EVALUATION OF HYDROXYCHLORIDE MINERAL INCLUSION ON BROILER AND LAYER PERFORMANCE

A Dissertation

by

AUSTIN THOMAS JASEK

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Jason Thomas Lee
Co-Chair of Committee,	Craig Daniel Coufal
Committee Members,	Christopher Bailey
	Stephen B. Smith
Head of Department,	David James Caldwell

August 2019

Major Subject: Poultry Science

ABSTRACT

The objective of this research was to evaluate improvements in bird performance parameters using various levels of hydroxychloride minerals in broiler and laying hen chickens. Experiment 1 consisted of 2 broiler trials. Diets for Trial 1 contained levels of Zn hydroxychloride (ZHC) at 40, 80, 120, and 160 ppm, and were compared to $ZnSO_4$ at 80 ppm, and the Trial 2 diets contained ZHC at 100, 125, and 150 ppm, compared to the 2 control diets containing $ZnSO_4$ at 90 ppm (inorganic Zn) with a coccidiostat (positive control (PC)) or without a coccidiostat (negative control (NC)). Dietary ZHC at 120 ppm Trial 1 increased feed intake (FI), increased body weight (BW), breast weight, and breast yield. The inclusion of ZHC at 150 ppm in Trial 2 increased carcass weight and breast yield. These results confirm the ability of ZHC to improve broiler performance and yield.

Experiment 2 evaluated increasing levels of manganese hydroxychloride (MnHCl) at 0, 40, 80, 120, and 160 ppm on performance parameters, Mn deposition, and tibia strength in broilers. Increasing levels of dietary MnHCl reduced feed conversion ratio (FCR) during the later stages of production. Elevated levels of MnHCl also led to linear and quadratic reductions in FCR and increases in tibia Mn deposition. These data indicate the benefit of feeding elevated levels of MnHCl on growth performance and mineral deposition in broilers.

Experiment 3 evaluated increasing levels of MnHCl in 45-wk-old White Leghorn laying hens on yolk and shell Mn content as a potential marker for trace mineral

requirements. Hens were depleted of manganese (Mn) for a 21 d period prior to the beginning of the experiment. Hens were then provided diets containing 0, 15, 30, 60, or 90 ppm MnHCl, which was compared to a non-depleted reference diet containing 70 ppm Mn oxide for a 35 day experimental period. Egg yolk Mn demonstrated higher sensitivity to changes in dietary Mn concentration than shell Mn. Replenishment of yolk Mn levels of layers consuming the reference diet was achieved as quickly as 10 d in hens consuming 90 ppm MnHCl, and 15 d in hens consuming 60 and 30 ppm. These results suggest egg yolk Mn concentration can provide a non-destructive method for determining Mn requirements in poultry.

In conclusion, improvements in performance and mineral deposition associated with increasing dietary concentration of hydroxychloride minerals are dependent on dose and time. Based on the results of these experiments, hydroxychloride minerals could serve as an alternative mineral source for use in livestock feed compared to traditional sulfates and oxides. When implementing alternative mineral sources into production, it is important to evaluate and potentially alter mineral requirements based on breed and target performance.

DEDICATION

I dedicate my dissertation to my family and close friends who have supported me unconditionally over the duration of my student career, from grade school through graduate school. These individuals have been significant in developing the person that I have become today.

To my father, Glen, and mother Debbie. Thank you for the endless love and support that you have provided and continue to provide. You have always been there in times of need to encourage and support. Without you, these experiences and opportunities would not have been possible. I cannot thank you enough for instilling my work ethic, drive, and determination at a young age.

To my sister, Taylor, thank you for always being there for me as a sister and a friend, especially while at Texas A&M. I can't thank you enough for your endless support throughout the years. Though we are not close in age, over the years we've motivated each other by example and desire for excellence.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Jason Lee, and committee co-chair, Craig Coufal, for providing me with this opportunity. I have learned so much from the both of your during my years at Texas A&M. Jason, the knowledge and experiences you have provided me in research, work ethic, and life are immeasurable. Without your patience, understanding, and dedication these opportunities would not have been possible. While under your direction the success and achievements accomplished by our lab are bar none. Lastly, the timeless hours dedicated to reviewing and editing manuscripts, reports, and presentations is greatly appreciated and I cannot thank you enough.

I would like to thank Dr. Coufal, your willingness to co-advise myself along with fellow lab mates upon Jason's departure is greatly appreciated. You made this transition seamless, and for this I am thankful. To my committee members Dr. Bailey and Dr. Smith, thank you for your guidance and support throughout the course of my research and graduate studies.

I would like to thank all my lab mates that I have had the opportunity to work with while apart of student research, you have become some of the greatest friends I could've asked for both at and outside of work. Rocky Latham, Cody Flores, Jake Pieniazek, Tucker Allcorn, Danny Portillo, Brooke Bodle, Trey Lester, Corey Johnson, Kyle Smith, Kyle Brown, and Hunter Walters – thank you, along with a more exhaustive list of undergraduate workers who helped with research. Together we were able to work

through long stressful hours spent weighing, sampling, and processing birds. Without all your hard work, dedication, and desire none of this is possible.

I would also like to thank Dale Hyatt, along with assistant farm managers and the rest of the farm crew at the Texas A&M Poultry Science Center for their time in preparing for research in the barns and feed mill.

Finally, to Ms. Brooke Bodle, your countless hours editing and reviewing the early drafts of manuscripts along with your selfless support through early mornings, late nights, and stressful days did not go unnoticed and is greatly appreciated. Thank you for your love and support through it all.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a dissertation committee consisting of Professor Jason Lee, Professor Craig Coufal, and Professor Christopher Bailey of the Department of Poultry Science and Professor Stephen Smith of the Department of Animal Science.

All work conducted for the dissertation was completed by the student independently.

Funding Sources

Funding for this research was made possible by contributing sponsorship from Micronutrients LLC.

NOMENCLATURE

BW	body weight
Cu	copper
d	day
DDGS	distillers' dried grains with solubles
FCR	mortality corrected feed conversion ratio
FI	feed intake
MBM	meat and bone meal
Mn	manganese
MnHCl	manganese hydroxychloride
MnO	manganese oxide
NC	negative control
PC	positive control
ppm	parts per million
SBM	soybean meal
WD	withdrawal
wk	week
ZHC	zinc hydroxychloride
Zn	zinc
$ZnSO_4$	zinc sulfate

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The use of various feed additives and supplements in the commercial poultry industry has steadily increased in recent years. Increasing use of feed additives is driven by rising feed ingredient prices which ultimately elevate feed cost. As nutritionists strive to reduce the cost of dietary formulations without compromising bird performance or offset the increased costs with improvements in performance, the use of feed additives make these objectives more obtainable. Feed additives can improve the availability of nutrients to the animals by weakening or destroying membranes responsible for encapsulating nutrients that cannot be digested or alter metabolic functions and system to improve the animals ability and efficacy to absorb and partition nutrients. The focus on improving feed cost has been directed on major ingredients that constitute large percentages of the feed (greater than 20%), however the need to maximize mineral bioavailability in both layers and broilers is continually increasing as the poultry industry strives to further improve performance, reduce mineral waste, and improve nutrient digestibility.

Importance of Trace Minerals

The importance of trace minerals is well understood and researched across a myriad of different fields of study. In a review, Suttle (2010) outlined 4 basic functions of minerals in animals that included; structural (1), classified by minerals ability to aid in architectural components of organs, tissue, bones, muscles and stability of membranes;

physiological (2), minerals present in various bodily fluids and tissues that help regulate osmotic pressure, acid-base balance, permeability, and nerve impulses; catalytic (3), function as catalysts in enzymatic function for metalloenzymes, hormones, or activation of cofactors or coenzymes; and regulatory (4), ability to regulate cell function, including replication and differentiation. In summary, minerals are an integral part of animal feeds and are pivotal for normal function, development, and potential growth improvement (Fernandes et al., 2008). However, the list of functions previously listed are based on macro and trace minerals commonly found in animal diets that include, but are not limited to calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), chloride (Cl), sulfur (S), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn). The focus of this paper is to provide deeper insight on trace minerals, specifically Zn and Mn.

Trace minerals are defined as “any group of metal ions present in minimal amounts in biological systems, which are required for their optimal activity” (Segen's, 2012). Commonly, trace mineral inclusion is classified by mg/kg or parts per million (ppm), whereas major or macro minerals are measured as a percent of the total diet (NRC, 1994). Trace minerals are usually supplemented in the diet through the use of a trace mineral premix, and commonly added in excess of recommendations to account for variability and ensure minimum requirements are achieved (Nollet et al., 2007). Nollet et al. (2008) classifies Cu, I, Fe, Mn, Se, Zn, and Co (as B_{12}, Co, or both) as essential trace minerals supplemented commonly in broiler diets. In agreement with Suttle (2010), Nollet et al. (2007) reports trace minerals importance in enzyme systems ultimately

effecting growth, bone development, feathering, enzyme structure, and appetite. In addition, trace minerals serve as constituents for hundreds of proteins associated with metabolism, hormone secretion, and immunity (Dieck et al., 2003). While trace mineral inclusion seems to be almost obsolete, as inclusion levels are minute, removing them could cause a countless problems. Trace mineral deficiencies can lead to insufficient performance, impair metabolic and physiological functions, and compromise the gastro-intestinal tract leading to the development of disease, health issues, and death.

Mineral Source

Minerals exist in many elemental forms and sources, these classifications contribute to differences in bioavailability between sources. Mineral source represents a determining factor for nutritionist's decision to supply dietary minerals that are efficient for the animal, while remaining affordable. Currently, inorganic trace minerals are primarily used, as they are easily obtainable and cost effective for use in least cost formulation. Cheap products allow nutritionist's the opportunity to include inorganic minerals in excess of the recommendation to provide a dietary safety margin (Bao et al., 2007; Yang et al., 2012), that ensures adequate growth (Zhao et al., 2010), without negatively influencing the cost of diet. Inal et al. (2001) noted that an excess of minerals 2 to 10 times the recommended levels is not uncommon. Furthermore, inorganic trace minerals that are commonly used in the industry include both oxides and sulfates, however vary in availability. McNaughton et al. (1974) reported ferric oxide and cuprous oxide only to be about 75% readily available as compared to their respective elements in sulfate form. Aksu et al. (2010) mentions a higher rate of mineral loss in

regards to inorganics because of dietary antagonisms. Prior research has shown inorganic mineral sources to be less bioavailable than other mineral sources (Wedekind et al., 1992; Manangi et al., 2015), such as organic (Kidd et al., 1996) and hydroxyl forms (Shaeffer et al., 2017). The chemical make-up of inorganic minerals can increase unwanted interactions and chelation with other minerals that form insoluble compounds resulting in reduced digestion, absorption, and bioavailability of both elements (Brooks et al., 2013; Manangi et al., 2015). Ultimately, these reductions in bioavailability are responsible for increases in the excretion of trace elements. Thus, the waste of heavy metal trace elements has increased over the years within broiler production as well as other sectors of livestock production. Dozier et al. (2003) described an environmental concern regarding Zn and Cu contamination reducing crop yield. Research is now focused on alternative strategies to reduce mineral pollution and improve the utilization of trace minerals within poultry diets.

The National Research Council (NRC) has established trace element requirements for both layers and broilers for Zn, Cu, and Mn. Requirements for Zn are 40 and 35 ppm, Cu is 8 and 4 ppm, and manganese is 60 and 30 ppm for broilers and layers, respectively (NRC, 1994). Mineral requirements were established over 20 years ago and many ingredients, additives, and most importantly birds have experienced changes that may deem these requirements inaccurate (Bao et al., 2007). Nutritionist use these values only as a guideline in feed formulations as values are easily altered based on bird age, performance targets, cost, and health. Research conducted by Coic and Coppenet (1989) reported that litter from broilers fed high concentrations of Zn and Cu,

contained 660 and 560%, respectively, in excess of crop requirements when applied on a nitrogen (N) basis.

Organic mineral sources is one of the alternative mineral sources available to replace or reduce the use of inorganics, and are commonly associated with a higher availability compared to inorganic minerals. Improvements in bioavailability of organics is due to differences in chemical structure. Research conducted by Ammerman et al. (1998) reports organic mineral structure stabilizes the mineral (Aksu et al., 2010), protecting itself from forming unwarranted complexes with various elements found in the digestive tract, allowing preferred absorption over complexed minerals. Similarly, Ao et al. (2009) outlines the reasons for improved availability including a ring structure that protects minerals from unwanted interactions (Wedekind et al., 1992; Wedekind et al., 1994), chelation that facilitates the absorption of other minerals (Miles and Henry, 2000), and multiple routes of absorption (Aldridge et al., 2007). Organic minerals added to diets in the poultry industry are normally chelated or chemically combined to amino acid complexes, proteins, or carbohydrates (Wedekind et al., 1992; Spears, 1996). Organic minerals have the ability to chelate other soluble dietary constituents, making digestion and absorption via different routes possible (Cao et al., 2000), compared to insoluble products combined to inorganic minerals, is the reason for improved bioavailability (Ao et al., 2009). Bao et al. (2007) determined that organic mineral inclusion could be reduced, decreasing mineral excretion without compromising broiler performance at d 29 (Brooks et al., 2012).

