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The Effects of Zinc Supplementation from Two Sources on Egg Quality and Bone Health in Laying Hens

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THE EFFECTS OF ZINC SUPPLEMENTATION FROM TWO SOURCES ON EGG QUALITY AND BONE HEALTH IN LAYING HENS

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The objective of this study was to compare zinc sources and levels of supplementation on laying hen performance. Bovan White Leghorn hens were fed one of six dietary treatments in a 2x3 factorial arrangement consisting of two zinc sources (Availa®Zn or zinc sulfate) and three levels (40, 80, or 120 ppm). Treatments were randomly assigned to 48 cages with five hens/cage. Blocks provided eight replicates/treatment. Hens were housed in a tiered manure belt housing system providing 97.2 cm^2/hen. Hens were given 110 g/hen/day of feed ad libitum. Feed intake, egg production, egg weights, egg components, eggshell strength, body weight, bone mineral density, keel bone scores, manure zinc content, and feather scores were measured. Data were analyzed using the Proc Glimmix procedure in SAS. No significant treatment effects were found for feed intake, egg production, feed conversion ratio, body weight gain, egg weight, percent eggshell, bone mineral density, or prevalence of keel bone injury. The 80 ppm level of zinc resulted in significantly greater ($P=0.048$) eggshell breaking strength during months 5 and 7 regardless of source. Hens fed Availa®Zn had higher percent yolk ($P=0.050$) and lower percent albumen ($P=0.031$) compared to hens fed zinc sulfate. There was a significant overall source effect ($P<0.0001$) for manure zinc content such that hens fed zinc sulfate had lower amounts of zinc excretion. There was a

significant level effect (P<0.0001) where hens consuming 120 ppm zinc excreted the greatest amount of zinc. A significant source by level interaction was observed (P<0.0001) for both the overall and individual analyses such that 120 ppm Availa®Zn showed the highest zinc excretion and both 40 ppm zinc sulfate and Availa®Zn showed the lowest zinc excretion. Hens fed 80 ppm zinc sulfate had significantly improved feather scores (P=0.031) over all other treatments, while hens fed 120 ppm Availa®Zn had the lowest feather scores. Based on these results Availa®Zn may improve eggshell breaking strength and percent egg yolk.

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Introduction

Zinc plays a vital role in growth and development of the chick so it is important to meet the minimum requirements when supplementing zinc in poultry diets. The NRC (1994) has reported the dietary requirement of zinc for leghorn-type chicks as 40 mg/kg of feed. The requirement decreases to 35 mg/kg of feed as the bird grows into an adult laying hen. The requirements for brown laying hens are slightly different. The NRC (1994) recommends 38 mg/kg of feed for brown chicks decreasing to 33 mg/kg of feed for growing brown hens. Roberson and Schaible (1958) showed that chicks fed a basal diet not supplemented with zinc grew poorly, did not feather properly, developed footpad dermatitis, and could not walk properly. Chicks that were fed the same basal diet with added zinc did not develop any of the deficiency symptoms seen in the first group. Their experiments showed the importance of zinc in growth, feather development, skin and foot health, and feed efficiency in poultry. Zinc is also important for stabilizing the structure of many enzymes and forms part of the active site of others. It is a required element in over 200 enzymes including carboxypeptidases, RNA polymerase III, alcohol dehydrogenase, and connective tissue, though the exact role of zinc in formation of connective tissue is still unknown. (Olhaberry et al., 1983; MacDonald, 2000). Zinc is also part of the enzyme carbonic anhydrase which is essential in supplying carbonate ions during eggshell formation in laying hens (Sahin et al., 2009). Overall, zinc is vital for growth and development, egg quality, bone health, and immune function in laying hens.

Absorption and Metabolism

The liver, pancreas, and small intestine all play an essential role in zinc homeostasis. These organs help maintain a balance in the body through absorption, reabsorption, and excretion of zinc. It is known that increased intake of zinc is correlated with increased uptake, but it is not known if greater uptake results in greater amounts of zinc being stored in tissues or pools that the body can pull from during times of zinc deficiency (Krebs, 2000). In some cases, excess zinc consumed over a short period of time can result in a decrease in fractional zinc absorption. This is most likely due to saturation of the available zinc transporters (Lönnerdal, 2000). Absorption also differs between zinc supplemented in the feed and zinc supplemented in water. In poultry production zinc is commonly added to the feed so only studies relating to this method of supplementation will be discussed. The specific cellular mechanisms of zinc uptake are still unknown, but several transporters have been identified. ZnT-1 is expressed mainly in the duodenum and jejunum of the small intestine and has been shown to function as an exporter of zinc. This transporter may help eliminate excess zinc from accumulation in tissues. The main roles and significance of transporters ZnT-2 and ZnT-3 are not yet known and require further investigation. ZnT-2 is expressed in the kidneys, testis, and intestines while ZnT-3 is expressed in the brain and testis. ZnT-4 is expressed in the brain, heart, lungs, liver, spleen, and kidneys. It is also thought to be expressed in mammary tissue and play a role in excretion of zinc into milk (Cousins and McMahon, 2000). Metallothionein (MT) has been identified as an intracellular metal binding protein. It's role in zinc absorption is still unclear. Synthesis of MT in the liver and small intestine is stimulated by zinc in the diet as well as zinc injection and inflammation (Krebs, 2000).

It is thought that MT may help limit concentrations of free zinc (Cousins, 1996). Divalent cation transporter 1 (DCT1) is a transmembrane polypeptide expressed in the small intestine that may also be involved in zinc uptake (Krebs, 2000). DCT1 is important in iron absorption and, therefore, it is up-regulated in response to low iron. This may cause increased uptake of zinc since DCT1 has a similar affinity for both iron and zinc (Cousins and McMahon, 2000). The primary site of zinc absorption is in the small intestine, and can be influenced by the presence of promoters or inhibitors. Other divalent cations in the diet such as calcium and magnesium can have an inhibitory effect on zinc absorption because of competition for transporters such as DCT1.

Some plant-based diets can inhibit absorption due to the presence of phytate. Phytate is composed of different forms of inositol phosphate and binds with cations, including zinc, to form very stable complexes, thereby reducing mineral absorption. The hexaphosphate and pentaphosphate forms seem to have the most negative effect on zinc absorption (Lönnerdal, 2000). Ao et al. (2007) showed that the addition of phytase to a diet known to contain phytate increased the bioavailability of zinc as well as feed intake and weight gain. Lönnerdal (2000) suggested that animal protein may also counteract the inhibitory effects of phytate. Further research into methods using phytase and animal protein together to decrease phytate inhibition of zinc absorption would prove beneficial to the poultry industry.

As mentioned earlier, consumption of excess zinc over a short period of time may inhibit absorption due to saturation of available transporters. There may also be a passive transport component to zinc absorption in which zinc can diffuse across a membrane, but the transporter-mediated method is likely the predominant mode of zinc transport

(Lönnerdal, 2000). Other factors that may influence zinc uptake include levels of cadmium and amount of protein in the diet. Cadmium at very high levels may inhibit zinc absorption. Protein positively impacts zinc absorption by increasing total zinc intake and increasing the bioavailability. The effects of ethylenediamine tetra-acetate (EDTA) are not as well known. In some cases it has been shown to positively impact zinc absorption in the presence of phytate, while in other instances it seems to negatively impact absorption and thus warrants further study (Lönnerdal, 2000). Maintenance of zinc levels in the body is dependent upon reabsorption and excretion as well as absorption from the diet. Sources of endogenous zinc may include bile and pancreatic secretions as well as duodenal secretions, though the regulation of these secretions is unknown (Krebs, 2000). Once absorbed, zinc is a factor in many processes essential to the body. MacDonald (2000) stated in a review that over 200 enzymes require zinc, and that these enzymes affect many major metabolic processes. Transcription of the enzyme thymidine kinase is regulated by zinc. This is part of the process that induces DNA synthesis. Both thymidine kinase mRNA activity and zinc-dependent protein binding to the promoter region of the gene are sensitive to low levels of zinc (Chesters et al., 1995). These two processes are essential to DNA synthesis. Zinc is also an important factor in gene transcription. A zinc binding protein has been identified, QM, that is one of the transcription factors that affects gene transcription through zinc availability (MacDonald, 2000).

Zinc affects both growth hormone (GH) and insulin-like growth factor-1 (IGF-1), which is why it is important for normal growth and development. IGF-1 regulates uptake of glucose and regulation of the cell cycle. A deficiency in zinc can cause a decrease in GH secretion from the pituitary, and since GH stimulates the secretion of IGF-1, this will cause a decrease in IGF-1 as well (Root et al., 1979). A deficiency in zinc will also directly affect IGF-1 concentrations as zinc increases IGF-1 synthesis (Yamaguchi and Hashizume, 1994). A decrease in both GH and IGF-1 will directly inhibit growth. In order to fully understand the role of zinc in cell growth and proliferation further study will need to be done to identify other binding and transport proteins.

Sources of Zinc: Organic and Inorganic

The standard source for zinc supplementation in poultry diets has been zinc-sulfate, but in recent years use of organic sources has become common due to their potential higher bioavailability and less environmental impact through manure loading. Bioavailability is defined as the percentage of nutrient utilized in the body for specific growth measurements. A greater bioavailability indicates greater absorption and deposition of the mineral in question. Inorganic sources of zinc in poultry diets include zinc-sulfate and zinc-oxide. Organic sources of zinc include zinc-methionine, zinc-polysaccharide complexes, zinc-lysine, zinc-proteinate, and zinc-amino acid complexes. Star et al. (2012) reported that with increasing dose levels of an organic zinc source, tibia zinc content was increased in broilers. Using 100% as the reference level of bioavailability of zinc-sulfate, they reported that the organic zinc source had a relative bioavailability of 164%. In their study, feed conversion ratio was also improved for the birds supplemented with the organic zinc source.

Other studies have had variable results when comparing bioavailability values of different organic and inorganic zinc sources. Cao et al. (2000) performed a study to evaluate the bioavailability of several different organic sources of zinc: zinc-methionine complex A and B, zinc-polysaccharide complex, zinc-lysine complex, zinc-amino acid chelate, and zinc-proteinate A, B, and C. They also evaluated the solubility of these zinc sources compared to zinc-sulfate. All eight sources had varying zinc contents and acidities. After three experiments they found that the only source with a significantly higher bioavailability was zinc-proteinate A. All other sources had similar bioavailability to zinc-sulfate. It was also stated that the best predictor for bioavailability estimates for chicks was solubility of the zinc source in a pH 5 buffer. Zinc-proteinate A had the lowest solubility in a pH 5 buffer. These studies show that other factors may be influencing bioavailability of inorganic and organic zinc sources, and that further research into this area may be useful.

There are also environmental concerns associated with zinc where organic sources might prove beneficial. Poultry manure is used in fertilizers and can have a negative impact in areas that are susceptible to heavy metal toxicity. Increased levels of zinc in soil has been linked to reduced crop yields (Giordano et al., 1975). Though the recommended level of zinc supplementation for poultry is 33 - 35 mg/kg, it is common for producers to supplement at higher levels to reduce the possibility of zinc deficiency. Burrell et al. (2004) showed that zinc excretion in broiler chickens increased with increasing concentrations of supplemental zinc regardless of source. Their study also showed that supplementation of an organic zinc complex in combination with zinc sulfate improved zinc utilization and decreased zinc excretion when compared to chickens supplemented with zinc sulfate alone. The addition of only the organic zinc source to the basal diet did not improve zinc retention or lower zinc excretion. Similar benefits of using both an organic and inorganic source of zinc were reported by Hudson et al. (2004).

The authors suggested that use of both sources of zinc in the diet may involve more intestinal transporters or absorption sites, thereby making the zinc more available to the animal. These results show that it may be possible to decrease the amount of zinc supplemented in the diet and decrease zinc excretions.

Deficiency and Toxicity

Zinc deficiency can be a major problem for producers and is more common than toxicity. Symptoms of zinc deficiency include abnormal feather development, bone deformities, dermatitis, and immune dysfunction. It can occur, even if the requirement for zinc in the diet is being met, due to other factors, such as phytase, that may inhibit zinc availability. Cook et al. (1984) encountered a commercially mixed diet that was sufficient in zinc, but caused symptoms of zinc deficiency in pheasant chicks. It was found that adding additional zinc to the commercial diet was successful in reducing the incidence of leg weakness and feather fraying. Possible reasons for the deficiency were hypothesized. A change in the type of pellet binder used may have inhibited zinc absorption. Excess calcium, phosphorus, copper, and iron in the diet may have negatively impacted zinc absorption as divalent cations compete for intestinal transporters. This study showed that it is important to consider other factors such as those mentioned above when determining availability of zinc in a commercially mixed diet. Zinc deficiency in young sexually immature rats has been shown to stunt growth and sexual development, cause hair loss and skin lesions, and decrease weight gain. Deficiency also resulted in decreased pituitary levels of GH in the young rats, explaining at least a partial cause of the decreased growth that was observed (Root et al., 1979).

Zinc toxicity occurs at intakes several times greater than those required for regular maintenance, and so is not a common concern for poultry producers. It has been seen in wild birds in areas where build-up of metals, including zinc, has occurred. Gasaway and Buss (1972) developed a study based on observations of high mortality of ducks, geese, and swans near an area in Idaho with known high concentrations of zinc. They supplemented mallard ducks at different levels of excess zinc to observe the effects and determine possible methods of detecting zinc toxicity in birds. They found that feed intake decreased rapidly and more severely with higher levels of zinc, and, as a consequence, body weight declined. Mortality rate was also high due to the severely decreased caloric intake. Other physical signs of zinc toxicity included leg paralysis and diarrhea. In their study it was determined that testing for zinc levels in the pancreas, liver, and kidneys may prove a useful diagnostic tool when determining if zinc toxicity has occurred. In a report by Holz et al. (2000) several orange-bellied parrots at an aviary facility in Victoria, Australia were dying of unknown causes. The birds were housed in a complex made of galvanized wire mesh. Necropsies were performed, and after several diseases and other causes of death were ruled out, tissue samples were analyzed for zinc concentrations. The tissues samples were taken from the kidney, liver, and pancreas. These samples were then compared with tissue samples from parrots from another population that had died of known causes. The average kidney zinc level of the birds in question was 154.3 µg/g dry weight which was significantly greater than the average level found in the birds from the other population, 65.3 µg/g dry weight. This led to all galvanized wire being replaced with a nylon mesh, after which mortality rates were significantly decreased. The authors of this study hypothesized that the birds did not die directly from zinc poisoning because of the lack of clinical signs and pathologic lesions typically associated with the condition. Rather, the most likely cause of death was determined to be secondary trauma from the birds running into walls or structures in the aviary. This was stated as the most likely cause based on previous knowledge that zinc toxicity can cause neurologic dysfunction.

While neither of these conditions is prevalent in poultry production, it is important to understand that zinc levels need to be maintained between certain values to avoid both deficiency and toxicity.

Egg Quality

Zinc is an important component of the egg. Egg breakage can occur at several stages of production. There are environmental factors involved as well as individual hen factors (Washburn, 1982). Broken eggs are a large financial loss to the poultry industry, so it is necessary for producers to understand how supplemental minerals, such as zinc, can help reduce the incidence of breakage. There are several layers that make up the egg shell and each is dependent on the others to function properly. These layers include: the mammillary layer, the palisade layer, the vertical or surface crystal layer, and the cuticle (Parsons, 1982; Solomon, 2010). There are also two layers of eggshell membranes that lie beneath the mammillary knob layer. The fibers within these membranes are mostly made up of protein. The membranes contribute to the overall strength of the eggshell, but their role in overall eggshell quality is still unclear (Parsons, 1982). The mammillary layer, the innermost layer, consists of mammillary knobs and contains the majority of the organic matter of the eggshell (Parsons, 1982). Studies have evaluated the organization and the density of these mammillary knobs to identify a correlation between these factors and the breaking strength and quality of the eggshell. While some have observed conflicting results, the overall consensus is that malformed mammillary bodies do, in fact, lead to lower quality eggshells (Solomon, 2010). This is, in part, because this layer provides the basis for all subsequent layers, and malformations there will mostly likely lead to poor construction of the following layers. The palisade layer consists of crystal columns oriented perpendicular to the surface of the eggshell, and is the thickest layer of the shell (Parsons, 1982; Solomon, 2010). The vertical or surface crystal layer lies just under the cuticle and is the final calcified layer of the eggshell. Finally, the surface of the egg is covered by an organic waxy coating, the cuticle. This layer functions mainly to protect the egg and its contents (Parsons, 1982). The pores present within the eggshell are important for environmental regulation and gas exchange (Parsons, 1982; Solomon, 2010). All of these elements make up the eggshell and can have a large impact on egg quality.

