and atoms with two or more are called polydents. Only polydents are able to form chelates since they are able to bind a metal in their electronic dents and claws. A coordinate bond, also known as a complex bond, consists of a metal and a ligand aligned in a way that allows for the available electrons from the donor to be close to the electrons of the metal. Mineral chelation is an important part of biological systems as they are necessary by many enzymes as a part of their structure.

Complexed micro minerals have been developed for use in animal nutrition for decades. Primarily the di- and trivalent metals have been chelated or complexed to amino acids and peptides, propionate, acetate, polysaccharides, and picolinate (Goff, 2018). Hydroxy trace minerals are also prepared for nutritional use but are inorganic in form. Organic trace minerals are found to be 1.1-2 times more efficient in absorption than their inorganic counter parts (Spears and Kegley, 2002; Wrightt and Spears, 2004; Pal et al., 2010). It is important to note that not all mineral complexes are absorbed and behave the same way in tests of solubility, and different modes of delivery are more effective than others.

In contrast to inorganic trace minerals, when minerals are bound to chelating agents such as amino acids or hydrolyzed proteins, they become more stable and less reactive in the digestive tract (Pal and Gowda, 2015). The categories for organic trace minerals are defined by the Association of American Feed Control Officials (AAFCO, 1998). The following mineral definitions are all according to Pal and Gowda (2015). Metal (specific amino acid) complexes are the products of complexing a soluble metal salt with a specific amino acid. One of the most common complexes of this kind is zinc methionine, which is created by complexing zinc sulfate and amino acid methionine. Another metal amino acid complexing way is achieved by having a metal ion (like zinc) complexed with several single amino acids. In this case each individual molecule is still one metal ion and one amino acid, but there are a variety of amino acids in the blend. Metal amino acid chelates are another form of organic minerals, where it is formed by the reaction of one mole of a metal ion from a soluble metal salt with two moles of amino acid to form coordinate-covalent bonds. Metal proteinates are achieved by the chelation of a soluble mineral salt with amino acids and/or hydrolyzed protein. The resulting final product may contain single amino acids, dipeptides, tripeptides, or other protein derivatives. Due to its big size and poor stability, metal proteinates can decrease the bioavailability of minerals. Metal polysaccharides complexes are prepared by coating the metal in polysaccharide molecules. These larger molecules based on chains of simple sugars are highly soluble in the digestive tract. Metal propionates are the result of combining soluble metals and soluble organic acids like propionic acid which are highly soluble. Yeast derivative complexes are minerals that are enriched by yeast, the most common is selenium yeast with selenium complexed with a methionine molecule (selenomethionine). All of these complexed minerals are used because they are shown to improve the bioavailability and increase the delivery of these minerals to the animal. These

minerals are more efficient because they do not react with other feedstuffs like inorganic trace minerals and are more bioavailable due to the pathways that they are delivered in the gut.

Encapsulated trace minerals

The process of encapsulation is where bioactive particles are protected from the environment by enclosing them in a physical barrier coating material. Coating materials can be made of various fats, waxes, carbohydrates, proteins, and synthetic compounds. Products that are encapsulated are cleverly designed to slowly release the enveloped compounds (like trace minerals) depending on the conditions to which they are exposed. The most common conditions that affect the release of the encapsulated materials are pH, temperature, and time (Poshadri and Kuna, 2010). Trace minerals that are complexed or encapsulated by organic compounds are more available than non-protected inorganic trace minerals that dissociate into chemically reactive cations when they are exposed to low pH aqueous phase in the upper digestive tract (Underwood and Suttle, 1999; Ghasemi et al., 2020). The encapsulation technique used in this manuscript is shown in detail in the Canadian patent WO2018089516 “Encapsulation of nutritional and/or compounds for controlled release and enhancing their bioavailability by limiting chemical or microbial exposure” (Ferket et al., 2017). The patent details “a controlled release lipid matrix consisting of at least one hydrogenated vegetable triglyceride selected from the group consisting of palm butter, sunflower oil, corn oil, rape oil, peanut oil, and soybean oil; or at least one animal triglyceride selected from the group consisting of bovine tallow and swine lard; and one or more nutrients encapsulated within the controlled release lipid matrix, wherein each of the one or more nutrients is selected from the group consisting of a vitamin, a mineral, and an amino acid” (Ferket et al., 2017)

Carriers are commonly added to nutritional blends to make premixes in order to minimize ingredient interactions and improve handling and stability characteristics. These non-nutritional carrier ingredients (like rice hulls, oat hulls, etc.) take up valuable space in diet formulations. With this specific microencapsulation technique, the encapsulation itself serves a nutritional function that allows for space in the formulation to be used for other nutrients. The composition of premixes affects the stability of vitamins, especially in the presence of inorganic trace minerals as they are highly reactive with vitamins reducing their bioavailability. Encapsulation of vitamins and trace minerals allows for premixes to be stored for longer periods and allows for slower vitamin degradation.

Trace Minerals Effects on Broiler breeders

Feeding broiler breeders a well-balanced diet where all of their nutritional requirements are met is very important. With the advancements in technology and nutrition, researchers are not just able to meet the mineral requirements of broiler breeders but are able to change the way these minerals are digested, absorbed, and utilized, making them even more bioavailable to the animal. Broiler breeder nutrition and feeding programs have an effect on fertile egg production and egg nutrient content (Hocking, 2009; Yenice et al., 2015), which has great importance on how robust the progeny will be (Araújo et al., 2019). Progeny growth, feed utilization, bone development, and immunological responses are all positive effects of better broiler breeder nutrition (Leksrisompong, 2010). It is hypothesized that with less antagonism in the gut, dietary supplementation of MITM decreases lipid peroxidation, decreases mineral excretion into the litter, improve footpad quality, improves maternal antibodies of progeny with better responses to vaccination, and improve gut morphology (Oviedo-Rondon et al., 2013).

Trace Mineral Feeding Effects on Progeny

The progeny of broiler breeders are the broilers that are raised for meat production, so better nutrition of these animals has been a popular topic of study in the past years. Nutritionists all over the world have worked tirelessly to formulate the best diet that optimizes the balance and amounts of trace minerals and other nutrients to meet the genetic potential of these broilers for maximum production and welfare. As the upper limit of nutrient intake is determined to optimize reproduction and growth performance characteristics, the influence of trace mineral nutrition of broiler breeders on the carry-over effects on their progeny has become a very important topic of study. In contrast to mammals, poultry have their progeny develop outside of the maternal womb; this creates a challenge for the progeny as everything that the embryo can use to develop has to be present in the egg (White, 1991).

Antagonistic interactions with minerals occur readily in the low pH environment of the upper gastrointestinal (GI), where TM also dissociates due to the acidic environment (Dibner et al., 2007; Underwood and Suttle, 1999). When minerals reach lower parts of the GI tract, the they can bind to other nutrients, phytate, minerals, and fiber which renders the minerals insoluble (Dibner et al., 2007). Insoluble forms of minerals are then excreted as they are not able to be absorbed by the animal. MITM provides stability for the complex in the acidic environment of the upper GI tract and should be resistant to dissociation in the crop, proventriculus, and gizzard, which allows for the TM to be delivered and absorbed by the epithelium of the small intestine (Leeson and Summers, 2001).

Dietary supplementation of minerals to broiler breeders that are more bioavailable, supports better hatching rate and progeny quality (Bhoyar, 2018). Better progeny quality, in this case, is defined by better immunological responses to disease and challenges. Oxidative stress

damage is most commonly observed as compromised gut morphology, and dietary supplementation of organic trace minerals often improves gut morphology. Inorganic trace minerals that catalyze lipid peroxidation in the gut and mucosal membranes can compromise mucosal gut health, supplementation with organic trace minerals has been observed to reduce the negative effects on the gut health. MITM is a possible way to improve gut health as they are hypothesized to behave like organic trace minerals in some ways as they are not fully organic in nature but do contain some aspects of protection offered by the lipid matrix. With changes to the villus height and crypt depth ratio, it has been determined that lower crypt depths indicate that there is less cellular turnover (Parsaie et al., 2007; Choct, 2009). In contrast, lower cellular turnover of the villi is indicative of less damage done to the villi, and with less villi turnover those nutrients can be used by the bird to improve performance (Parsaie et al., 2007; Choct, 2009). With organic trace minerals used as a base comparison, it is hypothesized that MITM fed to broilers will have a similar effect in the gut morphology.

Trace mineral nutrition for broiler breeders, besides supporting requirements of the hen, should supply minerals for embryo development (Richards, 1997; Kidd, 2003, Virden et al., 2003; Calini and Sirri, 2007; Torres et al., 2009). The egg composition is relatively constant (Calini and Sirri, 2007), but the TM content is dependent upon the maternal diet. Deficiency, imbalances, and excesses can affect the embryo development and hatching traits (Whitehead at al. 1985; Surai, 2002; Kidd, 2009). There is some evidence that substantially higher maternal dietary concentrations than are typically recommended or higher bioavailability of selenium, zinc and manganese may improve immune function, growth, and livability of broiler progeny (Richards, 1997; Virden et al., 2003; Kidd 2003, 2009).

Dietary supplementation of trace minerals for broiler breeders have an effect on broiler performance and livability (Bhoyar, 2018; Rebel et al., 2004; Leksrisompong, 2010; Moraes et al., 2011; Sun et al., 2012; Kidd et al., 1992; Kidd et al., 2003; Richards and Steele, 1987). Evidently, a carry-over effect of the maternal diet to progeny has been observed in many studies, attributed to the levels of trace minerals deposited in the egg (Richards and Packard, 1996). This carry-over effect is not observed in production parameters like body weight, but a carry-over effect is observed on livability and improved immunological response (Moraes et al., 2011; Kidd et al., 1992). With better TM uptake by the hen through the use of higher bioavailable sources, the better mineral utilization of those minerals by the embryo will produce more robust chicks (Latshaw, 1991).

Trace minerals play vital roles in growth and development of broiler chickens, not only in the formation of bones but also in the health and maintenance (Echeverry et al., 2016; M'Sadeq et al., 2018). Essential TM , including co-factors of antioxidant enzymatic systems, such as Se as a component of glutathione peroxidase, Zn, Cu, and Mn that activate superoxide dismutase, and Fe as part of catalase, are commonly provided in inorganic forms in poultry diets to meet the bird's nutritional needs (Sun et al., 2015; Wang et al., 2019). But as mentioned earlier, inorganic forms readily dissociate from inorganic salts when exposed to acidic pH in the upper section of gastrointestinal tract and may be antagonistically complexed with other dietary constituents in gastrointestinal tract. This could reduce their availability for absorption and, consequently increase their excretion into soil and water that contributes to the potential environmental pollution (Underwood and Suttle, 1999; Zhu et al., 2019).

Modern-day broilers have been genetically selected for fast growth, greater feed efficiency, and increased muscle development to meet the consumer demand for affordable meat

protein. However, the fast growth rate of modern broilers is often associated with increased incidence of skeletal/muscular abnormalities that adversely affect locomotion and meat quality (Angel, 2007). Skeletal malformations are common problems that are prevalent in the poultry industry due to the skeletal structure of the bird not being able to keep up with the muscular development (Angel, 2007; Dibner et al., 2007, Ferket et al., 2009). Due to the rapid rate of improvements made to the broilers' genetic potential, their growth rate and feed conversion ratio (FCR) have greatly increased. The intense genetic selection for fast growth has exacerbated metabolic problems in these birds (Angel, 2007). These metabolic problems may explain the skeletal problems observed since the birds are not able to properly allocate nutrients between bone development or building muscle mass. Implementing changes in the diet and management of these birds can lessen the prevalence of these conditions observed (Angel, 2007).

Trace Mineral Effects from Hen to Egg

As all of the future broilers come from eggs produced by the broiler breeders, it is important to note the importance of the eggshell and its properties from which the embryo will hatch (Bhoyar, 2018). If there are problems with the egg formation, there will be problems with how the embryo develops and thereby impacts chick growth performance and health. Eggshell quality is one of the most important problems in the poultry industry (Washburn, 1982; Singh and Singh, 2007), as shell quality affects the number of settable eggs and hatchability of those eggs, and consequently the profitability of the breeder-hatchery operation.

In particular Zn, Cu, and Mn have specific roles in shell formation. Deficiencies in these TM have resulted in detrimental effects on the production of quality hatching eggs by a number of authors. Inadequate dietary Zn supplementation decreases egg production and eggshell quality

(Nys et al., 1999) linked to its role as a cofactor of carbonic anhydrase, which is essential for eggshell deposition (Guimarães et al., 2013). This enzyme has its main activity in the shell gland (Zhang et al., 2017) and is involved in eggshell synthesis *via* the catalysis and interconversion of carbon dioxide and water to bicarbonate (Roberts, 2004). The appropriate formation of the shell is essential to maintain an acceptable production of settable eggs, since it provides structure and mechanical protection while serving as a source of calcium and other minerals to the embryo during development (Hunton, 1995; Vieira, 2007).

Copper also plays an important role in eggshell membrane formation, which in turn influences shell structure, texture, and shape. Copper is a cofactor of lysyl oxidase, which catalyzes the crosslinking of collagen and elastin in the membranes, and a deficiency can result in eggshell deformities (Baumgartner et al., 1978).

Manganese-deficient hens are reported to produce eggs with thinner shells with an alteration in the structure (Leach and Gross, 1983), reflecting the role of the minerals in the synthesis of chondroitin sulfate.

Selenium functions as a cofactor in the antioxidant function of enzymes like glutathione peroxidases and forms of thioredoxin reductase (Ferguson et al., 2011, Spears et al., 2007, Rotruck et al., 2010). In addition to its antioxidant function, Se affects internal and external egg quality (Fernandes et al., 2008). Pappas et al. (2005) showed that Se additions to layer diets mitigate the reduction of Haugh units during egg storage. Some studies concluded that adding selenium increased laying performance and egg quality (Skrivan et al., 2006; Sahin et al., 2008), whereas others reported no effects on laying performance and egg quality after supplementing selenium in diets (Gravena et al., 2011; Invernizzi et al., 2013). Asadi et al. (2017) reported that organic Se increased Se deposition in the egg and improved egg quality compared with the other

sources of Se (Asadi et al., 2017). Some researchers indicated Se yeast might be a superior organic source of selenium compared to other selenite sources because of the better utilization and absorption by the animal (Delezie et al., 2014, Attia et al., 2010, Han et al., 2017, Utterback et al., 2017). Qu et al. (2017) reported that adding selenium nanoparticles to the feed of laying hens had no effect on egg weight, Haugh units, yolk color, eggshell strength, and eggshell thickness. However, Se deposition in eggs from laying hens fed with different selenium sources and levels has not been clarified. Selenium is an important trace element with a well-established antioxidant function and mechanism (Asadi et al., 2017).

Trace mineral nutrition is important not only when looking at the quantity of the minerals fed, but also depending on which form they are supplemented and how available they are to the animal. The broiler breeder diet directly influences the mineral content of the egg, depending on nutrient concentration in the diet the hen deposits different amounts of trace minerals in the yolk and albumen to be used by the chick (Richards and Packard, 1996). Diets fed to broiler breeders with insufficient trace mineral levels have a negative impact on egg production, eggshell quality, increase embryonic mortality, and increase in unviable chicks (Araújo et al., 2019). Although some studies have contradictory results which indicate that maternal diet does not influence the fertility and hatchability of eggs (Hudson et al., 2004), there is a lot of literature that supports the findings that maternal diet does influence these traits. This discrepancy could be due to the variability of the minerals studied and how the data was analyzed.

In conclusion, TM nutrition has a significant impact on the health and productivity poultry. It is hypothesized that MITM will have an impact on the absorption capability of trace minerals. It will be less susceptible to trace mineral antagonism in the upper gut, and then be slowly released as the microencapsulation lipid matrix is digested in the small intestine where

absorption occurs by active transport. Another proposed hypothesis, is that higher levels of trace minerals are needed in order to meet the broiler breeders nutritional requirement as the most current NRC is outdated and may not reflect the modern broiler breeders requirements; furthermore, broiler breeders are on a skip a day feeding schedule thus making the hens go a long time without feed. With a slower release of trace minerals in the gut they could be able to absorb more of the trace minerals as they are released slower and further in the gut.

Aim of future chapters:

Antagonistic interactions with inorganic minerals are more prevalent in the low pH environment of the upper gastrointestinal (GI) tract, where ITM dissociate due to the acidic environment (Dibner et al., 2007; Underwood and Suttle, 1999; Virden et al., 2003). When minerals reach lower parts of the GI tract, the minerals can bind to other minerals, nutrients, phytate, and fiber which renders the minerals insoluble (Dibner et al., 2007; Richards et al., 2015; Kratzer, 1986). Insoluble forms of minerals are then excreted as they are not able to be absorbed by the animal.

With the reduced bioavailability of free inorganic trace minerals, the industry feeds broilers a much higher dose of minerals that exceed the animal's nutritional requirements (Swiatkiewicz et al., 2014; Aksu et al., 2010; 17.3), which increases excretion of minerals that have negative environmental impacts (Bao and Choct, 2009; Mezes et al., 2012). This interaction between these minerals is due to the coordinate covalent binding of all of these cations to phytic acid that forms insoluble complexes that cannot be absorbed by the bird (Dibner et al., 2007). MITM on the other hand, are hypothesized to release minerals slower and further in the gut of

the animal thus allowing for better absorption of these trace minerals and therefore less excretion.

HYPOTHESIS 1: We hypothesize that the inclusion of MITM in doses of 100% and 200% will improve the broiler breeder performance, jejunum histomorphology, tibia bone physical characteristics, and tibia bone composition though higher bioavailability and less adverse effects at higher doses in comparison to inorganic trace minerals.

HYPOTHESIS 2: We hypothesize that broiler breeders fed MITM will have a positive effect on internal and external egg quality, whole egg mineral composition, and shell mineral composition due to better mineral bioavailability and better mineral deposition in the eggs.

HYPOTHESIS 3: We hypothesize that there will be a carry-over effect from the broiler breeder fed MITM on progeny with improvements in performance, jejunum histomorphology, tibia bone characteristics, and tibia bone composition. This may be due to better mineral bioavailability and lessened adverse effects from higher dose inclusion of trace minerals. Additionally, we hypothesize that on top of the carry-over effect observed, feeding MITM to broilers will also improve these production parameters due to better bioavailability of trace minerals.

In order to test these hypotheses samples will be collected, and performance data will be analyzed. Broiler breeder egg production and minerals deposited, and its effects on progeny will be studied. Everything analyzed in these experiments is intertwined, the nutritional effects of the trace minerals will impact the amount of minerals deposited in the eggs, which in turn has effects on the pool of minerals the progeny can use to develop in the egg.

REFERENCES

AAFCO. 1998. Official publication of the Association of American Feed Control Officials incorporated (Ed. Paul. M. Bachman). pp. 237-238.

Abdallah, A., O. El-Husseiny, and K. Abdel-Latif. 2009. Influence of some dietary organic mineral supplementations on broiler performance. Int. J. Poult. Sci. 8:291–298.

Ammerman, C. B, D. H. Baker, and A.J. Lewis. 1995. Introduction to bioavailability of nutrients for animals. In: Ammerman, C. B, D. H. Baker, and A.J. Lewis, editors. Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. 1st ed. San Diego, CA, USA: Academic Press.

Angel, R. 2007. Metabolic disorders: Limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. App. Poult. Res. 16:138-149.

Araújo, C., R. Hermes, L. Bittencourt, C. Silva, L. Araújo, C, Granghelli, B. Leite. 2019. Different dietary trace mineral sources for broiler breeders and their progenies. Poult. Sci. 98:4716-4721.

Asadi F., Shariatmadari F., Karimitorshizi M.A., Mohiti-Asli M. Comparison of Different Selenium Sources and Vitamin E in Laying Hen Diet and Their Influences on Egg Selenium and Cholesterol Content, Quality and Oxidative Stability. Iran. J. Appl. Anim. Sci. 2017;7:83–89.

Attia Y.A., Abdalah A.A., Zeweil H.S., Bovera F., El-Din A.A.T., Araft M.A. Effect of inorganic or organic selenium supplementation on productive performance, egg quality and some physiological traits of dual-purpose breeding hens. Czech J. Anim. Sci. 2010;55:505–519. doi: 10.17221/1702-CJAS

Bai, S., L. Huang, Y. Luo, L. Wang, X. Ding, J. Wang, and K. Zjang. 2014. Dietary manganese supplementation influences the expression of transporters involved in iron metabolism in chickens. Biol. Tace. Ele. Res. 160:352-360.

Berwanger, E., S. L. Vieira, C.R. Angel, L. Kindlein, A. N. Mayer, M. A Ebbing, and M. Lopes. 2018. Poult. Sci. 97:2785-2797.

Bhoyar A., Why trace mineral supplements are pivotal for breeder efficiency. (2018). Retrieved February 14, 2021, from https://www.thepoultrysite.com/articles/why-trace-mineral-supplements-are-pivotal-for-breeder-efficiency

Bun, S., Y. Guo, F. Guo, F. Ji, and H. Cao. 2011. Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella. Poult. Sci. 90:1220–1226.

Burns, R.B. 1983. Antibody production suppressed in the domestic fowl (Gallus domesticus) by zinc deficiency. Avian Path. 12:141-146.

Calini, F., and F. Sirri. 2007. Breeder nutrition and offspring performance. Rev. Bras. Cienc. Avic. 9: 77-83.

Chen, H., G. Huang, T. Su., H. Gao, Z. K. Attieh, A. T. McKie, G. J. Anderson, and C. D. Vulpe. 2006. Decreased hephaestin activity in the intestine of copper-deficient mice causes systemic iron deficiency. J. Nutr. 136:1236-1241.

Chen. Y., J. T. Saari, and Y. J. Kang. 1994. Weak antioxidant defenses make the heart a target for damage copper-deficient rats. Free Radic. Biol. Med. 17:529-536.

Choct, M. 2009. Managing gut health through nutrition. Br. Poult. Sci. 50:9–15.

Constanzoa, M., L. Scolarob, G. Berlierc, A Marengob, S. Grecchia, C. Zancanaroa, M. Malatestaa, and S. Arpiccob. 2016. Cell uptake and intracellular fate of phospholipidic manganese-based nanoparticles. Int. J. Pharm. 508:83-91.