The second mineral source that could be used as an alternative to inorganic minerals is that of the hydroxy source, usually a hydroxychloride of which limited research has been conducted (Perez et al., 2017). Hydroxy minerals are classified as an inorganic, however alterations within the chemical structure resemble that of an organic. Cromwell et al. (1998) stated, Cu chlorides are less than one percent soluble in water, and should result in a less destructive Cu source to vitamins, additives, and dietary ingredients. Hydroxy minerals may also avoid interactions during digestion that compromise absorption (Shaeffer et al., 2017). Thus, creating the opportunity for reductions in dietary mineral inclusion, without negatively influencing growth or performance in broiler and layer flocks. Olukosi et al. (2018) reported growth performance and meat yield improvements in broilers fed hydroxychloride Zn and Cu compared to sulfate Zn and Cu. Nutritionist must evaluate these mineral sources and determine the option that will work best in their feeding program, balancing performance, growth, cost, and environmental impact.

Zinc

Zinc is an essential trace mineral in a multitude of biological functions in broiler and layer chickens. Zinc serves as a cofactor and/or constituent for over 200 enzymes and enzyme processes within the body, and is responsible for numerous functions (Ashmead and Zunino, 1993; Park et al., 2004; Salim et al., 2008; Star et al., 2012). Zinc is the only metal to have a significant role in all 6 metalloenzyme classes (transferase, lyase, ligase, hydrolase, isomerase, and oxidoreductase) (Vallee and Auld, 1990; Kidd et al., 1996). The role that Zn plays within these classes of enzymes is the reason of its

importance in metabolism, immunity, regulating homeostasis, and DNA and RNA synthesis (Salim et al., 2008; Suttle, 2010; Star et al., 2012). Furthermore, the involvement of Zn in protein synthesis (Suttle, 2010), carbohydrate, and energy metabolism (Kennedy et al., 1998), can lead to improvements in growth and muscle accretion. The function of Zn in immunity is definite, serving as a cofactor for thymulin, directly impacting T-lymphocyte production. T-lymphocytes are used as a mechanism to fight off threats to the body that include infection and cancerous cells (Fraker et al., 1986; Dardenne and Bach, 1993; Kidd et al., 1996). Previous studies have reported that increasing levels of Zn in broiler diets improved appetite (Ao et al., 2006b), growth, and bone development (Salim et al., 2008). Kennedy et al. (1998) noted a Zn deficiency in rats not only reduced appetite, but also altered preference toward fats over carbohydrates. In another study evaluating Zn deficiencies in rats, Kwun et al. (2007) observed reduction in growth rate and leptin mRNA levels. Sahu (2004), reported leptin is responsible for regulating the release of neuropeptide Y and corticotropin-releasing hormone, which up regulates appetite and down regulates food intake respectively when Zn is deficient. Rossi et al. (2007) associated Zn with epithelial cells and collagen synthesis reducing the risk of skin tearing, thus improving carcass quality. Furthermore, Lim and Paik (2003), noted that in layers, Zn was a constituent for carbonic anhydrase, which is a calcium binding protein important in providing the eggshell with the necessary carbonic ions for shell development and structure. Bone strength and integrity is critical in broilers and layers, Zn can help ensure proper bone structure and growth through collagen synthesis (Park et al., 2004; Brooks et al., 2013). Similarly, large

amounts of minerals are partitioned to the egg and eggshell for proper development (Park et al., 2004).

Zinc in Broilers

Zinc has been widely researched both alone and in combination with other minerals on the effects of broiler growth performance, processing yield, and meat quality characteristics. However, researchers have reported varying results with different forms and inclusion levels of Zn. Kucuk et al. (2003) observed improvements in d 42 live weight, weight gain, feed intake (FI), and feed conversion ratio (FCR) in broilers fed a diet containing a 30 ppm inclusion of $ZnSO_4$ reared under heat stress (34°C) compared to birds fed the control diet absent of Zn supplementation (approximately 45 ppm Zn). Kucuk further observed increases in processing parameters that included live weight, carcass wt., carcass yield, heart, liver, spleen, gizzard weights, and a reduction in fat pad weight in broilers consuming the diet supplemented with 30 ppm $ZnSO_4$. During the summer months of Egypt (daily house temperature of 33 to 36°C), Saleh et al. (2018) evaluated the inclusion of an organic Zn methionine product at 0, 25, 50, and 100 ppm on broiler growth performance, nutrient utilization, anti-oxidative properties and immune response over a 6 wk period. Saleh observed improvements in average daily gain (ADG), FCR, breast weight, and abdominal fat (reduction) when including Zn methionine at or above 50 ppm, with BW at termination being maximized at 50 ppm. These studies seem to agree with one another, in that the use of Zn in a stressful environment, in this case heat stress, can improve broiler performance characteristics. However, Bartlett and Smith (2003) evaluated dietary Zn at low (34 ppm), adequate (68

ppm), and high (181 ppm) levels on growth parameters over a 7 wk period in broilers subject to a thermo-neutral and heat stressed environment from day 21 to 49. Bartlett and Smith (2003) observed no differences in growth performance (BW and FI) with regards to the 3 dietary Zn concentrations during the 7 wk period. Interestingly, Bartlett and Smith (2003) low and adequate dietary Zn values were similar to those used by Kucuk et al. (2003), however were unable to illicit a performance response to Zn.

Though heat stress rearing is not uncommon in the southern United States, improvements in broiler housing and cooling systems have reduced the incidence in broiler production. Thus, the use of Zn in normal production settings is imperative for understanding the effects of these products. Sunder et al. (2008), evaluated increasing levels of Zn at 10, 20, 40, 80, 160, and 320 ppm in a corn and soybean meal (SBM) based diet, and observed no differences in BW, FI, or FCR in four wk-old broilers. In addition, Sunder et al. (2008) observed maximized Ca and P tibia concentrations in broilers fed the diet containing 40 and 80 ppm, thus increasing Zn concentration linearly increased Zn in bone, kidney, and liver with each elevation in inclusion. Star et al. (2012) conducted a study to determine the bioavailability of organic Zn (5, 10, and 15 ppm) compared to inorganic Zn (0, 5, 10, 15, 20, and 40 ppm) in broilers fed a corn, wheat and SBM based diet. Star et al. (2012) observed increases in tibia Zn concentration compared to the control diet absent of supplemental Zn at 15 ppm inorganic and 10 ppm organic leading to a linear improvement in bioavailability for the organic Zn compared to the inorganic, producing a relative bioavailability of the 164% compared to the inorganic source. The differences apparent in dietary Zn level and

source did not impact BW gain or FI, however organic Zn inclusion at 5 ppm produced the increased FCR, all other Zn levels and sources produced similar results to their counterparts (Star et al., 2012). Though these authors above reported differences in Zn, Ca, and P mineral concentrations in different tissues, effects on performance were minimal with the use of Zn.

However, Huang et al. (2007) evaluated increasing levels of supplemental inorganic Zn ($ZnSO_4*7H_2O$) from 0 to 140 ppm (20 ppm increments) on 21 d old broilers fed a corn soy diet (contained approximately 28 ppm Zn). Huang et al. (2007) observed maximum BW and FI in broilers supplemented with 20 ppm of Zn, however; elevated levels of Zn were required for optimum levels in pancreas (59 ppm) and bone (62 ppm) based on statistical modeling. Huang et al. (2007) concluded optimum dietary Zn level to be 84 ppm at 21 d of age based on parameters evaluated. In an experiment conducted by Dozier et al. (2003) evaluating dietary Zn inclusion of 40, 80, and 120 ppm from inorganic, organic, or the combination of both sources, no differences in BW, FCR, or mortality were observed amongst treatments in 17 d broilers. Dozier et al. (2003) concluded, though no differences in performance were observed, linear reductions in Zn excretion were observed as Zn addition was reduced. The lack of differences in the study conducted by (Dozier et al., 2003), may be due to the short longevity of the experiment. Interestingly, Huang et al. (2007) was able to observe differences in 21 d old broilers with the use of inorganic Zn at 20 ppm.

While inorganics are still widely used in poultry production, both organic and hydroxychloride minerals are being used more frequently in the industry. Brooks et al.

(2013) evaluated multiple levels (0, 6, and 12 ppm) of Zn from both inorganic and organic sources in broiler chicks from 8 to 21 d of age. The starter diet was semi-purified, containing soy protein concentrate and dextrose fed from d 0 to 7, and the basal diet fed from d 8 to 21 replaced some of dextrose with corn to add phytate in to the diet. Brooks et al. (2013) observed improved FCR with the inclusion of Zn, and dose dependent increases in FI, weight gain, and tibia Zn concentration regardless of Zn source. However when comparing improvements in weight gain, tibia Zn concentration, and total tibia Zn, Brooks et al. (2013) concluded improvements in bioavailability of 119, 116, and 116% respectively when feeding the organic propionate compared to the sulfate. In a study conducted by Ao et al. (2009), evaluating multiple combinations and sources (sulfate or chelated proteinate) of Cu and Zn at 0 ppm and 8 ppm for Cu and 20 ppm for Zn creating a 3 x 3 factorial, reported Zn supplementation regardless of source increased weight gain, gain:feed (G:F), and plasma Zn concentration. Ao et al. (2009) further observed improvements in tibia Zn concentration when including organic Zn, the addition of both Zn sources lowered liver Cu levels. Ao et al. (2006a), reported Zn requirements with respect to weight gain at 9.8 ppm and 20.1 ppm for organic and inorganic Zn respectively based on broken line analysis in 21 d old broilers fed diets supplemented with Zn at 0, 5, 10, 20, & 40 ppm of respective source. Batal et al. (2001) conducted 3 assays to determine Zn requirements of Zn sulfate (ZnSO4*7H2O) and ZnCl ($Zn_5Cl_2(OH)_8$) in chicks from 8 – 22 d of age. Chicks in assay 1 were fed additional sulfate at 0, 5.81, 10.81, 15.10, and 20.25 ppm Zn to a basal diet containing approximately 8.8 ppm, and determined 13.6 ppm of Zn supplementation was the

breaking point for weight gain, based on broken-line analysis. In assay 2 and 3, ZnCl was added at 5.38 and 10.81 in assay 2 and 5.41 and 10.82 ppm in assay 3. Addition of ZnCl in assay 2 produced a weight gain curve 102% of the $ZnSO_4$ value. Similarly, ZnCl produced a weight gain curve 111% to that of $ZnSO_4$ in assay 3. Results from assay 2 or 3 were not statistically different between Zn sources, however Zn level was significant in effecting weight gain and FI. These studies iterate the impact of Zn source on bioavailability and performance in broilers.