Zinc supplementation in the diet can have a visible effect on egg quality and hatchability. A study by Hudson et al. (2004) reported that supplementing broiler breeder hens with a combination of an organic and inorganic source of zinc, in this case a zinc amino acid complex (ZnAA) and zinc sulfate ($ZnSO_4$), reduced the incidence of cracked eggs and improved egg shell quality. They also showed that the hens supplemented with the ZnAA + $ZnSO_4$ diet had the highest hen-day egg production as well as the highest hen-housed chick production. This study clearly indicates benefits of using both organic and inorganic sources of zinc. Stefanello et al. (2014) found that supplementation of organic sources of the minerals Mn, Cu, and Zn resulted in greater shell thickness. They also found greater shell strength, indicating better eggshell formation, with increasing levels of the trace minerals regardless of source. Swiatkiewicz and Korelski (2008) found that use of an organic source of zinc, a zinc amino acid complex, may help increase the strength of the eggshell in older hens. Another study had similar results. Manangi et al. (2015) showed that use of an organic zinc supplement, in this case a zinc chelate, increased both eggshell thickness and eggshell strength in older hens. These findings could help decrease the amount of egg loss from breakage in commercial production.

Other studies have reported conflicting results about the benefits of zinc supplementation on egg production and egg quality. Lim and Paik (2003) found that hens supplemented with zinc methionine (Zn-Met) and hens supplemented with zinc manganese methionine (Zn-Mn-Met) both showed decreased egg production. The hens supplemented with the Zn-Mn-Met diet also had the highest incidence of soft shell and broken eggs. A possible reason for these results may be the antagonistic interactions of zinc and manganese since they are both divalent cations and compete for absorption sites. A study done by Stahl et al. (1986) also showed no benefits of supplemental zinc except for a reduction of feather fraying. In this study a basal diet containing 28 ppm was supplemented with zinc at four different levels: 0, 10, 20, and 40 ppm. There were no significant differences in egg production, feed intake, fertility, or hatchability, leading the researchers to conclude that the level of zinc in the basal diet was sufficient for normal production parameters and that supplementation was not necessary. Another study done by Abdallah et al. (1994) reported that removal of several different trace minerals from the diet of laying hens, including zinc, had no significant effect on egg weight, egg production, egg mass, percent shell, specific gravity, or hatchability. The study was only performed for 10 weeks, so it is possible that adverse effects may be seen if trace minerals, including zinc, were withheld for a longer period of time.

Bone Health

Another concern for egg producers is bone health. The egg development process requires an abundant supply of calcium from the diet and skeleton. As laying hens age, bone fragility and fracture can become a significant problem, subsequently leading to decreased egg production and increased mortality. Osteoporosis can become an issue in laying hens causing fragile bones. It occurs when there is an imbalance between the processes of remodeling, or bone resorption, and deposition of new bone (Whitehead and Fleming, 2000). The keel and humerus bones are among the more commonly fractured bones while spinal bones, though severely affected by osteoporosis, are rarely fractured. Still, the loss of integrity in the spinal bones is thought to be a main cause of paralysis in laying hens. This is a common symptom of caged layer fatigue (Whitehead and Fleming, 2000). Several studies have shown the benefits of movement and exercise in improving bone health. Housing systems such as aviaries allow for more movement than traditional battery cages, and so may be more desirable for maintaining bone strength and integrity. The mechanism by which exercise improves bone health is unknown, but is thought to involve stimulation of structural bone formation (Whitehead and Fleming, 2000).

Zinc has an important role in bone health. It is vital for proper bone development in growing chicks. Cook et al. (1984) observed long bone deformities in zinc-deficient pheasant chicks among other symptoms. The leg deformities and weaknesses were alleviated with addition of supplemental zinc to the diet. One study reported that βalanyl-L-histidinato zinc (AHZ) used in a cell culture of osteoblastic cells increased proteins that are involved in bone formation and cell proliferation and had a greater effect than zinc sulfate (Yamaguchi and Hashizume, 1994).

Still, there are varying results of zinc supplementation and its benefits in adult and older hens. Manangi et al. (2015) showed in their study that, although not statistically significant ($P = 0.10$), the use of chelated trace minerals zinc, copper, and manganese, resulted in the strongest bone strength values. These results make another positive argument for the use of organic sources of zinc as opposed to inorganic. Another study done with older hens, which tend to be more susceptible to osteoporosis, showed no significant effects of zinc supplementation on tibia bone parameters and bone ash content (Swiatkiewicz and Koreleski, 2008). The difference in these two studies may be due to the levels of zinc used, or may suggest that zinc works better to prevent osteoporosis when supplemented with other trace minerals known to have a positive effect on bone health such as copper and manganese.

Immunity

Zinc is also known to be important for normal immune system function in animals. The mineral is essential for proper functioning of mononuclear phagocytes, T lymphocytes, macrophages, neutrophils, natural killer cells, and heterophils. These cell types are important in immunity and disease resistance (Kidd et al., 1996; Prasad, 2009). Several studies have shown the positive effects of zinc supplementation on immune response in many species, including poultry. A study by Fernandes et al. (1979) showed the effects of zinc supplementation on immune response in mice. The mice were fed either a zinc deficient (Zn-) or zinc supplemented (Zn+) diet. Primary immune response was measured by the plaque-forming cell (PFC) assay. Response to the PFC assay was significantly lower in the Zn- mice after four weeks on the diet. Mice on the Zn- diet also showed reduced cytotoxic response and deficiency in generating killer T cells as well as a deficiency in natural killer cell activity. A significant reduction in T lymphocytes was also noted in the zinc deficient mice. This experiment showed the effects of zinc deficiency resulting in a decreased immune response, and, therefore, also showed the importance of zinc in immunity and health. Another, more recent study was done with humans and used zinc lozenges as a treatment for the common cold. The group taking the zinc lozenges had a shorter overall duration of cold, cough, and nasal discharge when compared to the placebo group. The symptom severity scores of the zinc group were also significantly decreased from those of the placebo group (Prasad, 2009). These results suggest that zinc may also act as an antiviral agent.

Recent studies show the increased benefits of organic zinc sources on immune function in poultry as opposed to commonly used inorganic sources. A study done by Hudson et al. (2004) showed enhanced immune response to phytohemagglutinin-P injections in broiler breeder hens fed a diet supplemented with a zinc amino acid complex. Higher antibody titers to Newcastle virus were also seen in the same group of hens. A more recent study by Manangi et al. (2015) showed a significant increase in IgG response in older hens fed a diet supplemented with chelated trace minerals, including zinc. In a 1996 review, Kidd et al. stated that zinc-methionine added to poultry diets has been shown to enhance cell functions which are important in disease resistance. They also noted several other studies which have shown that supplemental zinc in the diets of dams increased progeny immunity (Kidd et al., 1996). These studies make a compelling argument for the use of an organic zinc source instead of an inorganic source based on the potential benefits on hen immunity.

Antioxidant Properties of Zinc

An antioxidant is a substance or molecule that inhibits the oxidation of another molecule. While zinc does not completely fit into the definition of an antioxidant, it has been shown many times to have similar properties by exerting indirect effects on oxidants. As an antioxidant, zinc has both acute and chronic effects. In times of chronic deprivation of zinc, an organism can become more susceptible to injury by oxidative stress (Powell, 2000). Acute effects of zinc include stabilization of sulfhydryls by protecting them from oxidation as well as reducing the formation of OH from H_2O_2 and superoxide. This is important because several studies show that metal-catalyzed formation of OH can stimulate processes that are damaging to cells (Powell, 2000). Zinc has also been shown to reduce effects of ischemic damage and inhibit the process of protein oxidation. It is also a co-factor in the antioxidative enzyme copper-zinc-superoxide dismutase. All of these effects prove beneficial, yet the basic mechanisms for how zinc exerts antioxidant properties are still unknown.

Other Benefits in Production

Dietary zinc is vital to normal growth and development, but it also has many other beneficial uses in poultry production. A concern many producers have is their impact on the environment through ammonia losses in poultry manure. Zinc supplementation at levels higher than normal has been shown to decrease manure total nitrogen loss (Kim and Patterson, 2004). Zinc-sulfate has previously been shown to inhibit the activity of microbial uricase, an enzyme which breaks down uric acid, thereby increasing nitrogen retention. Kim and Patterson (2004) also showed that supplementing zinc at such high levels can reduce feed intake and, therefore, body weight. For these reasons further study in this area may be beneficial to determine which source of zinc and what levels optimize both feed intake and nitrogen retention, reducing the impact on the environment.

Supplemental trace minerals have also been shown to increase progeny livability when fed to breeders. A 2003 study reported that progeny from hens fed diets containing zinc and manganese amino acid complexes showed improved livability (Virden et al., 2003). These results may be correlated to improved immunity and disease resistance shown in other studies (Kidd et al., 1996; Hudson et al., 2004).

Another concern for many producers is heat stress. Poultry have a more difficult time maintaining a normal body temperature because they do not have sweat glands. An increase in body temperature beyond the normal range can have negative effects on production. A combination of zinc's antioxidant properties and its role in immune health make it a good supplement for stress reduction. The benefits of zinc and proven results of using it in heat stress situations are stated in Sahin and others' review (2009).

Poultry that are raised in floor pens with litter can have a higher incidence of foot pad dermatitis from contact with moisture and waste. A 2013 study reported that broiler chicks fed a diet containing zinc methionine had significantly less severe foot pad lesions than those that were fed a diet containing zinc oxide. They also noted that feeding excess biotin in combination with either zinc methionine or zinc oxide significantly reduced the severity of foot pad lesions (Abd El-Wahab et al., 2013). This information could be useful for producers that use this type of housing system and have concerns about foot pad dermatitis.

Zinc plays so many essential roles throughout the body that it is important to understand the effects of zinc supplementation. These studies show other benefits of zinc in poultry diets as well as promising areas for further research.

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Chapter 2: The Effects of Zinc Supplementation from Two Sources on Egg Quality and Bone Health in Laying Hens

INTRODUCTION

The poultry industry has long been working toward achieving increased egg production while maintaining egg quality, integrity, and hen health. The process of forming an egg takes a considerable amount of a hen's resources, so meeting nutritional needs is important. Traditionally, inorganic forms of trace minerals such as sulfates and oxides have been used in poultry premixes, but recent years have seen an increase in the use of organic forms of these minerals for their many proposed benefits.

Zinc is essential for many metabolic processes. It is a required component for over 200 enzymes that regulate bone resorption and DNA replication (Olhaberry et al., 1983; MacDonald, 2000). The NRC (1994) recommends 35 mg zinc per kg of feed in the diet for White Leghorn laying hens. In this study it was hypothesized that supplying supplemental zinc in excess of the requirement would increase egg quality and bone health, and that an organic form of zinc would be more efficiently utilized by the hen. Previous studies have shown higher bioavailabilities for organic trace minerals when compared to inorganic forms (Star et al., 2012). Other studies have shown no difference between bioavailability of organic and inorganic trace minerals (Cao et al., 2000). Supplementation of a form of zinc that is more readily available to the hen should help decrease excretion in the manure, keeping toxic levels of heavy metals out of fertilizers.

A main concern for egg producers is egg quality and integrity. Egg weight and egg breakage both affect a producer's income. Increased egg weight can mean increased profits, but if shell strength and shell thickness do not increase to compensate, eggs can be lost due to breakage, which can hurt profits. Zinc plays an important role in formation of the eggshell. It is a component of carbonic anhydrase which supplies carbonate ions during eggshell formation (Sahin et al., 2009). Increased zinc supplementation may help decrease egg loss from cracks and breaks. Some studies have shown increased eggshell strength and thickness and reduced egg breakage in hens supplemented with organic forms of zinc or a combination of organic and inorganic (Hudson et al., 2004; Stefanello et al., 2014). Older hens may continue to produce, but frequently the quality of the eggshell decreases as the hens age. Some studies have shown the benefits of supplemental zinc in increasing eggshell strength and thickness in older laying hens (Swiatkiewicz and Korelski, 2008; Manangi et al., 2015).

Bone health is a concern for all poultry producers. Bone fractures and breaks can mean lost birds and lost profit. It is also a welfare concern. Both the consumer and producer want healthy birds. Bone health is especially a concern in laying hens because the reproductive system requires many of the same nutrients needed for bone growth and maintenance. The goal is to supplement these nutrients at an economic level to maintain optimal bone health and egg quality. The importance of zinc for bone health can be observed in zinc deficient birds. A deficiency of zinc will typically cause long bone deformities which will inhibit normal growth and development (Cook et al., 1984). The benefits to bone growth and integrity may be more effective in younger chicks and pullets. Some studies have shown little to no benefit of increased zinc supplementation to bone health in older hens (Swiatkiewicz and Koreleski, 2008). One study reported promising results for older hens supplemented with organic trace minerals. Though not statistically significant, hens fed the organic trace minerals had stronger bone strength values than those fed inorganic trace minerals (Manangi et al., 2015). Supplementation of organic trace minerals versus inorganic trace minerals and the effects on bone health is an area in need of further research.

Availa®Zn is an organic zinc supplement produced by Zinpro Corporation (Eden Prairie, MN). It is a metal non-specific amino acid complex. It contains many essential amino acids, each bound to a zinc ion in a 1:1 ratio. A previous study done with the Availa®Zn product reported significantly higher bioavailability when compared to zinc sulfate (Star et al., 2012). This could indicate the potential for producers to supplement zinc at higher levels to achieve increased quality of production parameters without excess waste in the manure.

MATERIALS AND METHODS

Birds and Housing

Two hundred and forty Bovan White Leghorn laying hens were used in this trial from 19 to 60 weeks of age. The hens were provided access to 110 grams of feed per hen per day ad libitum. Water was also provided ad libitum through a nipple drinker system in each cage unit. The trial was conducted in a tunnel-ventilated building with evaporative cooling pads. When the trial began the hens were housed four per cage in 60 cages of a Farmer Automatic stacked manure belt housing unit (Farmer Automatic of America Inc., Register, GA). The dimensions for each cage unit were 16 inches tall in the front by 14 inches tall in the back by 19 inches wide by 17.25 inches deep, providing 81.9 cm^2/hen. Each cage unit was randomly assigned to one of six treatment diets with ten replicates per treatment. Blocking by row was done to reduce variability, and cages were randomly assigned within blocks. The trial began in June of 2014 and ran through March of 2015. The hens were maintained on a 16-hour light:8-hour dark photoperiod. Approximately five weeks into the trial the poultry facilities underwent a federal inspection by the University of Nebraska Institutional Animal Care and Use Committee (IACUC). The cages were determined to be too short in the back (14 inches) and did not allow adequate space per hen. Based on this recommendation the hens were moved during week six of the trial into a Big Dutchman stacked manure belt housing unit (Big Dutchman, Inc., Holland, MI). The cage dimensions in this unit were 18 inches tall in the front by 15.75 inches tall in the back by 24 inches wide by 20.25 inches deep, providing 97.2 cm^2/hen. A plan was made to move the birds while maintaining the assigned treatments and keeping hens and blocks of treatments together. In the Big Dutchman unit, the hens were housed five to a cage in 48 total cages. Eight of the original blocks were maintained, while blocks nine and ten had to be reorganized and birds distributed evenly to new cages with the same treatment. Hens were fed the experimental diets from 19 to 60 weeks of age. Feed intake was measured weekly and egg production was measured daily and calculated as percent production. All procedures were approved by the University of Nebraska IACUC.