Delezie E., Rovers M., Van der Aa A., Ruttens A., Wittocx S., Segers L. Comparing responses to different selenium sources and dosages in laying hens. Poult. Sci. 2014;93:3083–3090. doi: 10.3382/ps.2014-04301.

Dibner, J., J. Richards, M. Kitchell, and M. Quiroz. 2007. Metabolic challenges and early bone development. J. App. Poult. Res. 16, 126-137.

Downer, J.V., Hemken, R.W., Fox, J.D. and Bull, L.J. (1981) Effect of dietary iodine on tissue content of the bovine, journal of animal science 52, 413-417

Echeverry, H., A. Yitbarek, P. Munyaka, M. Alizadeh, A. Cleaver, and G. Camelo-Jaimes.2016. Organic trace mineral supplementation enhances local and systemic innate immune responses and modulates oxidative stress in broiler chickens. Poult. Sci. 95:518–527.

Favero, A. S., L. Vieira, C. R. Angel, F. Bess, H. S. Cemin, and T. L. Ward. 2013. Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. J. Appl. Poult. Res. 22:80–91.

Feng, J., and Z. G. Feng. 1998. Effect of Mn-deficiency on reproductive performance in egg-laying chickens. Acta Vet. Zoot. Sin. 29:499–505.

Ferguson L.R., Karunasinghe N., Zhu S., Wang A.H. Selenium and its' role in the maintenance of genomic stability. Mutat. Res. 2012;733:100–110. doi: 10.1016/j.mrfmmm.2011.12.011

Ferket, P. R., J. Fontaine, and J.-D. Bunod. 2017. Encapsulation of nutritional and/or compounds for controlled release and enhancing their bioavailability by limiting chemical or microbial exposure.

Ferket, P., Oviedo-Rondón, E., Mente, P., Bohórquez, D., Santos, A., Grimes, J., Felts, V. (2009). Organic trace minerals and 25-hydroxycholecalciferol affect performance characteristics, leg abnormalities, and biomechanical properties of leg bones of turkeys. Poult. Sci, 88, 118-131.

Ghasemi, H.A., Hajkhodadadi, I., Hafizi, M. et al. Effect of advanced chelate technology based trace minerals on growth performance, mineral digestibility, tibia characteristics, and antioxidant status in broiler chickens. Nutr Metab (Lond) 17, 94 (2020).

Gheisari, A. A., A. Sanei, A. Samie, M. M. Gheisari, and M. Toghyani. 2011. Effect of diets supplemented with different levels of manganese, zinc, and cuprum from their organic or inorganic sources on egg production and quality characteristics in laying hens. Biol. Trace Elem. Res. 142:557–571.

Goff JP. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. J Dairy Sci. 2018 Apr;101(4):2763-2813.

Gravena, R.A., Marques, R.H., Picarelli, J., Silva, J.D.T., Roccon, J., Hada, F.H., Queiroz, S.A. and Moraes, V.M.B., 2011. Supplementation in quail diet with organic minerals on performance and egg quality. Arq. Bras. Med. Vet. Zootec., 63: 1453-1460. https://doi-org.prox.lib.ncsu.edu/10.1590/S0102-09352011000600024

Han X.J., Qin P., Li W.X., Ma Q.G., Ji C., Zhang J.Y., Zhao L.H. Effect of sodium selenite and selenium yeast on performance, egg quality, antioxidant capacity, and selenium deposition of laying hens. Poult. Sci. 2017;96:3973–3980. doi: 10.3382/ps/pex216.

Hocking, P.M. 2009. Feed restriction. In: Hocking, P.M. (ed.), Biology of breeding poultry, pp. 307–330. CABI, Cambridge, MA.

Hopkins, P.S. (1975) The development of the foetal ruminant. Metabolism and digestion of the ruminant. Proceedings of the IVth international symposium on ruminant physiology, 7th edn. University of New England Publishing Unit, Armidale, Australia. Pp 1-14

Hudson, B. P., W. A. Dozier, J. L. Wilson, J. E. Sander, and T. L. Ward. 2004. Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic sources of zinc from hatching to 65 wk of age. J. Appl. Poult. Res. 13:349–359.

Invernizzi, G., Agazzi, A., Ferroni, M., Rebucci, R., Fanelli, A., Baldi, A., Dell'Orto V. and Savoini, G., 2013. Effects of inclusion of selenium-enriched yeast in the diet of laying hens on performance, eggshell quality, and selenium tissue deposition. Ital. J. Anim. Sci., 12: 131-133. https://doi. org/10.4081/ijas.2013.e1

Kabaija, E., and O. B. Smith. 1988. Trace element kinetics in the digestive tract of sheep fed diets with graded levels of dietary fibre. J. Anim. Physiol. Anim. Nutr. (Berl.) 59:218–224

Karimi, A., G. H. Sadeghi, and A. Vaziry. 2011. The effect of copper in excess of the requirement during the starter period on subsequent performance of broiler chicks. J. Appl. Poult. Res. 20:203-209.

Kidd, M. 2003. A treatise on chicken dam nutrition that impacts on progeny. Worlds Poult. Sci. J. 59:475-494.

Kidd, M. T., N. B. Anthony, and S. R. Lee. 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poult. Sci. 71:1201–1206.

Kidd, M.T., N. B. Anthony, L. A. Newberry, and S. R. LEE. 1993. Effect of supplemental Zinc in either a corn soybean or a milo and corn-soybean meal diet on the performance of young broiler breeders and their progeny. Poult. Sci. 72:1492-1499.

Laszlo, J. A. 1989. Effect of gastrointestinal conditions on the mineral-binding properties of dietary fibers. Adv. Exp. Med. Biol.249:133–145.

Latshaw, J. D.1991. Nutrition – mechanisms of immuno-suppression. Veterinary Immunology and Immunopathology. 30:111-120.

Leeson, S., and J. Summers. 2001. Nutrition of the Chicken. 4th ed. Univ Books, Guelph, Ontario, Canada

Leksrisompong, N. 2010. Effects of feeder space and feeding programs during rearing and laying on broiler breeder reproductive performance and livability. North Carolina State University (thesis), Raleigh, NC. 197p.

Lopes-Alonso, M. 2012. Trace minerals and livestock: not too much not too little. ISRN Vet Sci., p. 704825.

Lu, L., X. G. Luo, C. Ji, B. Liu, and S. X. Yu. 2007. Effect of manganese supplementation and source on carcass traits, meat quality, and lipid oxidation in broilers. J. Anim. Sci. 85:812-822.

M'Sadeq, S. A,S. B. Wu, M. Choct, R. A., and Swick. RA. 2018. Influence of trace mineral sources on broiler performance, lymphoid organ weights, apparent digestibility, and bone mineralization. Poult. Sci. 97:3176–3182.

Menedez, C., Baldelli, R., Camina, J.P., Escudero, B., Peino, R., Dieguez, C. and Casanueva, F.F. (2003) TSH stimulates leptin secretion by a direct effect on adipocytes. Journal of Endocrinology 176, 7-12

Moraes, V. M., E. O. Oviedo-Rondón, N.S. Leandro, M.J. Wineland, R.D. Malheiros, and P. Eusebio-Balcazar. 2011. Broiler breeder trace mineral nutrition and feeding practices on Embryo Progeny Development. Avian Biol. Res. 4:122-132.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. NAS-NRC. Washington, D.C.

Olgun, O. 2017. Manganese in poultry nutrition and its effect on performance and eggshell qua;lity. Worlds Pout. Sci. J. 73:45-56.

Oviedo-Rondon, E. O. 2012. Trace minerals for poultry. Minnesota Animal Nutrition Conference. 431p. Http:/conservancy.umn.edu/handle/11299/204341.

Oviedo-Rondon, E., N. M. Leandro, R. Ali, M. Koci, V. Moraes, and J. Brake. 2013. Broiler breeder feeding programs and trace minerals on maternal antibody transfer and broiler humoral immune response1. J. Appl. Poult. Res. 22:499–510.

Pal, D. T., & Gowda, N. S. (2015). Organic trace minerals for improving livestock production. Broadening Horizons, N17.

Pal, D. T., N. K. Gowda, C. S. Prasad, R. Amarnath, U. Bharadwaj, G. Suresh Babu, and K. T. Sampath. 2010. Effect of copper- and zinc-methionine supplementation on

bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. J. Trace Elem. Med. Biol. 24:89–94.

Pappas, A. C., Acamovic, T., Sparks, N. H. C., Surai, P. F., & McDevitt, R. M. (2005). Effects of supplementing broiler breeder diets with organic selenium and polyunsaturated fatty acids on egg quality during storage. Poultry Science, 84(6), 865-74.

Parsaie, S., F. Shariatmadari, M. J. Zamiri, and K. Khajeh. 2007. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers. Br. Poult. Sci, 48:594–600.

Peters, J., and D. Mahan. 2008. Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. J. Anim. Sci. 86:2247–2260.

Poshadri, A., and A. Kuna. 2010. Microencapsulation technology: A review. J. Res. ANGRAU 38:86–102

Qu W., Yang J., Sun Z.Z., Zhang R.H., Zhou F., Zhang K.C., Xia Y., Huang K.H., Mao D.N. Effect of Selenium Nanoparticles on Anti-Oxidative Level, Egg Production and Quality and Blood Parameter of Laying Hens Exposed to Deoxynivalenol. J. Anim. Res. Nutr. 2017;2 doi: 10.21767/2572-5459.100021.

Rajeswari, S., and S. Swaminathan. 2014. Role of copper in health and diseases. Int. J. Curr. Sci. 10:94–107.

Rebel, J.,J. Van Dam, B. Zekarias, F. Balk, J. Post, A. Flores miñambres, and A. Ter Huurne. 2004. Vitamin and trace mineral content in feed of breeders and their progeny: Effects of growth, feed conversion and severity of malabsorption syndrome of broilers. Brit. Poult. Sci. 45, 201-209.

Reevers, P.G., L. C. S. DeMars, W. T. Johnson, and H. C. Lukaski. 2005. Dietary copper deficiency reduces iron absorption and duodenal enterocyte hephaestin protein in male and female rats. J. Nutr. 135:92-98.

Richards, J. D., J. Zhao, R. J. Harrell, C. A. Atwell, and J. J. Dibner. 2010. Trace mineral nutrition in poultry and swine. Asian-Aust. J. Anim Sci. 23:1527-1534.

Richards, M. P., and N. C. Steele. 1987. Trace element metabolism in the developing avian embryo: A review. J. Exptl. Zool. Suppl. 1:39- 51.

Richards, M.P., and M.J. Packard. 1996. Mineral metabolism in avian embryos. Poult. Avian Biol. Rev. 7:143-161

Rotruck J.T., Pope A.L., Ganther H.E., Swanson A.B., Hafeman D.G., Hoekstra W.G. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 2010;179:588–590. doi: 10.1126/science.179.4073.588.

Roychoundhury S., S. Nath, P. Massanyi, R. Stawarz, M. Kacaniova, and A. Kolesarova. 2016. Copper-induced changes in reproductive functions: In vivo and vitro effects. Physiol. Res. 65:11-22.

Sahin, N., Onderci, M., Sahin, K. and Kucuk, O., 2008. Supplementation with organic or inorganic selenium in heat-distressed quail. Biol. Trace. Elem. Res., 122: 229-237. https://doi-org.prox.lib.ncsu.edu/10.1007/s12011-007-8075-6

Salim, H., C. Jo, and B. Lee. 2008. Zinc in broiler feeding and nutrition. Avian Biol. Res. 1:5–18

Singh, J. A., C. Ferrier.,and P. Singh. 2007. Measurement and analysis of the shocks generated during egg production. App. Pack. Res. 2: 45-59.

Skrivan, M., Simane, J., Dlouha, G. and Doucha, J., 2006. Effect of dietary sodium selenite, Se-enriched yeast and Se-enriched Chlorella on egg

Spears J.W., Weiss W.P. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 2008;176:70–76. doi: 10.1016/j.tvjl.2007.12.015.

Spears, J. W., and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80:2747–2752.

Subtle, N.F. 2010. Mineral Nutrition of Livestock (4th ed.,) CAB International Oxfordshire, United Kingdom, pp. 355-376.

Sun, J, D.Liu, R. Shi. 2015. Supplemental dietary iron glycine modifies growth, immune function, and antioxidant enzyme activities in broiler chickens. Livest. Sci. 176:129–134.

Sun, Q. J., Y. M. Guo, S. D. Ma, J. M. Yuan, S. Y. An, and J.H. Li. 2012. Dietary mineral sources altered lipid and antioxidant profiles in broiler breeders and posthatch growth of their offspring. Biol. Trace Elem. Res. 145:318–324.

Suttle, N. F. Mineral Nutrition of Livestock, edited by Suttle, N. 4th;4; ed. Wallingford, Oxfordshire, UK;Cambridge, MA;: CAB International, 2010.

Swiatkiewicz, S., A. Arczewska-Wlosek, and D. J´ozefiak. 2014. The efficacy of organic minerals in poultry nutrition: review and implications of recent studies. Worlds Poult. Sci. J. 70:475–486.

Tapiero, H., and K. D. Tew. 2003. Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed. Pharmacother. 57:399–411.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd Edn., CABI Publishing, Wallingford, UK, Pages: 497.

Utterback P.L., Parsons C.M., Yoon I., Butler J. Effect of supplementing selenium yeast in diets of laying hens on egg selenium content. Poult. Sci. 2005;84:1900–1901. doi: 10.1093/ps/84.12.1900.

Wang, G, L. J. Liu, W. J. Tao, Z. P. Xiao, X. Pei, and B. J. Liu. 2019. Effects of replacing inorganic trace minerals with organic trace minerals on the production performance, blood profiles, and antioxidant status of broiler breeders. Poult. Sci. 98:2888–2895.

Wang, G., L. Liu, Z. Wang, X. Pei, W. Tao, Z. Xiao, T. Ao. 2018. Comparison of inorganic and organically bound trace minerals on tissue mineral deposition and fecal excretion in broiler breeders. Biol. Trace Elem. Res. 189: 224-232.

Washburn, K.W. 1982. Incidence, cause, and prevention of eggshell breakage in commercial production. Poultr. Sci. 61: 205-2012.

White, H. B., III, 1991. Maternal diet, maternal proteins and egg quality. Pages 1–5 in: Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. D. C. Deeming and M.W.J. Ferguson, ed. Cambridge University Press, Cambridge, UK.

Wrightt, C. L., and J. W. Spears. 2004. Effect of zinc source and dietary level on zinc metabolism in Holstein calves. J. Dairy Sci. 87:1085–1091.

Xiao, J. F., S. G. Wu, H. J. Zhang, H. Y. Yue, J. Wang, F. Ji, and H. Qi. 2015. Bioefficacy comparison of organic manganese with inorganic manganese for eggshell quality in Hy-Line Brown laying hens. Poult. Sci. 94:1871:1878.

Xiao, J. F., Y. N Zhang,S. G. Wu, H. J. Zhang, H. Y. Yue, and H. Qi. 2014 Manganese supplementation enhances the synthesis of glycosaminoglycan in eggshell membrane: A strategy to improve eggshell quality in laying hens. Poult. Sci. 93: 380-388.

Xie, J. J., C. H. Tian, Y. W. Zhu, L. Y. Zhang, L. Lu, and X. G. Luo. 2014. Effects of inorganic and organic manganese supplementation on gonadotropin-releasing hormone-I and follicle stimulating hormone expression and reproductive performance of broiler breeder hens. Poult. Sci. 93:959–969.

Yang, Y. 2008. Adjustment of nutrition on the reproduction of poultry. J. Shanxi Agricult. Univ. 39:239–242.

Yenice, E., C. Mizrak, M. Gultekin, Z. Atik, and M. Tunca. 2015. Effects of dietary organic or inorganic manganese, zinc, copper and chrome supplementation on the performance, egg quality and hatching characteristics of laying breeder hens. Ankara. ¨Univ. Vet. Fak. Derg. 62:63–68.

Zhu, Z., L. Yan, S. Hu, S. An, Z. Lv, and Z. Wang. 2019. Effects of the different levels of dietary trace elements from organic or inorganic sources on growth performance, carcass traits, meat quality, and faecal mineral excretion of broilers. Arch. Anim. Nutr. 73:324–337.

CHAPTER 2

Production of Broiler Breeders Fed Diets Supplemented with Conventional Free or Lipid Microencapsulated Premix Forms of Trace Minerals at Standard or High Levels

ABSTRACT

Lipid matrix microencapsulation is hypothesized to improve bioavailability and reduce toxicity of broiler breeders fed diets supplemented with normal and excessive levels of inorganic trace mineral salts. At 27 weeks of age, 108 Aviagen broiler breeder females (Ross 708 and 24 males (Ross HY) were randomly distributed among 12 pens with 9 females and 2 males per floor pen in a temperature- and humidity-controlled room. Each pen was randomly assigned to one of 4 dietary treatments consisting of a factorial arrangement of 2 mineral premix forms (free and microencapsulated) and 2 mineral premix supplement dosages (100% and 200% of Aviagen recommendations): 1) Free 100% (FR100%); 2) Microencapsulated 100% (MI100%); 3) Free 200% (FR200%); and 4) Microencapsulated 200% (MI200%). Broiler breeder performance from 27 to 55 weeks of age eggs were collected twice daily and feed consumption measured weekly. At 54 weeks of age, approximately 200 eggs from each treatment were incubated in order to assess fertility. When the trial was terminated at 55 weeks of age, 15 hens per treatment were chosen randomly to access jejunum mucosal morphology and tibia bone. The breeders that received the microencapsulated trace minerals (MI100%) improved egg production parameters (HD%, HH%, feed conversion (kg feed/dozen eggs), and eggs per hen per week), but produced the lightest chicks at hatch. There we no significant differences observed in the histology parameters with regards to form of TM supplemented, but TM 100% dose greater villus height, crypt depth, and villi area than TM 200% ($P<0.5$). There were no significant treatment effects on

bone weight, length, and bone mass density (BMD). However, MITM supplementation treatments increased tibia bone thickness, and FRTM supplementation increased ash% and Cu content of tibias. Hens fed 200% TM had higher bone Mn content than 100% TM, whereas hens fed MI100% had the highest Fe content. It can be inferred that feeding lipid matrix microencapsulation trace minerals to broiler breeders does have some positive impacts on the production of eggs, but no effects were observed in the histomorphology of the jejunum or tibia bone parameters.

INTRODUCTION

Trace minerals (TM) hold key roles in the biological functions of animals, especially zinc, copper, manganese, and selenium. Trace minerals are so called due to small inclusion levels in the feed, which does not diminish their importance for the animal's performance.

Among trace minerals, zinc (Zn) plays an integral part in the activation of hundreds of enzymes (Bhoyar, 2018) and affects egg production in hens. Zinc nutrition affects biological functions like growth, tissue deposition, tissue repair, skeletal development, and immune competence (Salim et al., 2008; Abdallah et al., 2009; Richards et al., 2010; Burns, 1983; Dibner et al., 2007; Blanchard et al., 2001). Zinc also has antagonistic effects with excess Ca and P in the diet, which can lead to deficiency if the balance between these minerals is not taken into account (Underwood and Suttle, 1999; Cabell and Earle, 1965; Heath et al., 1966; O'Dell, 1984). Because broiler breeders are managed for a longer period than broilers, they require sufficient biological and immunological fitness to resist infectious and non-infectious diseases and challenges throughout their life, but especially during peak egg production. Animals that are raised with a Zn deficient diet have a lower humoral response (Burns, 1983) and compromised DNA and protein synthesis that results in poor growth, poor cell division, and poor keratin and collagen synthesis (Dibner et al., 2007; Blanchard et al., 2001). Gonadal hormone receptors (GHR) cannot be properly expressed if zinc is deficient in the diet (Tapiero and Tew, 2003). Moreover, marginal zinc deficiency of broiler breeders results in reduced deposition into their eggs (Richards and Steele, 1987), predisposing the progeny to compromised development and subsequent immune response (Kidd et al., 1992).

Along with zinc (Zn), manganese (Mn) and copper (Cu) play an important role in the broiler breeder performance and embryo development (Favero et al., 2013). Deficiencies in these

minerals result in low egg production rate, poor fertility, poor hatchability, reduction in eggshell strength, and bone strength (Favero et al., 2013). Copper is of major importance in the synthesis of LH by prostaglandin E2 (PGE2), and it also plays an important role in maintaining the concentration of LH and follicle-stimulating hormone (FSH) in serum (Rajeswari and Swaminathan, 2014; Sakumoto et al., 2014). FSH is the key hormone in the development and growth of the egg yolks in the ovaries, without its proper function infertility would become prevalent. Cholesterol metabolism is also regulated by the presence of manganese, in which cholesterol is the precursor of many steroid hormones (Xie et al., 2014), animals deficient in Mn have impaired reproductive capabilities (Hurley et al., 1984). Studies with layer hens pointed to the negative effects of Mn deficiency in the decrease of circulating hormones like progesterone, estradiol, LH, and FSH (Feng and Feng, 1998).

Minerals also play a very important role in the fertility of broiler breeders as they are involved in the regulation of many hormone excretions, immune defense systems, and intermediary metabolism (Peters and Mahan, 2008; Dieck et al., 2003). Luteinizing hormone (LH) is a key hormone in the female reproductive system, LH is produced in the anterior pituitary gland and triggers ovulation

Because trace minerals are included in the feed in small quantities, it is very important that these minerals are supplemented in the correct quantities and properly mixed throughout the feed as premixes. Conventional trace mineral premixes are blends of inorganic trace mineral salts with a limestone and non-nutritive carrier. However, a novel microencapsulation of nutrient premixes in a nutritive carrier lipid matrix (Ferket et al., 2017) has been shown to improve premix handling and stability characteristics, diminish losses due to oxidative stress, and modulate gut microbiome towards a symbiotic state in commercial broilers (Wedgaertner et al.,

2019). We hypothesized microencapsulation of trace mineral premixes will also benefit the reproductive performance of broiler breeder hens. Oxidative stress is of concern for broiler breeders, as they are fed a very high nutrient diet that causes antagonism. With the use of Zn, Cu, Mn, and Selenium (Se); oxidative stress can be managed using these external antioxidants (Willcox et al., 2004). Moreover, ileum histomorphological assessment revealed that birds fed OTM in the diet had a lower crypt depth and a higher villus height and crypt depth (VH/CD) ratio (Ma et al., 2011). Mucosal villi health is especially important when observing broiler breeders as they experience oxidative stress which affects the villi morphology.