Though broiler performance is of primary concern for nutritionists, companies strive to advance all aspects of the business to include processing characteristics when analyzing live performance. Saenmahayak et al. (2010) observed improvements in BW and FCR through d 42 and 48 in broilers that were fed diets supplemented with 40 ppm inorganic and 40 ppm organic Zn (80 ppm total) compared to those fed 80 ppm inorganic Zn. The performance results observed by Saenmahayak et al. (2010) when including organic Zn were similarly reflected when evaluating processing parameters as broilers fed an additional 40 ppm organic Zn to the 80 ppm inorganic Zn (120 ppm total) increased breast meat and total white meat yield compared to broilers fed inorganic Zn at 80 ppm. Improvements in foot pad dermatitis and skin lesions (sores, scabs, and scratches) were observed in broilers fed 40 ppm organic and 40 ppm inorganic Zn compared to those fed 80 ppm inorganic (Saenmahayak et al., 2010). Furthermore, in a study evaluating increasing levels of organic Zn (0, 15, 30, 45, and 60 ppm), Rossi et al. (2007) observed no differences in broiler growth performance through 42 d of age or improvements in processing yields associated with increasing levels of dietary organic

Zn. The reasoning for the lack of differences in Rossi et al. (2007) is due to the adequate supplementation of Zn in the basal diet of 1,500 ppm, which would minimize or completely mitigate the effect of additional Zn regardless of source. However, Olukosi et al. (2018) evaluated the effects of a high (80 ppm) and low (20 ppm) levels of Zn in conglomeration with Cu at 15 ppm between sulfate and hydroxychloride minerals. Olukosi et al. (2018), observed increases in broiler weight gain with the lower inclusion of Zn at 20 ppm regardless of mineral source from 21 to 35 d of age and cumulatively through d 35. The inclusion of hydroxychloride minerals regardless of Zn level improved FCR compared to sulfates from d 21 to 35 and cumulatively through 35 d. When evaluating processing parameters, Olukosi et al. (2018) reported improvements in carcass and breast meat yield with the low level of Zn inclusion, hydroxychloride Zn increased breast yield as compared to broilers fed sulfates. Liu et al. (2011) evaluated the effects of Zn from sulfate and 3 different organic sources (one amino acid and two proteinated sources) at 60, 120, and 180 ppm compared to a control absent of supplemental Zn. Liu et al. (2011) did not observe any differences in performance between the Zn source, however did report increases in FI and BW through d 21 and 42 with the inclusion of Zn at or above 60 ppm compared to the control. Improvements observed by Liu et al. (2011) in live performance were resembled in processing traits as carcass yield was increased in broilers fed diets containing at least 60 ppm Zn. The reason for the lack of Zn source effect on performance is not fully understood, however Liu et al. (2011) only raised a total of 468 birds across 13 treatments to total 6 replicates containing 6 birds each. The small amount of replicates and birds per replicate may have

been the reason for the lack of statistical differences among mineral source. The previous studies explain the effect Zn can have not only on live performance, but processing traits as well. Studies previously mentioned, indicate broiler carcass, breast meat yield or both can be increased when utilizing more available Zn sources or increasing dietary concentration. However, important things to consider when designing experiments is total number of birds, replicates per treatments, as well as number of birds per replicate. Reducing replicates and/or birds per replicate can reduce the power or your experimental design when analyzing statistics.

Minerals often compete with each other, whether it is for similar absorption sites or degradation of other minerals creating antagonisms between minerals. Pang and Applegate (2007) evaluated the effects of feeding different Cu sources (sulfate, lysinate, and tribasic Cu chloride) at 250 ppm on solubility of Cu, Ca, and Zn in the small intestine (duodenum and jejunum). Pang and Applegate (2007) noted an increase in Cu solubility with supplementation, regardless of source, at 250 ppm compared to the control diet containing 8 ppm Cu sulfate. While neither source nor level of Cu had an effect on Ca solubility, broilers fed 8 ppm Cu and Cu sulfate at 250 ppm improved Zn solubility compared to birds fed Cu lysinate (Pang and Applegate, 2007). The alterations in Zn solubility with different sources of Cu observed by Pang and Applegate (2007), may indicate the antagonism that exists between the two minerals.

Brooks et al. (2013) mentions increasing dietary corn can increase phytate concentration. Phytate is an antagonist to monogastric animals and can hinder digestion of trace minerals and other nutrients (Harland and Narula, 1999; Rimbach et al., 2008).

In the study previously mentioned, Ao et al. (2009) associated reductions in G:F ratio with the inclusion of $CuSO_4$ (8 ppm) to Zn (20 ppm) containing treatments with antagonism, however, feed:gain (F:G) in broilers fed organic Cu produce similar results to broilers fed Zn alone. Thus, signifying the challenges in feeding multiple minerals of varying sources in conglomeration with other ingredients that contain anti-nutritive factors impeding digestion and absorption of dietary nutrients.

Manganese

Similar to Zn, Mn is an essential trace mineral in many living systems aiding in functions necessary for life. Manganese is essential for bone development, both prenatal and post hatch (Richards et al., 2010), and is a constituent for multiple enzymes responsible for activation of metabolic processes that include carbohydrate, amino acid, and cholesterol synthesis (Nielsen, 1999). In the mitochondria, pyruvate carboxylase is activated by Mn (Medicine, 2001); pyruvate is carboxylated into oxaloacetate as the initial step of gluconeogenesis. In layers, Mn deficiencies can lead to multiple problems including decreased egg production, thinning egg shells (opaque), loss of eggs, abnormal ultrastructure of the egg, and morphology of mammillary knobs (Leach and Gross, 1983). Manganese has shown to improve initial eggshell quality by increasing hexosamine and hexuronic acid production, both of which are found in eggshell matrices (Longstaff and Hill, 1972). Gheisari et al. (2011) mentioned Mn can improve eggshell formation and breaking strength through increasing calcite crystal growth. Manganese fortifies skeletal integrity by improving calcification of the bone and formation of proteoglycans, which moderate compressive charges inhibiting growth of the epiphyseal

plate (Leach and Gross, 1983). Manganese superoxide dismutase (MnSOD) has very similar function to Cu-Zn SOD, however is located in the mitochondria opposed to the cytoplasm (Luo et al., 2007; Conly et al., 2012). This makes MnSOD an important enzyme in protecting normal mitochondrial function (Luo et al., 2007), by acting as an antioxidant and removing reactive oxygen species that can increase susceptibility of disease and modify metabolism (Holley et al., 2011).

Over the years Mn research has progressed, however initially Mn research primarily focused on reducing the incidence of slipped tendon in poultry. Van der Hoorn et al. (1938), reported Mn is necessary for growth and livability, as well as prevent perosis caused by a deficiency in Mn. Perosis, also known as slipped tendon or hock disease (Titus, 1932), is characterized by bowing of the leg at the tibia, flattening of the joint, and ultimately the "slipping" of the Achilles tendon from its original position (Insko et al., 1938). Perosis represents one of the classic signs of Mn deficiency in chickens (Gallup and Norris, 1939b; Wilgus Jr and Patton, 1939). Insko et al. (1938), reported an inclusion of Mn at 30 ppm to a ration containing 2.10% Ca and 1.25% P, and another including Mn at 5 ppm to a ration containing 3.04% Ca and 0.86% P significantly reduced the amount of slipped tendon, these same rations absent of Mn inclusion produced 87 and 70% slipped tendon, respectively. However, in current poultry production the addition of a mineral premix and nutrient profile of ingredients ameliorate the ability of a Mn deficiency.

Manganese in Broilers

Similar to other trace minerals, Mn concentration and bioavailability is of critical concern due to rapid growth rates which can potentially compromise bone strength and structure (Henry et al., 1989). Henry et al. (1986) evaluated the bioavailability of $MnSO_4$ and MnO supplemented at 40, 80, and 120 ppm over a 21 d assay period in young broilers. No differences in FI, weight gain, or FCR were observed among treatments, however based on regression analysis for tissue deposition of Mn, MnO was approximately 66% that of $MnSO_4$. In another study, Henry et al. (1989) evaluated the inclusion of Mn sulfate monohydrate, Mn oxide, or Mn-methionine at 0, 700, 1,400, and 2,100 ppm fed to broilers for a 21 d assay period. Henry et al. (1989), observed decreases in FI in all broilers supplemented with Mn, resulting in improved FCR for Mn-methionine fed broilers compared to all other treatments due to the amount that FI was reduced compared to all other treatments. After evaluating both bone and kidney Mn concentrations, Henry et al. (1989) determined oxides were less bioavailable than Mn sulfate, and Mn-methionine increased bioavailability compared to sulfate. In a study conducted by Smith et al. (1995), evaluating supplemental Mn at 0, 1,000, 2,000, and 3,000 ppm in inorganic ($MnSO_4*H_2O$ & MnO) and organic (Mn proteinate) form in broilers subjected to a thermo-neutral and heat distress environment for a 49 d assay period. Smith et al. (1995) observed improvements in Mn bioavailability in broilers fed the proteinate compared to the inorganic source. The presence of heat stress further improved the bioavailability of the proteinated source. The increases in bioavailability with the proteinate resulted in improvements in BW gain at 1,000 and 2,000 ppm

compared to MnO, however broilers consuming 3,000 ppm proteinate exemplified severe reductions in BW compared to other treatments (Smith et al., 1995). Brooks et al. (2012) evaluated the inclusion $MnSO_4$ and Mn proprionate at 0, 20, 100, and 500 ppm broiler chicks from 7 to 21 d of age following a 7 d depletion (d 0 to 7). Based on multiple linear regression analysis of tibia Mn concentration in relation to dietary Mn, Brooks et al. (2012) estimated Mn propionate relative bioavailability to be 139% compared to $MnSO_4$. Overall, each of these studies reported improvements in Mn bioavailability when utilizing organic forms of Mn compared to inorganic forms.

Wong-Valle et al. (1989), evaluated the effect of multiple inorganic sources of Mn (reagent grade (RG) $MnSO_4$*H2O, MnO RG, or feed grade oxide A, B, or C) at 0, 1,000, 2,000, or 3,000 ppm in 21 d old broilers. No differences in chick growth performance was observed with regards to source or inclusion level, however Mn content in bone and kidney were linearly correlated by Mn source increasing from MnO C, to B, to A, to RG, and maxing out with $MnSO_4$ RG. Increasing Mn level regardless of source from 0 to 1,000, 1,000 to 2,000, and 2,000 to 3,000 increased Mn content of both bone and kidney (Wong-Valle et al., 1989).

A requirement study was conducted by Li et al. (2011) to determine the $MnSO_4$ requirement in 21 d old broilers by evaluating growth performance and Mn and MnSOD concentration in various tissues, treatments consisted on increasing levels of Mn with 0, 20, 40, 60, 80, 100, 120, and 140 ppm. Through d 21, Mn inclusion had no impact on BW gain, FI, or F:G, however from d 15 to 21 Mn inclusion equal to or exceeding 80 ppm improved leg abnormality percentage compared to broilers supplemented with 0

and 20 ppm Mn. Linear improvements in heart MnSOD mRNA concentration, heart MnSOD activity, and Mn concentrations of heart, pancreas, and liver were observed in relation to Mn inclusion at d 7, 14, and 21. Overall, Li et al. (2011) estimated the 21 d broiler requirement for Mn to be approximately 130 ppm, based on the findings of this study.

Three studies were conducted by Conly et al. (2012), to determine the efficacy of tribasic Mn chloride compared to sulfates in broiler chicks. Experiment one evaluated Mn at 3,600, 4,500, and 5,400 ppm, on d 35 BW was negatively affected with the inclusion of Mn irrespective of source, however sulfate at 5,400 ppm decreased BW further compared to all other treatments. Similarly, blood plasma Mn was increased with all Mn inclusion compared to the basal, with the sulfate inclusion at 5400 ppm further increasing plasma concentration. In experiment 2 and 3, Mn source and inclusion (30 to 240 ppm) had no impact on growth performance for the duration (21 d), however in experiment 2, tibia Mn and liver Mg, Mn, and P were elevated with increases in dietary Mn concentration. These studies further discuss the differences in mineral absorption amongst various tissues associated with differences in Mn inclusion, although no major differences in performance were observed even with improvements in availability.

Though Mn effect on processing parameters is important, limited research has evaluated such effects. Lu et al. (2007) evaluated the effects of multiple sources of Mn supplemented at 100 or 200 ppm in the form of $MnSO_4*H_2O$, Mn chelated to an amino acid (MnAA) (1), MnAA (2), and a control absent of Mn (contained approximately 21 ppm Mn). Through d 21 and 42, Lu and collaborators observed no differences in daily

gain, FI, or G:F between mineral source or inclusion level, however the 200 ppm inclusion reduced leg abnormalities compared to broilers fed the control or the diet containing 100 ppm Mn. Similar to the performance data, no differences in breast muscle or thigh muscle were observed for Mn source or level, however abdominal fat was reduced in broilers fed supplemental Mn at either level. Reductions in abdominal fat may indicate Mn ability to spare energy for use in muscle accretion and maintenance, instead of inconsumable adipose tissue.

Manganese in Egg Producing Chickens (Laying Hens and Breeders)

Trace mineral inclusion levels are crucial in egg producing hens. Minerals serve as cofactors for enzymes that are responsible for supplying minerals and nutrients to the eggshell and yolk/embryo for extended periods of time. Trace mineral mobilization in different regions of the embryo is made possible by vitellogenin, a major transporter of trace minerals (Richards, 1997). In a previous study Lyons (1938), evaluated the impacts of Mn at low (7 ppm) and high (27 or 57 ppm) concentrations fed to laying hens and noted a reduced hatchability in eggs from hens fed the low level of Mn. Furthermore, Lyons (1938) reported a deficiency in Mn indicated by chick embryos was developed 10 to 14 d after feeding the low Mn diet. Swiatkiewicz and Koreleski (2008) conducted a 45 wk long study evaluating layer performance from 25 to 70 weeks of age in layers fed diets comprised partially, or completely, of organic Mn and Zn compared to layers fed solely inorganic. Swiatkiewicz and Koreleski (2008), reported mineral source had no impact on production rate, weight, mass, FI, feed conversion, shell thickness, shell %, or shell density at any point. However, in older laying hens (62 weeks) fed 50% organic

Mn, regardless of Zn inclusion, and organic Mn & Zn at 100% produced the highest egg shell breaking strength compared to the control diet absent of organic Mn and Zn. At 70 weeks of age, laying hens fed 100% organic Zn and Mn increased egg shell breaking strength compared to the diet absent of organic minerals and the layers fed the diet containing 50% organic Zn + 100% inorganic Mn (Swiatkiewicz and Koreleski, 2008).