Diets

Diets were formulated based on the NRC recommendations (1994). Zinc was excluded from the vitamin and mineral premix and added in separately based on the treatments for source and amount. The diets were organized in a 2 x 3 factorial arrangement. The variables were source: inorganic zinc (zinc sulfate; International

Nutrition, Omaha, NE) or organic zinc (Availa®Zn; Zinpro Corporation, Eden Prairie, MN) and level: 40, 80, or 120 ppm. Diet formulation for the trial is shown in Table 2.1. Feed buckets were weighed back weekly to determine feed intake and calculate feed conversion ratio (g feed/g egg mass).

Measurements

Measurements included weekly feed intake (FI), daily egg production (EP), biweekly egg weights (EW), monthly egg components (shell, yolk, and albumen) and eggshell breaking strength (BS), bimonthly hen weights (HW), as well as bone mineral density measurements and keel bone deformity scores at 18, 35, 50, and 60 weeks of age. Manure samples were also taken at ten week intervals and analyzed for zinc content. Feather scoring was done at the end of the study.

Weekly feed intake was calculated by subtracting the feed leftover in the feed troughs from the amount allotted for that cage at the beginning of the week. This number was then divided by the number of hens in the cage to calculate the feed intake per hen per day.

Egg production was averaged each week for each replicate cage. Egg weight was measured by weighing all eggs from each cage and calculating an average per cage every other week. Egg quality measurements included eggshell strength and component weights of the shell, yolk, and albumen. For these measurements two average size eggs were sampled from each cage once per month. Egg component measurements were done by first weighing the whole egg then cracking it open and separating the yolk from the albumen with a yolk separator. Then any remaining albumen was scraped out from the inside of the shell. The shell and yolk were weighed separately and albumen weight was calculated by subtracting the shell and yolk weight from the whole egg weight. Hens were weighed every other month together as a cage and divided by the number of hens in the cage to calculate an average hen weight. From these measurements body weight gain (BWG) was calculated.

Eggshell strength was measured using a Texture Analyzer (TA.XTPlus, Texture Technologies Corporation, Scarsdale, NY). Exponent software was used which measured and graphed the peak force required to crack the eggshell (Stable Micro Systems LTD., Surrey, UK). The eggs were tested using a compression test and were compressed at the rate of 10 mm/sec using 1 g of force (Anderson et al., 2004).

Bone mineral density was measured using a dual-energy x-ray absorptiometer (DEXA) (Model No. 476D014, Norland Medical Systems, Fort Atkinson, WI). Measurements were made of the tibia for bone area and bone mineral content (BMC), and then bone mineral density (BMD) was calculated. Six hens were randomly selected per treatment; one per replicate cage. Hens chosen were given a leg band for easy identification, and, when possible, the same hens were used for each measurement. In some cases, such as death of a hen, another hen from the same cage was selected as a replacement. The hens were comfortably placed dorsal on a foam pad and gently restrained with Velcro straps around the neck, breast including wings, legs, and feet. Each scan took approximately 12 minutes (Hester et al., 2004). The last BMD measurements were performed with excised tibia bone samples from one hen per cage (eight hens per treatment) at the conclusion of the trial. For these scans, a Lunar

PIXImus2 densitometer was used (GE Medical Systems, Chicago, IL) because the DEXA scanner used previously was no longer in working condition.

Keel bone scores were determined by palpating the breastbone with one or two fingers. Two hens per cage were randomly selected. The presence of indentations, fractures, and/or curves and their severities were noted and compiled to determine a score from one to four, with one being normal or containing very mild deformities and four being the existence of multiple severe deformities (Clark et al., 2008).

Manure samples were taken by sectioning off the manure belt by cage using spray paint and advancing the manure belt one cage at a time to collect the samples. One sample was collected from each cage every ten weeks. The samples were then dried in an oven at 100 ºC, ground, and sifted to eliminate feathers. Finally, the samples were sent to Midwest Labs (Midwest Laboratories, Omaha, NE) for analysis of zinc content.

Feather score was measured at the end of the study when the hens were 60 weeks of age. Hens were observed and assessed inside the cages to reduce handling and stress. An average score was given to the cage as a whole based on feather cover in each of these categories: wing, neck, breast, back, tail, and vent. Scores ranged from one to four, with one being no feather coverage and four being very good feather coverage (Blokhuis et al., 2007). A previous study used a slightly different scoring scale, but also averaged the scores for analysis and comparison (LaBrash, 2003).

Data Analysis

Data were analyzed using the Proc Glimmix procedure in SAS for a randomized complete block design with a 2 x 3 factorial arrangement. Analysis was performed for the factors: source and level, as well as any interactions between the factors. Repeated measures was used to evaluate treatment effects over time and look at any treatment by time interactions. FI, EP, EW, egg components, eggshell BS, BWG, BMD, KBS, and manure zinc content were all analyzed with repeated measures. The covariance structure was assumed to be first order auto-regression because of the use of repeated measures. A binomial distribution is assumed for EP and the model used was:

$$N_{ijktlm} = \mu + D_i + Y_j + DY_{ij} + T_t + TD_{it} + TY_{jt} + TYD_{ijt} + B_k + C_l + W_m$$

Where N_{ijktlm} is the linear predictor; μ is the overall mean; D_i is the effect of zinc source; Y_j is the effect of zinc level; DY_{ij} is the interaction between source and level; T_t is the effect of time; TD_{it} is the interaction between time and source; TY_{jt} is the interaction between time and level; TYD_{ijt} is the interaction between time, level, and source; B_k is the block effect; C_l is the normally distributed random cage effect; and W_m is the normally distributed covariance structure for the repeated measures within cages.

For FI, EW, egg components, BS, BWG, BMD, and manure zinc content normal distribution was assumed, and the following model was used:

$$N_{ijkt} = \mu + D_i + Y_j + DY_{ij} + T_t + TD_{it} + TY_{jt} + TYD_{ijt} + B_k + E_{ijk}$$

Where N_{ijktl} is the linear predictor; μ is the overall mean; D_i is the effect of zinc source; Y_j is the effect of zinc level; DY_{ij} is the interaction between source and level; T_t is the effect of time; TD_{it} is the interaction between time and source; TY_{jt} is the interaction between time and level; TYD_{ijt} is the interaction between time, level, and source; B_k is the block effect; and E_{ijk} is the normally distributed covariance structure for the repeated measures within cages. A similar analysis was performed for feather score with the removal of the time effect and repeated measures. A linear regression analysis was also performed for manure zinc content to test for linear response.

Keel bone scores were analyzed for odds and odds ratios of specific keel bone deformities. These results gave the odds of having a specific keel bone issue over not having that specific issue. Separate analysis was performed for curves, indentations, and fractures. Odds ratios served as a comparison between two specific treatment groups. For this analysis a binary distribution was assumed, and the following model was used:

$$N_{ijktlm} = \mu + S_i + D_j + SD_{ij} + T_t + ST_{it} + DT_{jt} + B_k + C_l + W_m$$

Where N_{ijktlm} is the linear predictor; μ is the overall mean; S_i is the effect of zinc source; D_j is the effect of zinc level; SD_{ij} is the interaction between source and level; T_t is the effect of time; ST_{it} is the interaction between source and time; DT_{jt} is the interaction between level and time; B_k is the block effect; C_l is the normally distributed random cage effect; and W_m is the normally distributed covariance structure for the repeated measures within cages.

RESULTS

No significant treatment effects were observed for egg production (Table 2). There was, however, a significant effect of time ($P < 0.0001$) such that egg production increased significantly between months 1 and 2 as the hens came into lay and began to decline after month 7 as the hens aged. There was a more noticeable drop in production during month 9. No specific dietary treatment was consistently higher or lower than the others (Figure 1).

There were no significant treatment effects on feed intake (Table 2). There was, again, a significant effect of time ($P < 0.0001$) such that feed consumption began to decline for all treatments after month 7 with a pronounced drop in consumption during month 9. Intake then increased to normal ranges for month 10. Feed intake for all treatments was greatest during months 6 and 7 (Figure 2). While not statistically significant, the hens fed 80 and 120 ppm zinc, regardless of source, had numerically higher feed intake from month 4 through month 10 of the study than those fed at the 40 ppm levels showing that the increase in zinc content did not affect palatability.

There was a significant source by time interaction for feed conversion ratio ($P = 0.008$, Table 2). Hens fed organic zinc had a lower conversion ratio during months 3 through 7 than the hens fed zinc sulfate (Figure 3a). The effect of time was also significant ($P < 0.0001$) such that all treatments had a significantly decreased feed conversion ratio from month 1 to month 2 (Figure 3b). Feed conversion stayed fairly consistent throughout the trial, except for a slight drop during month 9.

No significant treatment effects were found for body weight gain (Table 3). There was a significant effect of time ($P < 0.0001$) where all treatments showed weight gain through month 7 of the trial and then slight weight loss for months 9 and 10 (Figure 4). For egg weight there were no significant treatment effects (Table 3). There was a significant effect of time ($P < 0.0001$) as the eggs across all treatments increased in weight throughout the study (Figure 5).

There was a significant level by time interaction effect for eggshell breaking strength ($P = 0.048$, Table 3). The hens fed 80 ppm zinc, regardless of source, had eggs with varying degrees of breaking strength throughout the trial. During months 4, 6, and 10 the moderate level had eggs with the lowest breaking strength values, and during months 5, 7, 8, and 9 had eggs with the highest breaking strength values (Figure 6a). Hens fed both the low and high levels of zinc had eggs with fairly consistent breaking strength values throughout the study. Overall, the moderate level of supplementation (80 ppm) showed the greatest average breaking strength values. The effect of time was significant ($P < 0.0001$) with all treatments showing a downward trend in breaking strength values from month 7 through month 10. The source by level interaction approached significance ($P = 0.079$). Hens fed 40 ppm zinc sulfate had the highest breaking strength values when compared to those fed 80 and 120 ppm zinc sulfate. In contrast, hens fed 40 ppm organic zinc had noticeably lower breaking strength values than those fed 80 and 120 ppm organic zinc. Overall, hens fed 40 ppm zinc sulfate and hens fed 80 ppm organic zinc had the highest average eggshell breaking strength values (Figure 6b).

In the analysis of egg components, there were no significant treatment effects for percent eggshell (Table 4). However, the interaction between level of zinc and time was very nearly significant ($P = 0.053$) with 40 ppm zinc showing the highest percent eggshell during months 4 and 5 and, very noticeably, the lowest percent eggshell during month 10. Hens fed 80 ppm zinc had the highest numerical values for eggshell percent in months 6, 7, 9, and 10, but stayed fairly consistent throughout the trial. Hens fed 120 ppm zinc had eggs with relatively moderate values of percent eggshell except for month 2 when they were the highest, and months 5 and 8 when they were noticeably lower than the 40 and 80 ppm levels (Figure 7). There was also a significant effect of time (P <

0.0001) with all treatments showing a significant drop in eggshell percentage during month 8. In general, all treatments showed a trend toward decreasing eggshell percentage as the study progressed.

There was a significant source effect for percent egg yolk ($P = 0.05$, Table 4) such that hens fed organic zinc produced eggs with significantly more yolk as a percentage of the whole egg than did hens fed inorganic zinc (Figure 8a). There was also a significant effect of time ($P < 0.0001$) such that percent egg yolk increased for all treatments over the course of the study (Figure 8b). There was also a significant effect of source on percent egg albumen ($P = 0.031$, Table 4) where hens fed inorganic zinc had significantly greater percent albumen than did hens fed organic zinc (Figure 9a). The source by level by time interaction showed a trend toward significance ($P = 0.071$) with hens fed 40 ppm zinc sulfate having eggs with numerically higher albumen percentages during months 6, 9, and 10, and hens fed 40 ppm organic zinc having eggs with numerically lower albumen percentages during months 1 through 2 and months 4 through 7. The effect of time was also significant ($P < 0.0001$) with all treatments showing decreasing egg albumen percentage as the trial continued (Figure 9b).

There were significant treatment effects for manure zinc content (Table 5). Source and level both showed significant differences ($P < 0.0001$) such that the organic zinc source had higher average zinc excretion than did the inorganic zinc source. Regardless of source, the highest level of supplementation (120 ppm) showed the greatest amount of zinc excretion. There was also a significant source by level interaction ($P < 0.0001$) such that hens fed 40 ppm zinc sulfate had the lowest zinc excretion values while hens fed 120 ppm organic zinc had the highest zinc excretion values. For both the low and moderate levels of supplementation, both sources of zinc had similar excretion values. At the highest level of supplementation there is a significant difference between the two sources with hens fed the organic zinc source having significantly greater zinc excretion in the manure (Figure 10a). Both the source by time and level by time interactions approached significance ($P = 0.072$ and $P = 0.059$, respectively) indicating that both sources and all levels had decreased zinc excretion over time. Hens fed 40 ppm and 80 ppm zinc from both sources had similar excretion levels throughout the study, while there was significant difference between hens fed 120 ppm zinc sulfate and those fed 120 ppm organic zinc throughout the study. There was also a significant effect of time ($P < 0.0001$). For all treatments, overall zinc excretion decreased during the study (Figure 10b). When analyzed separately by month the source effect, level effect, and source by level interactions were all significant at each month ($P < 0.0001$). Hens fed 120 ppm organic zinc had the highest zinc excretion each month. Hens fed 40 ppm zinc sulfate had the lowest zinc excretion for months 3 and 6, while hens fed 40 ppm organic zinc had the lowest zinc excretion for months 8 and 10 (Figures 10c, 10d, 10e, and 10f). Overall, during each month, hens fed at the 40 and 80 ppm levels of zinc, regardless of source, had similar zinc excretion values. A linear regression analysis was also performed to predict manure zinc content based on dietary level of zinc (ignoring source). There was a significant linear effect of level of zinc in the diet on level of zinc in the manure ($P < 0.0001$; $R^2 = 0.78$). The analysis resulted in the following prediction equation:

$$\text{Manure Zn (ppm)} = -70.057 + 3.706 * \text{Diet Zn (ppm)}$$

This equation allows for future prediction of amount of zinc excretion given amount of zinc provided in the diet with good accuracy.

No significant treatment effects were observed for bone mineral density (Table 6), however, the source by level interaction for 19 weeks of age approached significance ($P = 0.061$). At 19 weeks of age, the hens fed 40 ppm inorganic zinc had significantly greater bone mineral density than the rest of the treatment groups. The hens fed 80 ppm organic zinc had the lowest bone mineral density (Figure 11). While not statistically significant, hens fed organic zinc had numerically higher bone mineral density at 35 and 60 weeks of age, and those hens also had more consistent bone mineral density throughout the study.

Keel bone scores were analyzed separately for prevalence of curves, indentations, and fractures (Table 7). There were no significant treatment effects found for incidence of keel bone curves. The effect of time, however, was significant ($P < 0.0001$) such that the odds of observing a curve in the keel bone increased as the hens aged (Figure 12a). No significant treatment effects were found for incidence of keel bone indentations. Again, the effect of time was significant ($P < 0.0001$) which showed that the odds of observing an indentation in the keel bone increased for most treatment groups as the hens aged. Hens fed 40 ppm organic zinc had reduced odds of keel bone indentation from 50 to 60 weeks of age. (Figure 12b). There were also no significant treatment effects observed for incidence of keel bone fractures. Though not statistically significant, hens fed 40 ppm organic zinc had the lowest numerical odds of keel bone fracture throughout the study (Figure 12c).

There was a significant source by level interaction ($P = 0.031$) for feather score (Table 8). Hens fed inorganic zinc supplemented at 80 ppm had the highest feather scores, while those supplemented at 40 ppm had the lowest feather scores. For hens fed organic zinc, those supplemented with 40 ppm zinc had the highest feather scores while those supplemented with 120 ppm zinc had the lowest feather scores. Overall, hens fed 80 ppm zinc sulfate had the best feather scores, and hens fed 120 ppm organic zinc had the worst feather scores (Figure 13).