Because of controlled feeding to manage body weight and associated reproductive performance, genetic companies and commercial poultry nutritionists recommend broiler breeders to be fed higher dietary supplementation levels of trace minerals than recommended by NRC (1994) to avoid marginal deficiencies (Favero et al., 2013). But excessive trace mineral supplementation lead to lower mineral bioavailability and potentially decrease animal performance because of marginal toxicity (Evans et al., 2015). Simply increasing the dietary level trace minerals will not have a positive impact on the animal's performance because more antagonism will take effect and negatively impact the animal's ability to properly digest these nutrients, which leads to excessive environmental emission.

Trace minerals not only impact their health and maintenance as a whole, but also have a profound effect in the quality of eggs and progeny robustness (Dibner et al., 2007; M'Sadeq et al., 2018). Early chick performance is influenced by egg source, since egg weight and chick weight at hatching are highly correlated (Halbersleben & Mussehl, 1922). Embryo size before hatching and at hatching may be altered by egg weight and incubation environment, regardless of

the avian species (Wilson, 1991). After hatching, however, the effect of egg weight decreases with the age of the progeny (O'Neil, 1955)

Therefore, supplementation of more bioavailable MITM may support not only the health and maintenance of the breeders but may also serve as a means to improve egg quality, hatchability, and progeny quality. Minerals including zinc (Zn) play an important role in ensuring biological fitness to sustain reproductive performance of broiler breeders. The biological and structural functions of trace minerals can only be realized if they are digestible and nutritionally bioavailable. Dietary supplementation of inorganic forms of trace minerals may form antagonistic complexes with other dietary components, which compromise digestibility and bioavailability and lead to excessive mineral emissions into the litter that is land applied (Bao and Choct, 2009; Mezes et al., 2012). In turn, feeding them protected minerals can increase the uptake and make it more bioavailable to the animal.

Broiler breeder nutrition is a very important aspect of the poultry industry with ultimate effects on broiler meat production. The aim of this present experiment is to evaluate the effect of high and commercial levels of protected and free trace minerals in broiler breeders by assessing production parameters and effects on the gut and skeletal parameters. We hypothesize that the inclusion of MITM in doses of 100% and 200% will improve the broiler breeder performance, jejunum histomorphology, tibia bone physical characteristics, and tibia bone composition though higher bioavailability and less adverse effects at higher doses in comparison to inorganic trace minerals. For this trial, in the protected mineral treatments the vitamins were also protected in order to show that the mineral-vitamin reactions are also diminished but no direct analysis to this effect are presented.

MATERIALS AND METHODS

Housing and Management

All experimental procedures on live animals used in this experiment were approved by the North Carolina State University Animal Care and Use Committee. The birds used in this experiment were obtained when they were 27 weeks of age after being raised by standard husbandry practices for broiler breeder pullets and cockerels. One-hundred-eight Ross 708 females, and 24 Ross HY males were randomly distributed among 12 pens (1.8 m by 1 m; about .163 m^2 per bird) with 9 females and 2 males per pen, so there were 3 replicate pens per treatment. Hens were housed in an environmentally controlled room maintained at about 25°C and 77% relative humidity. Each pen had a nest box with 6 nest spaces. Water was available *ad libitum* by a Plasson bell drinker. Each pen was supplied with a female feeder that had a screen to exclude males from consuming the female feed, and a male feeder raised to a level out of reach for the females to consume the male feed. The amount of feed was issued daily to each feeder in accordance to the breeder recommendations. The floor pen litter was composed of soft pine shavings that was renewed when necessary and supplemented with fresh shavings twice per week or as needed to maintain litter quality and maintain environmental ammonia emission below 10-15 ppm. The broiler breeders received 16 hours of incandescent light (30-60 lux) and 8 hours of dark. Eggs were collected manually twice daily, and egg production was recorded by pen. At 54 weeks, approximately 200 eggs from each treatment (50 eggs per pen) were incubated ChickMaster^{tm} incubator with 1800 eggs maximum capacity in order to assess fertility of eggs set by candling at 10 days of incubation and then transferred to the hatcher at 17 days. After hatch, individual body weights of the chicks were recorded and the percentage of early dead, late dead, and piped was determined for each treatment group.

To test the hypothesis, this experiment was designed to evaluate 4 dietary treatments consisting of a factorial arrangement of 2 vitamin and mineral premix forms (free and protected) and 2 mineral premix supplement dosages (100% and 200% of Aviagen recommendations): 1) Free 100% (FR100%); 2) Microencapsulated 100% (MI100%); 3) Free 200% (FR200%); and 4) Microencapsulated 200% (MI200%). The free premix forms were prepared as typically done by blending free active ingredients with a non-nutritive ground rice hull carrier and limestone. The protected premix form was prepared by microencapsulating active ingredients in a hydrogenated vegetable oil matrix as described by Ferket et al. (2018) and supplied by Jefo, Inc. (Saint Hyacinthe, Quebec, Canada). The dietary treatment feeds were manufactured from a common basal feed that did not include the vitamin and mineral premixes, palmitic acid, and an non-nutritive filler (vermiculite), to which the respective vitamin premixes and filler was added as illustrated in Tables 1, 2 and 3. Palmitic acid was added to the FR200%, FR100% and MI100% diet at an inclusion rate to standardize all diets to contain the same dietary level of hydrogenated vegetable oil as provided by the MI200% diet.

Tables 1, 2, and 3 illustrate the three feed phases for each treatment group, which was formulated to meet or exceed the breeder nutrient recommendation for the respective sexes (Ross Parent stock Handbook, 2018) and supplied to the birds in mash form. The formulation of each dietary treatment for the hen feed was balanced to have identical nutrient composition except for the trace mineral dose level. All roosters received the same balanced diet formulated to meet or exceed the recommendations from AVIAGEN guide of ROSS Broiler Breeders 708 (2700 kcal Metabolizable Energy/kg, 16.2% Crude Protein, 0.58% Ca, and 0.76% P.

Jejunum Mucosal Histomorphology

At the end of the trial when the hens were 55 weeks of age, 5 productive hens were randomly selected form each pen (15 hens per treatment) and euthanized by cervical dislocation. Within 5 minutes of confirmed death of the sampled bird, approximately 5 cm section from the middle of the jejunum (identified as the section between the end of the duodenum loop and the Meckel's diverticulum) were removed opened longitudinally, and fixed immediately in a buffered 10% formalin solution for 48 h. Samples were then washed in 70% ethanol to remove the fixing solution, dehydrated in increasing alcohol concentrations, clarified in xylol, and embedded in paraffin. Semiseriated 5-mm transverse thick histological sections were stained with hematoxilin-eosin (Behmer et al., 1976), and microscope slides were assembled with Canada balsam. Light-microscope (LEICA-DMR; Leica Camera AG, Solms, Germany) was used to visualize stained sections on slides at 4X magnification, and images were captured by Image Tools to measure the villus height (VH), upper villi width, bottom villi width, crypt depth (CD), and muscularis mucosae thickness using AmScope™ x86 software (AMSCOPE, Orange County, CA) . The villi surface was calculated using 10 readings per replicate per variable, according to the formula: VH/CD and villi surface = [(upper villi width + bottom villi width)/2] X villus height in (Solis de los Santos et al., 2005).

Bone mineral composition and characteristics

After the jejunum sample was collected within the critical 5-minute time period after confirmed death for each of 15 hens per treatment as described above, the left tibia was dissected and frozen at -20°C. Then the flesh was removed from the bones, weighed, and measured for

bone length and diaphysis diameter using digital calipers. Scale used had a sensitivity to .01 mm. Bone strength and bending moment were then determined by the TA.HDPlus texture analyzer machine (Stable Micro Systems, Hamilton, MA) with a 250kg load cell. The bone mass density was assessed using dual-energy x-ray absorptiometry (DEXA, SCHICK, accuDEXA BMD Assessment System, Long Island City, NY). The mineral composition of these bones was then determined by the procedure as described by AOAC (1995), and trace mineral composition determined by inductively coupled plasma mass spectrometry (ICP-MS, 5800 ICP-OES, Agilent, Santa Clara, CA).

Statistical analysis

All data were statistically analyzed as a 2x2 factorial randomized design with 3 replicate pens as the experimental unit per treatment group. Data was analyzed by 1-way ANOVA (JMP 15 software; SAS Inst. Inc.) and the means were then statistically distinguished using Tukey's multiple range test. The main and interaction factor effects and treatment group effects were considered significant at $P<.05$.

RESULTS

Full table of results can be found in Table 4. Broiler breeder performance was assessed using HD, HH, kg feed/dozen eggs, eggs per hen per week. There were no significant premix form X trace mineral dose interaction effects nor main effects of trace mineral dose observed on any of the performance measurements. For all of these parameters there were statistically significant ($P<.05$) differences where the microencapsulated form of the supplemented trace minerals offered better values, regardless of the dose. Hen Day (HD) egg production offered

better results for the microencapsulated form of the trace minerals than the free form (71.7% *vs.* 66.2%, P<.005), but no statistically significant differences were observed between the doses or treatments. Hen House (HH) egg production yielded a similar treatment response, where the microencapsulated form offered better results when compared to free trace minerals (71.7% *vs.* 66.0%, P<.005). Microencapsulation significantly improved feed conversion (kg feed/dozen eggs) by about 9%, but supplementation level did not. The number of eggs per hen per week was also significantly (P<.005) improved by 8% for hens supplemented with the microencapsulated than the free premix forms, whereas the supplementation level of trace minerals did not. There was a difference of 11 eggs per hen in favor of the MITM treatments over the FRTM treatments as illustrated in Figure 1. The weights of chicks from hens subjected to the MITM treatments were significantly less than hens subjected to the FRTM treatments (41.9 g versus 43.2 g, P<.05) Percentage egg fertility and hatchability (of fertile eggs set) by treatment combinations are presented in figure 2. Although statistical analysis could not be done, the results indicate that the higher dose of TM supplementation reduced fertility and hatchability.

The effect of premix form and TM dose on the jejunum mucosa histomorphology is presented in Table 5. There were no significant form X dose interaction effects nor premix form effects observed, However, highly significant (P>.005) TM dose effects were observed on villus crypt depth and villus surface area. Hens fed the high dose of TM (200% of industry recommendations) had 25% lower villus height, 23% lower crypt depth, and 31% lower villus surface area than hens fed the standard dose of TM (100% of industry recommendations). Villus tip width was marginally (P<0.15) reduced by the high TM dose. There were no significant treatment effects observed on villus bottom width, villus height:crypt depth ratio, and muscularis thickness.

The effect of premix form and TM dose on the physical characteristics of tibia bone is presented in Table 6. In general, there were no significant form X TM dose effects or TM main effects on tibia bone characteristics observed in sampled hens at the termination of the study period. However, it is noteworthy that there was a marginal treatment interaction effect ($P<0.15$ and $P< 0.20$, respectively) on tibia bending moment and breaking strength. At the normal TM dose level, the MITM treatment increased tibia bone bending moment and breaking strength, but the opposite was observed at the high TM dose. The main effect of TM form on tibia thickness as significant, favoring the MITM treatments over the FRTM treatments (7.82 mm versus 7.62 mm, $P<0.05$). There was only a marginal TM dose effect ($P<0.15$) observed on tibia length, favoring the normal TM dose. There were no treatment effects observed on fresh tibia weight, cortical bone thickness, or bone mass density determined by DEXA.

The effect of premix form and TM dose on tibia bone ash and select mineral content is presented in table 7. P, Ca, and Zn content for the tibia bone of the broiler breeders yielded no statistically significant ($P<.05$) treatment effects. Although there were no significant treatment interaction effect nor main effects of TM dose on percentage bone ash, there was a significant main effect of premix form, favoring the free form over the microencapsulated form (35.8% vs 24.0%, $P<.05$). Likewise, birds fed diets with the free premix form had bones with significantly higher Cu content than those fed the microencapsulated form of premix (.000407 mg Cu/g bone *vs.* .000237 mg Cu/g bone, $P<.05$. Only a significant Form X Dose interaction effect was observed on Fe content in the broiler breeder bones, whereas this interaction effect approached significance for Mn content. At normal TM dose levels, Fe and Mn content of bone increased by microencapsulation, but decreased at high dietary TM dose. Interestingly, only Mn content in the bones of broiler breeders was significantly increased as TM dose increased.

DISCUSSION

This study demonstrated that the most important effect of the broiler breeders fed diets supplemented with MITM was the effect on performance parameters, independent of dietary TM dose. As egg production, feed conversion rate, fertility rate, and hatchability rate was significantly improved by the premix microencapsulation treatments, the benefit protecting reactive inorganic trace minerals in a lipid matrix demonstrate that it is a promising technology for broiler breeders. Indeed, the economic return from the increased production efficiency and chick yield my greatly surpass the added cost of the microencapsulating the premix. This is especially important as these are broiler breeders who produce broilers for the meat industry. With a small investment in the feed mineral ingredient, we can see that these hens have greater egg production, which in turn will result in more broilers for the meat industry. In comparison to the other treatments that had a higher level of minerals or are free mineral diets, the superior breeder hen reproductive performance of the MITM fed hens may be due to less antagonistic effects of inorganic trace minerals. In this trial broiler breeders fed MITM produced a mean of 11 more eggs per hen than hens fed FRTM; this is a substantial difference observed where each hen has an increased potential for progeny production. Additionally, Evans et al. (2015) reported feeding higher levels of trace minerals could potentially decrease animal performance. However, the results reported herein does not fully agree with the findings of Evans et al (2015), as the performance parameters observed were not affected significantly by the dose of TM supplemented. In contrast, egg fertility and chick hatchability indicate that hens fed 100% levels of TM (regardless of form) could lead to better hatchability and fertility in contradiction to Evans et al. (2015) findings. The progeny from hens fed MITM weighed a mean of 1.2 g less than the

progeny from FRTM fed broiler breeders (P<.05). The effects of the breeder treatments on progeny performance will be the subject of a subsequent chapter in this thesis.

The effect of MITM on broiler breeder performance are in accordance with Favero et al. (2013), where amino acid-mineral complexes were used as a means of protection for the minerals and showed improvements in broiler breeder performance. As MITM is a novel form of protection, little references are available for comparison and other forms of protections are used as a basis for comparison.

Enterocytes are highly prolific and have a high turn-over rate relative to other cells in the body. They serve a critical barrier function and are essential for digestion and nutrient assimilation, so histomorphological evaluation of the enteric villi is a means to assess mucosal distress (Favero et al., 2013). Previous studies (Parsaie et al., 2007; Choct, 2009) have attributed higher VH/CD ratios, which is indicative of a lower rate of villus tip sloughing of enterocytes relative to their generation in the crypts. Similarly, Ma et al. (2011) observed that birds fed OTM had lower crypt depths and higher VH/CD ratios in the ileum as compared to inorganic (free) trace minerals. In contrast, we did not observe significant differences among TM form treatments for jejunal VH, CD, or VH/CD ratio, which may be a different response than other researchers who only evaluated the ileum. However, dietary TM dose did affect mucosal histomorphology. For this trial the dose effect observed was improvements in villus height, deeper crypt, and bigger villi area. With an increase of 44% of the villi area, it means that there is more villi area for nutrients to be absorbed in the gut. Although MITM are inorganic, they have an organic coating matrix that protects the trace minerals from reactions in the gut while still retaining their inorganic characteristics.

Bone is a primary body reserve for minerals, particularly in egg-laying birds that require diurnal mobilization of minerals for proper eggs shell formation. The physical characteristics of tibia bone and mineralization is typically measured to assess bioavailability of dietary minerals. No significant treatment effects were observed on the physical characteristics of tibia bone, including length, weight, bending moment, bone breaking strength, and BMD (Table 6). It is noteworthy that tibia bone thickness was significantly greater in the microencapsulated form than free form of trace minerals. As bone ash and mineral content of bone (Table 7) are the primary indicators of dietary mineral bioavailability, it is noteworthy that microencapsulation of trace minerals significantly reduced bone ash and bone copper content in comparison to free form of trace minerals. This observation implies that the microencapsulation of trace minerals may reduce the bioavailability of inorganic trace minerals (Evans et al., 2015), regardless of dose. Only Mn content of tibia was increased as trace mineral dose increased. However, a significant mineral form X dose interaction was observed only on iron content of bone. Bone iron content was significantly increased by microencapsulation of trace minerals at the normal dose of trace minerals but not at the high dose level. This observation may be related to increased bone marrow observed among the MI treatments as suggested by the increased tibia bone width also observed.

In conclusion, feeding MITM to broiler breeders has great potential to improve broiler breeder egg production and efficiency. Although, microencapsulation may compromise bioavailability slightly, this is more than offset by the benefits in productivity. This is a novel mode of encapsulation that needs to be studied further, but this trial provided good indications that it may improve broiler breeder performance. Possible benefits of MITM can be observed for

broiler breeder nutrition and associated positive effects on progeny and egg quality, which will both be discussed in subsequent chapters.

REFERENCES

Abdallah, A., O. El-Husseiny, and K. Abdel-Latif. 2009. Influence of some dietary organic mineral supplementations on broiler performance. Int. J. Poult. Sci. 8:291–298. and diseases. Int. J. Curr. Sci. 10:94–107.

Association of official analytical chemists - AOAC. Official methods of analysis. 16.ed. Washington, D.C.: 1995. 1094p.

Bao, Y. M., and M. Choct. 2009. Trace mineral nutrition for broiler chickens and prospects of application of organically complexed trace minerals: a review. Anim. Prod. Sci. 49:269–282.

Bhoyar A., Why trace mineral supplements are pivotal for breeder efficiency. (2018). Retrieved February 14, 2021, from https://www.thepoultrysite.com/articles/why-trace-mineral-supplements-are-pivotal-for-breeder-efficiency

Blanchard RK, Moore JB, Green CL, Cousins RJ. Modulation of intestinal gene expression by dietary zinc status: effectiveness of cDNA arrays for expression profiling of a single nutrient deficiency. Proc Natl Acad Sci USA. 2001;98:13507–13513.

Burns, R.B. (1983) Antibody production suppressed in the domestic fowl (Gallus domesticus) by zinc deficiency, Avian Pathology, 12: 141—146.

Cabell CA, Earle IP. Additive effect of calcium and phosphorus on utilization of dietary zinc. J Anim Sci. 1965;24:800–804.

Choct, M. 2009. Managing gut health through nutrition. Br. Poult. Sci. 50:9–15.

Dibner JJ, Richards JD, Kitchell ML, Quiroz MA. Metabolic challenges and early bone development. J Appl Poult Res. 2007;16:126–137.

Dibner, J. J., J. D. Richards., M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126–137.

Dieck, H. T., F. Doring, H. P. Roth, and H. Daniel. 2003. Changes in rat hepatic gene expression in response to zinc deficiency as assessed by DNA arrays. J. Nutr. 133:1004–1010.

Evans, J., Richards, J., Fisher, P., & Wedekind, K. (2015). Greater bioavailability of chelated compared with inorganic zinc in broiler chicks in the presence or absence of elevated calcium and phosphorus. Open Access Animal Physiology, 97. doi:10.2147/oaap.s83845

Favero, A. S., L. Vieira, C. R. Angel, F. Bess, H. S. Cemin, and T. L. Ward. 2013. Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. J. Appl. Poult. Res. 22:80–91.

Feng, J., and Z. G. Feng. 1998. Effect of Mn-deficiency on reproductive performance in egg-laying chickens. Acta Veterinaria et Zootechnica Sinica 29:499–505.

Heth DA, Becker WM, Hoekstra WG. Effect of calcium, phosphorus and zinc on zinc-65 absorption and turnover in rats fed semipurified diets. J Nutr. 1966;88:331–337.

Hurley, L. S., C. L. Keen, and D. L. Baly. 1984. Manganese deficiency and toxicity: Effects on carbohydrate metabolism in the rat. Neurotoxicology 5:97–104.

Kidd, M.T., Anthony, N.B. & Lee, S.R (1992) Progeny performance when dams and chicks are fed supplemental zinc. Poultry Science, 71: 120—1206.

M'Sadeq, S. A., S. B.Wu, M. Choct, and R. A. Swick. 2018. Influence of trace mineral sources on broiler performance, lymphoid organ weights, apparent digestibility, and bone mineralization. Poult. Sci. 0:1–7.

Ma, W., H. Niu, Y. Wang, and J. Feng. 2011. Effects of zinc glycine chelate on oxidative stress, contents of trace elements, and intestinal morphology in broilers. Biol. Trace Elem. Res. 142:546–556.

Mezes M, Erdélyi M, Balogh K (2012) Deposition of organic trace metal complexes as feed additives in farm animals. Euro Chemi Bull 1(10):410–413

Muszy´nski, S., E. Tomaszewska, M. Kwiecie´n, P. Dobrowolski, and A. Tomczyk. 2018. Effect of dietary phytase supplementation on bone and hyaline cartilage development of broilers fed with organically complexed copper in a cu-deficient diet. Biol. Trace Elem. Res. 182:339–353.

O'Dell BL. Bioavailability of trace elements. Nutr Rev. 1984;42: 301–308.

Parsaie, S., F. Shariatmadari, M. J. Zamiri, and K. Khajeh. 2007. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers. Br. Poult. Sci. 48:594–600.

Peters, J., and D. Mahan. 2008. Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. J. Anim. Sci. 86:2247–2260.

Rajeswari, S., and S. Swaminathan. 2014. Role of copper in health

Richards, J. D., J. Zhao, R. J. Harrell, C. A. Atwell, and J. J. Dibner. 2010. Trace mineral nutrition in poultry and swine. Asian Australas. J. Anim. Sci 23:1527–1534.

Richards, M. P., and N. C. Steele, 1987. Trace element metabolism in the developing avian embryo: A review. J. Exptl. Zool. Suppl. 1:39–51.

Sakumoto, R., K. G. Hayashi, and T. Takahashi. 2014. Different expression of PGE synthase, PGF receptor, TNF, Fas and oxytocin in the bovine corpus luteum of the estrous cycle and pregnancy. Reprod. Biol. 14:115–121.

Salim, H., C. Jo, and B. Lee. 2008. Zinc in broiler feeding and nutrition. Avian Biol. Res. 1:5–18.

Solis de los Santos, F., Farnell, M., Tellez, G., Balog, J., Anthony, N., Torres-Rodriguez, A., . . . Donoghue, A. (2005). Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. *Poultry Science, 84*(7), 1092-1100. doi:10.1093/ps/84.7.1092

Tapiero, H., and K. D. Tew. 2003. Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed. Pharmacother. 57:399–411.