Xiao et al. (2015) evaluated the effects of feeding organic or inorganic Mn at 0, 25, 50, 100 and 200 ppm in 50 wk-old laying hens fed a diet depleted of Mn for 4 weeks and supplemented Mn diets for 8 weeks. Supplementation of Mn had no impact on egg production, egg weight, or feed efficiency; however when evaluating main effects, organics minerals and elevated inclusion increased breaking strength. At termination of the study, supplementation of inorganic Mn more than 25 ppm and organic Mn at all levels increased shell Mn content compared to layers fed the control absent of Mn and the diet containing 25 ppm inorganic Mn (Xiao et al., 2015). To conclude, Xiao et al. (2015) estimated bio-efficacy of organic Mn 357%, 406%, and 470% more than that of $MnSO_4$ for shell thickness, breaking strength, and eggshell Mn, respectively. Similar to mineral source effects in broilers, these studies indicate further improvements with the use of non-inorganic Mn sources in layers.

A series of studies was conducted by Longstaff and Hill (1972) to evaluate Mn impact on hexosamine and uronic acid levels along with shell quality characteristics. Longstaff and Hill (1972) reported reduced levels of hexosamine and uronic acid in thin egg shells and those classified as soft and reported elevated levels of hexosamine in layers fed higher levels of Mn. Dietary Mn may also be associated with the duration of

time that eggs spend in the shell gland (Longstaff and Hill, 1972). Manganese role in eggshell development may be important for producers, as egg producers strive for exterior egg quality to maintain interior quality to consumers.

The egg yolk and shell both contain Mn. Hopcroft et al. (2018) evaluated the changes in embryonic mineral content overtime of development at d 0, 6.5, 13.5 and 17.5. Hopcroft et al. (2018) did not report any differences in embryonic Mn concentration for the duration of the incubation period, and implies further research is needed to better understand mechanisms and mode of action of Mn transportation and partitioning in the embryo during incubation.

Trace Mineral Combination in Poultry Production

In laying hens, trace minerals have shown to be essential in regulating processes responsible for growth and development of bone and eggshells (Richards et al., 2010). Due to their role in enzymatic reactions and/or interact with calcium crystal deposition and formation, trace minerals have shown to have an impact on shell quality (Fernandes et al., 2008). In commercial situations, the use of minerals is commonly through a trace mineral premix added during feed manufacturing. While research evaluating single minerals alone is important, understanding the effect of multiple minerals in poultry is more applicable to commercial industry. Mabe et al. (2003) evaluated the inclusion of inorganic or organic Mn, Zn, and Cu at 30, 30, 5 and 60, 60, 10 ppm respectively compared to the absence of minerals in various aged layers. Mabe et al. (2003) observed an increase in Mn yolk composition with the supplementation of the high levels of inorganic and organic minerals compared to the layers fed the diet absent in

supplemental minerals. When evaluating Zn yolk composition, the organic minerals, regardless of level, and the high level of inorganics increased composition compared to the layers absent of mineral supplementation. Overall in layers 69 to 82 weeks, the supplementation of minerals regardless of source and level reduced egg weight. Similarly, layers 60 to 73 weeks old absent of mineral supplementation produced the heaviest egg weights compared to those supplemented with trace minerals. Furthermore, layers 69 to 82 weeks of age fed the high level of organic minerals increased egg breaking strength compared to the low level of organic minerals. Likewise, layers 60 to 73 weeks old that were fed high levels of minerals, regardless of source, had increased egg breaking strength compared to layers absent of mineral supplementation. (Mabe et al., 2003).

In broilers, Bao et al. (2007) evaluated the impact of using organic trace minerals at 3 different levels compared to a mineral deficient diet and a diet compromised of inorganic trace minerals. Bao et al. (2007) observed reductions in FI, thus reducing BW in broilers fed the diets deficient in trace minerals. Yang et al. (2011) observed the effects of including Cu, Fe, Zn, and Mn at four different levels to create a 4 x 4 factorial arrangement. Yang et al. (2011) reported a negative impact on cumulative FCR in broilers fed Mn at or above 80 ppm. Yang et al. (2011) also mentions the elevated levels of trace mineral composition of common feed stuffs due to elevated excretion amounts and infiltration into ground water. The increase in crop mineral composition may allow for the reduction in dietary mineral supplementation as mentioned by Yang et al. (2011). Nollet et al. (2007) evaluated the effects of replacing inorganic Zn, Mn, Fe, and Cu at

37, 70, 45, and 12 ppm respectively with organic forms at 10 ppm for Zn, Mn, and Fe and 2.5 ppm Cu. Nollet et al. (2007) observed no differences in performance parameters at the end of the study (39 d). However, Nollet et al. (2007) did note a reduction in mineral excretion for Zn, Mn, Fe, and Cu of 63, 46, 73, and 55% respectively in broilers fed organics compared to inorganics. These data support the claim that reductions in dietary mineral concentration is possible without negatively impacting performance and reducing excretion when using organic minerals.

Mondal et al. (2010) evaluated the effects of inorganic Cu (15 ppm), Fe (90 ppm), Mn (90 ppm), Zn (80 ppm), I (2 ppm), and Se (0.3 ppm), organic Cu (2.5 ppm), Fe (15 ppm), Mn (15 ppm), Zn (13.33 ppm), and Cr (0.23 ppm), and the absence of minerals in 39 d old broilers. Trace mineral inclusion had no impact on FI, carcass weight, frame weight, or carcass yield, however organic trace minerals improved BW and FCR (39 d) compared to the control fed broilers. Inorganic minerals seemed to maximize breast and leg weight when evaluating carcass traits, however organic minerals produce intermediate results in regards to leg weight. Interestingly, organic trace minerals improved or maintained live performance and most processing characteristics when feed at a fraction of the inclusion compared to inorganic mineral sources. Similarly, M'Sadeq et al. (2018) conducted a study comparing inorganic, organic, yeast organic, and hydroxychloride Cu, Fe, I, Se, Mn, Zn, and Cr in broilers 38 d of age. Each trace mineral premix contained varying amounts of minerals with the inorganic containing the highest concentration for all minerals (absent of Cr), the yeast organic premix contained identical calculated mineral concentrations to the

hydroxychloride minerals, and the organic premix contained the lowest levels compared to all other premixes. Broilers consuming organic and hydroxychloride minerals produced the heaviest d 39 BW compared to the inorganic broilers, and both organic and hydroxychloride fed broilers improved FCR compared to the inorganic fed broilers. Tibia Mn concentration was reduced with the inclusion of either organic or hydroxychloride minerals compared to inorganic fed broilers. Collins and Moran (1999) evaluated the effects of feeding $MnSO_4$ (0 or 180 ppm) and/or $ZnSO_4$ (0 or 150 ppm) in 2 commercial broiler strains for a 49 d study. Ultimately, Collins and Moran (1999) observed no differences in live growth performance or processing yields amongst dietary treatments.

When feeding trace minerals, understanding the effects of dietary ingredients and nutrients can impact dietary efficacy, as some may alter the availability and absorption of others. Many plant derived ingredients contain phosphorus in the form of phytate, which is indigestible to monogastrics due to the absence of endogenous phytase. Phytate (myo-inositol hexaphosphate) molecules have a high binding affinity for cations (Zn, Fe, Ca, Cu, & Mn) forming insoluble salts (Harland and Narula, 1999), further impeding digestion of both P and other trace minerals. Sebastian et al. (1996) conducted a study to evaluate the supplementation of phytase on broiler performance and trace mineral (Ca, P, Cu, and Zn) utilization. Sebastian et al. (1996) reported an increase in male and female broiler BW separately, with the inclusion of phytase in a low P diet compared to a low P diet absent of phytase inclusion in 21 d old broilers. Furthermore, Sebastian also reported phytase supplementation increased P retention 12.4% in males, Ca retention by

12.2% in males, Cu retention 19.3% in males, and Zn retention 62.3 and 44.3% in males and females respectively. To conclude, it is important to reevaluate mineral requirements, especially Zn, when using a phytase in feed formulation (Sebastian et al., 1996; Santos et al., 2015).

The strategic use of trace minerals and feed additives are frequently altered and evaluated in poultry industries to maximize production and efficacy. The emerging production of alternative forms of trace minerals offers nutritionists new strategies and opportunities to improve dietary formulations Therefore, the series of experiments described in this document were undertaken with the goal of evaluating: (1) the effect of zinc hydroxychloride (ZHC) on broiler growth performance and processing yield, (2) the effect of MnHCl level on broiler growth performance and tibia strength, and (3) evaluate the effect of increasing levels of MnHCl in 45-wk-old White Leghorn layers on shell and yolk Mn deposition.

CHAPTER II

EVALUATION OF A ZINC HYDROXYCHLORIDE ON BROILER GROWTH PERFORMANCE AND PROCESSING YIELD

Introduction

The need to improve mineral bioavailability in broilers is increasing as the industry strives to further improve performance and nutrient digestibility, and reduce mineral waste. Trace minerals that are of principal focus in poultry diet formulation include, but are not limited to, Zn, Cu, and Mn. Trace minerals in poultry ensure necessary biological functions are functioning at near optimum levels (Nollet et al., 2007). Trace minerals are crucial for life as they serve as catalysts and cofactors to numerous enzymes and hormones, which effect animal growth (Arias and Koutsos, 2006), improve bone development and mineralization (Banks et al., 2004), and improve tissue growth, feathering, enzyme structure, and appetite (Nollet et al., 2007). Not only do trace minerals support life, they also affect the quality of life via improved growth and immunity. Important for biological functions, trace minerals can also affect many areas of performance in broilers and layers that include increased BW, meat production, and egg yield.

Zinc is considered to be an essential trace mineral for broiler and layer chickens as it serves as a cofactor or constituent for over 200 enzymes and enzyme processes responsible for numerous functions ranging from immunity to growth (Ashmead and Zunino, 1993; Park et al., 2004; Salim et al., 2008; Star et al., 2012). Zinc is the only

metal to play a significant role in all 6 metalloenzyme classes (transferase, lyase, ligase, hydrolase, isomerase, and oxidoreductase) (Vallee and Auld, 1990; Kidd et al., 1996). The importance that Zn plays within these classes of enzymes is the reason of its significance in metabolism, immunity, regulating homeostasis, and DNA and RNA synthesis (Salim et al., 2008; Suttle, 2010; Star et al., 2012). Furthermore, Zn plays an important role in protein synthesis (Suttle, 2010), carbohydrate, and energy metabolism (Kennedy et al., 1998); these responsibilities of Zn result in its ability to influence growth and muscle accretion. Zinc also plays an important role in immunity as it is a cofactor for thymulin, directly impacting T-lymphocyte production, which serves as a mechanism utilized to fight off threats to the body that include infection and cancerous cells (Fraker et al., 1986; Dardenne and Bach, 1993; Kidd et al., 1996). In previous studies, increasing levels of Zn in broiler diets have shown to improve appetite (Ao et al., 2006b), growth, and bone development (Salim et al., 2008). Given the increasing growth rate and yield of commercial broilers, Zn requirement should be well considered.

Similar to Zn, Cu is also an essential trace mineral that serves as a cofactor for multiple enzymes. Copper is important for lysyl oxidase, which is responsible for collagen crosslinking (Rucker et al., 1998); leading to improvements in skin, bone, intestine, and tendon strength (Richards et al., 2010). Of all trace minerals, Cu has the broadest range of functions and attributes related to not only growth and enzyme activation, but to health. Copper contains many antibacterial and bacteriostatic properties (Arias and Koutsos, 2006; Karimi and Zhandi, 2015), which have resulted in Cu being

used in excess of requirements as an alternative to antibiotics and alternative growth promoters (Pesti and Bakalli, 1996).

Several sources exist for both Cu and Zn and extend across 3 mineral sources that include inorganic, organic, and hydroxychloride (hydroxy). Hydroxy minerals have garnered attention over recent years due to the potential as an alternative to inorganic and organics minerals. Most inorganic minerals are provided in the form of sulfates, oxides, or the combination of both. It is important to understand the differences in bioavailability between sources. Inorganic minerals are comprised of cations that can create irreversible bonds with phytate groups as well as other anti-nutritive elements (Brooks et al., 2013). Hydroxychloride minerals are classified as inorganics, however, contain a chloride bond that resembles that of an organic structure. The chloride bond stabilizes the mineral and limits some of the undesirable interactions with anti-nutritive elements found in feed ingredients.