DISCUSSION

In this study the use of an inorganic zinc supplement was compared to the use of an organic zinc supplement in laying hen diets. The hypothesis was that the organic source of zinc (Availa®Zn) would be more available to the hen and would have a positive impact on production parameters. No significant differences in feed intake were observed which indicates that the higher levels of supplementation did not affect palatability. There were also no significant differences in feed conversion ratio or egg production. These results agree with the findings of Stefanello et al. (2014) who reported no effect of supplemental trace minerals in laying hens on feed intake, feed conversion, or egg production. Manangi et al. (2015) also reported no effect of organic trace minerals versus inorganic trace minerals on feed intake, feed conversion, or egg production. The sudden drop in feed intake and egg production during month 9 of the trial may be explained by severe winter weather that the area experienced that month. During one weekend in particular, there was a winter storm that temporarily knocked out the power to the building in which the birds were housed, causing a drop in temperature and no lighting. This may explain the decrease in feed intake and egg production. This was likely also correlated to the weight loss that was observed in month 9. All three parameters seemed to recover in month 10.

The egg weights that were observed were very consistent throughout the trial with no significant treatment effects. The average egg weight trended upwards throughout the trial as the birds came into lay and continued into peak lay. There were significant differences observed in eggshell strength. Hens supplemented with 80 ppm zinc regardless of source showed significantly higher eggshell breaking strength at several different time points. Hens fed 80 and 120 ppm organic zinc had greater eggshell breaking strength than did hens fed 80 and 120 ppm inorganic zinc. These results are supported by previous studies that reported greater eggshell strength values for eggs laid by hens fed organic trace minerals when compared to eggs laid by hens fed inorganic trace minerals (Swiatkiewicz and Korelski, 2008; Manangi et al., 2015). Other studies have shown no difference in eggshell breaking strength between hens supplemented with organic trace minerals and hens supplemented with inorganic trace minerals (Hudson et al., 2004; Stefanello et al., 2014). In contrast, the 40 ppm level of supplementation had the highest breaking strength values for zinc sulfate, very similar to the values obtained with 80 and 120 ppm organic zinc. The 40 ppm level of organic zinc had significantly lower eggshell breaking strength values than all other treatments. The reason for this is unclear, and this may be an area in need of further research. Overall, eggshell strength decreased steadily during the last half of the study which is supported by the results reported by Swiatkiewicz and Korelski (2008) who saw a decline in eggshell breaking strength with increasing hen age.

The results for percent eggshell were greatly varied, with no treatment showing consistently higher or lower eggshell percentages. These findings also agree with the study done by Swiatkiewicz and Korelski (2008) who reported no significant differences in eggshell percent between hens fed organic trace minerals and hens fed inorganic trace minerals. All treatments showed a drop in eggshell percentage during month 8. This occurred around the same time that there was a decrease in eggshell strength. The 40 ppm level of zinc, regardless of source, showed significantly lower eggshell percentages during the last month of the trial indicating that higher levels of zinc supplementation may help increase eggshell deposition and strength in older hens.

Significant differences were observed for both egg yolk and albumen percentages. Hens fed organic zinc had significantly higher egg yolk percentages than hens fed zinc sulfate. Yolk is a main source of nutrients in the egg so this information may be valuable to egg and breeder producers. As expected from the previous results, hens fed zinc sulfate had significantly higher egg albumen percentages than hens fed organic zinc. Albumen is a large source of protein in the egg so this information may be useful to egg producers looking to increase protein content and reduce fat in their eggs.

A concern associated with increasing levels of zinc supplementation is excess zinc excretion in the manure. Since poultry manure is commonly used as a fertilizer, increased levels of certain trace minerals, such as zinc, can be a concern. Significant differences were found between treatments for manure zinc content with hens fed 40 ppm zinc sulfate having the lowest excretion levels and hens fed 120 ppm organic zinc having the highest excretion levels. The highest level of supplementation (120 ppm) was correlated to a high zinc excretion for both sources. This indicates that the hens are not retaining a large portion of the zinc that is ingested. Since absorption was not measured in this study, conclusions about the overall bioavailability are difficult. Further study in this area to measure absorption and storage in tissues would help give a better view of how the hens are utilizing this higher level of zinc. Overall, the zinc content of the manure decreased for all treatments from the beginning to the end of the study. This could indicate that as the hen's body adapts to the diet it can better utilize the nutrients in the feed. These results partially agree with a previous study by Burrell et al. (2004) who reported that increasing levels of supplemental zinc in the diet resulted in increasing levels of zinc excretion in the manure. The same result of increased levels of zinc supplementation causing increased levels of manure zinc excretion was also reported by Kim and Patterson (2004). The linear regression analysis showed a strong linear relationship between zinc level in the diet and zinc level in the manure. The prediction equation will be valuable to producers who may be interested in feeding higher levels of zinc for the proposed benefits, but also want to keep zinc levels in the manure low. The prediction equation generated from this study will be a useful tool for producers and agronomists.

Bone health in laying hens is always a concern for producers. In this study, no significant treatment effects were found for bone mineral density. Previous studies also found no effect of organic trace minerals on bone health properties (Swiatkiewicz and Korelski, 2008; Manangi et al., 2015). The first scans were done at the beginning of the trial when the hens were 19 to 20 weeks of age and just beginning to lay. This measurement was done shortly after the hens had been introduced to the dietary treatments. The scans during month 4 were done when the hens were 35 weeks of age and in peak lay. Hens in peak lay divert many bodily resources to egg production, so this is a time when bone health can suffer and cage layer fatigue becomes a concern. This may explain the slight decrease in bone mineral density observed between the first two measurements. There were no significant treatment effects observed at 35 weeks indicating that neither source of zinc significantly improved bone mineral density. Bone mineral density improved throughout the remainder of the study, though no source of zinc had more impact than the other. The last bone mineral density scans were done on excised tibia bone samples that were taken at 60 weeks of age. These scans show a great improvement in bone mineral density which is inconsistent with previous scans and with expectations for bone density of older hens. The differences may be due, in part, to scanning the leg bones ex vivo as opposed to the previous scans which were in vivo. Differences may also be due to a new scanner that was used for the last measurement. A Lunar PIXImus2 scanner was used for the 60-week bone samples since the previous machine that had been used (Norland Medical Systems DEXA scanner) had broken down beyond repair. If all measurements had been performed in the same manner on the same machine, there would likely have been less inconsistency. Because of this the time effect could not be compared. Further research in the area of zinc supplementation on bone mineral density may be interesting and useful to poultry producers.

In the analysis of keel bone scores, no significant treatment effects were observed. These results are supported by previous studies that also found no effect of organic trace minerals compared to inorganic trace minerals on bone health parameters (Swiatkiewicz and Korelski, 2008; Manangi et al., 2015). The data were analyzed separately for prevalence of curves, indentations, and fractures. There was a significant effect of time for keel bone curves and indentations showing that the incidence of both increased over time. This loss of bone health is likely due to calcium being pulled from skeletal stores for egg production. The incidence of fractures was not impacted by any of the treatments, but the highest level of supplementation (120 ppm) for both sources showed a numerical decrease in keel bone fractures over time, indicating that higher levels of zinc supplementation may help repair bone fractures and decrease the chances of severe injury. Since these measurements were performed on the same hens throughout the study, change in keel bones, including healing, was observed. Of considerable note is the trend observed for the hens fed 40 ppm organic zinc. Hens fed 40 ppm zinc, regardless of source, had consistently low incidence of fracture and showed a decrease in incidence of indentation which may be correlated with decreased egg production that was observed in this treatment group during the last few months of the trial. As they diverted more bodily resources to bone healing, the reproductive system compensated with a decrease in egg production. This could be an interesting area for further study to observe specific hens with keel bone damage to see if egg production and keel bone healing are inversely correlated with one another. At 60 weeks of age all hens fed organic zinc had the lowest numerical odds of keel bone fracture. This may demonstrate proposed higher bioavailability and a higher requirement of zinc for rebuilding and healing any skeletal injuries.

Feather cover is an important part of health and welfare in poultry. Feather loss, aside from molt, can indicate nutrient deficiencies or other health concerns and can prevent heat conservation which may directly impact productivity (Deschutter and Leeson, 1986). There was a significant source by level interaction for feather score such that hens fed 80 ppm zinc sulfate had the highest average feather cover scores. Hens fed 40 ppm organic zinc had the second highest average feather scores; much higher than hens fed 40 ppm zinc sulfate. This may indicate an area where the organic source of zinc is more readily available and utilized by the bird. The inconsistency with this theory is shown with the lowest average feather scores from hens fed 120 ppm organic zinc. A previous study done by Stahl et al. (1986), who reported decreased feather fraying with increasing levels of zinc in the diet, is supported by the result seen in the hens fed 80 ppm zinc sulfate. The results here are interesting, and it is unclear why these differences are present. This could be an area of further research into which supplements in the diet would achieve better feather cover.

In conclusion, the majority of egg production parameters were not affected by zinc source or level of supplementation. The analysis of eggshell strength showed promising results for organic zinc supplemented at higher levels. This could be an area for future research to determine the optimum level of supplementation to achieve the greatest eggshell quality. The analysis of manure zinc content did not produce the expected result of decreased excretion from hens fed organic zinc because of improved bioavailability. Instead, organic zinc had higher zinc excretion at all levels, indicating that such high levels are not retained. Past studies have shown benefits of using a combination of both organic and inorganic trace mineral sources in the diet (Burrell et al., 2004; Hudson et al., 2004) with the hypothesis that this method may involve more absorption sites and transporters, and, therefore, allow for better absorption and utilization of trace minerals such as zinc. This would be an interesting area for further research to determine the benefits of a combination of sources on egg quality, bone health, mineral excretion, and immunity. Normal levels of organic zinc supplemented in the feed resulted in manure zinc content similar to those observed for normal levels of zinc sulfate. There were several benefits to using an organic zinc source shown in this study. The organic zinc source was shown to improve eggshell strength when supplemented at higher levels as well as percent egg yolk, and had a positive impact on feed conversion ratio, percent eggshell, and incidence of keel bone indentations and fractures.

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Table 1. Composition of Diets.

Ingredient	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Corn (%)	55.40	55.40	55.40	55.40	55.40	55.40
Soybean Meal (%)	27.70	27.70	27.70	27.70	27.70	27.70
Oil (%)	4.45	4.45	4.45	4.45	4.45	4.45
Dical Phosphate (%)	1.60	1.60	1.60	1.60	1.60	1.60
Ca Carbonate (%)	4.96	4.96	4.96	4.96	4.96	4.96
Limestone (%)	4.96	4.96	4.96	4.96	4.96	4.96
Salt, white (%)	0.46	0.46	0.46	0.46	0.46	0.46
Lysine (%)	0.02	0.02	0.02	0.02	0.02	0.02
Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20
Trace Min Premix[1] (%)	0.10	0.10	0.10	0.10	0.10	0.10
Vit Premix[2] (%)	0.05	0.05	0.05	0.05	0.05	0.05
Zinc Sulfate (ppm)	40.00	80.00	120.00	0.00	0.00	0.00
Availa®Zn[3] (ppm)	0.00	0.00	0.00	40.00	80.00	120.00
Nutrient analysis[4]	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Protein (%)	18.50	19.00	19.00	19.30	19.40	19.20
Phosphorus (%)	0.60	0.69	0.65	0.71	0.62	0.61
Calcium (%)	4.64	4.91	4.66	4.51	4.16	4.66
Zinc (ppm)	89.30	115.00	162.00	86.60	131.00	160.00

[1]Trace mineral premix provided the following per kilogram: Cu ($CuSO_4$, 3,180 mg); I (I_2, 480 mg); Fe ($FeSO_4$, 26,700 mg); Mn ($MnSO_4$, 10,000 mg); Se (Na_2O_3Se, 380 mg); and limestone (59,260 mg).
[2]Vitamin premix provided the following per kilogram: Vitamin A (1,322,751.3 IU); vitamin D3 (1,322,7751.3 IU); vitamin E (2,204.6 IU), vitamin B12 (1.75 mg); biotin (11 mg); menadione (110.25 mg); thiamine (275.55 mg); riboflavin (771.6 mg); D-Pantothenic acid (1,102.3 mg); vitamin B6 (220.45 mg); niacin (4,850.1 mg); folic acid (44.1 mg); and phytase (60,000 FYT).
[3]Zinpro Corporation, Eden Prairie, MN.
[4]Conducted at Midwest Labs, Omaha, NE.

Table 2. Dietary effects of zinc source and level on egg production, feed intake, and feed conversion ratio.

Diets		Egg	Feed Intake	Feed
	Supplemental	Production	(g/hen/day)	Conversion
Source	**Level (ppm)**	**(%)**		**(g feed: g egg)**
Zinc Sulfate	40	90.84	98.62	1.848
Zinc Sulfate	80	88.99	97.56	1.899
Zinc Sulfate	120	92.10	98.44	1.897
Organic Zinc[1]	40	91.58	96.49	1.889
Organic Zinc[1]	80	89.87	99.64	1.905
Organic Zinc[1]	120	91.35	98.54	1.924
SEM[2]		0.01	1.75	0.10
Main Effects				
Source				
Zinc Sulfate		90.64	98.21	1.881
Organic Zinc[1]		90.93	98.22	1.906
P-value		0.781	0.994	0.755
Level				
40 ppm		91.21	97.56	1.868
80 ppm		89.43	98.60	1.902
120 ppm		91.73	98.49	1.910
P-value		0.090	0.807	0.901
Time				
P-value		< 0.0001	< 0.0001	< 0.0001
Interactions			**P-value**	
Source x Level		0.689	0.490	0.984
Source x Time		0.519	0.962	0.008
Level x Time		0.113	0.760	0.767
Source x Level x Time		0.712	0.998	0.223

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Standard Error of Means.

Table 3. Dietary effects of zinc source and level on hen weight, egg weight, and shell strength.

Diets Source	Supplemental Level (ppm)	Body Weight Gain (g)	Egg Weight (g)	Eggshell Breaking Strength (N)
Zinc Sulfate	40	21.36	60.90	63.64
Zinc Sulfate	80	29.35	59.90	62.02
Zinc Sulfate	120	19.44	59.50	60.84
Organic Zinc[1]	40	23.48	59.94	59.54
Organic Zinc[1]	80	18.97	60.28	63.81
Organic Zinc[1]	120	28.60	60.35	62.69
SEM[2]		6.50	0.50	4.50
Main Effects				
Source				
Zinc Sulfate		23.38	60.10	62.17
Organic Zinc[1]		23.68	60.19	62.01
P-value		0.955	0.822	0.901
Level				
40 ppm		22.42	60.42	61.59
80 ppm		24.16	60.09	62.91
120 ppm		24.02	59.93	61.77
P-value		0.957	0.608	0.632
Time				
P-value		< 0.0001	< 0.0001	< 0.0001
Interactions			**P-value**	
Source x Level		0.323	0.184	0.079
Source x Time		0.685	0.748	0.569
Level x Time		0.897	0.768	0.048
Source x Level x Time		0.205	0.830	0.515

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Standard Error of Means.

Table 4. Dietary effects of zinc source and level on percent eggshell, percent yolk, and percent albumen.

Diets Source	Supplemental Level (ppm)	Eggshell (%)	Yolk (%)	Albumen (%)
Zinc Sulfate	40	11.61	26.40	62.01
Zinc Sulfate	80	11.58	26.91	61.51
Zinc Sulfate	120	11.61	26.58	61.81
Organic Zinc[1]	40	11.70	27.21	61.09
Organic Zinc[1]	80	11.73	26.99	61.28
Organic Zinc[1]	120	11.66	26.69	61.65
SEM[2]		0.18	0.20	0.24
Main Effects				
Source				
Zinc Sulfate		11.60	26.63	61.78
Organic Zinc[1]		11.70	26.96	61.34
P-value		0.271	0.050	0.031
Level				
40 ppm		11.65	26.81	61.55
80 ppm		11.66	26.95	61.40
120 ppm		11.64	26.63	61.73
P-value		0.983	0.302	0.394
Time				
P-value		< 0.0001	< 0.0001	< 0.0001
Interactions			**P-value**	
Source x Level		0.908	0.135	0.227
Source x Time		0.834	0.499	0.685
Level x Time		0.053	0.387	0.496
Source x Level x Time		0.152	0.236	0.071

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Standard Error of Means.

Table 5. Dietary effects of zinc source and level on manure zinc content.