Underwood EJ, Suttle FR100%. The Mineral Nutrition of Livestock. 3rd ed. New York, NY, USA: CABI; 1999.

Willcox, J., S. Ash, and G. Catignani. 2004. Antioxidants and prevention of chronic disease. Crit. Rev. Food Sci. Nutr. 44:275–295.

Xie, J. J., C. H. Tian, Y. W. Zhu, L. Y. Zhang, L. Lu, and X. G. Luo. 2014. Effects of inorganic and organic manganese supplementation on gonadotropin-releasing hormone-I and follicle stimulating hormone expression and reproductive performance of broiler breeder hens. Poult. Sci. 93:959–969.

Figure 1. Total egg difference when broiler breeder hens are fed microencapsulated vs free trace minerals from week 27-55

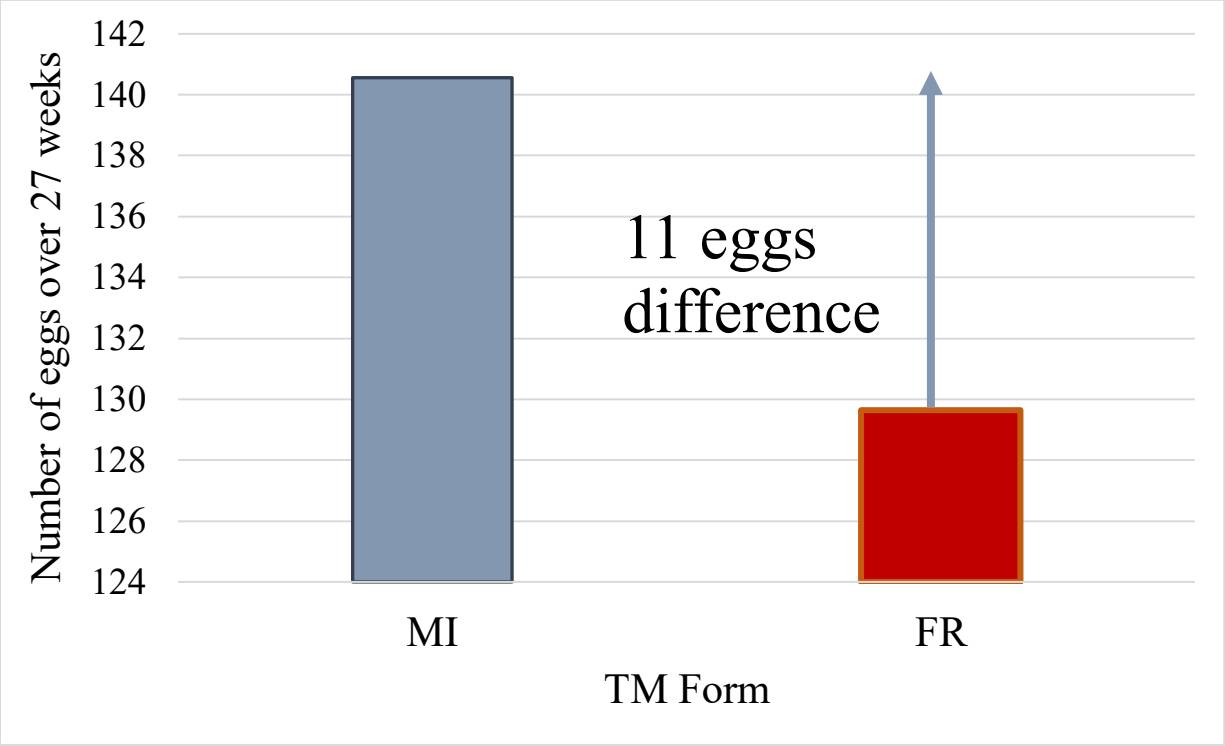

Figure 2. Percentage of chicks hatched of fertile eggs and percent fertility of broiler breeders fed microencapsulated (MI) and free (FR) trace minerals at 100% and 200% dose levels.

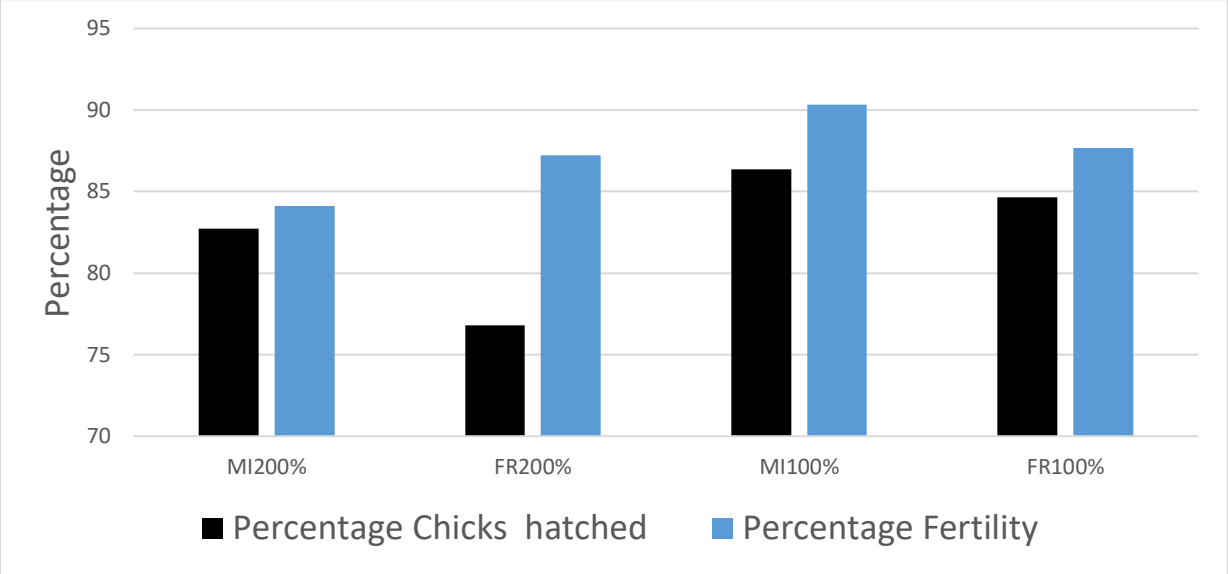

Figure 3. Free trace mineral and vitamin premix *vs* microencapsulated trace mineral and vitamin premix at zero magnification and 40X magnification.

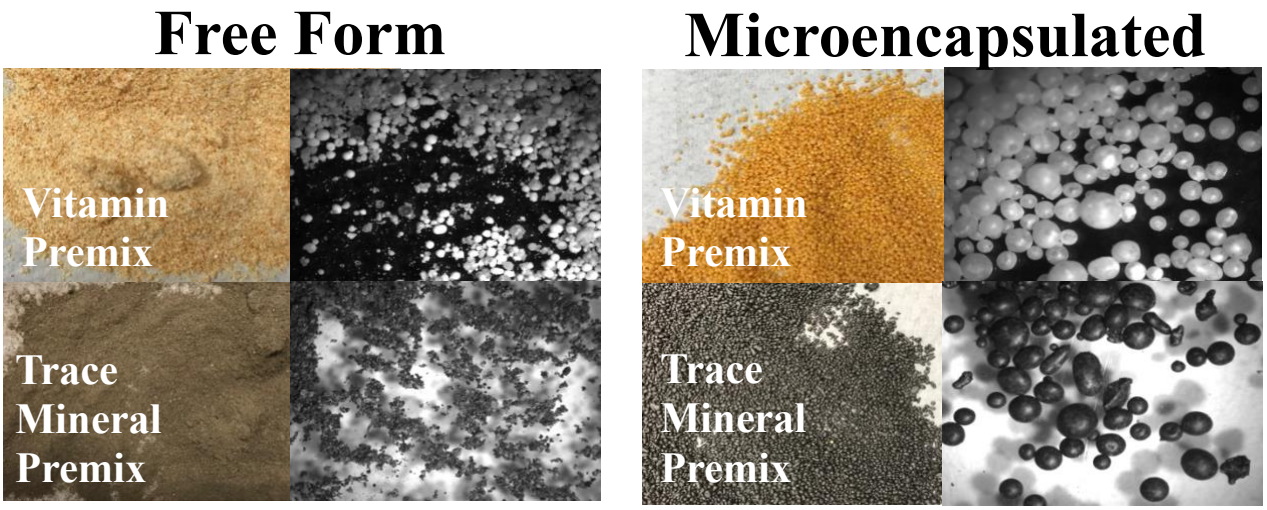

Figure 4. Distinction of how villi measurements were taken for jejunum histomorphology measurements.

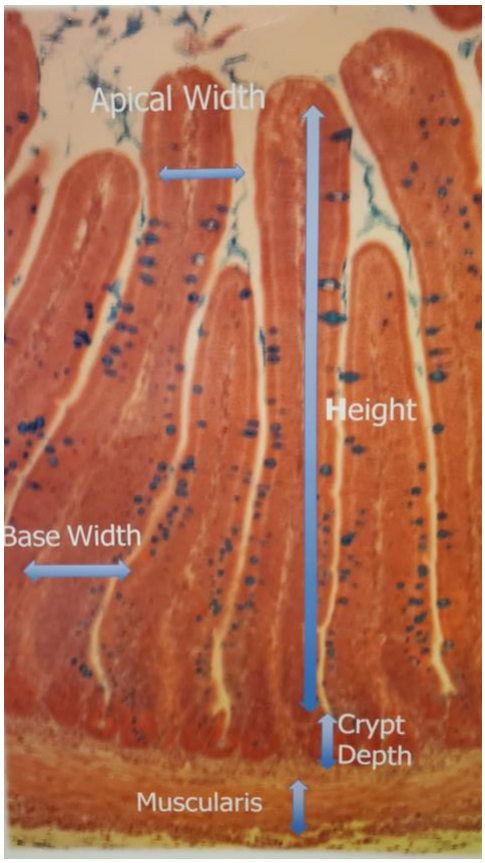

Table 1. Experimental diets fed to broiler breeders from 27 to 34 weeks of age

Ingredient	Experimental Treatments			
	MI200%	FR200%	MI100%	FR100%
	---------- % of Diet ----------			
Corn	65.18	65.18	65.18	65.18
Soybean Meal (47.5% CP)	19.12	19.12	19.12	19.12
Limestone fine	7.06	7.06	7.06	7.06
Wheat Middlings	6.55	6.55	6.55	6.55
Poultry Fat	0.50	0.50	0.50	0.50
Mono-Dicalcium Phosphate	0.48	0.48	0.48	0.48
Salt	0.31	0.31	0.31	0.31
DL-Methionine	0.17	0.17	0.17	0.17
Sodium Bicarbonate	0.14	0.14	0.14	0.14
Choline Chloride (60%)	0.06	0.06	0.06	0.06
L-Threonine	0.03	0.03	0.03	0.03
Quantum Blue Phytase 750 FTU	0.02	0.02	0.02	0.02
	0	0	0	0
Protected Vitamin (PV)	0.078[1]	0	0.078[1]	0
Protected Mineral (PM)	**0.306[3]**	**0**	**0.092[2]**	**0**
Free Vitamin (FV)	0	0.05[1]	0	0.05[1]
Free Mineral (FM)	0	**0.20[3]**	0	**0.06**[2]
Palmitic Acid	0	0.134	0.117	0.134
Vermiculite	0	0.0	0.097	0.140
Total Ingredients	100.0	100.0	100.0	100.0
Metabolize Energy, kcal/kg	2800	2800	2800	2800
Crude Protein, %	15.0	15.0	15.0	15.0
Dig Lysine, %	0.65	0.65	0.65	0.65
Dig Methionine + Cyst(e)ine, %				
Dig Threonine, %				
Crude Fat (ether extract), %	3.28	3.28	3.28	3.28
Ca, %	2.85	2.85	2.85	2.85
Non-phytate P, %	0.58	0.58	0.58	0.58

[1]Each kilogram of PV at a dietary inclusion of 0.078% or FV at a dietary inclusion of 0.05% supplied the following per kg of complete feed: vitamin A, 13,000 IU; cholecalciferol, 5,850 IU; alpha-tocopherol, 84.5 IU; niacin, 78 mg; pantothenic acid, 23 mg; riboflavin, 8.5 mg; pyridoxine, 4.2 mg; menadione, 4 mg; folic acid, 2.5 mg; thiamin, 3.25 mg; biotin, 0.234 mg; vitamin B_{12}, 0.02 mg; ethoxyquin.

[2]Each kilogram of PM at a dietary inclusion of 0.092% or FM at a dietary inclusion of 0.06% supplied the following per kg of complete feed: 78 mg Zn as ZnO; 78 mg Mn as MnO; 33 mg Fe as $FeSO_4.H_2O$; 9 mg Cu as CuSO4; .9 mg I as $Ca(IO_3)_2$.

[3]Each kilogram of PM at a dietary inclusion of 0.306% or FM at a dietary inclusion of 0.20% supplied the following per kg of complete feed: 255 mg Zn as ZnO; 255 mg Mn as MnO; 108 mg Fe as $FeSO_4.H_2O$; 30 mg Cu as CuSO4; .2.85 mg I as $Ca(IO_3)_2$.

Table 2. Experimental diets fed to broiler breeders from 35 to 49 weeks of age

Ingredient	Experimental Treatments			
	MI200%	FR200%	MI100%	FR100%
	---------- % of Diet ----------			
Corn	66.25	66.35	66.35	66.35
Soybean Meal (47.5% CP)	17.80	17.80	17.80	17.80
Limestone fine	7.65	7.65	7.65	7.65
Wheat Middlings	6.24	6.24	6.24	6.24
Poultry Fat	0.50	0.50	0.50	0.50
Mono-Dicalcium Phosphate	0.39	0.39	0.39	0.39
Salt	0.28	0.28	0.28	0.28
Sodium Bicarbonate	0.19	0.19	0.19	0.19
DL-Methionine	0.15	0.15	0.15	0.15
Choline Chloride (60%)	0.06	0.06	0.06	0.06
Selenium Premix NCSU	0.05	0.05	0.05	0.05
Quantum Blue Phytase 750 FTU	0.02	0.02	0.02	0.02
L-Threonine	0.02	0.02	0.02	0.02
	0	0	0	0
Protected Vitamin (PV)	0.078[1]	0	0.078[1]	0
Protected Mineral (PM)	**0.306[3]**	**0**	**0.092[2]**	**0**
Free Vitamin (FV)	0	0.05[1]	0	0.05[1]
Free Mineral (FM)	0	0.20[3]	0	0.06[2]
Palmitic Acid	0	0.134	0.117	0.134
Vermiculite	0	0.0	0.097	0.140
Total Ingredients	100.0	100.0	100.0	100.0
Metabolize Energy, kcal/kg	2800	2800	2800	2800
Crude Protein, %	14.4	14.4	14.4	14.4
Dig Lysine, %	0.62	0.62	0.62	0.62
Dig Methionine + Cyst(e)ine, %				
Dig Threonine, %				
Crude Fat (ether extract), %	3.28	3.28	3.28	3.28
Ca, %	3.05	3.05	3.05	3.05
Non-phytate P, %	0.58	0.58	0.58	0.58

[1]Each kilogram of PV at a dietary inclusion of 0.078% or FV at a dietary inclusion of 0.05% supplied the following per kg of complete feed: vitamin A, 13,000 IU; cholecalciferol, 5,850 IU; alpha-tocopherol, 84.5 IU; niacin, 78 mg; pantothenic acid, 23 mg; riboflavin, 8.5 mg; pyridoxine, 4.2 mg; menadione, 4 mg; folic acid, 2.5 mg; thiamin, 3.25 mg; biotin, 0.234 mg; vitamin B_{12}, 0.02 mg; ethoxyquin.

[2]Each kilogram of PM at a dietary inclusion of 0.092% or FM at a dietary inclusion of 0.06% supplied the following per kg of complete feed: 78 mg Zn as ZnO; 78 mg Mn as MnO; 33 mg Fe as $FeSO_4.H_2O$; 9 mg Cu as CuSO4; .9 mg I as $Ca(IO_3)_2$.

[3]Each kilogram of PM at a dietary inclusion of 0.306% or FM at a dietary inclusion of 0.20% supplied the following per kg of complete feed: 255 mg Zn as ZnO; 255 mg Mn as MnO; 108 mg Fe as $FeSO_4.H_2O$; 30 mg Cu as CuSO4; .2.85 mg I as $Ca(IO_3)_2$.

Table 3. Experimental diets fed to broiler breeders from 50 to 55 weeks of age

Ingredient	Experimental Treatments			
	MI200%	FR200%	MI100%	FR100%
	---------- % of Diet ----------			
Corn	67.17	67.17	67.17	67.17
Soybean Meal (47.5% CP)	17.97	17.97	17.97	17.97
Limestone fine	8.20	8.20	8.20	8.20
Wheat Middlngs	4.73	4.73	4.73	4.73
Poultry Fat	0.50	0.50	0.50	0.50
Mono-Dicalcium Phosphate	0.37	0.37	0.37	0.37
Salt	0.28	0.28	0.28	0.28
Sodium Bicarbonate	0.19	0.19	0.19	0.19
DL-Methionine	0.15	0.15	0.15	0.15
Choline Chloride (60%)	0.06	0.06	0.06	0.06
Selenium Premix NCSU	0.05	0.05	0.05	0.05
Quantum Blue Phytase 750 FTU	0.02	0.02	0.02	0.02
L-Threonine	0.02	0.02	0.02	0.02
Protected Vitamin (PV)	0.078[1]	0	0.078[1]	0
Protected Mineral (PM)	**0.306[3]**	**0**	**0.092[2]**	**0**
Free Vitamin (FV)	0	0.05[1]	0	0.05[1]
Free Mineral (FM)	0	0.20[3]	0	0.06[2]
Palmitic Acid	0	0.134	0.117	0.134
Vermiculite	0	0.0	0.097	0.140
Total Ingredients	100.0	100.0	100.0	100.0
Metabolize Energy, kcal/kg	2800	2800	2800	2800
Crude Protein, %	15.0	15.0	15.0	15.0
Dig Lysine, %	0.65	0.65	0.65	0.65
Dig Methionine + Cyst(e)ine, %				
Dig Threonine, %				
Crude Fat (ether extract), %	3.28	3.28	3.28	3.28
Ca, %	2.85	2.85	2.85	2.85
Non-phytate P, %	0.58	0.58	0.58	0.58

[1]Each kilogram of PV at a dietary inclusion of 0.078% or FV at a dietary inclusion of 0.05% supplied the following per kg of complete feed: vitamin A, 13,000 IU; cholecalciferol, 5,850 IU; alpha-tocopherol, 84.5 IU; niacin, 78 mg; pantothenic acid, 23 mg; riboflavin, 8.5 mg; pyridoxine, 4.2 mg; menadione, 4 mg; folic acid, 2.5 mg; thiamin, 3.25 mg; biotin, 0.234 mg; vitamin B_{12}, 0.02 mg; ethoxyquin.

[2]Each kilogram of PM at a dietary inclusion of 0.092% or FM at a dietary inclusion of 0.06% supplied the following per kg of complete feed: 78 mg Zn as ZnO; 78 mg Mn as MnO; 33 mg Fe as $FeSO_4.H_2O$; 9 mg Cu as CuSO4; .9 mg I as $Ca(IO_3)_2$.

[3]Each kilogram of PM at a dietary inclusion of 0.306% or FM at a dietary inclusion of 0.20% supplied the following per kg of complete feed: 255 mg Zn as ZnO; 255 mg Mn as MnO; 108 mg Fe as $FeSO_4.H_2O$; 30 mg Cu as CuSO4; .2.85 mg I as $Ca(IO_3)_2$.

Table 4. Determined values of mineral content in broiler breeder diets with trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 200%)[1]

Mineral	MI200%	FR200%	MI100%	FR100%
Sodium (Na, %)	0.156	0.195	0.168	.197
Calcium (Ca, %)	2.17	3.53	3.26	3.48
Phosphorus (P, %)	0.464	0.543	.514	.532
Magnesium (Mg, %)	0.153	0.184	.190	.186
Potassium (K, %)	0.635	0.630	.615	.587
Copper (Cu, ppm)	30.7	51.7	14.8	26.2
Iron (Fe, ppm)	361	533	365	493
Manganese (Mn, ppm)	269	458	129	180
Zinc (Zn, ppm)	256	468	127	186
Selenium (Se, ppm)	0.969	1.38	.604	.646

[1]Values were evaluated through Trouw Nutrition Laboratory (Amersfoort, Netherlands)

Table 5. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 200%) on broiler breeder performance from 26 to 55 weeks of age.[1]

		Egg Production %		Feed Conversion		
		HD[1]	HH[2]	kg feed/dozen of eggs	Eggs/hen (per week)	Chick weight (g)
TM Form						
MI		71.71[a]	71.66[a]	2.63[b]	5.02[a]	41.94[b]
FR		66.21[b]	66.01[b]	2.85[a]	4.63[b]	43.17[a]
TM Dose						
100%		69.95	69.78	2.70	4.89	42.39
200%		67.98	67.89	2.78	4.76	42.70
Treatment	**Dose**					
MI	200%	70.93	70.93	2.69	4.96	45.86
FR	200%	66.60	64.86	2.87	4.66	47.35
MI	100%	72.87	72.39	2.57	5.10	44.64
FR	100%	67.69	67.16	2.82	4.74	45.34
		----------*P-value*----------				
TM Form		**.0019**	**.0014**	**.0140**	**.0019**	**0.0422**
TM Dose		.2618	.2843	.3386	.2618	0.5824
TM Form*TM Dose		.8154	.8099	.6115	.8169	0.1442
SEM		1.24	1.24	.062	.087	0.43

[a,b]Means within a column with a different superscript differ significantly (P<0.05).
[1]Means are an average of 3 pens per treatment, with each pen containing ea. 9 hens and 2 roosters.
[2]Hen Day Egg production = 100((eggs produced)/(# hens))
[3]Hen House Egg Production = 100((eggs produced)/(# hens housed))
[4]Standard error of the mean with 8 degrees of freedom.