The increase in antibiotic free (ABF) poultry production in recent years is well known (Cervantes, 2015). However, it offers a challenge to the industry that used antibiotics and ionophores for decades to prevent and treat flock health issues. As previously mentioned, both Zn and Cu have therapeutic properties ranging from improvements in immune health to bactericidal effects, and may be used as a possible aid in ABF production (Rao, 2017).

Previous research iterating the importance of trace minerals and of possible improvements in bioavailability led to the following two trials which evaluated the effect

of increasing levels ZHC alone and in unison with a tribasic Cu chloride source on growth performance and breast meat yield.

Materials and Methods

Trial 1

Experimental Design

Trial 1 consisted of 5 experimental treatments containing increasing levels of Zn in the form on ZHC at 40, 80, 120, and 160 ppm and a control containing Zn in the form of $ZnSO_4$ at 80 ppm. There were 12 replicates per treatment, each containing 36 male broilers for a total of 2,160 chicks. Chicks were placed in floor pens for a 48 d trial. Prior to placement, birds were vaccinated with a live oocyst vaccine[1] at d-of-hatch.

Experimental Diets

Diets were corn and SBM-based and contained phytase[2] throughout all phases (Table 1). The diets included distillers' dried grains with solubles (DDGS) at 3.0, 4.0, 5.0, and 9.0% during the starter, grower, finisher, and withdrawal (WD) phases, respectively. Meat and bone meal (MBM) was included at 5.5% in the starter, 5.0% in the grower, 3.0% in finisher, and 2.0% in the WD phase. Diets were manufactured from one large basal batch and Zn was then added to replace corn starch to achieve target Zn level and source. All diets were fed as a pellet with the exception of the starter diet, which was pelleted and then crumbled. The starter diet was fed from d 1 to 14, grower from d 15 to d 28, finisher from d 29 to d 41, and WD from d 42 to d 48.

---

[1] Advent® – Huvepharma® – Peachtree City, Georgia
[2] Quantum Blue® – AB Vista – Marlborough, Wiltshire

Animals and Management Practice

In Trial 1, 2,160 Ross 708 male broilers with an average initial BW of 34.4 g were randomly allotted to floor-pens and dietary treatments based on weight at d of hatch. The study consisted of a total of 60 pens, each containing 36 chicks at d of age placed in 3.34 m^2 floor pens with nipple drinkers and a tube feeder. Feed and water were available *ad libitum.* Chicks were provided age appropriate supplemental heat and were exposed to an industry-type lighting program. The lighting program consisted of: d 1 to d 8, 24 h of light at 2 foot candles; d 9 to d 18, 16 h of light at 0.75 foot candles; d 19 to d 32, 18 h of light at 0.1 foot candle; and d 33 to termination, 20 h of light at 0.05 foot candle. Broilers were reared on pine shavings that had been used by 3 previous flocks of birds.

Broilers and feed were weighed on d 14, 28, 41, and 48 to determine average BW, FCR, and FI. Mortality was recorded daily. On d 50, 6 broilers were randomly selected following an 8 h feed WD, and processed for determination of carcass weights, yield, and severity of woody breast (WB) and white striping (WS) in breast tissue indicated by score 0 to 3 for WB and 0 to 2 for WS, with 0 indicating no incidence. All animal husbandry procedures were conducted in accordance with an approved Texas A&M University animal use protocol (IACUC).

Trial 2

Experimental Design

Trial 2 consisted of 5 experimental treatments. The positive control (PC) contained salinomycin[3], 125 ppm Cu sulfate, and 90 ppm Zn from an inorganic source. The negative control (NC) was the same as the PC group, but the NC diet contained no salinomycin. Copper supplementation was removed from the control diets after 28 d of age. These 2 treatments were included as an attempt to mimic current industry methods with industry use of Cu that is removed prior to the third or fourth feeding phase. The 3 experimental treatments each contained Cu at 125 ppm in the form of Cu hydroxychloride, and were supplemented with increasing levels of Zn in the form of ZHC at 100, 125, and 150 ppm, respectively. There were 10 replicates per treatment, each containing 35 feather-sexed broilers (18 females and 17 males), resulting in a total of 1,750 Ross 708 chicks placed in floor pens for a 49 d trial.

Experimental Diets

Diets were corn and SBM-based and contained phytase[4] throughout all phases (Table 2). Diets included DDGS at 5.0% throughout the trial and MBM at 5.0% through d 28 and 2.5% from d 28 to d 49. The control diets were formulated to represent diets currently used in commercial production. The PC diet was supplemented with 125 ppm Cu from Cu sulfate and 90 ppm Zn from Zn oxide (60 ppm) and $ZnSO_4$ (30 ppm). The

---

[3] Sacox®60 – Huvepharma® – Peachtree City, Georgia
[4] Axtra®Phy – DuPont® – Marlborough, Wiltshire

NC diet was similar to the PC diet, however was absent of salinomycin[3]. The 3 experimental treatments each contained Cu at 125 ppm in the form of Cu hydroxychloride, and were supplemented with increasing levels of Zn in the form of ZHC at 100, 125, and 150 ppm, respectively. All diets were pelleted with the exception of the starter, which was pelleted and then crumbled. The starter diet was fed from d 1 to 14, grower from d 15 to 28, finisher from d 29 to 42, and WD from d 43 to 49.

Animals and Management Practice

One thousand seven hundred fifty straight-run Ross 708 broilers with an average initial BW of 41 g were randomly allotted to floor-pens based on initial BW on d of hatch. The study consisted of 50 total pens, each containing 35 chicks at d of age placed 3.34 m^2 floor pens with nipple drinkers and a tube feeder. Feed and water were available *ad libitum.* Chicks were provided age appropriate supplemental heat and were exposed to an industry type lighting program. The lighting program consisted of: d 1 to 8, 24 h of light at 2 foot candles; d 9 to 18, 16 h of light at 0.75 foot candles; d 19 to 32, 18 h of light at 0.1 foot candle; and d 33 to termination, 20 h of light at 0.05 foot candle. Broilers were reared on pine shavings that had been used previously by 3 flocks of birds.

Birds and feed were weighed on d 14, 28, 42 and 49 to determine average BW; FCR, and FI. Mortality was observed and reported daily. On d 50, following an 8-hour feed withdrawal, 8 birds (4 males/4 females) were randomly selected from each replicate pen to determine carcass, breast, and tender yield. All animal husbandry procedures were conducted in accordance with an approved Texas A&M University animal use protocol (IACUC).

Statistical Analysis

All data were subject to a one-way Analysis of Variance (ANOVA) using the GLM model (SPSS software). Means were deemed significantly different at $p \leq 0.05$ and were further separated by Duncan's multiple range test. In Trial 1, linear and quadratic regression analysis was conducted on ZHC treatments only to determine the correlation between increasing levels of hydroxychloride Zn on FI, BW, FCR, processing weights and yields.

Results

Trial 1

Diets were analyzed for Zn concentration (**Table 1**). Analyzed Zn concentrations were separated by a 30 to 40 ppm difference, which correlates with the 40 ppm interval that was used when supplementing ZHC. The 2 treatments containing 80 ppm Zn (treatments 2 & 5) had similar Zn concentrations. Zinc concentrations were about 40 ppm higher than supplemented values which can be contributed to Zn contained in the feed ingredients.

Table 1. Ingredient profile and nutrient concentration of diets fed to male broilers in a study evaluating varying levels of Zn[1] (Trial 1).

Ingredient	Starter	Grower	Finisher	WD
Corn	61.74	65.96	68.29	68.65
SBM (48%)	27.55	22.91	20.51	16.41
Pork MBM	5.50	5.00	5.00	2.00
DDGS	3.00	4.00	3.00	9.00
Soy Oil	0.50	0.52	1.18	1.89
Limestone	0.45	0.47	0.75	0.91
Sodium Chloride	0.41	0.38	0.38	0.34
DL-Methionine (98%)	0.27	0.24	0.21	0.17
Lysine HCL	0.16	0.18	0.18	0.19
Biofos 16/21P	0.12	0.05	0.25	0.22
L-Threonine	0.08	0.07	0.06	0.05
Choline Chloride	0.07	0.06	0.05	0.04
Zn Free Trace Mineral[2]	0.05	0.05	0.05	0.05
Vitamin Premix[3]	0.05	0.04	0.35	0.25
Cu[4]	0.02	0.02	0.02	0.02
Phytase[5]	0.01	0.01	0.01	0.01
Xylanase[6]	0.01	0.01	0.01	0.01

[1] Target Zn inclusions for each treatment were 40 ppm ZHC, 80 ppm ZHC, 120 ppm ZHC, 160 ppm ZHC, and 80 ppm $ZnSO_4$. Analyzed dietary Zn levels in the starter were 109, 123, 161, 203, and 129 ppm respectively. Grower levels were 102, 124, 168, 191, and 130 ppm respectively. Finisher levels were 82, 124, 155, 197, and 106 ppm respectively. Withdrawal levels were 76, 117, 153, 176, and 107 ppm respectively.

[2] Zinc Free Mineral Premix added at this rate (1.0 lbs per ton) yields 100 ppm Mn, 20 ppm iron, 3 ppm Cu, 0.75 ppm iodine, and 0.3 ppm selenium.

[3] Vitamin premix added at this rate (0.5 lbs per ton) yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[4] IntelliBond® C – Micronutrients – Indianapolis, IN

[5] Quantum Blue® - AB Vista – Marlborough, Wiltshire

[6] Econase® - AB Vista – Marlborough, Wiltshire

Table 1. Continued

Ingredient	Starter	Grower	Finisher	WD
Calculated Nutrient Content (%)				
Protein	22.38	20.53	18.74	17.38
Calcium	1.05	0.97	0.90	0.84
Available Phosphorus	0.50	0.46	0.42	0.38
AME Poultry (kcal/lb)	1380	1400	1425	1450
Dig Methionine	0.56	0.51	0.46	0.40
Dig Lysine	1.10	1.00	0.90	0.80
TSAA	0.84	0.86	0.78	0.63
Tryptophan	0.24	0.21	0.19	0.17
Threonine	0.86	0.78	0.71	0.64
Arginine	1.37	0.98	1.09	0.97
Sodium	0.23	0.22	0.21	0.20
Analyzed Nutrient Content (%)				
Protein	17.9	17.8	15.7	14.4
Crude Fat	4.22	4.04	4.28	5.64
Crude Fiber	3.50	3.10	2.90	3.00
Sodium	0.16	0.19	0.15	0.17
Mn (ppm)	142	133	114	147
Cu (ppm)	100	123	141	96

During the starter and WD phases, neither inclusion level nor source of Zn affected FI (g/bird/d) **(Table 2)**. Diets containing 120 ppm ZHC increased FI ($p < 0.05$) compared to diets with less ZHC (40 and 80 ppm) and the diet containing $ZnSO_4$ during the grower phase and through d 28 and d 41. In the finisher phase, the addition of ZHC at 120 and 160 ppm and 80 ZnSO4 increased ($p < 0.05$) FI compared to diets containing 40 ZHC. The inclusion of ZHC at 120 ppm increased ($p<0.05$) in broiler FI compared to all other dietary treatments. During the grower and finisher phase and cumulatively through d 28 and 41, a linear increase in FI was observed with an increase in ZHC **(Table 2)**.

Table 2. Feed intake (g/b/d) for male broilers fed varying levels of Zn by phase and cumulatively in Trial 1.