Diets Source	Supplemental Level (ppm)	Zinc Level (ppm) Month 3	Month 6	Month 8	Month 10	Average
Zinc Sulfate	40	285.63	243.63	268.13	238.75	259.03
Zinc Sulfate	80	418.00	366.13	344.00	344.12	368.06
Zinc Sulfate	120	494.50	465.63	463.88	420.00	461.00
Organic Zinc[1]	40	326.88	255.50	245.63	230.12	264.53
Organic Zinc[1]	80	416.50	404.13	369.63	334.00	381.06
Organic Zinc[1]	120	647.13	618.50	591.00	556.00	603.16
SEM[2]		14.39	8.22	8.21	8.97	5.43
Main Effects						
Source						
Zinc Sulfate		399.38	358.46	358.67	334.29	362.70
Organic Zinc[1]		463.50	426.04	402.08	373.37	416.25
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001
Level						
40 ppm		306.25	249.56	256.87	234.44	261.78
80 ppm		417.25	385.13	356.81	339.06	374.56
120 ppm		570.81	542.06	527.44	488.00	532.08
P-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001
Time						
P-value						
Interactions				**P-value**		
Source x Level		<0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Source x Time						0.072
Level x Time						0.059
Source x Level x Time						0.083

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Standard Error of Means.

Table 6. Dietary effects of zinc source and level on bone mineral density.

Source	**Diet Supplemental Level (ppm)**	**BMD (g/cm²) 19 wks**	**35 wks**	**50 wks**	**60 wks[3]**
Zinc Sulfate	40	0.294	0.224	0.249	0.301
Zinc Sulfate	80	0.268	0.215	0.248	0.283
Zinc Sulfate	120	0.252	0.217	0.234	0.302
Organic Zinc[1]	40	0.251	0.217	0.240	0.304
Organic Zinc[1]	80	0.238	0.213	0.235	0.278
Organic Zinc[1]	120	0.269	0.244	0.242	0.305
SEM[2]		0.01	0.01	0.02	0.02
Main Effects					
Source					
Zinc Sulfate		0.271	0.218	0.244	0.295
Organic Zinc[1]		0.253	0.225	0.239	0.296
P-value		0.090	0.392	0.748	0.979
Level					
40 ppm		0.273	0.221	0.244	0.303
80 ppm		0.253	0.214	0.241	0.281
120 ppm		0.260	0.230	0.238	0.303
P-value		0.313	0.229	0.937	0.302
Interactions		**P-value**			
Source x Level		0.061	0.110	0.816	0.964

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Standard Error of Means.
[3]Performed on ex vivo bone samples using a Lunar PIXImus2 scanner.

Table 7. Dietary effects of zinc source and level on keel bone scores.

Diet Source	Supplemental Level (ppm)	Curve Mean	Curve CI[2]	Indentation Mean	Indentation CI[2]	Fracture Mean	Fracture CI[2]
				Odds			
Zinc Sulfate	40	0.26	0.11-0.50	0.48	0.31-0.66	0.17	0.07-0.36
Zinc Sulfate	80	0.45	0.24-0.67	0.68	0.49-0.82	0.08	0.03-0.24
Zinc Sulfate	120	0.32	0.16-0.54	0.55	0.35-0.73	0.14	0.05-0.32
Organic Zinc[1]	40	0.23	0.09-0.45	0.45	0.28-0.64	0.04	0.01-0.15
Organic Zinc[1]	80	0.49	0.24-0.74	0.63	0.44-0.79	0.10	0.04-0.27
Organic Zinc[1]	120	0.38	0.19-0.61	0.54	0.36-0.72	0.12	0.04-0.30
				Odds Ratio			
Zinc Sulfate vs. Organic Zinc[1]		0.93	0.41-2.09	1.13	0.60-2.13	1.65	0.63-4.27
	40 vs. 80	0.37	0.13-1.04	0.46	0.22-1.00	0.84	0.24-2.94
	40 vs. 120	0.61	0.23-1.62	0.74	0.34-1.58	0.59	0.17-2.04
	80 vs. 120	1.64	0.62-4.34	1.58	0.72-3.47	0.71	0.21-2.34
Main Effects							
Source				**Odds**			
Zinc Sulfate		0.34		0.57		0.12	
Organic Zinc[1]		0.36		0.54		0.08	
P-value		0.851		0.692		0.298	
Level							
40 ppm		0.24		0.47		0.08	
80 ppm		0.47		0.65		0.09	
120 ppm		0.35		0.54		0.13	
P-value		0.163		0.144		0.680	
Time				**Odds**			
P-value		< 0.0001		< 0.0001		0.136	
Interactions				**P-value**			
Source x Level		0.892		0.967		0.262	
Source x Time		0.461		0.671		0.381	
Level x Time		0.700		0.243		0.756	
Source x Level x Time		0.776		0.192		0.983	

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Confidence Interval.

Table 8. Dietary effects of zinc source and level on feather scores.

Diets		Feather Score (1-4)[3]
Source	**Supplemental Level (ppm)**	
Zinc Sulfate	40	2.79
Zinc Sulfate	80	3.31
Zinc Sulfate	120	2.83
Organic Zinc[1]	40	3.17
Organic Zinc[1]	80	2.85
Organic Zinc[1]	120	2.67
SEM[2]		0.15
Main Effects		
Source		
Zinc Sulfate		2.98
Organic Zinc[1]		2.90
P-value		0.511
Level		
40 ppm		2.98
80 ppm		3.08
120 ppm		2.75
P-value		0.099
Interactions		**P-value**
Source x Level		0.031

[1]Availa®Zn; Zinpro Corporation, Eden Prairie, MN.
[2]Standard Error of Means.
[3]Score 1 = little to no feather coverage; Score 4 = very good feather coverage.

Figure 1. Effect of zinc source and level on egg production over time.

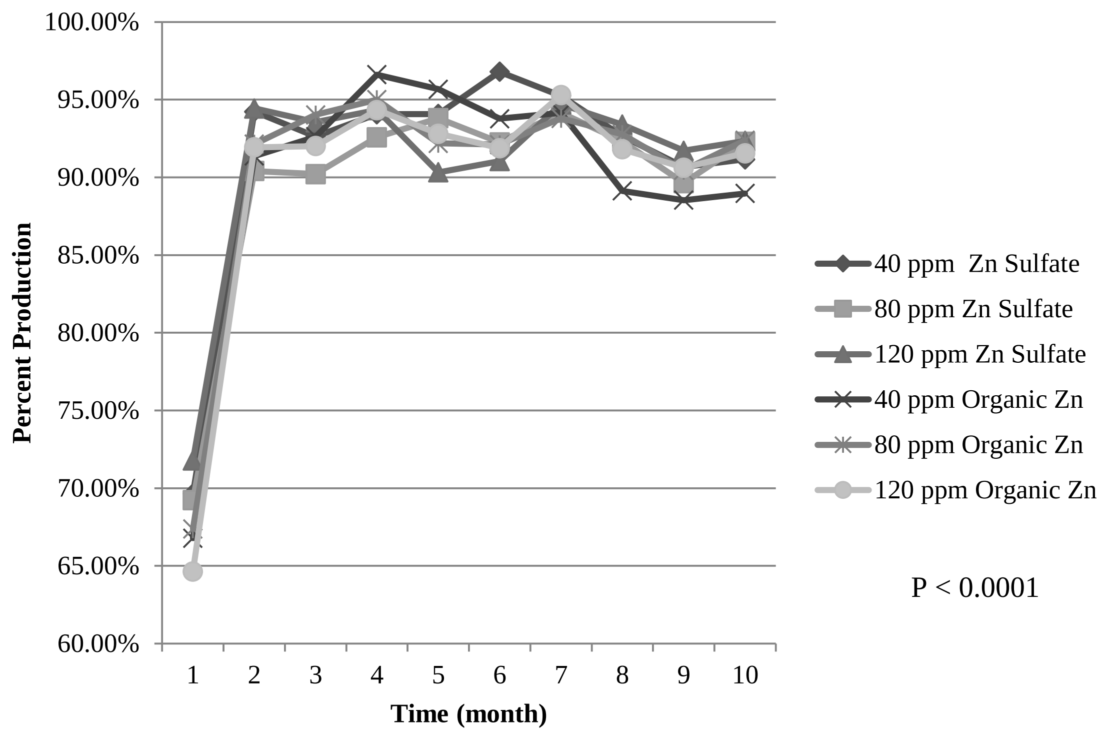


Figure 2. Effect of zinc source and level on feed intake over time.

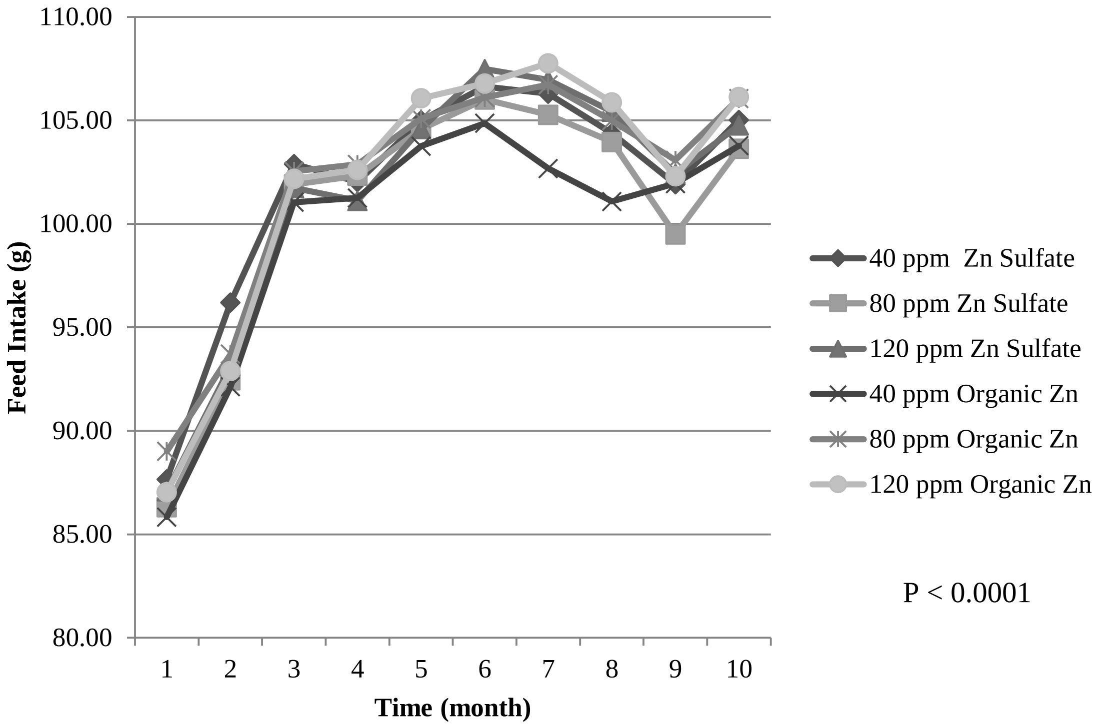


Figure 3a. Effect of zinc source and level on feed conversion ratio: Source by Time Interaction.

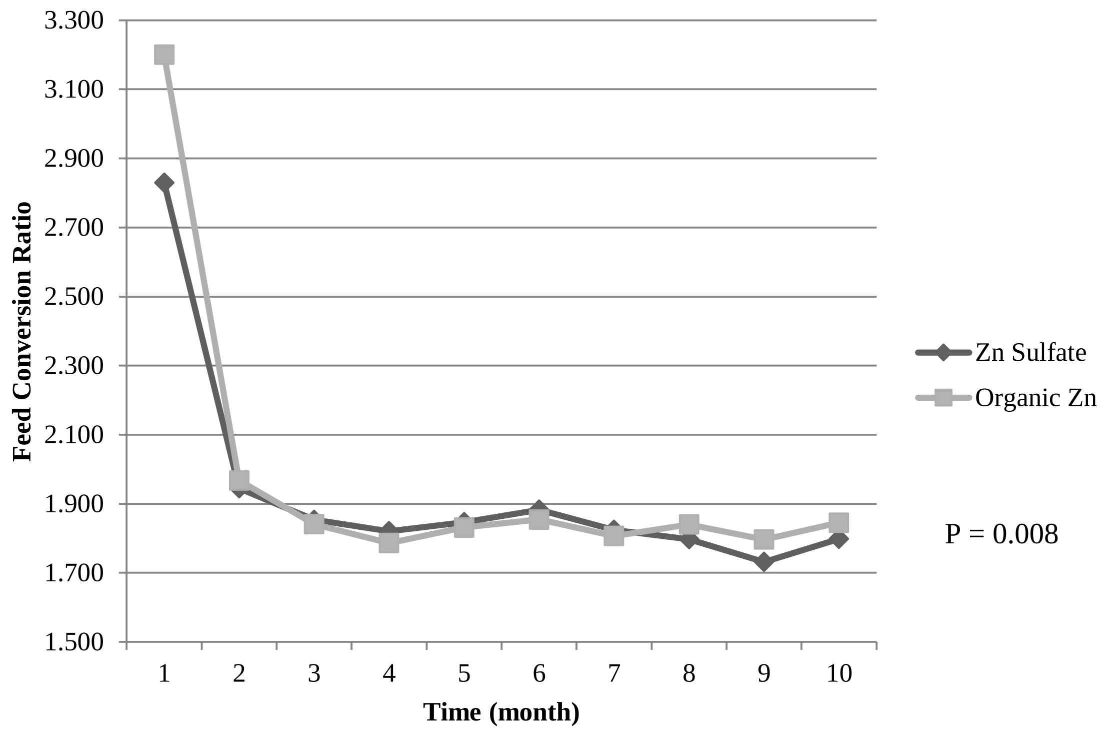


Figure 3b. Effect of zinc source and level on feed conversion ratio over time.

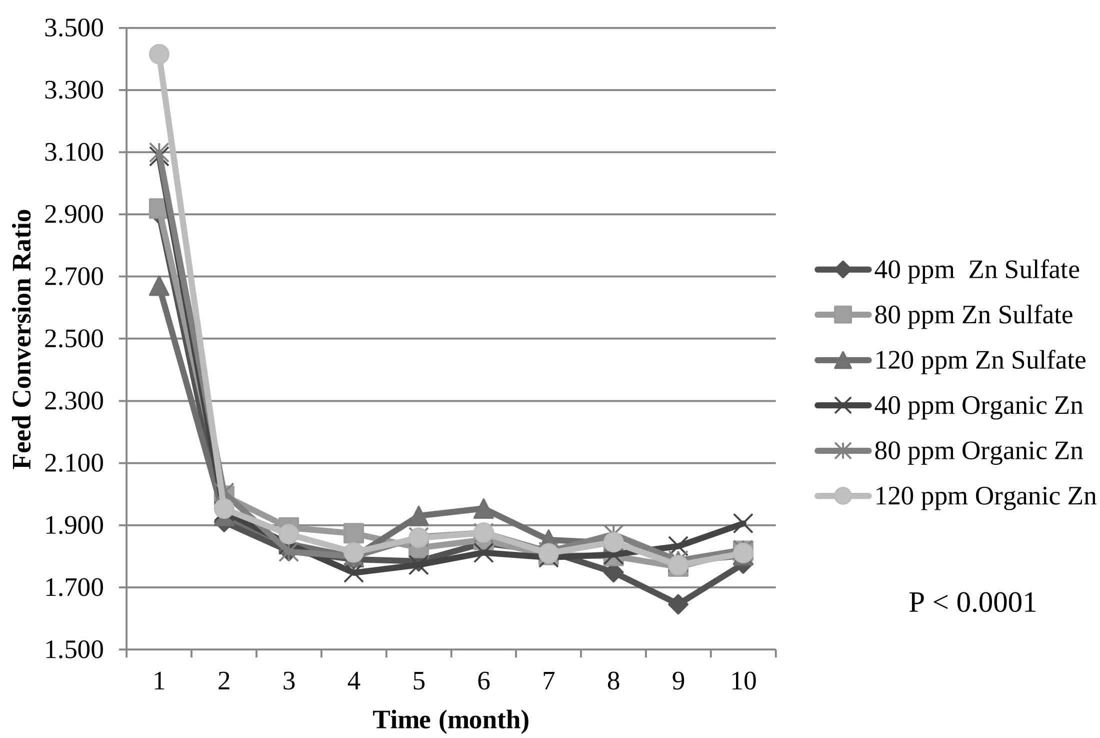


Figure 4. Effect of zinc source and level on body weight gain over time.