Table 6. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 200%) on jejunum mucosa histomorphology of broiler breeders[1]

		Villus Height (VH, μm)	Villus tip width (μm)	Villus bottom width (μm)	Crypt depth (CD, μm)	Muscularis (μm)	Area (μm^2)	Ratio (VH/CD)
TM Form								
MI		857.04	159.48	186.90	128.38	203.89	154637	6.91
FR		834.17	156.97	170.95	132.75	210.22	141458	6.43
TM Dose								
100%		968.46[a]	166.74	186.91	147.73[a]	212.54	175103[a]	6.76
200%		722.76[b]	149.72	170.94	113.40[b]	201.57	120992[b]	6.58
Treatment	**Dose**							
MI	200%	733.35	152.76	183.65	112.52	187.09	129606	6.78
FR	200%	712.16	146.67	158.22	114.28	216.06	112377	6.38
MI	100%	980.74	166.20	190.15	144.24	220.69	179668	7.04
FR	100%	956.18	167.27	183.68	151.21	204.39	170539	6.49
		----------*P-value*----------						
TM Form		.6641	.8255	.3037	.6262	.7376	.4742	.2997
TM Dose		**<.0001**	.1391	.3029	**.0003**	.5624	**.0045**	.6801
TM Form*TM Dose		.9744	.7534	.5397	.7713	.2340	.8256	.8729
SEM		37.05	8.02	10.86	6.31	13.30	12934	.32

[a,b]Means within a column with a different superscript differ significantly ($P<0.05$).
[1]Means are an average of 15 birds per treatment with 10 villi measurements per hen.

Table 7. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 200%) on the physical characteristics of tibia bone of broiler breeders at 55 weeks of age [1]

		Length (mm)	Diaphysis Diameter (mm)	Fresh weight (g)	Bending moment (mm)	Bone strength (N)	BMD[2]
TM Form							
MI		116.95	7.82[a]	18.04	.1276	350.39	.6907
FR		117.98	7.62[b]	18.05	.1299	351.17	.6871
TM Dose							
100%		118.19	7.76	17.91	.1280	347.84	.6847
200%		116.69	7.69	18.18	.1296	353.92	.6933
Treatment	**Dose**						
MI	200%	116.24	7.81	18.16	.1248	342.35	.6975
FR	200%	117.18	7.57	18.2	.1347	366.28	.6888
MI	100%	117.66	7.84	17.91	.1304	358.43	.6839
FR	100%	118.71	7.68	17.90	.1255	337.15	.6856
		----------*P-value*----------					
TM Form		.3088	**.0354**	.9757	.6671	.9280	.8731
TM Dose		.1340	.4829	.5120	.7614	.6616	.7050
TM Type*TM Level		.9571	.6635	.9529	.2027	.1316	.8142
SEM		.68	.067	.29	.0040	1.1	.015

[a,b]Means within a column with a different superscript differ significantly (P<0.05).
[1]Means are an average of 15 bones per treatment.
[2]Bone Mass Density

Table 8. Effect of dietary supplementation of trace minerals in two forms (microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 200%) on tibia bone ash content and mineral composition of broiler breeders at 55 weeks of age[2]

Treatment		Ash%	P	Ca	Cu	Fe	Mn	Zn
			----------mg/g[3]----------					
TM Form								
MI		34.02[b]	18.97	42.03	.000237[b]	.0153	.0019	.0549
FR		35.80[a]	18.27	41.06	.000407[a]	.0150	.0020	.0574
TM Dose								
100%		34.69	18.73	42.17	.000345	.0152	.0018[b]	.0565
200%		35.10	18.51	40.92	.000292	.0151	.0020[a]	.0558
Treatment	**Dose**							
MI	200%	34.50	19.06	42.13	.00017	.01427[ab]	.00191	.05320
FR	200%	35.74	17.93	39.62	.00029	.01614[ab]	.00220	.05850
MI	100%	33.53	18.87	41.94	.00021	.01640[a]	.00188	.05660
FR	100%	35.85	18.60	42.41	.00032	.01400[b]	.00176	.05647
			----------*P-value*----------					
TM Form		**.0330**	.1298	.2880	**.0143**	.0787	.4123	.0985
TM Dose		.5990	.6036	.1781	.3077	.9991	**.0216[1]**	.6346
TM Form*TM Dose		.5075	.3510	.1211	.3336	**.0113[1]**	.0595	.0851
SEM		.5703	.3208	.6657	.00004769	.0005625	.00007190	.0010

[a,b]Means within a column with a different superscript differ significantly (P<0.05).
[1]Means are an average of 15 bones per treatment.
[2]Means are an average of 3 pens per treatment, with each pen containing ea. 9 hens and 2 roosters.
[3]mg of mineral per g of ash

CHAPTER 3

Hatching Egg Quality of Broiler Breeders Fed Diets Supplemented with Conventional Free or Lipid Microencapsulated Premix Forms of Trace Minerals at Standard or High Levels

ABSTRACT

Lipid matrix microencapsulation is hypothesized to improve bioavailability and reduce toxicity of broiler breeders fed diets supplemented with normal and excessive levels of inorganic trace mineral salts. The objective of the study was to evaluate the effect of microencapsulated trace minerals premix on internal and external quality and mineral composition of eggs. Twelve pens with 9 females and 2 males were randomly assigned to one of 4 dietary treatments consisting of a factorial arrangement of 2 mineral premix forms (free and microencapsulated) and 2 mineral premix supplementation dosages (100% and 200% of Aviagen recommendations): 1) Free 100% (FR100%); 2) Microencapsulated 100% (MI100%); 3) Free 200% (FR200%); and 4) Microencapsulated 200% (MI200%). 18 eggs per treatment were collected from broiler breeders at 1, 6, 10, 13, 21, and 27 weeks lay. Egg quality parameters evaluated included egg weights, Haugh unit, yolk color, shell strength, shell elasticity, vitelline membrane strength, and vitelline membrane elasticity. Mineral composition of eggshell and whole egg was also assayed. Microencapsulation showed better results for Haugh Unit scores during 10th week of lay but offered lower values for egg weights during the 21st week of lay and yolk color during 1st week of lay. Higher TM dose improved values for egg weights during 1st week of lay, yolk color in 6th and 21st week of lay, and shell strength during 21st week of lay. There were also some significant interactions when looking at egg weights, shell strength and elasticity, vitelline membrane strength and elasticity. Where MI100% presented with better results for egg weights, vitelline membrane strength and elasticity during 13th week of lay. In contrast, MI100% presented with

the lowest shell strength results during the 6th week of lay. Lastly, FR200% showed highest shell elasticity during the 13th week of lay. In conclusion, premix form or dose had marginal effects on external or internal egg quality, or mineral composition of hatching eggs.

INTRODUCTION

Broiler breeders produce eggs that are hatched to produce the commercial meat broilers, so eggshell and internal composition are critical for embryonic development and chick quality (Bhoyar, 2018). Eggshell quality is one of the most important aspects in the poultry industry (Washburn et al.,1982; Singh et al., 2007; Harms et al., 2007), as shell quality affects the number of settable eggs and hatchability. Broiler breeder nutrition and feeding programs do have an effect on fertile egg production and egg nutrient content (Leksrisompong et al., 2006; Robinson et al., 2007; Romero-Sanchez et al., 2008; Hocking, 2009; Abbas et al., 2010; Leksrisompong, 2010; Yenice et al., 2015), which has great importance on progeny robustness (Kidd, 2003, Dibner et al., 2007). Progeny growth, feed utilization, bone development, and immunological responses are all dependent on broiler breeder nutrition (Kemp et al., 2001; Calini and Sirri, 2007; Bozkurt et al., 2008; Fisher and Gous, 2009; Eusebio-Balcazar et al., 2020; Favero et al., 2013). Trace mineral bioavailability is an important aspect of broiler breeder nutrition and may depend upon dietary supplementation levels and the form of premix supplemented. Depending on the nutrient concentration and bioavailability of the hen's diet, most of the trace minerals are deposited in the yolk, which is used by the chick through perinatal development (Richards and Packard, 1996). Diets fed to broiler breeders with insufficient trace mineral (TM) levels have a negative impact on egg production, eggshell quality, increased embryonic mortality, and an increase in unviable chicks (Kidd, 2003; Dibner et al., 2007).

When considering "egg quality", calcium is the primary mineral of interest, as calcium carbonate is the main structure of the egg and holds all of the egg components together. Not only are calcium (Ca) and phosphorus (P) important in the eggshell formation, but it has been well documented that trace minerals also play a large role in the eggshell mineralization process

(Darvishi et al., 2020). Zinc (Zn) supplementation to hens is required for eggshell deposition, as it is an integral structural component of the carbonic anhydrase enzyme (Guimaraes et al., 2013). This enzyme has an activity in the shell gland (Zhang et al., 2017) and is involved in eggshell synthesis *via* the catalysis and interconversion of carbon dioxide and water to bi-carbonate (Roberts, 2004). Zamani et al. (2015) and Guo et al. (2002) reported that supplementation of additional Zn in the diet has positive effects in the eggshell thickness, where in contrast Stevenson (1985) found that high dietary levels of Zn had no beneficial impact on egg quality and shell thickness. The eggshell provides a barrier against pathogens and prevents water loss from inside of the egg. The eggshell is a sufficient source of calcium for the developing embryo, which supports a strong skeletal system formation (Harms et al., 1996; Singh et al., 2007). Eggshells provide 70-90% of the calcium required for the embryo to develop (Packard and Packard, 1991; Hunton, 1995; Vieira. 2007), which elevates the importance of a good, strong shell to support the embryo's developmental needs.

Egg mineral deposition by the hen is achieved by including TM in the yolk and albumen. The yolk is the most nutrient-rich part of the egg where the majority of the TM are deposited; the albumen only stores some TM. The transfer of TM from hen to egg involves two pathways: mostly through the ovary into the egg yolk; some *via* the oviduct to the albumen (Richards, 1997). In order to achieve this targeted delivery of trace minerals to the ovary and subsequently to the yolk, the estrogen-induced precursor yolk protein, vitellogenin, plays very important role as a transporter of TM from hen to the egg (Richards, 1997). Vitellogenin is produced in the liver of the hen and then secreted into the blood (Richards, 1989). As levels of vitellogenin rise in the blood, more TM metals (Zn, Cu, Fe) are bound and mobilized into the yolk (Hill, 1974). The second mode by which TM are stored in the egg is the albumen. Estrogen, along with other

hormones like progesterone and testosterone, stimulate the albumen protein synthesis in the magnum (Richards, 1997). Ovalbumin, one of the proteins synthesized for this process, binds trace minerals like selenium (Se), zinc (Zn), copper (Cu), and manganese (Mn) (Palmer and Guillette, 1991). Conalbumin, which is another protein synthesized to facilitate the TM binding process, has two iron binding sites at the amino and carboxyl ends (Palmer and Guillette, 1991). Conalbumin, and other egg albumen proteins alike, functions as a means to bind and limit the availability of iron and other metals, which inhibits microbial growth and serves as an antimicrobial agent in the egg (Richards, 1997)

When minerals reach lower parts of the GI tract, they can bind to other nutrients, phytate, minerals, and fiber, which renders the minerals insoluble (Dibner et al., 2007). Insoluble forms of minerals are then excreted as they are not able to be absorbed by the animal. The formation of insoluble complexes can be minimized by supplementing the diet with forms of TM that are less susceptible to complexing, such as organic trace mineral supplements (Dibner et al., 2007). Alternatively, microencapsulation in lipid matrix may be another means to minimize the formation of insoluble TM complexes and reduce lipid peroxidation (Ferket et al., 2017). Microencapsulated trace mineral salts (MITM) may be more resistant to dissociation and complexing in the acidic environment of the crop, proventriculus, and gizzard, thus allowing for more TM to be delivered and absorbed by the epithelium of the small intestine. The hypothesis tested in this study was supplemental trace mineral premix form and dietary supplementation levels for broiler breeders affect the internal/external quality and mineral content egg of hatching eggs.

MATERIALS AND METHODS

One-hundred-eight Ross 708 broiler breeder hens and 24 Ross HY males were randomly assigned to 12 treatment pens and managed from 28 weeks to 55 weeks of production as described in chapter 3. To test the hypothesis, this experiment was designed to evaluate 4 dietary treatments consisting of a factorial arrangement of 2 mineral premix forms (free and protected) and 2 mineral premix supplement dosages (100% and 200% of Aviagen recommendations): 1) Free 100% (FR100%); 2) Microencapsulated 100% (MI100%); 3) Free 200% (FR200%); and 4) Microencapsulated 200% (MI200%). The formulation details of the trace mineral premixes and dietary treatments are also presented in Chapter 3. Eggs collected during 1, 6, 10, 13, 21, and 27 weeks of lay were labeled by pen, transported, and stored at 7°C for immediate analysis at the Prestage Department of Poultry Science Egg Quality Laboratory (NC State University. Raleigh, NC 27695). Twenty-four settable quality eggs were randomly selected from each treatment so that 6 eggs could be subjected to each egg quality assay as described below.

Egg quality

Egg weights, Haugh unit, yolk color, shell strength, shell elasticity, vitelline membrane strength, vitelline membrane elasticity was analyzed as a part of egg quality. Egg weights, albumen height, and yolk color were determined using TSS TECHNICAL SERVICES (Technical Services and Supplies Ltd (TSS), York, England). Haugh Unit is calculated by the computer where it takes into account the albumen height and egg weight. Yolk color is analyzed by a machine using DSM Yolk Color Fan (Roche Fan). Shell strength and elasticity were determined by using a Stable microsystems (TA.HD.plus C texture analyzer) machine with a 250 kg load cell.

Through the use of the Stable Microsystems equipment (TA.HD.plus C texture analyzer), the vitelline membrane strength was determined using a 500g load cell. With a sensitivity of 0.1g and applied pressure rate of 3.2mm per second, and instrument set at 10g full scale. A 1mm wide rounded-end tip was used to apply direct pressure to the membrane (Keener et al., 2006).

Whole egg mineral analysis

To determine egg mineral composition, the liquid contents (albumen+yolk) of 6 eggs from each replicate collection period were stomached from each replicate in order to evaluate the mineral contents and were then weighed. These eggs were dried at 50°C for 48 hours in order to attain the dry weight and then digested with acid (HCl). The mineral composition of these eggs was determined by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (ICP-MS, 5800 ICP-OES, Agilent, Santa Clara, CA).

Shell mineral analysis

Six eggshells per replicate from the whole liquid egg mineral analysis collected at 1 week of lay. The shells were ground and then digested in 2 ml of 6 N HCl to solubilize minerals prior of composition analysis (Reference method). The mineral composition of the eggshells was determined by using Inductively coupled plasma mass spectrometry (ICP-MS) (ICP-MS, 5800 ICP-OES, Agilent, Santa Clara, CA).

Statistical analysis

All data were statistically analyzed as a 2x2 factorial randomized design with 3 replicate pens as the experimental unit per treatment group. Data was analyzed by 1-way ANOVA (JMP

15 software; SAS Inst. Inc.) and the means were then compared using Tukey's test. Data was considered significant when P<.05.

RESULTS

The effect of dietary supplementation level and form of trace mineral premixes on the internal and external egg quality measurements are presented in tables 1 to 7.

Egg weight quality data collected for the 1st week of lay through 27th week of lay is summarized in Table 1. During the first week of lay egg weights were significantly (P<.05) increased as dietary TM supplementation dose increased (56.67g *vs*.58.78g, respectively). At 13 weeks of lay, the low dietary TM supplementation level premix form had no significant effect on egg weights, but egg weights were decreased by microencapsulation at the higher TM supplementation level. Egg weights of hens fed the microencapsulated premix was significantly lower than the free form, regardless of the TM dose (64.56 vs 66.79 g, respectively; P<.05). No significant differences were observed during 6th, 10th, and 27th weeks of lay.

Haugh Unit quality data collected for the 1st week of lay through 27th week of lay is summarized in Table 2. Haugh unit was significantly greater in eggs from hens fed the microencapsulated than the free TM premix (82.91 vs 86.24, respectively; P<0.05), regardless of supplementation dose level. No significant differences were observed during 1st, 6th, 13th, 21st, and 27th weeks of lay.

Yolk color data collected for the 1st week of lay through 27th week of lay is summarized in Table 3. Regardless of dietary TM dose, yolk color was significantly higher from hens fed the free form than microencapsulated form of TM premix during the first week of lay (8.66 vs 7.95, respectively; P<0.005). In contrast to what was observed during the first week of lay, yolk color

score increased as dietary TM dose increased (8.22 vs 8.43, respectively; P<0.05). Yolk color increased significantly (P<.05) as the TM dose level increased (8.53 *vs.* 9.08), however the premix form may have some biological impact on the yolk color for the 21st week as it was close to significance (*P*=.0620). No significant differences were observed during 10th, 13th, and 27th weeks of lay.

Egg shell strength data collected for the 1st week of lay through 27th week of lay is summarized in Table 4. A significant premix form X TM dose effect was observed on eggshell strength for eggs collected during the 6th week of lay. At the high dietary TM supplementation level, premix form had no significant effect on eggshell strength; but eggshell strength was decreased in eggs produced from hens fed premix microencapsulation at the lower supplementation level (3853.79g *vs.* 3216.96g, P<0.05). during the 6th week of lay there was also a noteworthy biological effect that although it was not quite significant at .0586 p value, the hens fed FR form minerals showed an increase of 8.9% in the eggshell strength. Shell strength increased significantly (P<.05) as the TM dose level increased, regardless of the form of premix. No significant differences were observed during 1st, 10th, 13th, and 27th weeks of lay.

Egg shell elasticity data collected for the 1st week of lay through 27th week of lay is summarized in Table 5. During the 13th week of lay, eggshell elasticity was significantly decreased by microencapsulation when compared to the high dose of free TM, but the free TM dose level offered the lowest values for eggshell elasticity. Some biological effects may be observed during the 27th week of lay when referring to eggshell elasticity, where it was close to significance (P=.0719) and the higher TM dose resulted in greater shell elasticity. No significant differences were observed during 1st, 6th, 10th, 21st, and 27th weeks of lay.

Vitelline membrane strength and elasticity data collected for the 1st week of lay through 27th week of lay is summarized in Tables 6 and 7, respectively. During the 13th week of lay vitelline membrane strength was significantly increased by microencapsulation at the lower TM dose and free TM supplementation offered the lowest values for vitelline membrane strength (1.99g *vs.* 1.52g respectively). No significant differences were observed during 1st, 6th, 10th, 21st, and 27th weeks of lay. Likewise, vitelline membrane elasticity at 13 weeks of lay was significantly increased by microencapsulation at the lower TM dose and free TM supplementation offered the lowest values for vitelline membrane elasticity (3.42mm *vs.* 2.63mm respectively). No significant differences were observed during 1st, 6th, 10th, 21st, and 27th weeks of lay.

There were no significant treatment effects observed on the whole egg content for calcium (Ca), phosphorus (P), and iron (Fe) during any of the weeks of measure (Tables 7, 8, and 10 respectively). However, some significant main treatment effects were observed on the Cu, Mn, and Zn content of whole eggs sampled at 21 weeks of lay (Tables 9, 11, and 12, respectively). At that egg sample time, Cu, Mn, and Zn content was lower significantly lower among hens fed the microencapsulated premix than the free form, regardless of the TM supplementation level. Only Zn content of whole eggs increased significantly as TM supplementation level increased.

There were no significant treatment effects on mineral content of eggshells from hens at 10 weeks of lay (Table 13).

DISCUSSION

As observed in the literature, the sampled egg weights increased as weeks of lay progressed through the 27-week study period (Richards and Packard, 1996). However, treatment effects on egg weights and egg quality analysis were not consistent in statistically significance ($P<.05$); some differences were observed due to TM form while others due to TM dose, so drawing clear conclusions is difficult. According to Darvishi et al. (2020), dietary inclusion of organic Zn increased egg weights in comparison to inorganic Zn. Therefore, microencapsulated inorganic trace mineral did not respond like an organism TM as expected in terms of egg weight, although egg production rate was observed to increase (chapter 2). Haugh unit, eggshell strength and elasticity, and vitelline membrane strength and elasticity all showed decreasing tendencies as the hens aged. These decreasing tendencies were expected as multiple bodies of work have detailed these incidences. As hens age, the egg size and weight increase and the shells become thinner. With a thinner eggshell, more gaseous exchange occurs in the egg, causing the albumen to become more liquid and less dense. Additionally, with more gaseous exchange in the egg the egg loses moisture more readily which decreases the total volume of the egg. With a less dense albumen, the egg yolk accumulates more water from the albumen through osmosis, which increases pressure on the vitelline membrane and decreases its strength. There were no consistent statistically significant ($P<.05$) treatment differences in the Haugh unit, eggshell strength and elasticity, or vitelline membrane strength and elasticity. Due to these inconsistent differences little inferences can be made about the effect of the form, dose, and treatment effect on these parameters.

Additionally, no consistent differences in the whole egg or eggshell mineral analysis were observed. The findings of this experiment disagree with the findings of Zamani et al.

(2005) and Guo et al. (2002), where they found that Zn over supplementation did have an effect on the eggshell thickness and strength. The findings by Stevenson (2005) where high dietary levels of Zn supplemented to hens had no effect on eggshell thickness is in accordance to the findings of this trial due to the fact that eggshell thickness was not affected by form of TM or dose.

In conclusion, feeding broiler breeders microencapsulated trace minerals do have some effects on the internal and external egg quality properties. Although the changes are inconsistent through the different testing periods, changes in the progeny quality and productivity will be explored in the next chapter. Some differences in the progeny productivity parameters were observed, even if no consistent changes in the eggs were observed.

REFERENCES

Abbas, S.A., Gasm Elseid, A.A. and Ahmed, M.-K.A. (2010) Effect of body weight uniformity on the productivity of broiler breeder hens. Int. J. Poult. Sci., 9, 225– 230.

Bhoyar A., Why trace mineral supplements are pivotal for breeder efficiency. (2018). Retrieved February 14, 2021, from https://www.thepoultrysite.com/articles/why-trace-mineral-supplements-are-pivotal-for-breeder-efficiency

Bozkurt, M., C¸ abuk, M. and Alc¸ic¸ek, A. (2008) Effect of dietary fat type on broiler breeder performance and hatching egg characteristics. J. Appl. Poultry. Res., 17, 47– 53.

Calini, F. and Sirri, F. (2007) Breeder nutrition and offspring performance. Braz. J. Poult. Sci., 9, 77– 83.