Treatment	Starter	Grower	d 28	Finisher	d 41	WD	Total
40 ZHC[1]	33.1	116.0^{b}	74.1^{b}	188.1^{b}	110.0^{c}	220.1	125.6^{b}
80 ZHC	33.2	117.1^{b}	74.8^{b}	190.2^{ab}	111.1^{bc}	215.1	126.0^{b}
120 ZHC	33.7	120.1^{a}	76.5^{a}	193.4^{a}	113.4^{a}	224.1	129.2^{a}
160 ZHC	33.4	118.3^{ab}	75.3^{ab}	193.6^{a}	112.1^{ab}	216.3	126.7^{b}
80 ZnSO4	32.7	116.5^{b}	74.1^{b}	192.7^{a}	111.4^{bc}	220.7	126.9^{b}
ANOVA							
Pooled SEM	0.137	0.432	0.269	0.963	0.439	1.539	0.542
P-value	0.124	0.004	0.007	0.005	0.007	0.120	0.019
Regression							
Linear	0.291	0.047	0.017	0.038	0.047	0.876	0.235
Quadratic	0.493	0.038	0.018	0.108	0.061	0.915	0.233

[a-c] Means within a column with different superscripts differ at $p < 0.05$

[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN

Body weight was influenced with the increasing levels and source of Zn in the diet. Body weight was more sensitive to Zn inclusion and source early in growth phases as consumption impacted the differences in BW. The inclusion of ZHC at 80, 120, and 160 increased d 14 BW compared to $ZnSO_4$ at 80 ppm and the ZHC at 40 ppm **(Table 3)**. On d 28, the 120 and 160 ppm ZHC diets increased ($p < 0.05$) BW compared to broilers fed 40 ppm inclusion of ZHC and the 80 ppm inclusion of $ZnSO_4$, while the inclusion of ZHC at 80 ppm produced intermediate results. Similarly, on d 41, the inclusion of 120 and 160 ppm ZHC increased BW compared to the broilers fed the diet containing 40 ppm of ZHC, while the 80 ppm ZHC and the 80 ppm inclusion of $ZnSO_4$ produced intermediate results. Linear improvements in BW were observed with increasing levels of ZHC at 14, 28, and 41 d of age **(Table 3)**. On d 14 and d 28 a significant quadratic correlation was observed as well for BW.

Table 3. Average BW (kg) for male broilers fed varying levels of Zn at the termination of each growth phase in Trial 1.

Treatment	d 14	d 28	d 41	d 48
40 ZHC[1]	0.399[b]	1.482[c]	2.899[b]	3.611
80 ZHC	0.411[a]	1.524[ab]	2.944[ab]	3.626
120 ZHC	0.416[a]	1.531[a]	2.963[a]	3.676
160 ZHC	0.411[a]	1.529[a]	2.966[a]	3.653
80 $ZnSO_4$	0.399[b]	1.497[bc]	2.920[ab]	3.630
ANOVA				
Pooled SEM	0.002	0.005	0.011	0.018
P-value	<0.001	0.002	0.012	0.409
Regression				
Linear	0.004	0.004	0.043	0.313
Quadratic	<0.001	0.002	0.090	0.535

[a-c] Means within a column with different superscripts differ at $p < 0.05$
[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN

Similar to BW, FCR was sensitive to Zn level and source and was also more sensitive in younger birds as differences in FCR were lost in the WD phase and cumulatively. However, unlike BW, FCR did not improve beyond the 80 ppm ZHC inclusion rate for any evaluated period. During the starter phase, the inclusion of ZHC at 80, 120, and 160 ppm improved ($p < 0.05$) FCR compared to the lower inclusion of ZHC (40 ppm), while the diet containing 80 ppm $ZnSO_4$ produced intermediate results **(Table 4)**. In the grower phase, the addition of 80 ppm ZHC improved ($p < 0.05$) FCR compared to the dietary inclusion of ZHC at 40 and 120 ppm. Through d 28 the dietary inclusion of ZHC at 80 ppm improved ($p < 0.05$) FCR compared to all other diets containing ZHC, while 80 ppm $ZnSO_4$ produced intermediate results. The inclusion of ZHC at 40 ppm improved ($p < 0.05$) FCR compared to broilers fed ZHC at 120 ppm and $ZnSO_4$, while the 80 and 160 ppm ZHC produced intermediate results during the finisher phase. No differences in FCR were observed between dietary treatments containing different levels or source of Zn during the WD phase or cumulatively for the study. Through d 41 the addition of ZHC at 80 ppm improved ($p < 0.05$) FCR compared to all other dietary treatments. Linear improvements in FCR were less apparent than those observed in BW, however increases in ZHC reduced FCR linearly during the finisher phase **(Table 4)**.

Table 4. Mortality corrected feed conversion ratio (FCR) for male broilers fed varying levels of Zn in Trial 1.

Treatment	Starter	Grower	d 1-28	Finisher	WD	d 1-41	d 1-48
40 ZHC[1]	1.291[a]	1.502[ab]	1.448[a]	1.734[c]	2.200	1.588[b]	1.706
80 ZHC	1.247[b]	1.478[c]	1.419[b]	1.739[bc]	2.247	1.575[c]	1.698
120 ZHC	1.254[b]	1.511[a]	1.445[a]	1.772[a]	2.242	1.603[a]	1.724
160 ZHC	1.265[b]	1.493[abc]	1.435[a]	1.759[abc]	2.257	1.592[ab]	1.713
80 ZnSO4	1.273[ab]	1.487[bc]	1.433[ab]	1.768[ab]	2.197	1.596[ab]	1.712
ANOVA							
Pooled SEM	0.004	0.003	0.003	0.005	0.017	0.002	0.003
P-value	0.006	0.006	0.002	0.032	0.642	0.001	0.061
Regression							
Linear	0.122	0.856	0.634	0.030	0.353	0.100	0.107
Quadratic	0.006	0.914	0.302	0.070	0.601	0.257	0.272

[a-c] Means within a column with different superscripts differ at $p < 0.05$
[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN

Similar to cumulative average bird weight, the inclusion of ZHC at 120 and 160 ppm increased ($p<0.05$) live weight of the subsample of birds processed and carcass weight compared to the broilers consuming diets containing ZHC at 40 ppm, with 80 ppm ZHC and 80 $ZnSO_4$ yielding intermediate carcass weights **(Table 5)**. Broilers fed diets that included ZHC at 80, 120, and 160 ppm increased ($p<0.05$) tender weight compared to broilers fed diets with 40 ppm of ZHC. The inclusion of ZHC at 120 ppm increased ($p<0.05$) breast weight compared to the 40 ppm inclusion of ZHC and the 80 ppm $ZnSO_4$, while the 80 ppm ZHC had similar breast weight compared to the 120 ppm ZHC. Elevated carcass and breast weights with increasing supplementation of ZHC led to linear correlations for these parameters associated with increases in ZHC. The $ZnSO_4$ diet decreased carcass yield compared to all other dietary treatments containing ZHC. The dietary inclusion of ZHC at 80 and 120 ppm increased ($p<0.05$) breast meat yield

compared to diets with 40 ppm ZHC and 80 ppm $ZnSO_4$. The increases in breast meat yield associated with the ZHC at 80 and 120 ppm led to a significant quadratic correlation **(Table 5)**. No significant differences in severity of WB, WS, or tender yield were observed among all dietary treatments even with the increases in breast weight and yield at ZHC levels of 80 and 120 ppm **(Table 5)**.

Table 5. Processing weights and yield of 49 d old male broilers fed varying levels of Zn in Trial 1.

Treatment	Live Wt.	WOG	WOG Yield	Tender wt.	Breast wt.	WB	WS	Tender Yield	Breast Yield
	g	g	%	g	g	score	score	%	%
40 ZHC[1]	3603.3[b]	2812.8[b]	78.06[a]	142.0[b]	690.1[c]	1.23	1.17	5.05	24.51[b]
80 ZHC	3670.5[ab]	2866.0[ab]	78.10[a]	148.1[a]	723.2[ab]	1.48	1.24	5.17	25.22[a]
120 ZHC	3733.3[a]	2919.7[a]	78.21[a]	148.4[a]	738.4[a]	1.31	1.25	5.09	25.28[a]
160 ZHC	3724.8[a]	2908.9[a]	78.12[a]	148.0[a]	723.1[ab]	1.33	1.25	5.09	24.85[ab]
80 ZnSO4	3669.4[ab]	2844.3[ab]	77.53[b]	144.2[ab]	704.1[bc]	1.24	1.20	5.07	24.68[b]
ANOVA									
Pooled SEM	20.6	15.0	0.10	0.9	5.2	0.05	0.02	0.02	0.09
P-value	0.058	0.03	0.057	0.007	0.004	0.376	0.292	0.342	0.011
Regression									
Linear	0.032	0.019	0.755	0.038	0.021	0.774	0.164	0.864	0.285
Quadratic	0.071	0.039	0.906	0.028	0.005	0.584	0.242	0.418	0.008

[a-c] Means within a column with different superscripts differ at $p < 0.05$

[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN

Trial 2

Mineral analysis for feed included Cu and Zn concentration **(Table 6)**. Zinc values were within the acceptable range as treatments 3, 4 and 5 contained increasing level of Zn with a separation of approximately 20 ppm between each. Copper concentration in the starter and grower phase remained relatively constant between the 5 dietary treatments. In the finisher and WD phase Cu concentration was reduced to approximately 7 ppm in the control diets, caused by the removal of supplemental $CuSO_4$ in these diets. The Cu level in treatments 3, 4 and 5 remained constant at approximately 120 ppm, which correlates with the supplementation of 125 ppm Cu hydroxychloride to these treatments.

Table 6. Ingredient profile and nutrient content of diets fed to straight-run broilers in a study evaluating varying levels and sources of Zn[1] with and without the use of an ionophore (Trial 2).

Ingredient	Starter	Grower	Finisher	WD
Corn	53.31	64.36	69.69	72.25
SBM (48%)	32.8	21.61	18.5	16.2
DL-Methionine (98%)	0.27	0.26	0.21	0.18
Lysine HCL	0.025	0.218	0.23	0.22
L – Threonine	--	0.075	0.085	0.075
FAT, A/V Blend	2.4	2.25	2.55	2.3
Limestone	0.59	0.61	0.67	0.68
Biofos 16/21P	0.005	--	0.010	0.03
Sodium Chloride	0.35	0.36	0.40	0.40
Trace Mineral Premix[2]	0.05	0.05	0.05	0.05
Vitamin Premix[3]	0.025	0.02	0.012	0.012
DDGS	5.0	5.0	4.99	5.0
Pork MBM	5.0	5.0	2.5	2.5
Phytase[4]	0.008	0.008	0.007	0.007
Choline Cl-60	0.075	0.088	0.04	0.052
Cu Sulfate	0.05	0.05	--	--
salinomycin[5]	0.05	0.05	0.05	0.05

[1]Target Zn inclusions for each treatment were 90, 90, 100, 125, and 150 ppm Zn. Analyzed dietary Zn levels in the starter were 112, 113, 136, 178, and 153 ppm respectively. Grower levels were 120, 129, 140, 160, and 201 ppm respectively. Finisher levels were 131, 113, 138, 147, and 160 ppm respectively. WD levels were 115, 116, 129, 162, and 170 ppm respectively.
[2]Zinc Free Mineral Premix added at this rate (1.0 lbs per ton) yields 100 ppm Mn, 20 ppm iron, 3 ppm Cu, 0.75 ppm iodine, and 0.3 ppm selenium.
[3]Vitamin premix added at this rate (0.5 lbs per ton) yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet
[4]Axtra®Phy – DuPont® – Marlborough, Wiltshire
[5]Sacox® 60 - Huvepharma® - Peachtree City, Ga

Table 6. Continued

Ingredient	Starter	Grower	Finisher	WD
Calculated Nutrient Content (%)				
Protein	24.16	19.90	17.45	16.52
Calcium	0.88	0.85	0.66	0.66
Available Phosphorus	0.44	0.42	0.33	0.33
AME Poultry (kcal/lb)	1375	1420	1450	1455
Dig Methionine	0.60	0.54	0.47	0.43
Dig Lysine	1.18	1.04	0.92	0.86
TSAA	1.02	0.90	0.80	0.75
Tryptophan	0.27	0.21	0.18	0.17
Threonine	0.88	0.78	0.71	0.66
Arginine	1.58	1.23	1.05	0.98
Sodium	0.18	0.18	0.18	0.18
Analyzed Nutrient Content (%)				
Protein	24.56	19.63	17.4	16.3
Crude Fat	5.95	6.12	5.66	5.14
Crude Fiber	3.10	2.70	3.70	2.6
Calcium	0.97	0.92	0.58	0.61
Phosphorus	0.68	0.62	0.48	0.46
Sodium	0.17	0.17	0.16	0.17
Ash %	5.89	5.13	3.41	3.63

No differences in BW occurred on d 14, d 28, or d 49 **(Table 7)**. However, on d 42 the inclusion of salinomycin decreased ($p < 0.05$) BW compared to the control diet without salinomycin and all other dietary treatments containing increasing levels of ZHC and 125 ppm CuHCl **(Table 7)**. No differences in overall mortality (%) were observed among dietary treatments **(Table 7)**.

Table 7. Average BW (kg.) and cumulative mortality (%) of straight-run broilers fed varying levels and sources of Zn in Trial 2.