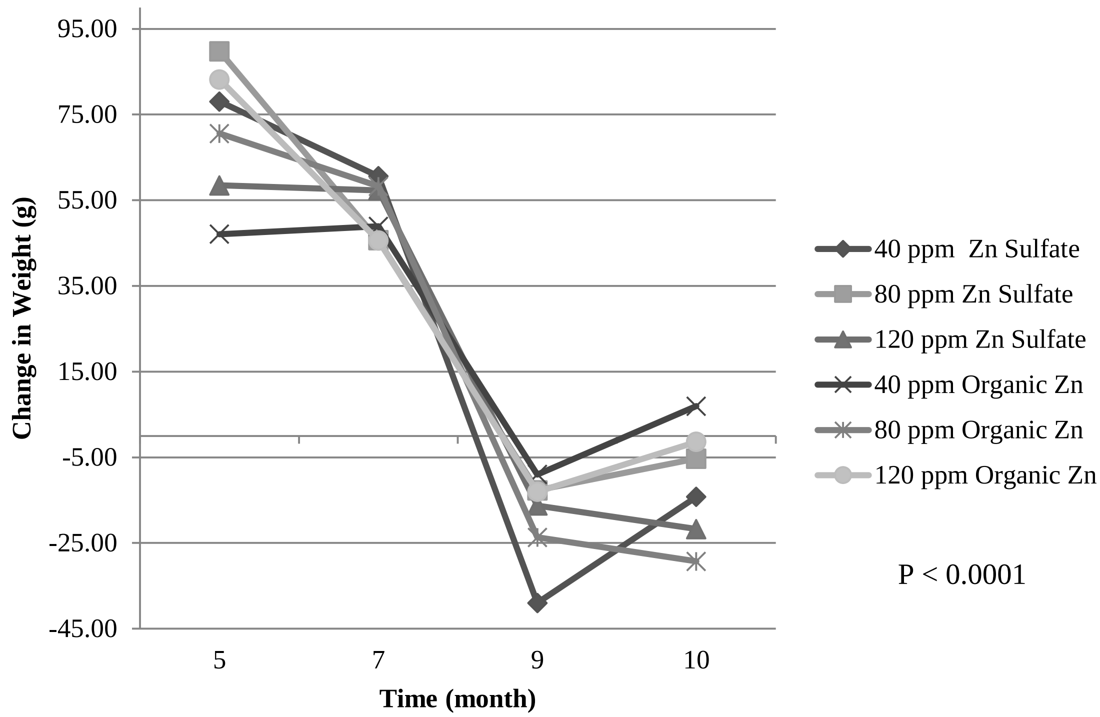


Figure 5. Effect of zinc source and level on egg weight over time.

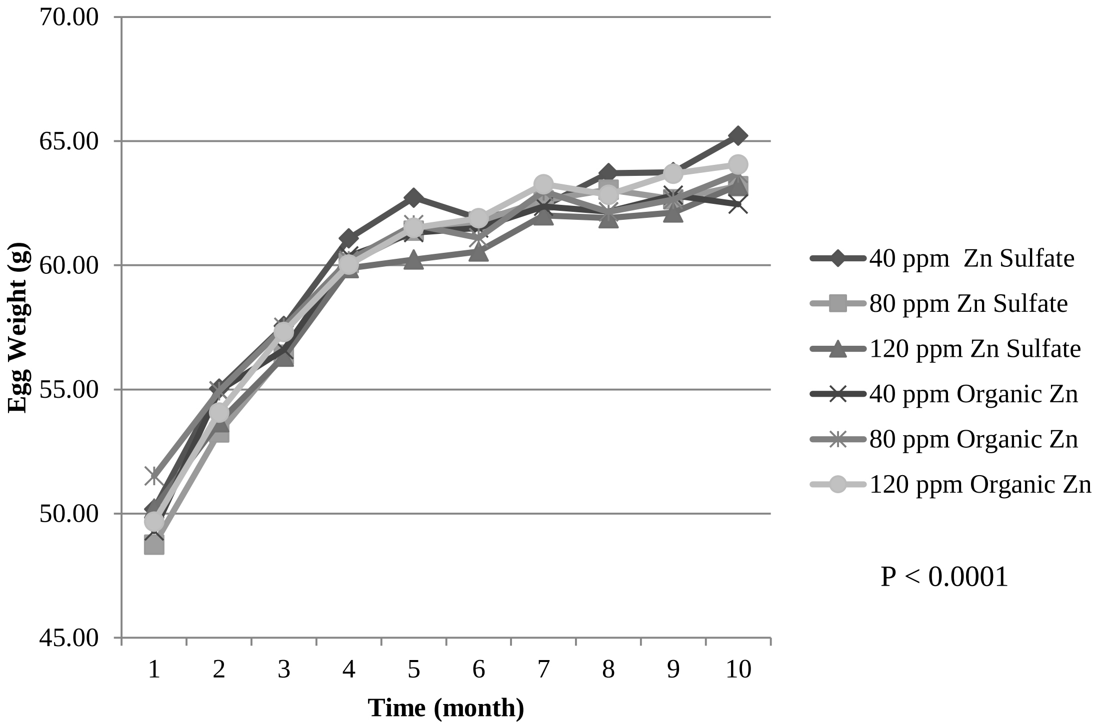


Figure 6a. Effect of zinc source and level on eggshell strength: Level by Time Interaction.

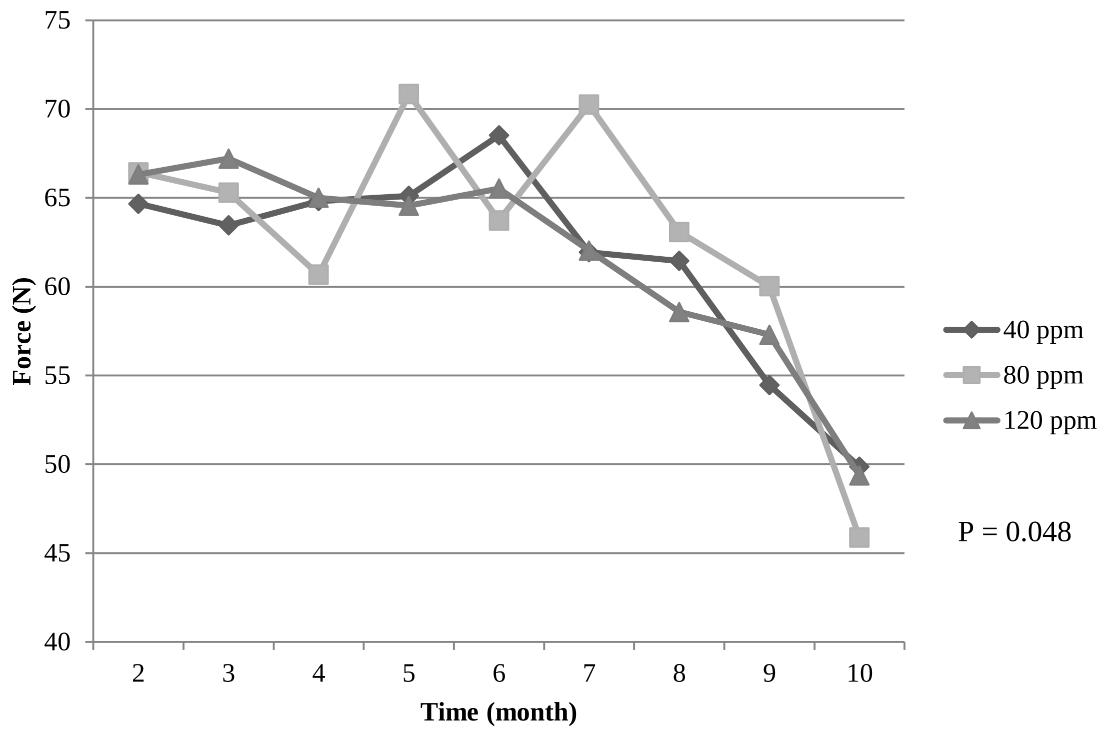


Figure 6b. Effect of zinc source and level on eggshell strength: Source by Level Interaction.

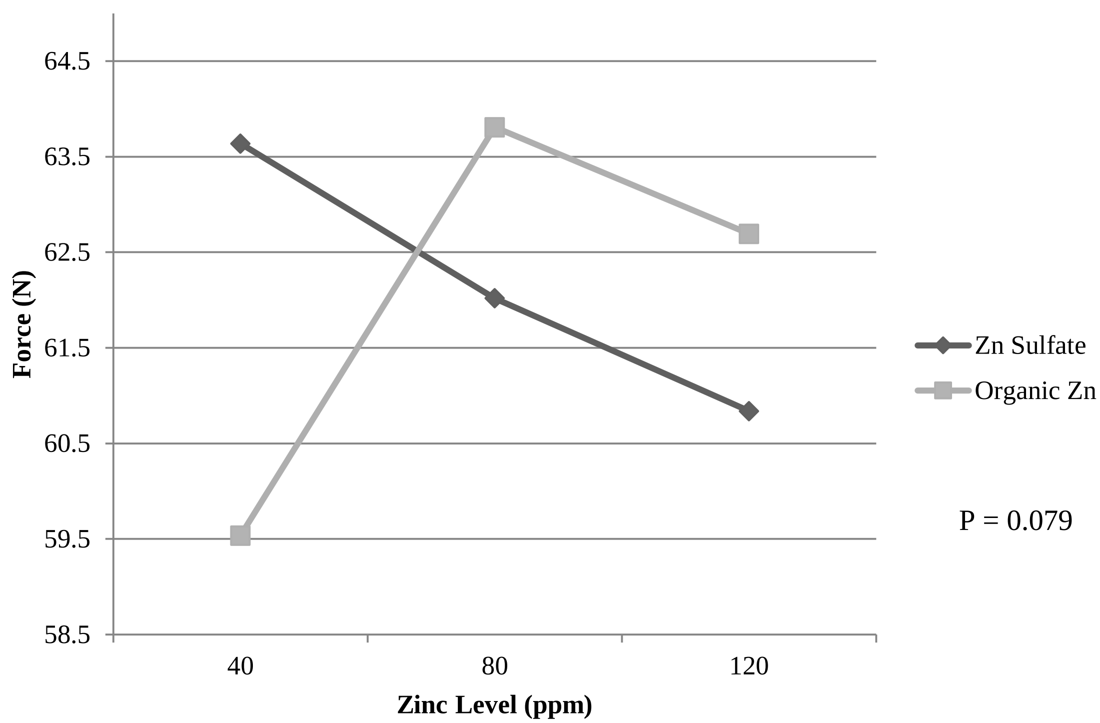


Figure 7. Effect of zinc source and level on percent eggshell: Level by Time Interaction.

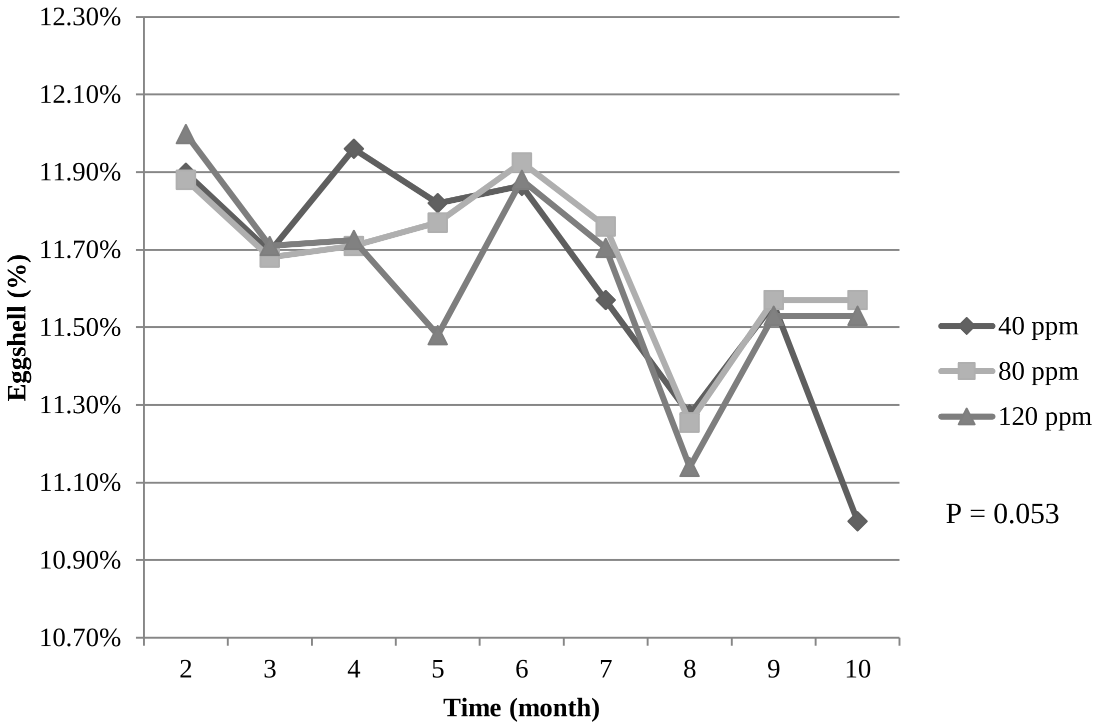


Figure 8a. Effect of zinc source and level on percent yolk: Source Effect.

Figure 8b. Effect of zinc source and level on percent yolk over time.

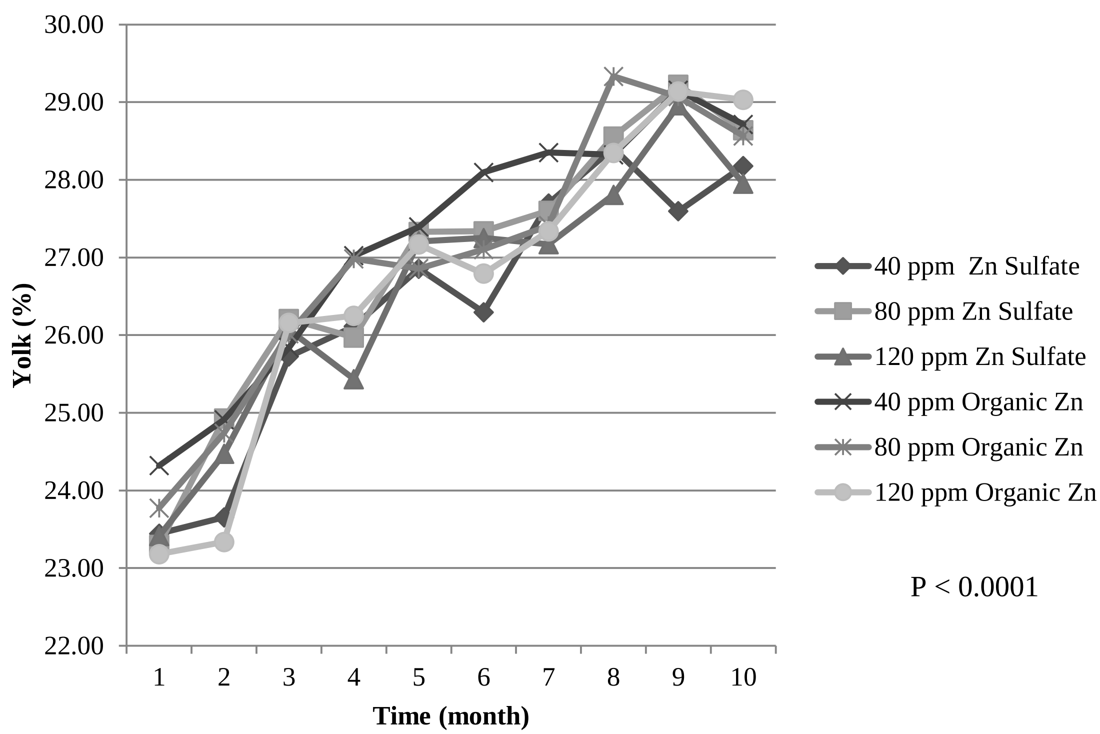


Figure 9a. Effect of zinc source and level on percent albumen: Source Effect.