Darvishi, Y., M. Shams Shargh, S. Hassani.Effect of organic or inorganic Zinc and Manganese sources on performance and egg quality traits of laying hens. J Adv Pharm Edu Res 2020;10(S1):22-28.

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126–137.

Eusebio-Balcazar, P.E., Oviedo-Rondo´ n, E.O., Mitchell, A., Brake, J., Wineland, M.J., Moraes, V. and Leandro, N. (2010) Effects of breeder feeding and trace mineral source on leg health and bone traits of broiler progeny. Poult. Sci., 89(Suppl. 1), Abstract 177, p. 223.

Favero, A. S., L. Vieira, C. R. Angel, F. Bess, H. S. Cemin, and T. L. Ward. 2013. Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. J. Appl. Poult. Res. 22:80–91.

Favero, A., S. L. Vieira, C. R. Angel, F. Bess, H. S. Cemin, and T. L. Ward. 2013. Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. J. Appl. Poult. Res. 22:80–91.

Fisher, C. and Gous, R.M. (2009) Protein and amino acid responses. In: Hocking, P.M. (ed.), Biology of breeding poultry, pp. 331– 359. CABI, Cambridge, MA.

Guimaraes, A. C. T., N. K. Sakomura, L. Hauschild, B. A. Loureiro,and D. C. Z. Donato. 2013. Microminerais complexados aamino´acidos no desempenho reprodutivo de matrizes pesadas eresposta da progˆenie. Cienc. Rural 43:1044–1049.

Guo Y.M., Yang R., Yuan J., Ward T.L., Fakler T.M. (2002): Effect of Availa Zn and ZnSO4 on laying hen performance and egg quality. Poultry Science, 81 (Suppl.), 40.

Harms RH, Douglas CR, Sloan DR. Midnight feeding of commercial laying hens can improve eggshell quality. J. Applied Poult. Res. 1996; 5: 1±5.

Hill, R., 1974. Changes in circulating copper, manganese and zinc with the onset of lay in the pullet. Pages 632–634 in: Trace Elements in Man and Animals. Vol. 2. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, and W. Mertz, ed. University Park Press, Baltimore, MD.

Hocking, P.M. (2009) Feed restriction. In: Hocking, P.M. (ed.), Biology of breeding poultry, pp. 307–330. CABI, Cambridge, MA.

Hudson, B. P., W. A. Dozier, J. L. Wilson, J. E. Sander, and T. L. Ward. 2004. Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic sources of zinc from hatching to 65 wk of age. J. Appl. Poult. Res. 13:349–359.

Hunton, P. 1995. Understanding the architecture of the egg shell.Worlds Poult. Sci. J. 51:141–147.

Keener, K., McAvoy, K., Foegeding, J., Curtis, P., Anderson, K., & Osborne, J. (2006). Effect of testing temperature on internal egg quality measurements. Poultry Science, 85(3), 550-555. doi:10.1093/ps/85.3.550

Kemp, C., Wylie, L. and Fisher, C. (2001) Broiler breeder nutrition, nutrient transfer and broiler performance. Paper presented at 13th European Symposium on Poultry Nutrition, pp.61 – 67. Blankenberghe, Belgium.

Kidd, M. T. 2003. A treatise on chicken dam nutrition that impacts on progeny,. Worlds Poult. Sci. J. 59:475–494.

Lavelin I, Meiri N, Pines M. New insight in eggshell formation. Poult. Sci. 2000; 79: 1014±7. PMID: 10901204

Leksrisompong, N. (2010) Effects of feeder space and feeding programs during rearing and laying on broiler breeder reproductive performance and livability. North Carolina State University (thesis), Raleigh, NC. 197p.

Leksrisompong, N., Plumstead, P.W., Romero-Sanchez, H. and Brake, J. (2006) Effect of feeding program during rearing and onset of lay on reproductive performance of broiler breeder females. Poult. Sci., 85(Suppl. 1), 180 (Abstr.)

Packard, M. J., and G. C. Packard. 1991. Patterns of mobilization of calcium, magnesium, and phosphorus by embryonic yellow headed blackbirds (*xanthocephalus xanthocephalus*). J. Comp. Physiol. B 160:649-654

Palmer, B. D., and L. J. Guillette, Jr., 1991. Oviductal proteins and their influence on embryonic development in birds and reptiles. Pages 29–46 in: Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. D. C. Deeming and M.W.J. Ferguson, ed. Cambridge University Press, Cambridge, UK.

Panic, B., L. Bezbradica, N. Nedeljkov, and A. G. Istwari, 1974. Some aspects of trace element metabolism in poultry. Pages 635–637 in: Trace Element in Man and Animals. Vol. 2. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, and W. Mertz, ed. University Park Press, Baltimore, MD.

Pavlovski Z,S Ï krbić Z, Lukić M, Vitorović D, Lilić S, Petričević V. Shell quality‹everlasting problem in the today poultry science. Biotech. Ani. Husb. 2012; 28: 393±404.

Richards, M. (1997). Trace mineral metabolism in the avian embryo. *Poultry Science, 76*(1), 152-164. doi:10.1093/ps/76.1.152

Richards, M. P., 1989. Influence of egg production on zinc, copper and iron metabolism in the turkey hen (Meleagris gallopavo). Comp. Biochem. Physiol. 93A:811–817.

Richards, M. P., and M. J. Packard. 1996. Mineral metabolism in avian embryos. Poult. Avian biol. Rev. 7:143-161

Roberts, J. R. 2004. Factors affecting egg internal quality and eggshell quality in laying hens. J. Poult. Sci. 41:161–177.

Robinson, F.E., Zuidhof, M.J. and Renema, R.A. (2007) Reproductive efficiency and metabolism of female broiler breeders as affected by genotype, feed allocation, and age at photostimulation. 1. Pullet growth and development. Poult. Sci., 86, 2256– 2266.

Romero-Sanchez, H., Plumstead, P.W., Leksrisompong, N, Brannan, K.E. and Brake, J. (2008) Feeding broiler breeder males. 4. Deficient feed allocation reduces fertility and broiler progeny body weight. Poult Sci., 87, 805–811.

Singh JA, Ferrier C, Singh P. Measurement and analysis of the shocks generated during egg production. App. Pack. Res. 2007; 2: 45±59.

Stevenson M.H. (1985): Effect of added cassava root meal, ZnO and their interaction on the production and quality of eggs from laying hens. Journal of the Science of Food and Agriculture, 36, 909–914

Vieira, S. L. 2007. Chicken embryo utilization of egg micronutrients.Rev. Bras. Cienc. Avic. 9:1–8.

Washburn, K.W. (1982) Incidence, cause, and prevention of eggshell breakage in commercial production. Poultry Science 61: 205-2012.

Webster, A.B. (2004) Welfare implications of avian osteoporosis. Poultry Science 83: 184-192.

Whitehead, C.C. and FLEMING, R.H. (2000) Osteoporosis in laying hens. Poultry Science 79: 1033-1041.

Yenice, E., C. Mizrak, M. G¨ultekin, Z. Atik, and M. Tunca. 2015. Effects of dietary organic or inorganic manganese, zinc, copper and chrome supplementation on the performance, egg quality and hatching characteristics of laying breeder hens. Ankara ¨Univ. Vet. Fak. Derg. 62:63–68.

Zamani A., Rahmani H.R., Pourreza H.R. (2005): Eggshell quality is improved by excessive dietary zinc and manganese. In: Proceedings 15th European Symposium on Poultry Nutrition, 25–29 September, Balatonfured, Hungary, 520–522

Zhang, Y. N., H. J. Zhang, J. Wang, H. Y. Yue, X. L. Qi, S. G.Wu, and G. H. Qi1. 2017. Effect of dietary supplementation of organic or inorganic zinc on carbonic anhydrase activity in eggshell formation and quality of aged laying hens. Poult. Sci. 96:2176–2183.

Table 1. Egg weight parameter quality of eggs at 1-27 weeks of lay from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		1 week of lay	6 weeks of lay	10 weeks of lay	13 weeks of lay	21 weeks of lay	27 weeks of lay
		----------Egg weight (g)----------					
TM Form[1]							
MI		56.98	58.89	62.00	61.34	64.56[b]	67.36
FR		58.46	59.35	61.55	62.24	66.79[a]	68.44
TM Dose[2]							
100%		56.67[b]	58.82	60.97	61.98	55.17	67.56
200%		58.78[a]	59.42	62.59	61.60	66.18	68.24
Form[3]	**Dose[3]**						
MI	200%	58.84	58.45	63.33	59.97[b]	64.34	66.85
FR	200%	58.70	60.38	61.83	63.22[a]	68.01	69.62
MI	100%	55.12	59.33	60.66	62.71[a]	64.76	67.86
FR	100%	58.21	58.30	61.26	61.25[ab]	65.57	67.26
Source of Variation		----------*P-value*----------					
Form		.1213	.6571	.6477	.3489	**.0315**	.3460
Dose		**.0297**	.5584	.1053	.6903	.3248	.5573
FormXDose		.0921	.1479	.2897	**.0156**	.1643	.1453
SEM		.66	.71	.70	.67	.72	.81

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.

Table 2. Haugh Unit internal quality of eggs at 1-27 weeks of lay from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		1 week of lay	6 weeks of lay	10 weeks of lay	13 weeks of lay	21 weeks of lay	27 weeks of lay
		----------Haugh Unit----------					
TM Form[1]							
MI		92.68	91.16	86.24[a]	81.60	80.75	83.32
FR		93.86	90.74	82.91[b]	81.86	81.86	80.57
TM Dose[2]							
100%		94.09	91.11	84.60	82.88	79.66	82.48
200%		92.45	90.80	84.54	80.58	82.96	81.41
Form[3]	**Dose[3]**						
MI	200%	91.11	90.79	87.00	81.61	81.79	83.05
FR	200%	93.79	90.80	82.08	79.54	84.12	79.76
MI	100%	94.25	91.54	85.48	81.58	79.71	83.57
FR	100%	93.94	90.66	83.72	84.17	79.60	81.37
Source of Variation		----------*P-value*----------					
Form		.3174	.7591	**.0188**	.8964	.5608	.0948
Dose		.1677	.8288	.9649	.2539	.0861	.5125
FormXDose		.2091	.7531	.2584	.2482	.5223	.7378
SEM		.83	.99	.98	1.41	1.34	1.14

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.

Table 3. Yolk Color internal quality of eggs at 1-27 weeks of lay from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		1 week of lay	6 weeks of lay	10 weeks of lay	13 weeks of lay	21 weeks of lay	27 weeks of lay
		----------Yolk Color----------					
TM Form[1]							
MI		7.95[b]	8.42	8.69	8.36	9.06	9.44
FR		8.66[a]	8.44	8.94	8.06	8.56	9.67
TM Dose[2]							
100%		8.21	8.22[b]	8.91	8.08	8.53[b]	9.50
200%		8.42	8.64[a]	8.72	8.33	9.08[a]	9.61
Form[3]	**Dose[3]**						
MI	200%	8.083	8.77	8.50	8.55	9.38	9.61
FR	200%	8.750	8.50	8.94	8.11	8.77	9.61
MI	100%	7.833	8.05	8.88	8.16	8.72	9.27
FR	100%	8.583	8.38	8.94	8.00	8.33	9.72
Source of Variation		----------*P-value*----------					
Form		**.0035**	.8875	.3756	.2016	.0620	.2744
Dose		.3684	**.0368**	.4902	2951	**.0387**	.5835
FormXDose		.8566	.1229	.4902	.5597	.6746	.2744
SEM		.16	.14	.20	.17	.19	.14

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.

Table 4. Egg Shell Strength external quality of eggs at 1-27 weeks of lay from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		1 week of lay	6 weeks of lay	10 weeks of lay	13 weeks of lay	21 weeks of lay	27 weeks of lay
		----------Egg Shell Strength (N) ----------					
TM Form[1]							
MI		45.35	33.50	36.39	34.31	34.05	36.30
FR		44.5	36.46	34.96	34.71	34.82	37.14
TM Dose[2]							
100%		44.65	34.64	35.58	33.59	32.43	36.96
200%		45.20	35.29	35.77	35.44	36.44	36.48
Form[3]	**Dose[3]**						
MI	200%	45.74	35.44[ab]	37.83	34.56	35.92	36.20
FR	200%	44.65	35.14[ab]	33.71	36.30	36.97	36.76
MI	100%	44.96	31.55[b]	34.96	34.06	32.18	36.40
FR	100%	44.34	37.79[a]	36.21	33.12	32.67	37.52
Source of Variation		----------*P-value*----------					
Form		.7384	.0586	.4130	.7824	.5814	.6481
Dose		.8309	.6765	.9171	.2109	**.0055**	.7965
FormXDose		.9261	**.0300**	.1277	.3586	.8424	.8777
SEM		183.53	106.56	125.62	104.89	100.99	132.66

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.

Table 5. Egg Shell Elasticity external quality of eggs at 1-27 weeks of lay from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		1 week of lay	6 weeks of lay	10 weeks of lay	13 weeks of lay	21 weeks of lay	27 weeks of lay
		----------Egg Shell Elasticity (mm) ----------					
TM Form[1]							
MI		0.249	0.220	0.220	0.220	0.240	0.300
FR		0.254	0.217	0.210	0.230	0.220	0.290
TM Dose[2]							
100%		0.253	0.224	0.200	0.210	0.240	0.250
200%		0.250	0.213	0.220	0.240	0.220	0.330
Form[3]	**Dose[3]**						
MI	200%	0.247	0.216	0.236	0.213[ab]	0.218	0.348
FR	200%	0.253	0.210	0.211	0.263[a]	0.228	0.315
MI	100%	0.252	0.224	0.204	0.219[ab]	0.269	0.245
FR	100%	0.255	0.224	0.205	0.207[b]	0.205	0.258
Source of Variation		----------*P-value*----------					
Form		.5042	.7976	.3644	.1792	.2591	.8172
Dose		.6329	.3918	.1660	.0801	.5634	.0719
FormXDose		.8734	.8149	.3436	**.0287**	.1271	.6060
SEM		.0045	.0088	.0097	.0098	.017	.031

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.

Table 6. Vitelline Membrane Strength Internal quality of eggs at 1-27 weeks of lay from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		1 week of lay	6 weeks of lay	10 weeks of lay	13 weeks of lay	21 weeks of lay	27 weeks of lay
		----------Vitelline Membrane Strength (N) ----------					
TM Form[1]							
MI		.0227	.0201	.0180	.0182	.0177	.0191
FR		.0227	.0198	.0173	.0164	.0190	.0173
TM Dose[2]							
100%		.0231	.0201	.0165	.0173	.0186	.0185
200%		.0222	.0198	.0188	.0174	.0182	.0178
Form[3]	**Dose[3]**						
MI	200%	.0216	.0190	.0189	.0168	.0168	.0189
FR	200%	.0226	.0205	.0185	.0177	.0195	.0166
MI	100%	.0235	.0212	.0171	.0195	.0187	.0191
FR	100%	.0228	.0190	.0159	.0149	.0185	.0178
Source of Variation		----------*P-value*----------					
Form		.9584	.8389	.5505	.1690	.3077	.0745
Dose		.4566	.8014	.0980	.9516	.7143	.4764
FormXDose		.5297	.1986	.7519	**.0332**	.2312	.5768
SEM		.10	.10	.097	.092	.088	.074

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.

Table 7. Vitelline Membrane Elasticity Internal quality of eggs at 1-27 weeks of lay from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		1 week of lay	6 weeks of lay	10 weeks of lay	13 weeks of lay	21 weeks of lay	27 weeks of lay
		----------Vitelline Membrane Elasticity (mm) ----------					
TM Form[1]							
MI		3.97	3.64	3.04	3.16	1.17	1.31
FR		3.99	3.47	3.12	2.87	1.34	1.09
TM Dose[2]							
100%		4.06	3.67	2.82	3.03	1.28	1.25
200%		3.90	3.44	3.35	3.01	1.24	1.15
Form[3]	**Dose[3]**						
MI	200%	3.88	3.36	3.16	2.93[ab]	1.05	1.28
FR	200%	3.92	3.50	3.53	3.12[ab]	1.41	1.01
MI	100%	4.05	3.92	2.91	3.42[a]	1.28	1.33
FR	100%	4.06	3.42	2.71	2.63[b]	1.26	1.16
Source of Variation		----------*P-value*----------					
Form		.9158	.4831	.8008	.2445	.2468	.0746
Dose		.5250	.3400	.1114	.9352	.7843	.3989
FormXDose		.9373	.1973	.4005	**.0429**	.1928	.6869
SEM		.17	.18	.23	.17	.10	.085

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.

Table 8. Calcium content of whole eggs from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		Week 6 of lay	Week 10 of lay	Week 13 of lay	Week 21 of lay	Week 27 of lay
TM Form				mg Ca/g		
MI		46.25	30.54	34.22	32.25	32.91
FR		37.88	37.12	27.62	36.98	29.37
TM Dose						
100%		42.24	33.22	28.83	32.28	33.11
200%		41.90	34.43	33.12	36.95	28.83
Form	**Dose**					
MI	200%	39.27	29.80	36.87	32.96	29.24
FR	200%	36.50	39.06	28.63	40.95	28.49
MI	100%	44.52	31.27	31.05	31.54	35.97
FR	100%	47.99	35.18	26.60	33.01	30.26
Source of Variation		----------*P-value*----------				
Form		0.1886	0.166	0.09	0.0694	0.3253
Dose		0.954	0.7951	0.2815	0.072	0.2006
FormXDose		0.6064	0.5666	0.5984	0.2006	0.4482
SEM		4.12	3.24	2.44	1.74	2.21

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters

Table 9. Phosphorus content of whole eggs from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		Week 6 of lay	Week 10 of lay	Week 13 of lay	Week 21 of lay	Week 27 of lay
TM Form				mg P/g		
MI		157.32	110.33	117.23	119.89	116.35
FR		132.90	134.09	98.29	130.67	102.33
TM Dose						
100%		145.72	119.21	102.18	117.93	116.04
200%		144.50	125.22	113.70	132.63	101.39
Treatment	**Dose**					
MI	200%	151.61	109.01	124.38	122.55	103.60
FR	200%	137.39	141.43	100.88	142.71	99.55
MI	100%	163.03	111.65	108.66	117.23	126.97
FR	100%	128.41	126.76	95.71	118.64	105.11
Source of Variation		----------*P-value*----------				
Form		0.2112	0.1757	0.1474	0.1687	0.2684
Dose		0.9474	0.7262	0.3963	0.0659	0.2185
FormXDose		0.5859	0.615	0.6659	0.2287	0.4432
SEM		12.71	11.97	8.67	5.34	7.84

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters

Table 10. Copper content of whole eggs from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		Week 6 of lay	Week 10 of lay	Week 13 of lay	Week 21 of lay	Week 27 of lay
TM Form				mg Cu/g		
MI		0.0100	0.0092	.00027	0.0242[b]	0.0255
FR		0.0033	0.0183	.0024	0.0300[a]	0.0250
TM Dose						
100%		0.0100	0.0142	.0025	0.0258	0.0258
200%		0.0033	0.0133	.0027	0.0283	0.0245
Treatment	**Dose**					
MI	200%	0.0001	0.0100	.0027	0.0233	0.0260
FR	200%	0.0067	0.0167	.0026	0.0333	0.0233
MI	100%	0.0200	0.0083	.0026	0.0250	0.0250
FR	100%	0.0000	0.0200	.0023	0.0267	0.0267
Source of Variation		----------*P-value*----------				
Form		0.5447	0.1550	.4572	**0.0131**	0.8666
Dose		0.5447	0.8945	.4572	0.2566	0.6956
FormXDose		0.2415	0.6912	.8372	0.0658	0.4697
SEM		0.0105	0.0044	.0002	0.0015	0.0021

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters

Table 11. Iron content of whole eggs from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		Week 6 of lay	Week 10 of lay	Week 13 of lay	Week 21 of lay	Week 27 of lay
TM Form				mg Fe/g		
MI		1.99	1.43	1.52	1.31	1.25
FR		1.61	1.65	1.45	1.51	1.26
TM Dose						
100%		1.79	1.54	1.48	1.32	1.27
200%		1.81	1.54	1.49	1.50	1.23
Treatment	**Dose**					
MI	200%	1.92	1.36	1.62	1.33	1.16
FR	200%	1.69	1.73	1.35	1.67	1.29
MI	100%	2.05	1.51	1.40	1.29	1.32
FR	100%	1.53	1.57	1.55	1.35	1.23
Source of Variation		----------*P-value*----------				
Form		0.1725	0.2986	0.7765	0.0607	0.9081
Dose		0.9537	0.9708	0.9714	0.0969	0.8008
FormXDose		0.5783	0.4405	0.3332	0.173	0.5282
SEM		0.177	0.143	0.149	0.072	0.116

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters

Table 12. Manganese content of whole eggs from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		Week 6 of lay	Week 10 of lay	Week 13 of lay	Week 21 of lay	Week 27 of lay
TM Form				mg Mn/g		
MI		0.03833	0.02417	0.03000	0.02250[b]	0.02273
FR		0.03167	0.02833	0.03000	0.02833[a]	0.02667
TM Dose						
100%		0.03167	0.02417	0.02900	0.02250[b]	0.02083
200%		0.03833	0.02833	0.03091	0.02833[a]	0.02909
Treatment	**Dose**					
MI	200%	0.04000	0.02500	0.03333	0.02500	0.02400
FR	200%	0.03667	0.03167	0.02800	0.03167	0.03333
MI	100%	0.03667	0.02333	0.02600	0.02000	0.02167
FR	100%	0.02667	0.02500	0.03200	0.02500	0.02000
Source of Variation		----------*P-value*----------				
Form		0.2191	0.255	0.945	**0.0335**	0.4844
Dose		0.2191	0.255	0.7308	**0.0335**	0.1613
FormXDose		0.5237	0.49	0.2506	0.7477	0.319
SEM		0.0035	0.0025	0.0033	0.0018	0.0037

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters

Table 13. Zinc content of whole eggs from broiler breeders fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

		Week 6 of lay	Week 10 of lay	Week 13 of lay	Week 21 of lay	Week 27 of lay
TM Form				mg Zn/g		
MI		1.337	0.924	1.010	0.882[b]	0.838
FR		1.193	1.090	0.875	1.028[a]	0.816
TM Dose						
100%		1.217	0.959	0.886	0.863[b]	0.830
200%		1.313	1.055	1.000	1.047[a]	0.823
Treatment	**Dose**					
MI	200%	1.307	0.933	1.083	0.915	0.816
FR	200%	1.320	1.177	0.900	1.178	0.828
MI	100%	1.367	0.915	0.922	0.848	0.857
FR	100%	1.067	1.003	0.850	0.877	0.803
Source of Variation		----------*P-value*----------				
Form		0.3534	0.2218	0.2082	**0.0332**	0.7696
Dose		0.5251	0.4746	0.294	**0.0091**	0.9108
FormXDose		0.313	0.5622	0.5758	0.0801	0.6397
SEM		0.1029	0.0930	0.0675	0.0451	0.0476

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters

Table 14. Mineral content of eggshells from broiler breeders at 10 weeks of lay fed diets supplemented free or lipid microencapsulated premixes at 100% and 200% of Ross 708 TM recommendations.