	BW				Mortality
Treatment	**d 14**	**d 28**	**d 42**	**d 49**	**d 1-49**
Control + salinomycin	0.393	1.313	2.519[b]	3.090	3.985
As 1 – salinomycin	0.402	1.383	2.646[a]	3.153	4.607
125ppm CuHCl[2] + 100ppm ZHC[1] 1-49 d	0.401	1.367	2.657[a]	3.280	4.999
125ppm CuHCl + 125ppm ZHC 1-49 d	0.399	1.371	2.683[a]	3.243	2.224
125ppm CuHCl + 150ppm ZHC 1-49 d	0.404	1.392	2.728[a]	3.234	5.404
ANOVA					
One-way P-value	0.154	0.449	0.003	0.409	0.185
Pooled SEM	0.002	0.015	0.027	0.039	0.457

[a,b] Means in columns with different superscripts differ significantly at $p \leq 0.05$
[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN
[2] CuHCl – Intellibond Cu, Micronutrients USA LLC, Indianapolis, IN

None of the dietary treatments had a significant impact on mortality corrected FCR during the starter, grower, finisher phase, d 1 to 28, and d 1 to 42 **(Table 8)**. During the WD phase, the inclusion of salinomycin improved ($p < 0.05$) FCR compared to the control diet without salinomycin and the diet containing the highest level of ZHC at 150 ppm. Birds fed lower levels of ZHC (100 and 125 ppm) produced similar FCR to that of the Control diet containing salinomycin during the WD phase. Cumulatively from d 1 to 49, the inclusion of salinomycin improved ($p < 0.05$) FCR compared to the diet

containing the highest level of ZHC at 150 ppm. The control without salinomycin and the inclusion of ZHC at 100 and 125 ppm produced similar results to that of the control with salinomycin. The differences in FCR may be a result of higher BW (180 g) observed in treatments containing increasing levels of ZHC, naturally increasing these birds maintenance requirement.

Table 8. Mortality corrected feed conversion ratio (FCR) for straight-run broilers fed varying levels and sources of Zn in Trial 2.

Treatment	Starter	Grower	Finisher	WD	d 1-28	d 1-42	d 1-49
Control + salinomycin	1.240	1.636	1.828	2.202[b]	1.513	1.644	1.737[b]
As 1 – salinomycin	1.248	1.542	1.852	2.541[a]	1.461	1.641	1.776[ab]
125ppm CuHCl[2] + 100ppm ZHC[1] 1-49 d	1.233	1.568	1.834	2.443[ab]	1.474	1.645	1.752[ab]
125ppm CuHCl + 125ppm ZHC 1-49 d	1.223	1.546	1.848	2.477[ab]	1.458	1.647	1.777[ab]
125ppm CuHCl + 150ppm ZHC 1-49 d	1.236	1.528	1.860	2.656[a]	1.449	1.647	1.793[a]
ANOVA							
One-way P-value	0.603	0.696	0.979	0.026	0.761	0.995	0.049
Pooled SEM	0.005	0.026	0.020	0.049	0.017	0.005	0.007

[a,b] Means in columns with different superscripts differ significantly at $p \leq 0.05$
[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN
[2] CuHCl – Intellibond Cu, Micronutrients USA LLC, Indianapolis, IN

During the starter and WD phase, no differences in FI were observed among dietary treatments **(Table 9)**. Though during the grower phase and through d 28 and 49, the control diet absent of salinomycin and all diets containing ZHC exhibited increased broiler FI compared to the control plus salinomycin **(Table 9)**. Through d 42 and during the finisher phase the control diet without salinomycin and the dietary treatments containing ZHC increased ($p < 0.05$) FI compared to the control with salinomycin, with the diet containing 150 ppm ZHC consuming the greatest amount of feed and

significantly ($p < 0.05$) increasing consumption compared to both control diets and the diet containing 100 ppm ZHC.

Table 9. Feed consumption (g/bird/d) for straight-run broilers fed varying levels and sources of Zn in Trial 2.

Treatment	Starter	Grower	Finisher	WD	d 1-28	d 1-42	d 1-49
Control + salinomycin	31.4	104.0^{b}	153.7^{c}	175.6	67.6^{b}	96.0^{c}	106.7^{b}
As 1 – salinomycin	32.7	107.3^{a}	164.9^{b}	176.6	69.3^{a}	100.8^{b}	111.3^{a}
125ppm CuHCl[1] + 100ppm ZHC[2] 1-49d	31.8	106.7^{a}	167.5^{b}	186.8	69.2^{a}	101.6^{b}	113.9^{a}
125ppm CuHCl + 125ppm ZHC 1-49d	31.6	106.8^{a}	172.4^{ab}	183.9	69.0^{a}	103.4^{ab}	115.3^{a}
125ppm CuHCl + 150ppm ZHC 1-49d	32.7	107.8^{a}	175.7^{a}	180.9	69.5^{a}	104.7^{a}	115.2^{a}
ANOVA							
One-way P-value	0.194	0.015	<0.001	0.871	0.042	<0.001	0.003
Pooled SEM	0.215	0.552	2.078	3.416	0.315	0.841	1.139

[a-c] Means in columns with different superscripts differ significantly at $p \leq 0.05$
[1] CuHCl – Intellibond Cu, Micronutrients USA LLC, Indianapolis, IN
[2] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN

No differences in tender weight, white meat weight, carcass yield, tender yield, or white meat yield were observed between all dietary treatments **(Tables 10 & 11)**. The diet containing 150 ppm ZHC increased ($p = 0.05$) live and carcass weight compared to the control diet with salinomycin, while the control minus salinomycin and treatments containing 100 and 125 ppm ZHC produced intermediate results. These improvements are related to the improvements observed in consumption and BW. Breast weight was maximized in broilers consuming 150 ppm ZHC, and increased weights compared to the control absent of salinomycin and the diet containing 100 ppm ZHC. When evaluating processing data birds from the same pen were separated by sex (male and female), for evaluation of the interaction of broiler sex on processing parameters. Male broilers

increased ($p < 0.05$) carcass, breast, tender, and white meat weights. Female broilers increased ($p < 0.05$) tender and total white meat yield **(Table 11)**. No differences were observed between male and female broilers when evaluting carcass and breast yield **(Table 11)**. However, increasing level of ZHC led to elevations in breast yield, with ZHC inclusion at 150 ppm maximizing breast meat yield compared to the low ZHC (100 ppm) and the control absent of salinomycin, control with salinomycin and ZHC at 125 ppm produced intermediate results.

Table 10. Processing weights (kg) for 50 d old male (M) and female (F) broilers fed varying levels and sources of Zn in Trial 2.

Treatment	Sex	Live Wt	WOG Wt	Breast	Tender	White Meat
Control + salinomycin	M	3.416	2.720	0.731	0.144	0.875
Control + salinomycin	F	2.791	2.210	0.590	0.127	0.718
As 1 – salinomycin	M	3.504	2.751	0.706	0.141	0.847
As 1 – salinomycin	F	2.884	2.286	0.611	0.131	0.742
125ppm CuHCl[2] + 100ppm ZHC[1] 1-49d	M	3.512	2.790	0.730	0.143	0.872
125ppm CuHCl + 100ppm ZHC 1-49d	F	2.890	2.282	0.602	0.129	0.731
125ppm CuHCl + 125ppm ZHC 1-49d	M	3.590	2.865	0.755	0.150	0.905
125ppm CuHCl + 125ppm ZHC 1-49d	F	2.840	2.266	0.599	0.133	0.732
125ppm CuHCl + 150ppm ZHC 1-49d	M	3.571	2.825	0.756	0.147	0.903
125ppm CuHCl + 150ppm ZHC 1-49d	F	2.983	2.347	0.643	0.134	0.777
Main Effects						
Treatment						
Control + salinomycin		3.103[b]	2.465[b]	0.661	0.135	0.796
As 1 – salinomycin		3.194[ab]	2.518[ab]	0.658	0.136	0.794
125ppm CuHCl + 100ppm ZHC 1-49d		3.201[ab]	2.536[ab]	0.666	0.136	0.802
125ppm CuHCl + 125ppm ZHC 1-49d		3.195[ab]	2.550[ab]	0.673	0.141	0.814
125ppm CuHCl + 150ppm ZHC 1-49d		3.277[a]	2.586[a]	0.699	0.140	0.840
Sex						
Male		3.514[a]	2.786[a]	0.734[a]	0.145[a]	0.878[a]
Female		2.870[b]	2.274[b]	0.607[b]	0.131[b]	0.738[b]
P-value						
Treatment		0.050	0.050	0.088	0.208	0.096
Sex		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment X Sex		0.701	0.576	0.362	0.826	0.403

[a-b] Means in columns with different superscripts differ significantly at $p \leq 0.05$

[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN

[2] CuHCl – Intellibond Cu, Micronutrients USA LLC, Indianapolis, IN

Table 11. Processing yields (%) for 50 d old male (M) and female (F) broilers fed varying levels and sources of Zn in Trial 2.

Treatment	Sex	WOG Yield	Breast Yield	Tender Yield	White Meat Yield
Control + salinomycin	M	79.68	26.78	5.28	32.05
Control + salinomycin	F	79.32	26.66	5.76	32.42
As 1 – salinomycin	M	78.56	25.60	5.12	30.72
As 1 – salinomycin	F	79.29	26.65	5.73	32.38
125ppm CuHCl[2] + 100ppm ZHC[1] 1-49d	M	79.45	26.11	5.11	31.22
125ppm CuHCl + 100ppm ZHC 1-49d	F	79.00	26.35	5.63	31.98
125ppm CuHCl + 125ppm ZHC 1-49d	M	79.86	26.36	5.23	31.59
125ppm CuHCl + 125ppm ZHC 1-49d	F	79.82	26.40	5.85	32.25
125ppm CuHCl + 150ppm ZHC 1-49d	M	79.18	26.66	5.20	31.86
125ppm CuHCl + 150ppm ZHC 1-49d	F	78.85	27.32	5.68	33.00
Main Effects					
Treatment					
Control + salinomycin		79.50	26.72[ab]	5.52	30.39
As 1 – salinomycin		78.93	26.12[b]	5.43	31.55
125ppm CuHCl + 100ppm ZHC 1-49d		79.23	26.23[b]	5.33	31.60
125ppm CuHCl + 125ppm ZHC 1-49d		79.84	26.38[ab]	5.56	31.94
125ppm CuHCl + 150ppm ZHC 1-49d		79.02	26.99[a]	5.44	32.44
Sex					
Male		79.34	26.28	5.19[b]	31.47[b]
Female		79.29	26.64	5.74[a]	32.38[a]
P-value					
Treatment		0.065	0.050	0.255	0.067
Sex		0.685	0.074	< 0.001	<0.001
Treatment X Sex		0.445	0.351	0.891	0.403

[a-b] Means in columns with different superscripts differ significantly at $p \leq 0.05$
[1] ZHC – Intellibond Zn, Micronutrients USA LLC, Indianapolis, IN
[2] CuHCl – Intellibond Cu, Micronutrients USA LLC, Indianapolis, IN

Discussion

The Zn requirement for broilers as stated in the NRC (1994) is 40 ppm, while traditional diets meet or exceed this concentration with the addition of most mineral premixes. Mineral sources and chelates with increased bioavailability are being evaluated compared to traditional inorganic minerals found in poultry diets which are typically provided in the form of sulfates or oxides. Newer sources include, but are not limited to, hydroxychloride minerals which are modified inorganics, as well as organic mineral sources usually chelated to an amino acid or proteinate. The majority of previous research evaluates mineral effects on broilers at concentrations similar to requirements stated by the NRC (0 to 60 ppm). These studies attempt to establish a requirement for inorganics as well as different mineral sources compared to inorganics. Huang et al. (2007) observed an initial bump in performance with the supplementation of 20 ppm $ZnSO_4$ (equivalent to 48.37 ppm dietary Zn) compared to a diet without Zn supplementation in 21 d old broilers. Huang et al. (2007) did not observe any additional improvements in performance with 20 ppm incremental increases of Zn up to 140 ppm. In a 17 d study conducted by Dozier et al. (2003) evaluating the effects of feeding inorganic and organic Zn at 40, 80, and 120 ppm, no differences in performance were observed between sources. The lack of differences observed by Huang et al. (2007) and Dozier et al. (2003) was probably due to the short duration of these experiments at an early stage in production, however the data do correlate with NRC recommendations.