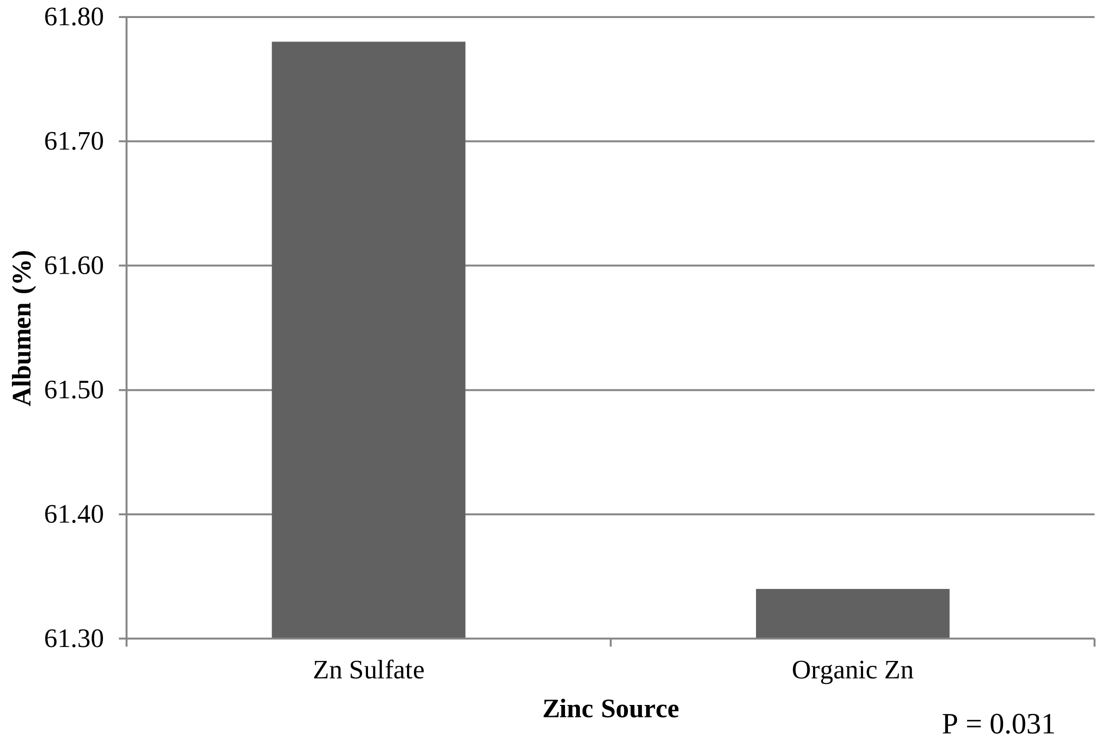


Figure 9b. Effect of zinc source and level on percent albumen over time.

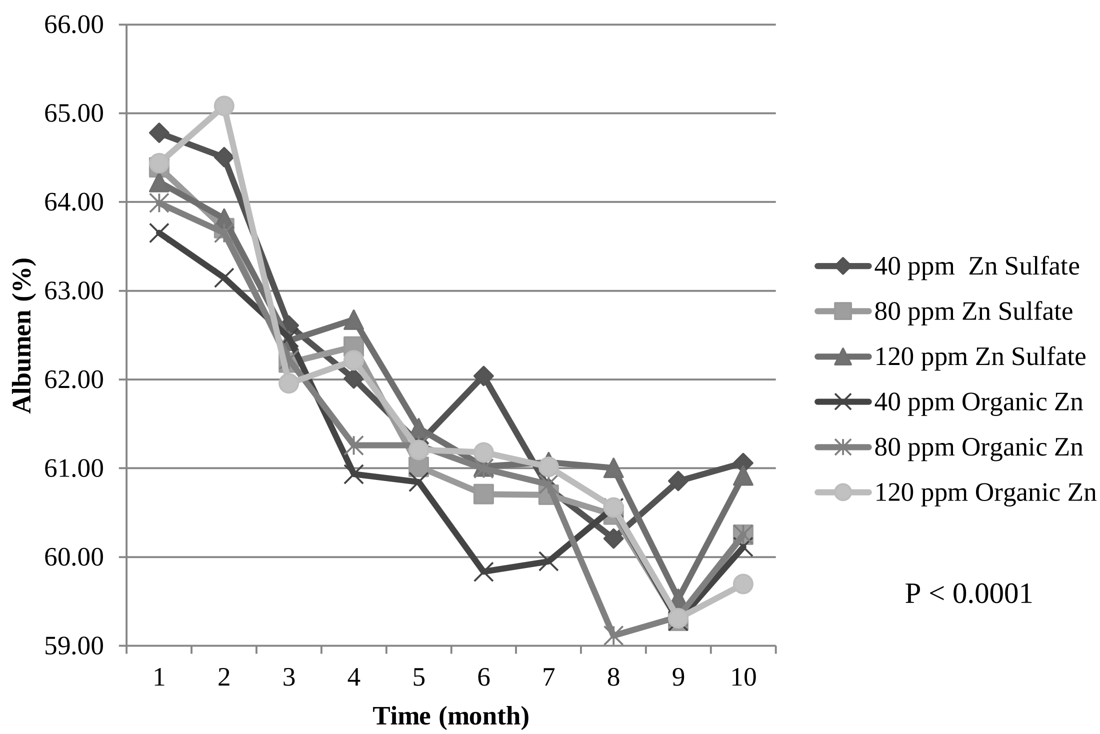


Figure 10a. Effect of zinc source and level on manure zinc content: Source by Level Interaction.

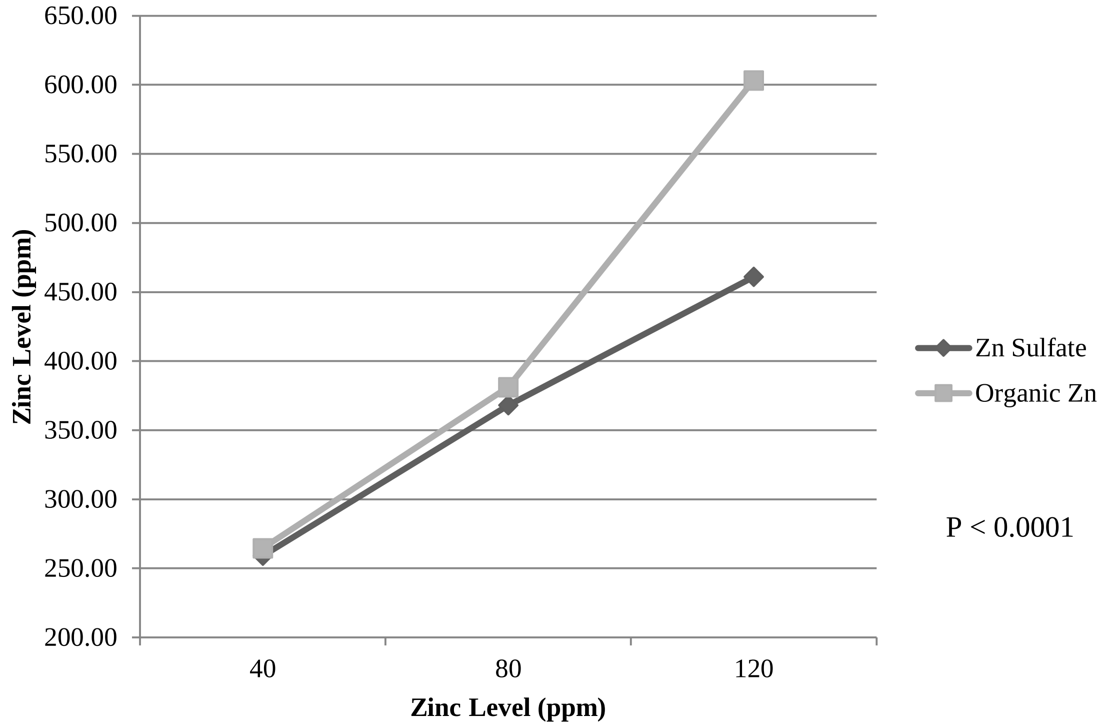


Figure 10b. Effect of zinc source and level on manure zinc content over time.

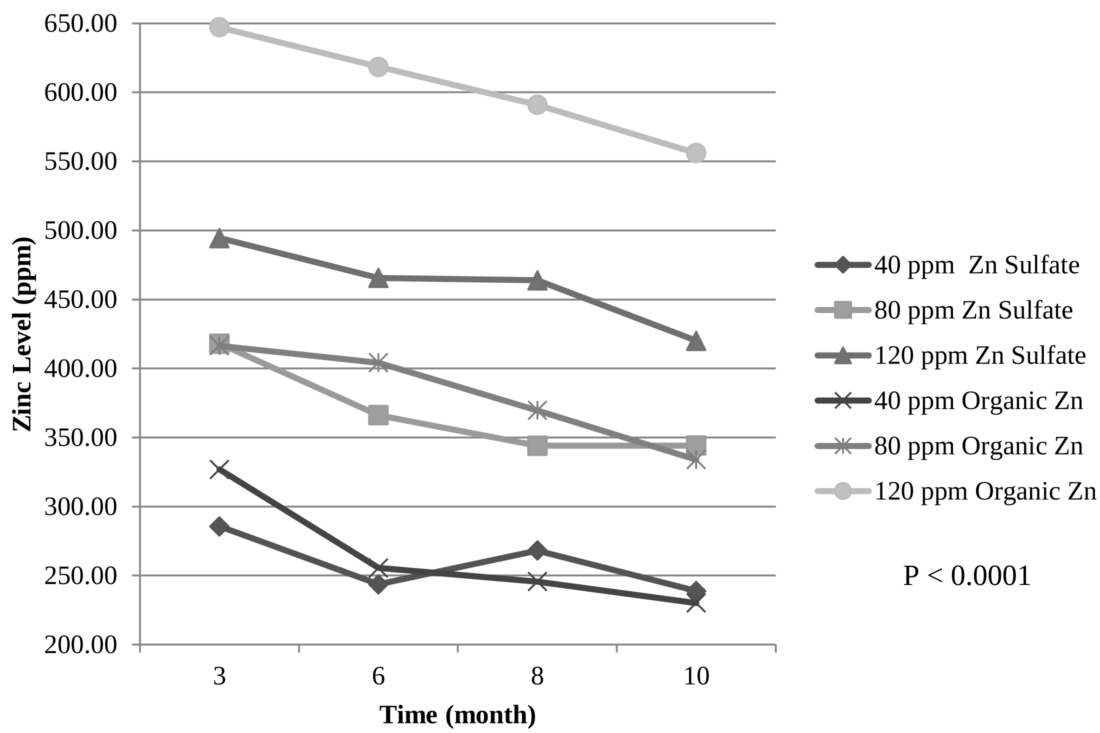


Figure 10c. Effect of zinc source and level on manure zinc content: Source by Level Interaction - Month 3.

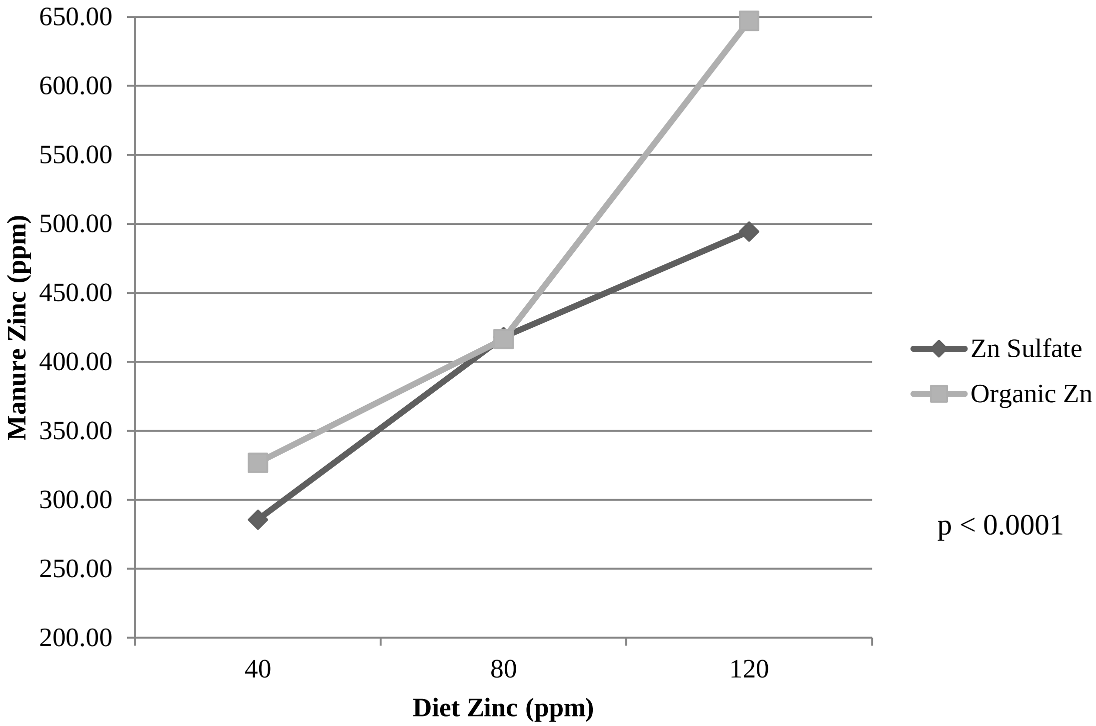


Figure 10d. Effect of zinc source and level on manure zinc content: Source by Level Interaction - Month 6.

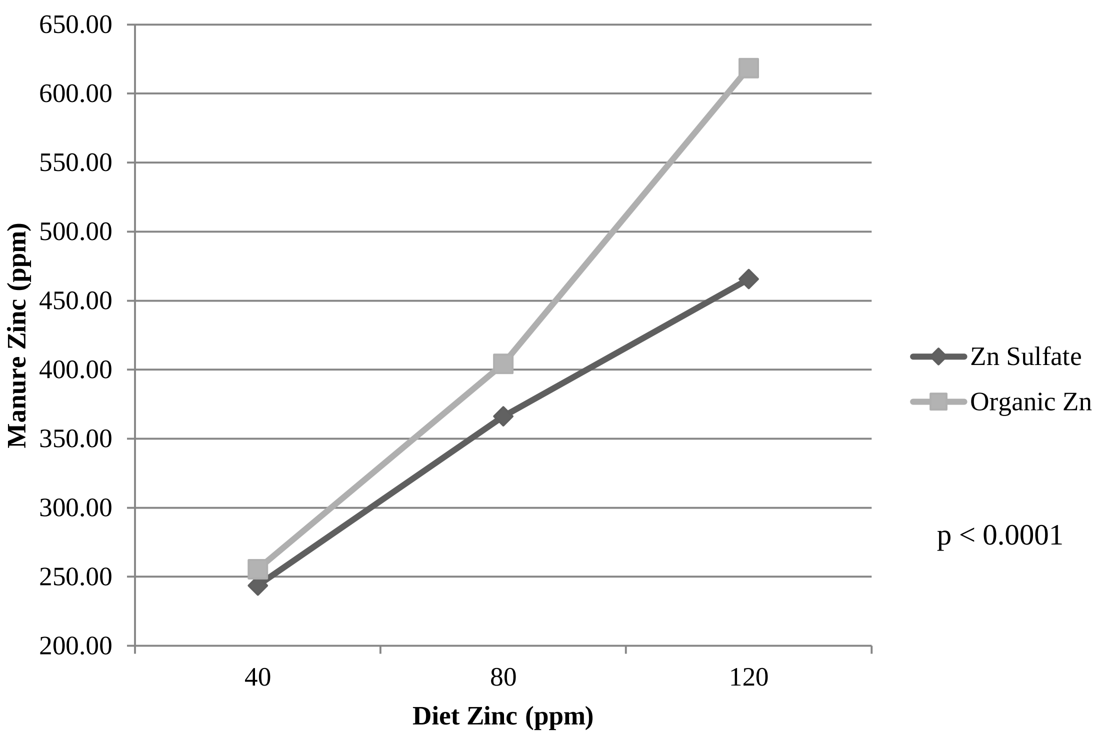


Figure 10e. Effect of zinc source and level on manure zinc content: Source by Level Interaction - Month 8.