Shell

		Ca	P	Cu	Fe	Mn	Zn
		----------mg/g----------					
TM Form							
MI		353.6020	1.2433	0.0010	0.0148	0.0024	0.0029
FR		344.4340	1.1600	0.0014	0.0162	0.0030	0.0047
TM Dose							
100%		349.5640	1.1980	0.0010	0.0150	0.0021	0.0034
200%		349.3270	1.2117	0.0013	0.0158	0.0032	0.0040
Treatment	**Dose**						
MI	200%	356.0833	1.2233	0.0012	0.0159	0.0033	0.0038
FR	200%	342.5700	1.2000	0.0014	0.0157	0.0031	0.0041
MI	100%	351.1200	1.2633	0.0008	0.0136	0.0015	0.0019
FR	100%	347.2300	1.1000	0.0013	0.0170	0.0030	0.0055
Source of Variation		----------*P-value*----------					
Form		0.4351	0.3951	0.4095	0.4217	0.395	0.1437
Dose		0.9889	0.7793	0.511	0.7974	0.1925	0.8482
FormXDose		0.661	0.5187	0.6902	0.3774	0.2469	0.2069
SEM		7.8358	0.0687	0.0003	0.0012	0.0004	0.0009

[1]Premix form main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[2]Trace mineral supplementation dose main effect means are an average of 12 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters.
[3]Treatment means are an average of 6 eggs sampled from each of 3 replicate pens per treatment containing ca. 9 hens and 2 roosters

CHAPTER 4

Carry-over broiler breeder effect supplementation of trace minerals in two forms (lipid microencapsulated (MI) and free (FR) trace minerals) and two doses (100%, 200%) on their progeny fed microencapsulated in two doses (100%, 200%)

ABSTRACT

Microencapsulated inorganic trace minerals (MITM) have been observed to improve egg production and feed efficiency of broiler breeders, and may also improve the performance, histology, and bones of their progeny, especially the progeny that are also fed normal or excessive dietary levels of MITM. Broiler breeders were assigned to 4 dietary treatments consisting of a factorial arrangement of 2 mineral premix forms (free and protected) and 2 mineral premix supplement dosages (100% and 200% of Aviagen recommendations). Sixty chicks from each of these four broiler breeder treatment groups were randomly assigned to 10 replicate alternative design cages and fed one of 2 dietary levels of MITM (100% or 200% of Aviagen trace mineral recommendations. Growth performance at 7, 14, and 21 days, jejunum histomorphology, bone characteristics, and bone mineral composition were all assessed and statistically analyzed as a 4X2 factorial. No statistically significant ($P<.05$) treatment effects were observed on growth performance. Progeny fed MI100% had higher villi height and greater bone elasticity and lower bone ash than those fed MI200%. Progeny from broiler breeder that received diets supplemented with free TM at 200% level had the highest value for diaphysis diameter, weight, and ash%. Carry-over effects for ash% and BMD were observed, where progeny from broiler breeders fed free minerals at 100% and 200% and then fed 200% microencapsulated trace mineral presented with higher as%. There were no treatment effects on

tibia bone mineral composition. This study demonstrated broiler breeders fed MITM may improve skeletal and enteric development of the progeny, especially when progeny is also fed MITM.

INTRODUCTION

Broilers are raised for meat production; therefore, better feeding of these animals has been a popular topic of study in the past years in order to increase profits and improve growth. Worldwide, animal nutritionists have worked towards the goal of formulating the best diet that optimizes nutrition that meets the genetic potential of broilers for maximum production and feed utilization. With a plateau reached on how much feed can ameliorate production; the need to look back into the carry-over effects of broiler breeder feeding manipulation and effects upon broilers has become a very important topic of study. Poultry, in contrast to mammals, have their progeny develop outside of the maternal womb which creates a challenge for the progeny as everything that the embryo can use for development has to be present in the egg (White, 1991; Richards, 1997; Kidd, 2003; Virden et al., 2003; Calini and Sirri, 2007; Torres et al., 2009). It has been studied that if the broiler breeders are deficient in trace minerals (TM), they are not able to deposit any of these trace minerals in the egg and the progeny will also be deficient (Richards and Steele, 1987); showing the importance of feeding breeders trace minerals that meet all of their and the progenies requirements.

Free inorganic trace minerals have reduced bioavailability for the animal; thus, the industry feeds broilers a much higher dose of minerals than the animal's nutritional requirements (Swiatkiewicz et al., 2014; Aksu et al., 2010; 17.3), which results in increased environmental emissions that compromises environmental sustainability (Bao and Choct, 2009; Mezes et al., 2012). In contrast, microencapsulation is hypothesized to release minerals at a slower rate that does not overwhelm active transport absorption mechanisms and limits chelation, thus allowing for better absorption and less excretion of trace mineral.

In the past, researchers have been focused on the amount of minerals in the eggs; whereas now, researchers are focused on the chemical form that the minerals are in and how readily the embryo is able to mobilize and use these minerals (Dibner and Richards, 2005). For embryos that are deficient in trace minerals it has been observed that they have impaired growth, abnormal organ development, and death in cases of extreme deficiencies (Richards and Steele, 1987). It has been studied that the embryos have a limited source of all of the essential minerals, except for Calcium (Ca) which can be readily found by the embryo in the eggshell (Angel, 2007). Some studies report that up to 90% of the Ca and 30% of the Magnesium used by the embryo's development comes from the eggshell which serves as a storage for these minerals (Packard and Packard, 1991). With respect to Ca being fed in the diet, the more Ca fed the less Zinc (Zn), Copper (Cu), and Manganese (Mn) are absorbed (Dibner et al., 2007) due to trace mineral interactions in the gut. This interaction between these minerals is due to the coordinate covalent binding of all of these cations to phytic acid that forms insoluble complexes that cannot be absorbed by the bird (Dibner et al., 2007).

Moreover, modern-day broilers have been engineered to be fast growers and build muscle at a fast rate in order to meet consumer demands for protein. With the genetic selection for fast muscle growth, comes some skeletal problems. Skeletal malformations are common problems that are prevalent in the poultry industry due to the skeletal structure not being able to keep up with the muscular development (Angel, 2007; Dibner et al., 2007). Modern broilers' skeletal systems are characterized as having poor calcification and high bone porosity (Swiatkiewicz and Arczewska-Wlosek, 2012; Williams et al., 2000), which can lead to damage and deformities. As a result of these skeletal issues, trace minerals are frequently fed in higher doses that exceed the NRC recommended requirements. Although minerals are being fed in a higher dose, these

skeletal problems persist and the persistence of these problems could be due to mineral-mineral antagonism and poor mineral bioavailability (National Research Council (US), 1994; Richards et al., 2015; Kratzer, 1986).

With a hypothesized better mineral uptake by the hen through the use of microencapsulated inorganic trace minerals (MITM), better mineral utilization of those minerals by the progeny may produce more robust chicks. Through this carry-over change caused by the better utilization of minerals, the progeny of these broiler breeders fed the slower release MITM may show better trace mineral utilization, bone development, and immunological responses. We hypothesize that there will be a carry-over effect from broiler breeder fed MITM on progeny with improvements in performance, jejunum histomorphology, tibia bone characteristics, and tibia bone composition due to better mineral bioavailability and lessened adverse effects from higher dose inclusion of trace minerals. Additionally, we hypothesize that on top of the carry-over effect observed, feeding MITM to broilers will also improve these production parameters due to better bioavailability of trace minerals. The objective of this study was to evaluate the carry-over effect of dietary supplementation of standard and high levels (100% and 200% of breeder recommendations) of inorganic trace minerals for broiler breeders that are either microencapsulated in a conventional free form premix on the performance, histology, and bones of their progeny that received diets supplemented with standard or high levels of microencapsulated inorganic trace minerals.

MATERIALS AND METHODS

Housing and Management

All experimental procedures on live animals used in this experiment approved by the North Carolina State University Animal Care and Use Committee. The broiler breeders used in this experiment were obtained when they were 27 weeks of age after being raised by standard husbandry practices for broiler breeder candidates. One-hundred-eight Ross 708 females, and 24 Ross HY males were randomly distributed among 12 pens (1.8 m by 1 m; about .163 m^2 per bird) with 9 females and 2 males per pen, so there were 3 replicate pens per treatment. Four dietary treatments arranged as a 2 X 2 factorial of 2 forms of trace mineral premixes (microencapsulated (MI) or Free (FR)) and 2 supplementation levels (100% and 200% of Aviagen Recommendations) as follows: Microencapsulated 200% (MI200%), Free 200% (FR200%), Microencapsulated 100% (MI100%), Free 100% (FR100%). The formulation information of these dietary treatments is presented in detail in Chapter 2.

The broiler breeders were housed in a room maintained at about 25°C and 77% relative humidity. Each pen had a nest box with 6 nest spaces. Water was available *ad libitum* by a Plasson bell drinker. Each pen was supplied with a female feeder that had a screen to exclude males from consuming the female feed, and a male feeder raised to a level out of reach for the females to consume the male feed. The amount of feed was issued daily to each feeder in accordance to breeder recommendations. The floor pen litter was composed of soft pine shavings that was renewed when necessary and supplemented with fresh shavings twice per week or as needed to maintain litter quality and minimize environmental ammonia emission below 10-15 ppm. The broiler breeders received 16 hours of fluorescent light (30-60 lux) and 8 hours of dark.

The eggs collected during 54 weeks of lay were incubated according to standard protocols using ChickMaster™ with a maximum capacity of 1800 eggs. Sixty unsexed chicks from each of the four broiler breeder treatment groups were randomly assigned to 10 replicate battery cages (.37 m^2 each) (Alternative Design, Springdale, AR), and fed one of 2 dietary levels of MITM (100% or 200% of Aviagen trace mineral recommendations. This resulted in 8 experimental treatments (Table 1) arranged as a 4X2 factorial of 4 broiler breeder diets and 2 levels of MITM supplemented to the diets of progeny. Each of these 8 treatments were randomly assigned to 5 replicate cages containing 6 chicks. Group body weight data (per cage) was collected at the beginning of the trial, and the homogeneity of chick weights was taken into account when placing the birds.

The birds were maintained in a controlled environment, the temperature, humidity, and light schedule were in accordance with the flock manual guidelines. Water was available for *ad libitum* consumption by nipple drinkers. The dietary treatments were supplied to the chicks in mash form. The dietary treatment formulations were isocaloric and isoproteic and met or exceeded NRC (1994) recommendations (Table 2). Vitamin levels were kept constant through all of the different treatments and only the inclusion levels and forms of the minerals were changed. Weekly body weights and feed consumption were recorded and feed conversion ratio (FCR = Feed/Gain) was calculated.

Jejunum Histomorphology

At the end of the trial when the chicks were 21 days of age, a total of 9 chicks were randomly selected from each of the 8 treatments and euthanized by cervical dislocation. Within 5 minutes of confirmed death of the sampled bird, approximately 5 cm section from the middle

of the jejunum (identified as the section between the end of the duodenum loop and the Meckel's diverticulum) were removed opened longitudinally, and fixed immediately in a buffered 10% formalin solution for 48 h. Samples were then washed in 70% ethanol to remove the fixing solution, dehydrated in increasing alcohol concentrations, clarified in xylol, and embedded in paraffin. Semiseriated 5-mm transverse thick histological sections were stained with hematoxilin-eosin (Behmer et al., 1976), and microscope slides were assembled with Canada balsam. Light-microscope (LEICA-DMR; Leica Camera AG, Solms, Germany) was used to visualize stained sections on slides at 4X magnification, and images were captured by Image Tools to measure the villus height (VH), upper villi width, bottom villi width, crypt depth (CD), and muscularis mucosae thickness using AmScope™ x86 software (AMSCOPE, Orange County, CA) . The villi surface was calculated using 10 readings per replicate per variable, according to the formula: VH/CD and villi surface = [(upper villi width + bottom villi width)/2] X villus height in (Solis de los Santos et al., 2005).

Bone mineral composition and characteristics

At 21 days, 15 chicks per treatment were euthanized by the IACUC approved method of cervical dislocation and their right tibias were dissected, weighed, and measured as follows. The bone length and middle (diaphysis) diameter were measured in millimeters using digital calipers. Bone strength and bending moment were then determined by TA.HDPlus texture analyzer machine with a 250 kg (Stable Micro Systems, Hamilton, MA) load cell. The two support points for the bones were two inches apart. The bone mass density (BMD) was assessed using dual-energy x-ray absorptiometry (DEXA, SCHICK, accuDEXA BMD Assessment System, Long Island City, NY). The mineral composition of these bones was then determined by Inductively

coupled plasma mass spectrometry (ICP-MS, 5800 ICP-OES, Agilent, Santa Clara, CA) with 6 broiler bones per treatment being assessed.

Statistical analysis

The experimental design was a 4x2 factorial design with 5 replicates per treatment. The replicate pens were the experimental units. This experiment was designed in such way so that the focus was the carry-over effects from broiler breeder to progeny, and how the performance of the progeny would change when fed the low and high levels of MITM. Data was analyzed by 1-way ANOVA (JMP 15 software; SAS Inst. Inc.) the means were then compared using Tukey's test. Data were considered significant when $P<.05$.

RESULTS

Table 3 illustrates the treatment effects on progeny broiler growth performance approximated breeder standards. There were no significant treatment effects observed on body weights, feed intake, and FCR of progeny broilers. There were no significant carry-over effects from the broiler breeder dietary treatments, nor progeny dietary treatments effects.

The results of premix form and TM dose on jejunum intestinal mucosa histology is reported in Table 4. The only statistically significant ($P<.05$) treatment effect on jejunum mucosal histology was observed on villus height of 21 d chicks. The progeny fed standard (100% of Aviagen recommendations) level of microencapsulated minerals had 11% longer villi than those fed the higher level (200% of Aviagen recommendations) of microencapsulated mineral treatments. Although statistically significant broiler breeder X progeny diet effects were observed on histology, it is noteworthy that a near significant ($P=0.06$) interaction effect was

observed on villi surface area. Progeny from broiler breeders fed the FR100% treatment had greater villi surface area when fed the MI100% treatment than the MI200%, but progeny from broiler breeders fed the MI200% treatment had greater villi surface area when fed the MI200% treatment.

The effect of premix form and TM dose on progeny tibia bone results can be found in Table 5. Unlike the growth performance data and mucosal histology data, there were significant treatment effects on several bone quality attributes. The progeny of the MI100% treatment group had significantly ($P<.05$) greater tibia length than progeny in MI200% treatment group. Tibia length was not significantly affected by the broiler breeder dietary treatments. In contrast, no carry-over effects were detected. Birds that were from the FR200% diet from the broiler breeders, presented with higher values for diaphysis diameter, fresh weight, and ash%. Progeny that were fed MI100% diets presented with higher values for bone bending moment. Inversely, progeny fed MI100% diets presented with lower values for ash%. Progeny birds presented with statistically significant ($P<.05$) Ash% and DEXA carry-over effects. Progeny that received FR100%*MI200%, FR200%*MI100%, and FR200%*MI200% treatments showed higher values for ash% showing a carry-over effect. Treatment FR200%*MI200% showed the highest values for bone density whereas FR200%*MI100% showed the lowest values for DEXA, pointing to a carry-over effect.

The effect of premix form and TM dose on bone mineral content can be found in Table 4. No significant differences were observed when looking at the mineral content of the tibia and no carry-over effects were observed ($P<.05$).

DISCUSSION

Feeding trace minerals to broiler breeders has an effect on offspring broiler performance and livability (Bhoyar, 2018; Rebel et al., 2004; Kemp et al., 2001; Kidd, 2009; Calini and Sirri, 2007; Bozkurt et al., 2008; Fisher and Gous, 2009; Eusebio-Balcazar et al., 2010; Lekrinsompong, 2010; Moraes et al., 2011; Sun et al., 2012; Kidd et al., 1992; Kidd, 2003; Richards and Steele, 1987). Hence, there is an observed carry-over effect from parent to progeny in many studies dealing with the effects of maternal diet on the amount of minerals deposited in the egg (Richards and Packard, 1996). This observed effect is not directly observed in production parameters like body weight, but the carry-over effect is observed by the differing livability and improved immunological responses (Moraes et al., 2011; Kidd et al., 2003).

No statistically significant ($P<.05$) interactions effects among broiler breeder treatments were observed on the progeny performance at 7, 14, and 21 days. Nevertheless, there were differences found in the villi height on birds fed treatments MI100%. Jejunum histomorphological analysis provides an insight into the differences that are observed between the treatments. Villi height showed some significant differences by treatments. Villi height was longer in the broilers fed MI100%, but no carry-over effects were observed pertaining to other histology parameters. Previous studies (Choct, 2009; Parsaie et al., 2007) have attributed higher VH/CD ratios to the better mucosal health and absorptive capacity. Lower crypt depths are indicative of lower villi cellular turnover, which represents a lower demand for nutrients for repairs and better use of nutrients for performance (Choct, 2009; Parsaie et al., 2007). Higher free form trace mineral supplementation levels were expected to have the greatest crypt depths as their antagonism in the gut would cause oxidative stress and villi deterioration.

Due to the rapid improvements made to the broilers' genetic potential, their growth rate and feed conversion ratio (FCR) have greatly improved. However, this rapid genetic selection for fast growth has exacerbated metabolic problems in these birds (Angel, 2007). These metabolic problems can be the explanation for the skeletal problems observed as the birds are not able to properly allocate nutrients for bone development as the nutrients are going towards building muscle mass. Implementing changes in the diet and management of these birds can lessen the prevalence of these skeletal conditions observed (Angel, 2007). Organic trace minerals have been shown to increase ash content of bones (Vieira et al., 2020), so there is evidence that mineral feeding has an effect on the bone structure of poultry. Using alternative nutrients such as MITM in the pre-starter feed may allow for optimal skeletal development, this is especially important in the starter phase as the chicks have a high requirement for trace minerals due to growth at an accelerated rate. Significant differences in the tibia bones were found among treatments. With respect to the tibia measurement parameters like length, thickness, weight, and DEXA statistical differences were observed. No significant breeder treatment carry-over effects on progeny bone bending moment and breaking strength also not observed.

Antagonistic interactions with inorganic minerals have great prevalence in the low pH environment of the upper gastrointestinal (GI) tract, where ITM dissociate due to the acidic environment (Dibner et al., 2007; Underwood and Suttle, 1999; Virden et al., 2003). When minerals reach lower parts of the GI tract, the minerals can bind to other minerals, nutrients, phytate, and fiber which renders the minerals insoluble (Dibner et al., 2007; Richards et al., 2015; Kratzer, 1986). Insoluble forms of minerals are then excreted as they are not able to be absorbed by the animal. These findings are contradictory to what is expected, as treatments with protected minerals were expected to show more bioavailability for the broiler breeders and better

bone calcification was expected. However, in one experiment where broiler bones were assessed, the ideology of feeding more bioavailable forms of trace minerals would result in stronger bones being supported (Richards et al., 2015; Kratzer, 1986).

In conclusion, this study showed indications that broiler breeders fed with MITM ameliorates the bone structure of the progeny. Further studies on this new mode of delivering TM and its carry-over effects on broilers needs to be studied further to get a better understanding on how this changed the carry-over effect from maternal diet to progeny.

REFERENCES

Aksu, D., T. Aksu, B. ¨ Ozsoy, and E. Baytok. 2010. The effects of replacing inorganic with a lower level of organically complexed minerals (Cu, Zn and Mn) in broiler diets on lipid peroxidation and antioxidant. Asian-Australasian J. Anim. Sci. 23: 1066–1072.

Ammerman CB, Baker DH, Lewis AJ. Introduction to bioavailability of nutrients for animals. In: Ammerman CB, Baker DH, Lewis AJ, editors. Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and Vitamins. 1st ed. San Diego, CA, USA: Academic Press; 1995.

Angel, R. 2007. Metabolic disorders: Limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. App. Poult. Res. 16:138-149.

Bao, Y. M., and M. Choct. 2009. Trace mineral nutrition for broiler chickens and prospects of application of organically complexed trace minerals: a review. Anim. Prod. Sci. 49:269–282.

Bhoyar A., Why trace mineral supplements are pivotal for breeder efficiency. (2018). Retrieved February 14, 2021, from https://www.thepoultrysite.com/articles/why-trace-mineral-supplements-are-pivotal-for-breeder-efficiency

Bozkurt, M., C¸ abuk, M. and Alc¸ic¸ek, A. (2008) Effect of dietary fat type on broiler breeder performance and hatching egg characteristics. J. Appl. Poultry. Res., 17, 47– 53.

Bun, S., Y. Guo, F. Guo, F. Ji, and H. Cao. 2011. Influence of organic zinc supplementation on the antioxidant status and immune responses of broilers challenged with Eimeria tenella. Poult. Sci. 90:1220–1226.

Calini, F. and Sirri, F. (2007) Breeder nutrition and offspring performance. Braz. J. Poult. Sci., 9, 77– 83.

Choct, M. 2009. Managing gut health through nutrition. Br. Poult. Sci. 50:9–15.

Dibner, J. J., and J. D. Richards. 2005. Mineral metabolism and chelated minerals for hatchlings. Pages 51-72 in Recent Advances in Animal Nutrition. P. C. Garnsworthy and J. Wiseman, ed. Nottingham Univ. Press. UK

Dibner, J. J., J. D. Richards, M. L. Kitchell, and M. A. Quiroz. 2007. Metabolic challenges and early bone development. J. Appl. Poult. Res. 16:126–137.

Eusebio-Balcazar, P.E., Oviedo-Rondo´ n, E.O., Mitchell, A., Brake, J., Wineland, M.J., Moraes, V. and Leandro, N. (2010) Effects of breeder feeding and trace mineral source on leg health and bone traits of broiler progeny. Poult. Sci., 89(Suppl. 1), Abstract 177, p. 223.

Fisher, C. and Gous, R.M. (2009) Protein and amino acid responses. In: Hocking, P.M. (ed.), Biology

Kemp, C., Wylie, L. and Fisher, C. (2001) Broiler breeder nutrition, nutrient transfer and broiler performance. Paper presented at 13th European Symposium on Poultry Nutrition, pp.61 – 67. Blankenberghe, Belgium.

Kidd, M. T., N. B. Anthony, and S. R. Lee. 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poult. Sci. 71:1201–1206.

Kidd, M.T. (2003) A treatise on chicken dam nutrition that impacts on progeny. World Poult. Sci. J., 59, 475– 494.

Kidd, M.T. (2009) Vitamins, minerals, and micronutrients. In: Hocking, P.M. (ed.), Biology of breeding poultry, pp. 361– 374. CABI, Cambridge, MA.

Kratzer FH, Vohra P. *Chelates in Nutrition*. Boca Raton, FL, USA: CRC Press; 1986.

Leksrisompong, N. (2010) Effects of feeder space and feeding programs during rearing and laying on broiler breeder reproductive performance and livability. North Carolina State University (thesis), Raleigh, NC. 197p.

Mezes M, Erdélyi M, Balogh K (2012) Deposition of organic trace metal complexes as feed additives in farm animals. Euro Chemi Bull 1(10):410–413

Moraes, V. M., Oviedo-Rondón, E. O., Leandro, N. S., Wineland, M. J., Malheiros, R. D., & Eusebio-Balcazar, P. (2011). Broiler breeder trace mineral nutrition and feeding practices on Embryo Progeny Development. *Avian Biology Research, 4*(3), 122-132. doi:10.3184/175815511x13147937995611

National Research Council (US), Subcommittee on Poultry Nutrition. *Nutrient Requirements of Poultry*. 9th ed. Washington, DC, USA: National Academy Press; 1994.

Packard, M. J., and G. C. Packard. 1991. Patterns of mobilization of calcium, magnesium, and phosphorus by embryonic yellow headed blackbirds (*xanthocephalus xanthocephalus*). J. Comp. Physiol. B 160:649-654

Parsaie, S., F. Shariatmadari, M. J. Zamiri, and K. Khajeh. 2007. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers. Br. Poult. Sci. 48:594–600.

Rebel, J., Van Dam, J., Zekarias, B., Balk, F., Post, J., Flores miñambres, A., & Ter Huurne, A. (2004). Vitamin and trace mineral content in feed of breeders and their progeny: Effects of growth, feed conversion and severity of malabsorption syndrome of broilers. *British Poultry Science, 45*(2), 201-209. doi:10.1080/00071660410001715803

Richards, J., Evans, J., Fisher, P., & Wedekind, K. (2015). Greater bioavailability of chelated compared with inorganic zinc in broiler chicks in the presence or absence of elevated calcium and phosphorus. *Open Access Animal Physiology,* 97. doi:10.2147/oaap.s83845

Richards, M. P., and N. C. Steele, 1987. Trace element metabolism in the developing avian embryo: A review. J. Exptl. Zool. Suppl. 1:39–51.

Richards, M.P. (1997) Trace mineral metabolism in the avian embryo. Poult. Sci., 76, 152– 164.

Richards, M.P., and M.J. Packard. 1996. Mineral metabolism in avian embryos. Poult. Avian Biol. Rev. 7:143-161

Solis de los Santos, F., Farnell, M., Tellez, G., Balog, J., Anthony, N., Torres-Rodriguez, A., Donoghue, A. (2005). Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. Poultry Science, 84(7), 1092-1100. doi:10.1093/ps/84.7.1092

Sun QJ, Guo YM, Ma SD, Yuan JM, An SY, Li JH (2012) Dietary mineral sources altered lipid and antioxidant profiles in broiler breeders and post hatch growth of their off springs. Biol Trace Elem Res 145:318–324

Swiatkiewicz, S., A. Arczewska-Wlosek, and D. J´ozefiak. 2014. The efficacy of organic minerals in poultry nutrition: review and implications of recent studies. Worlds Poult. Sci. J. 70:475–486.

Swiatkiewicz, S., & Arczewska-Wlosek, A. (2012). Prebiotic fructans and organic acids as feed additives improving mineral availability. *World's Poultry Science Journal, 68*(2), 269-279. doi:10.1017/s0043933912000323

Torres, C.A., Vieira, S.L., Reis, R.N., Ferreira, A.K., Silva, P.X. and Furtado, F.V.F. (2009) Productive performance of broiler breeder hens fed 25-hydroxycholecalciferol. R. Bras. Zootec., 38, 1286– 1290.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd Edn., CABI Publishing, Wallingford, UK, Pages: 497.

Vieira R, Ferket P, Malheiros R, Hannas M, Crivellari R, Moraes V, Elliott S. Feeding low dietary levels of organic trace minerals improves broiler performance and reduces excretion of minerals in litter. Br Poult Sci. 2020 Oct;61(5):574-582.

Virden, W. S., J. B. Yeatman, S. J. Barber, C. D. Zunnwalt, T. L. Ward, A. B. Johnson, and M. T. Kidd. 2003. Hen mineral nutrition impacts progeny livability. J. Appl. Poult. Res. 12:411– 416.

Virden, W.S., Yeatman, J.B., Barber, S.J., Zumwalt, C.D., Ward, T.L., Johnson, A.B. and Kidd, M.T. (2003) Hen mineral nutrition impacts progeny livability. J. Appl. Poult. Res., 12, 411– 416.

Wang, G., Liu, L., Wang, Z., Pei, X., Tao, W., Xiao, Z., . . . Ao, T. (2018). Comparison of inorganic and organically bound trace minerals on tissue mineral deposition and fecal excretion in broiler breeders. *Biological Trace Element Research, 189*(1), 224-232. doi:10.1007/s12011-018-1460-5

White, H. B., III, 1991. Maternal diet, maternal proteins and egg quality. Pages 1–5 in: Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. D. C. Deeming and M.W.J. Ferguson, ed. Cambridge University Press, Cambridge, UK.

Williams, B., Solomon, S., Waddington, D., Thorp B. and Farquharson, C. (2000) Skeletal development in the meat type chicken. British Poultry Science 41: 141-149.

Table 1. Progeny treatment distribution from broiler breeders fed trace minerals in two forms and two doses (microencapsulated (MI) and free (FR) trace minerals, and (100%, 200%)) with progeny fed two levels of microencapsulated (MI) levels (100%, 200%)

Treatment (1-9)	Broiler breeder Treatment	Progeny Treatment	Concise form
T1	Microencapsulated 200%	Microencapsulated 200%	MI200%/MI200%
T2	Microencapsulated 200%	Microencapsulated 100%	MI200%/MI100%
T3	Free 200%	Microencapsulated 200%	FR200%/MI200%
T4	Free 200%	Microencapsulated 100%	FR200%/MI100%
T5	Microencapsulated 100%	Microencapsulated 200%	MI100%/MI200%
T6	Microencapsulated 100%	Microencapsulated 100%	MI100%/MI100%
T7	Free 100%	Microencapsulated 200%	FR100%/MI200%
T8	Free 100%	Microencapsulated 100%	FR100%/MI100%

Table 2. Experimental diets fed to broiler from 0 to 21 days of age

Ingredients (% of diets)	MI100%	MI200%
Corn	50.70	50.70
Soybean meal	40.65	40.65
Poultry fat	4.49	4.49
Limestone	1.37	1.37
Mono-Dicalcium phosphate	1.51	1.51
Salt NaCl	0.24	0.24
Soda bicarbonate	0.16	0.16
L-Lysine HCl 98%	0.17	0.17
DL-Methionine 99.0%	0.34	0.34
L-Threonine 98.5%	0.10	0.10
TEST PREMIX SPACE	0.20	0.20
Choline chloride 60%	0.07	0.07
Total	**100.00**	**100.00**
Protected Vitamin (PV)	0.078[1]	0.078[1]
Protected Mineral (PM)	**0.092[2]**	**0.306[3]**
Free Vitamin (FV)	0	0
Free Mineral (FM)	0	0
Palmitic Acid	0.117	0
Vermiculite	0.097	0
Total Ingredients	100.0	100.0
ME POULTRY, Kcal ME/kg	3000	3000
Crude Protein, %	23.26322	23.26322
LYS DIG, %	1.28	1.28
Ca App Phytase, %	0.96	0.96
NON PHYT P, %	0.48	0.48
DIG LYS MG	11471.48	11471.48
DIG LYS:ME	3.823828	3.823828
EE, %	6.804345	6.804345
CA, %	0.96	0.96
Total P, %	0.727788	0.727788

[1]Each kilogram of PV at a dietary inclusion of 0.078% or FV at a dietary inclusion of 0.05% supplied the following per kg of complete feed: vitamin A, 13,000 IU; cholecalciferol, 5,850 IU; alpha-tocopherol, 84.5 IU; niacin, 78 mg; pantothenic acid, 23 mg; riboflavin, 8.5 mg; pyridoxine, 4.2 mg; menadione, 4 mg; folic acid, 2.5 mg; thiamin, 3.25 mg; biotin, 0.234 mg; vitamin B_{12}, 0.02 mg; ethoxyquin.

[2]Each kilogram of PM at a dietary inclusion of 0.092% or FM at a dietary inclusion of 0.06% supplied the following per kg of complete feed: 78 mg Zn as ZnO; 78 mg Mn as MnO; 33 mg Fe as $FeSO_4.H_2O$; 9 mg Cu as CuSO4; .9 mg I as $Ca(IO_3)_2$.

[3]Each kilogram of PM at a dietary inclusion of 0.306% or FM at a dietary inclusion of 0.20% supplied the following per kg of complete feed: 255 mg Zn as ZnO; 255 mg Mn as MnO; 108 mg Fe as $FeSO_4.H_2O$; 30 mg Cu as CuSO4; .2.85 mg I as $Ca(IO_3)_2$.

Table 3. Effect of dietary supplementation of encapsulated and free trace minerals on broiler breeder progeny performance with carry over effects from broiler breeders fed trace minerals in two forms and two doses (microencapsulated (MI) and free (FR) trace minerals, and (100%, 200%))

	7d			14d			21d		
	Body Weight (g)	Feed intake (kg)	FCR	Body Weight (g)	Feed intake (kg)	FCR	Body Weight (g)	Feed intake (kg)	FCR
Broiler breeder (BB)									
FR100%	0.185	1.36	1.66	0.458	3.84	1.57	0.867	1.24	1.52
MI100%	0.180	1.30	1.63	0.443	3.67	1.54	0.874	1.22	1.47
FR200%	0.192	1.29	1.50	0.468	3.68	1.49	0.900	1.25	1.47
MI200%	0.186	1.29	1.55	0.459	3.73	1.51	0.908	1.24	1.44
Broiler (P)									
MI100%	0.182	1.31	1.63	0.451	3.66	1.52	0.894	1.23	1.46
MI200%	0.189	1.31	1.54	0.463	3.80	1.53	0.881	1.24	1.49
BB * P									
FR100%*MI100%	0.186	1.49	1.80	0.454	3.79	1.56	0.893	1.25	1.48
FR100%*MI200%	0.181	1.29	1.62	0.448	3.73	1.55	0.888	1.25	1.48
MI100%*MI100%	0.187	1.21	1.47	0.467	3.50	1.43	0.909	1.22	1.42
MI100%*MI200%	0.173	1.23	1.63	0.436	3.64	1.55	0.885	1.21	1.45
FR200%*MI100%	0.184	1.24	1.52	0.463	3.90	1.58	0.842	1.24	1.56
FR200%*MI200%	0.179	1.31	1.65	0.439	3.61	1.53	0.859	1.19	1.46
MI200%*MI100%	0.196	1.36	1.52	0.468	3.86	1.55	0.891	1.27	1.52
MI200%*MI200%	0.198	1.35	1.47	0.483	3.83	1.47	0.932	1.26	1.42
	----------*P-value*----------								
BB	0.3755	0.923	0.1265	0.4505	0.0966	0.8668	0.7238	0.8095	0.5028
P	0.8016	0.7199	0.2138	0.7436	0.4136	0.727	0.8115	0.9287	0.6553
BB*P	0.6282	0.0595	0.1577	0.6329	0.2085	0.6279	0.7843	0.627	0.6935
SEM	.006	.04	.04	.01	.05	.04	.02	.03	.02

Table 4. Effect of dietary supplementation of microencapsulated trace minerals (100%, 200%) on progeny jejunum histomorphology at 21 days with carry over effects from broiler breeders fed trace minerals in two forms and two doses (microencapsulated (MI) and free (FR) trace minerals, and (100%, 200%))

	Villus Height (VH, μm)	Villus tip width (μm)	Villus bottom width (μm)	Crypt depth (CD, μm)	Muscularis (μm)	Area (μm^2)	Ratio (VH/CD)
Broiler breeder (BB)							
FR100%	918.74	191.44	214.86	121.31	141.25	187861.79	7.62
MI100%	894.73	177.13	199.65	130.48	128.09	166275.44	6.87
FR200%	833.00	181.34	208.79	130.62	126.79	163413.80	6.62
MI200%	840.59	179.10	193.61	123.70	123.58	160674.50	6.93
Broiler (P)							
MI100%	922.51[a]	178.03	198.60	121.31	129.65	177632.56	7.29
MI200%	821.02[b]	186.47	209.85	130.48	130.21	161480.20	6.73
BB * P							
FR100%*MI100%	999.19	196.95	224.41	118.90	138.75	214217.59	8.47
FR100%*MI200%	838.30	185.93	205.31	123.72	143.76	161506.00	6.78
MI100%*MI100%	940.02	160.20	185.99	134.50	128.07	160411.11	6.99
MI100%*MI200%	849.44	194.06	213.32	126.45	128.11	172139.77	6.76
FR200%*MI100%	936.73	183.61	211.40	132.50	130.44	187178.36	7.14
FR200%*MI200%	729.26	179.06	206.18	128.74	123.14	139649.23	6.09
MI200%*MI100%	814.10	171.38	172.61	126.80	121.33	148723.18	6.55
MI200%*MI200%	867.09	186.82	214.61	120.61	125.83	172625.82	7.31
		----------*P-value*----------					
BB	0.3507	0.7737	0.5174	0.4624	0.0929	0.4099	0.2303
P	**0.0122**	0.4159	0.2985	0.5183	0.913	0.1971	0.1203
BB*P	0.1077	0.4108	0.1719	0.8205	0.8191	0.0637	0.0881
SEM	39.00	10.21	10.98	5.03	3.60	12673	.35

[a,b]Means within a column with a different superscript differ significantly ($P<0.05$).
[1]Means are an average of 9 birds per treatment with 10 villi measurements per broiler

Table 5. Effect of dietary supplementation of microencapsulated trace minerals on progeny tibia bone characteristics at 21 days with carry over effects from broiler breeders fed trace minerals in two forms and two doses (microencapsulated (MI) and free (FR) trace minerals, and (100%, 200%)) [1]

	Length (mm)	Diaphysis diameter (mm)	Fresh weight (g)	Bending moment (mm)	Bone strength (N)	Ash %	BMD[2]
Broiler breeder (BB)							
FR100%	76.42	6.250^{b}	8.671^{ab}	0.0383	172.89	61.63^{b}	0.3308
MI100%	77.15	6.250^{b}	8.270^{b}	0.0387	173.87	59.59^{b}	0.3370
FR200%	77.53	6.657^{a}	9.292^{a}	0.0391	177.50	64.25^{a}	0.3251
MI200%	77.71	6.362^{ab}	8.519^{ab}	0.0403	165.73	59.55^{b}	0.3277
Broiler (P)							
MI100%	77.86^{a}	6.477	8.816	0.0407^{a}	177.60	60.10^{b}	0.3331
MI200%	76.55^{b}	6.283	8.560	0.0375^{b}	167.30	62.41^{a}	0.3273
BB * P							
FR100%*MI100%	77.18	6.214	8.759	0.0381	173.58	57.50^{c}	0.3509^{ab}
FR100%*MI200%	75.67	6.287	8.583	0.0386	172.11	65.76^{a}	0.3107^{cd}
MI100%*MI100%	78.19	6.471	8.787	0.0393	181.62	59.57^{c}	0.3438^{abc}
MI100%*MI200%	76.11	6.029	7.753	0.0381	166.03	59.61^{c}	0.3302^{abcd}
FR200%*MI100%	77.58	6.636	9.141	0.0404	177.30	63.28^{ab}	0.2985^{d}
FR200%*MI200%	77.48	6.677	9.443	0.0377	177.60	65.22^{a}	0.3518^{a}
MI200%*MI100%	78.47	6.587	8.577	0.0452	177.89	60.06^{bc}	0.3391^{abc}
MI200%*MI200%	76.94	6.137	8.460	0.0354	153.57	59.04^{c}	0.3163^{bcd}
	----------*P-value*----------						
BB	0.3019	**0.0180**	**0.0164**	0.8405	0.7487	**<.0001**	0.8012
P	**0.0126**	0.0609	0.2682	**0.0458**	0.186	**0.0001**	0.5165
BB*P	0.5678	0.1176	0.2241	0.1291	0.6302	**<.0001**	**0.0018**
SEM	.36	.10	.23	.002	.79	.59	.009

[a,b]Means within a column with a different superscript differ significantly (P<0.05).
[1]Means are an average of 15 birds per treatment
[2]Bone Mass Density

Table 6. Effect of dietary supplementation of microencapsulated trace minerals on progeny tibia bone mineral composition at 21 days with carry over effects from broiler breeders fed trace minerals in two forms and two doses (microencapsulated (MI) and free (FR) trace minerals, and (100%, 200%)) [1]

	Ca	P	Cu	Fe	Mn	Zn
	----------mg/g----------					
Broiler breeder (BB)						
FR100%	347.17	175.83	0.0020	0.3573	0.0097	0.3473
MI100%	368.26	183.88	0.0023	0.4172	0.0123	0.3844
FR200%	341.55	170.68	0.0020	0.3846	0.0106	0.3655
MI200%	338.91	167.77	0.0020	0.3093	0.0106	0.4151
Broiler (P)						
MI100%	341.40	170.99	0.0020	0.3609	0.0108	0.3698
MI200%	356.54	178.09	0.0021	0.3733	0.0107	0.3863
BB * P						
FR100%*MI100%	357.26	180.81	0.0020	0.3615	0.0117	0.3642
FR100%*MI200%	337.08	170.85	0.0020	0.3532	0.0077	0.3303
MI100%*MI100%	327.00	163.88	0.0020	0.3425	0.0123	0.3520
MI100%*MI200%	409.52	203.87	0.0025	0.4918	0.0122	0.4168
FR200%*MI100%	339.99	170.83	0.0020	0.4197	0.0090	0.3483
FR200%*MI200%	343.11	170.53	0.0020	0.3495	0.0122	0.3827
MI200%*MI100%	341.35	168.45	0.0020	0.3200	0.0103	0.4147
MI200%*MI200%	336.47	167.10	0.0020	0.2985	0.0108	0.4155
	----------*P-value*----------					
BB	0.7143	0.6589	0.11	0.204	0.3639	0.08
P	0.4467	0.4652	0.151	0.7342	0.9041	0.3795
BB*P	0.2692	0.2724	0.11	0.1746	0.121	0.2856
SEM	13.93	9.62	.00008	.04	.001	.02

[1]Means are an average of 6 bones per treatment

CHAPTER 5

Final Conclusions

Dietary inclusion of microencapsulated trace minerals (MITM) indeed positively impacts broiler breeder egg production rate and production efficiency, regardless of dietary supplementation level. MITM fed birds produced about 11 more setable eggs per hen during 27 weeks of lay than hens fed the conventional free trace mineral premix form (FRTM). This increased productivity of MITM hens interestingly occurred without increasing feed intake, thus significantly decreasing FCR (kg feed/dozen eggs). The jejunum histomorphology showed an increase in villi area for hens fed 100% levels of trace minerals (TM) independent of the premix form (MITM or FRTM), where increased values for villus height, crypt depth, and villi area were significantly different. Tibia bone characteristics and mineral composition did not show any consistent changes independently of TM form or dose.

Hens fed MITM only had marginal and and inconsistent effects on the internal and external egg quality and specific mineral contents. Although egg quality parameters were evaluated in the 1st, 6th, 10th, 13th, 21st, and 27th weeks of lay, more frequent egg quality assessment is recommended for future research in this topic. With more frequency of egg quality parameters, it will be possible to determine if MITM or FRTM feeding did have an impact on some of these egg quality characteristics by week, or if they were due to random chance.

Dietary supplementation of MITM for broiler breeders and progeny has interesting results on progeny performance and welfare. There were no differences in the performance parameters observed during the 21day-day trial. The most consistent effects of MITM feeding to broilers were in tibia bone characteristics. Broiler breeders fed MITM had greater tibia length and bending moment when the broilers were fed MI100%. Progeny from FR200% broiler

breeder treatment demonstrated the greatest diaphysis diameter and fresh weights of tibia. The interaction of broiler breeder (FR200%) in combination with progeny treatment (MI200%) resulted in the greatest tibia bone mass density (BMD) and ash content. Interestingly, there were no differences in the tibia bone mineral composition of the progeny for any of the minerals analyzed.

Feeding MITM to broiler breeders increased the number of eggs produced throughout the period of lay. TM form and dose did not have consistent effects on internal and external egg quality, and mineral composition of eggs. Progeny chick weight at hatch was also impacted, where the lightest chicks at hatch were from treatments where from the breeders fed MITM. Additionally, no effects in progeny performance parameters (BW, feed intake, FCR) were observed up to 21 days. MITM may be an alternative for broiler breeders as there is an increase in fertile egg production and a lower FCR (kg feed/dozen eggs). If each hen is able to produce 11 eggs more, lets assume that from those eggs 8 hatch. Each of those can be sold for roughly $.60, that is roughly $5 more per hen. Multiply that figure by a ten thousand broiler breeder flock, and that is nearly $50,000 more profitability per flock. Which has highly significant economic impacts for the industry, as more fertile eggs are produced per hen, and consequently more broilers to market increases integrated corporate profitability.