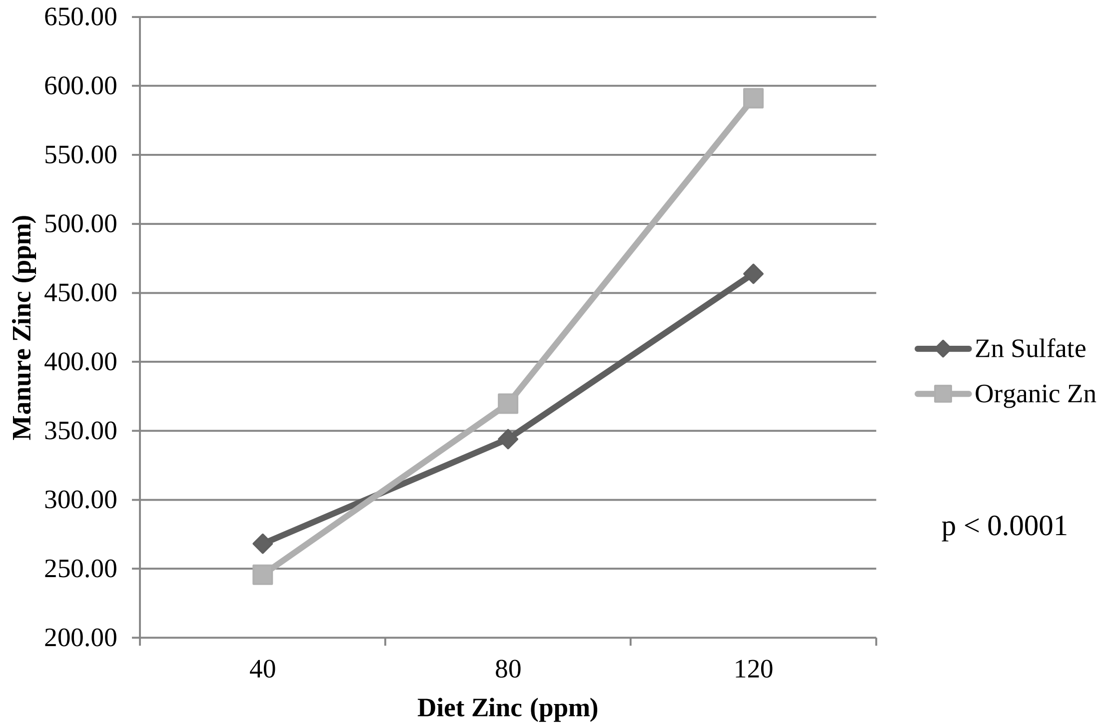


Figure 10f. Effect of zinc source and level on manure zinc content: Source by Level Interaction - Month 10.

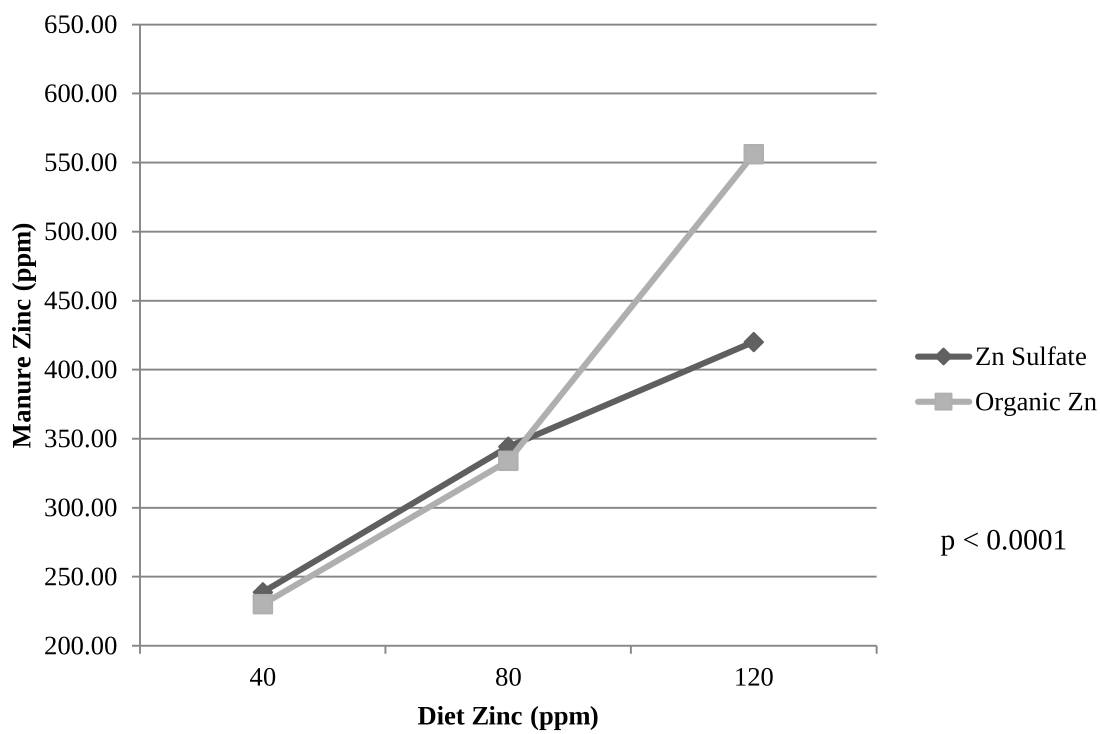


Figure 11. Effect of zinc source and level on bone mineral density: Source by Level Interaction - Age 19 wks.

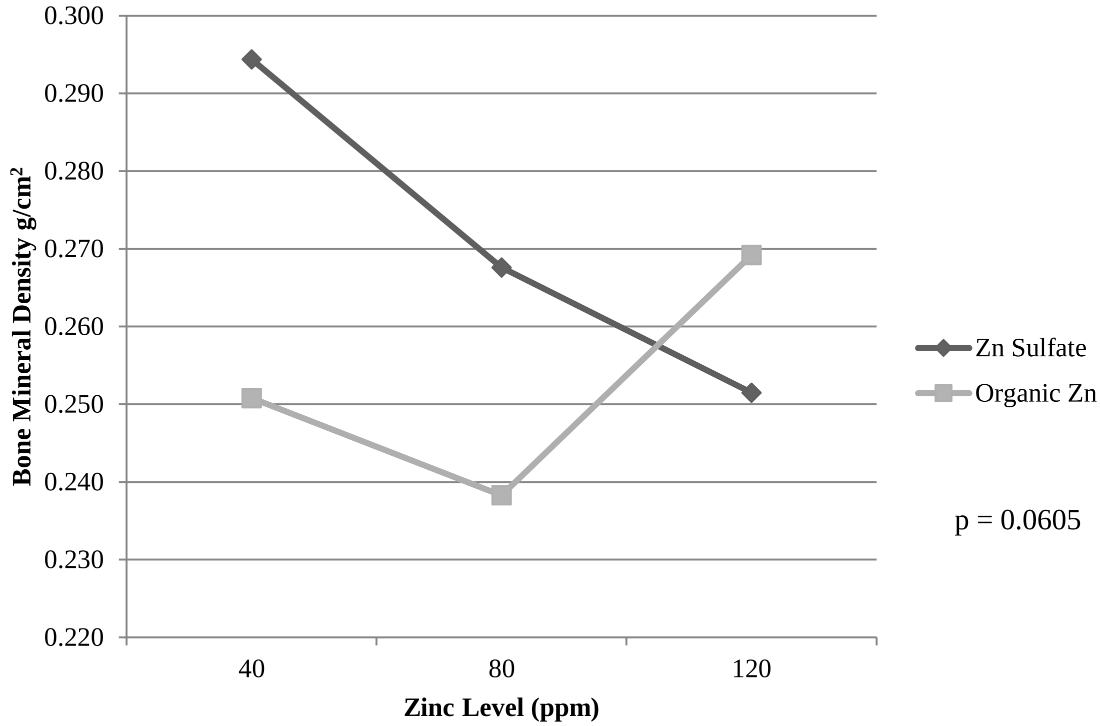
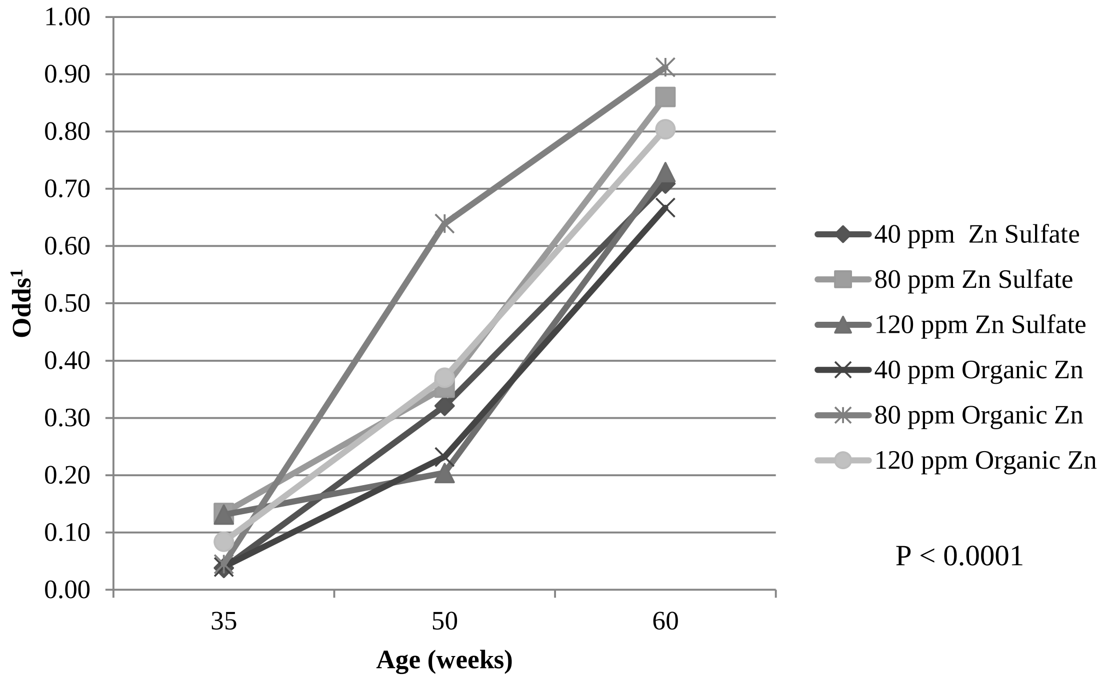
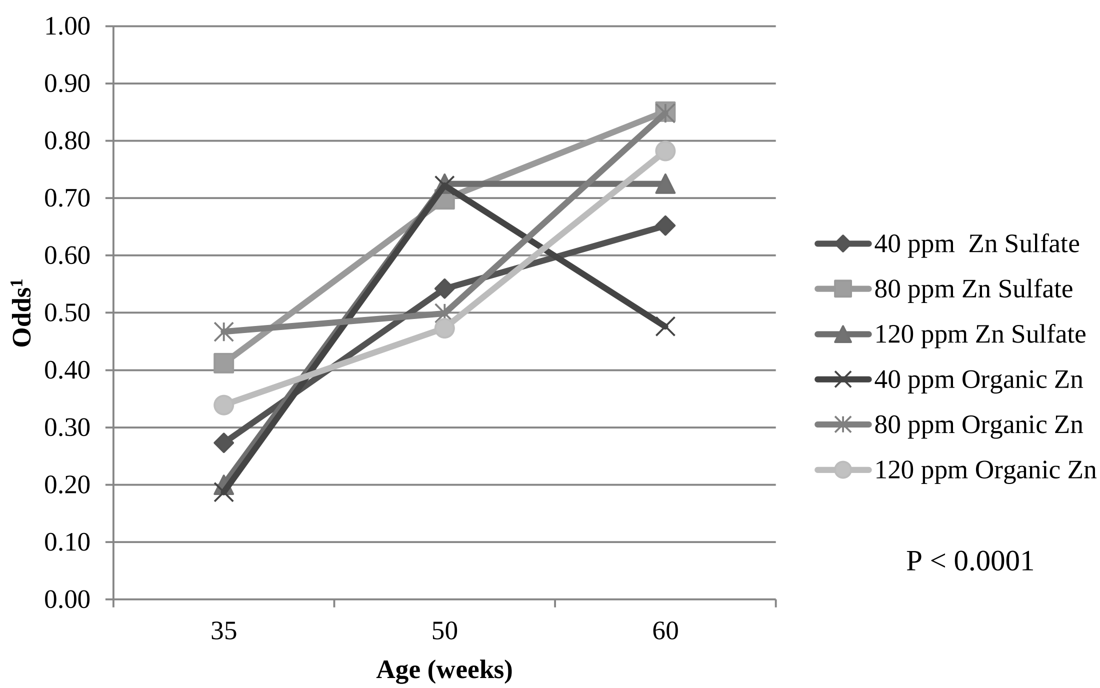


Figure 12a. Effect of zinc source and level on keel bone scores over time - Curve.



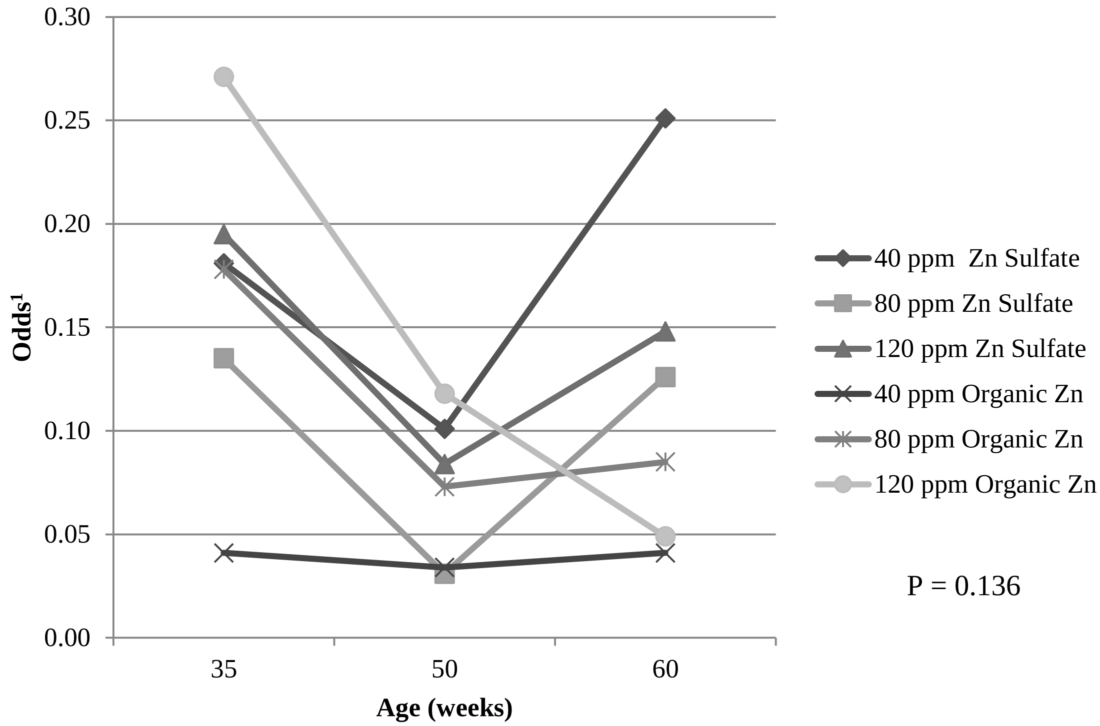
[1]Odds of observing keel bone curvature.

Figure 12b. Effect of zinc source and level on keel bone scores over time - Indent.



[1]Odds of observing keel bone indentation.

Figure 12c. Effect of zinc source and level on keel bone scores over time - Fracture.



[1]Odds of observing keel bone fracture.

Figure 13. Effect of zinc source and level on feather score: Source by Level Interaction.