Ao et al. (2006b) compared the inclusion of $ZnSO_4$ and an organic form of Zn, and established an inorganic requirement of 20.1 ppm supplemental Zn and an organic

requirement of 9.8 ppm, basal diet contained 23 ppm Zn. The parameter establishing this requirement was a weight gain from placement to d 21 of age. In 2009, Ao et al. reported improvements in performance with the inclusion of Zn compared to a control absent in Zn, but no statistical differences in performance were observed between inorganic and organic Zn. The lack of differences may be caused by the low level of inclusion at 20 ppm for both sources to a basal diet containing over 30 ppm. Bao et al. (2007) fed organic sources of Zn, Cu, Fe, and Mn, Zn levels were at 20, 40, and 80 ppm compared to an inorganic inclusion at 50 ppm and a control absent of mineral supplementation. Bao et al. (2007) reported no extended growth benefit with increased supplementation of organic Zn past 40 ppm in 29 d old broilers and reported decreased Zn excretion in 21 d old broilers fed organic Zn at all levels compared to birds fed inorganic at 50 ppm. Similarly, Liu et al. (2011) observed improvements in weight gain and intake through 42 d of age with Zn supplementation of 60 ppm compared to 0 ppm, and no improvement at 120 or 180 ppm. When supplementing diets with 10 ppm organic Zn (with other organic minerals) compared to 37 ppm inorganic (with other inorganic minerals), Nollet et al. (2007) did not observe any differences in performance between sources. The reduction in mineral supplementation with the use of organics allowed for reductions in mineral excretion as well. Previous research implies that when using organic mineral sources inclusion can be reduced compared to inorganic mineral inclusion without impacting performance. Similarly, most studies did not observe added improvements in growth parameters evaluated past initial inclusion of Zn. These studies indicate the requirement and potentially the maximum efficacy of mineral inclusion as

no further improvements were observed in most studies. However, it is important to note that all except one of these studies evaluated younger birds (less than 30 d).

Research evaluating the effects of hydroxychloride Zn on broilers is limited. A few experiments have been conducted in the past two decades, however only observe 2 levels of ZHC inclusion. Olukosi et al. (2018) evaluated two levels of hydroxychloride at 200 and 400 ppm compared to sulfate at the same levels. Similarly, Cao et al. (2000) evaluated Zn chloride at 200 and 400 ppm compared to multiple varieties of $ZnSO_4$ at the same levels and Zn oxide at 400 ppm. Batal et al. (2001) took a different approach, evaluating ZHC compared to $ZnSO_4$ at much lower supplemental levels between 5 and 11 ppm. At these levels of inclusion it is difficult to understand and determine the effects of ZHC at varying levels.

The effect dietary Zn inclusion had on FI was relatively consistent between both experiments, with a positive correlation being observed overall. This effect on broiler consumption is well documented, as multiple studies have shown increasing levels of Zn to increase consumption (Batal et al., 2001; Ao et al., 2006b; Huang et al., 2007; Ao et al., 2009). In a study conducted by Ao et al. (2009), an increase in FI was observed with the inclusion of Cu and Zn compared to a basal diet absent of additional Zn and Cu inclusion. Inclusion of ZHC in Trial 1 at 120 ppm increased intake through d 41 and termination. Similarly, the inclusion of ZHC and Cu hydroxychloride in Trial 2 increased intake compared to the control diet containing salinomycin. These trials agree with the observations of previous literature mentioning the effect of Zn on increasing appetite (FI) (Batal et al., 2001; Huang et al., 2007; Salim et al., 2008).

In Trial 1, the increasing levels of dietary Zn increased BW through d 14, 28, and 41. BW seemed to peak at ZHC inclusion of 120 ppm as no further BW improvements were observed at 160 ppm. Following a similar trend as observed with peak intake at 120 ppm. Past research has reported similar results with minimal improvements being observed in excess of the initial inclusion of hydroxychloride (Batal et al., 2001; Olukosi et al., 2018) and organic Zn (Ao et al., 2006b; Zhao et al., 2016; Olukosi et al., 2018; Saleh et al., 2018), however these studies report Zn concentrations much lower than the current trial (< 60ppm). In a study conducted by (Ao et al., 2006b), increasing the supplemental Zn inclusion in the diet to 5, 10, 20 and 40 ppm increased weight gain linearly with $ZnSO_4$ and quadratic with an organic proteinate. The basal diet in this study contained 23 ppm Zn to which the Zn products were then added. Ao et al. (2006b) concluded via a broken-line analysis, an additional Zn inclusion of 9.8 ppm proteinate and 20.1 ppm $ZnSO_4$ to represent the requirement based on weight gain for the study. Brooks et al. (2013) reported a statistical increase in BW through 21 d of age with increasing inclusions of $ZnSO_4$ and propionate at 6 to 12 ppm compared to a control diet absent of supplemental Zn inclusion. The change in BW was experienced over a 14 d period (d 7 to 21) following the feeding of a Zn-deficient diet for 7 d. Similar to the current studies, in Trial 2, the increasing concentration of ZHC only numerically increased d 42 and 49 BW compared to the control diet absent of salinomycin (approximately 80 g).

The improvements in FCR associated with increases in dietary hydroxychloride Zn concentration in the current studies agree with prior reports for inorganic (Zhao et al.,

2016) and organic Zn (Star et al., 2012; Zhao et al., 2016; Saleh et al., 2018). Olukosi et al. (2018) reported improvements in feed efficiency with the inclusion of hydroxychloride minerals during the grower phase and cumulatively compared to broilers fed sulfates. Literature evaluating the impacts of multiple levels of hydroxychloride minerals on performance is limited as most of the research only utilizes 1 or 2 different inclusions at levels not more than 80 ppm (Batal et al., 2001; Olukosi et al., 2018).

Rossi et al. (2007) observed no differences in carcass, drumstick, thigh, or breast meat yield in broilers fed increasing levels of organic Zn from 0 to 60 ppm. Contrary to Rossi et al. (2007), Liu et al. (2011) reported improvements in carcass yield from multiple Zn sources at 60 ppm. Similar to the current trials, Saleh et al. (2018) noted increases in breast yield and reductions in fat pad yield with organic Zn supplementation of 50 ppm. Olukosi et al. (2018) observed an improvement in breast meat yield when supplementing broilers with hydroxychloride mineral sources compared to inorganic. The findings of these studies support the results of Trial 1, with improvements in carcass yield, and both trials with breast meat yield increasing as inclusion of ZHC is increased. The level of ZHC increase in the current trial was greater than those used in previous studies indicating a further improvement in processing parameters past the levels of Zn that was used. The impact of Zn on breast myopathies (WB and WS) is not well documented. Some reports have eluded increases in yield and growth rate to negatively impact or increase the incidence and severity of these myopathies (Sihvo et al., 2014; Bodle et al., 2018). Contrary to these statements, heavier breast weight (30 to 40 g) and

improvements in breast yield (0.6 to 0.7%) associated with elevated levels of ZHC did not negatively influence the presence or severity of these myopathies.

In both Trials, broilers consuming diets that contained elevated levels of ZHC, increased overall FI. Improvements in FI, led to heavier BW and in Trial 1 heaver breast weights. Overall, elevated level of ZHC increased breast meat yield in both studies.

CHAPTER III

EVALUATION OF INCREASING MANGANESE HYDROXYCHLORIDE LEVEL ON MALE BROILER GROWTH PERFORMANCE AND TIBIA STRENGTH

Introduction

Manganese effect on broiler performance has been well established, however the majority of the research was completed over a decade ago (Gallup and Norris, 1939b; Ji et al., 2006; Lu et al., 2007). In production, broilers from multiple genetic lines have experienced a magnitude of change that includes increases in growth rate and yield improvements compared to their counter parts from decades prior (Bodle et al., 2018). The forms of Mn available have also undergone some change, while inorganic and organic minerals are still commonly used, hydroxychloride forms of trace minerals are becoming more popular.

Manganese is an essential trace mineral to sustain optimal growth and health in livestock. Classic signs of a Mn deficiency in broilers include perosis (slipped tendon/enlarged hock) (Gallup and Norris, 1939b; Wilgus Jr and Patton, 1939), reduced growth, impaired metabolism, ataxia, and abnormal reproductive function (Henry, 1995). Manganese serves as a constituent for multiple enzymes involved in the metabolism of carbohydrates, amino acids, and cholesterol. Of these enzymes, pyruvate carboxylase (Ashmead and Zunino, 1993) is important for glucose metabolism, acting in the mitochondria of the cell creating oxaloacetate (start of Krebs cycle) from pyruvate

(end of glycolysis). Manganese superoxide dismutase found in the mitochondria requires Mn (Conly et al., 2012) and functions as an antioxidant to protect the cell from reactive oxygen species that can cause disease and modify metabolic function (Holley et al., 2011).

Inorganic and organic sources of Mn are utilized exclusively or in combination in livestock diets to meet Mn requirements. Bioavailability differences between sources impact the decision on Mn selection for dietary formulation. Historically, organic minerals tend to be more bioavailable to the bird (Henry et al., 1989), which can lower inclusions without impacting performance (M'Sadeq et al., 2018). Common forms of inorganic sources of minerals include sulfates and oxides. The hydroxychloride form of inorganic minerals have been researched thoroughly since the 1990's (Cromwell et al., 1998; Miles et al., 1998; Batal et al., 2001) initially starting with Cu chloride. The chloride bond helps stabilize the targeted mineral by preventing unwanted chelation and interaction with other minerals that further reduce availability to the bird (Espinosa et al., 2017).

The inorganic matrix of the bone is the primary storage site for Mn (Conly et al., 2012), which is primarily comprised of calcium and phosphorus. Smaller portions of Mn are deposited in the organic matrix of bone, though small, this Mn is important for the formation of proteoglycans. The deposition of Mn and formation of proteoglycans can improve bone structure, increase bone density, and strengthen bones (Leach, 1986; Gheisari et al., 2011). Conly et al. (2012) reported increasing dietary Mn concentration of the diet corresponded with elevated Mn concentrations in tissue and bone. Previous

findings indicate the importance of Mn for metabolic function, skeletal integrity, and the limited research conducted on the efficacy and potential requirement of broilers fed hydroxychloride Mn led to the current experiment (Experiment 2). The objective was to evaluate the effects on increasing MnHCl on growth performance and skeletal strength in broilers.

Materials and Methods

Experimental Design

The experimental design consisted of 5 dietary treatments in a randomized complete block design with the first treatment absent of any supplementary Mn, while the remaining 4 treatments contained increasing levels of MnHCl at 40, 80, 120, and 160 ppm.

Experimental Diets

Diets for Experiment 2 were corn and SBM based, containing phytase[5] (500 FTU/kg) throughout all phases **(Table 12)**. Diets included DDGS at 5.0% throughout the experiment. A Mn free custom mineral premix was utilized to ensure no supplemental Mn was added during the mixing stage. The basal diet plus the inclusion of corn starch was used for the diet containing "0" ppm supplemental Mn. Manganese hydroxychloride was then added to the basal diet at the expense of corn starch to achieve the target Mn concentration for the respective treatment. This process created the 5

[5] OptiPhos® - Huvepharma – Peachtree City, Georgia

dietary treatments fed throughout the duration of Experiment 2 containing 0, 40, 80, 120, and 160 ppm of supplemental MnHCl. All diets were fed as a pellet with the exception of the starter, which was pelleted and then crumbled. Diets were pelleted at 180° F and conditioned for 12 to 15 s prior to pelleting. The starter diet was fed from d 1 to 14, grower from d 15 to 28, finisher from d 29 to 42, and finisher II from d 43 to 49, and finisher III d 50 to 55. Nutrient content of the basal diet from each phase and Mn concentration for all experimental treatments were analyzed **(Table 12)**. The diet absent of Mn supplementation contained approximately 40 ppm of Mn, this was from Mn present in other ingredients utilized in dietary formulation (Table 1). Manganese concentration increased by approximately 40 ppm as target MnHCl inclusion increased by 40 ppm per treatment.

Animals and Management Practice

Two thousand and one hundred Ross 708 male broilers with an average initial BW of 43.9 g were randomly allotted to floor-pens and dietary treatments based on initial BW on d of hatch. Experiment 2 consisted of a total of 60 pens, each containing 35 chicks at d of age. Floor pens were 3.34 m^2 and contained nipple drinkers and a tube feeder. Feed and water were available *ad libitum.* Chicks were provided age appropriate supplemental heat and were provided an industry type lighting program. The lighting program consisted of: d 1 to 8, 24 h of light at 2 foot candles; d 9 to 18, 16 h of light at 0.75 foot candles; d 19 to 32, 18 h of light at 0.1 foot candle; and d 33 to termination, 20 h of light at 0.05 foot candle. Broilers were reared on pine shavings that had been used previously by 3 flocks.

Table 12. Ingredient profile and nutrient content for diets fed male broilers evaluating the manganese[1] hydroxychloride requirement in Experiment 2.

Ingredient	Starter	Grower	Finisher	Finisher II	Finisher III
DL-Met98	5.95	5.10	4.45	4.00	3.20
Lysine HCL	5.15	4.80	3.90	3.80	4.00
L-Threonine 98.5%	1.80	1.60	1.40	1.20	1.05
Fat, Blended AV	66.00	63.00	74.00	86.00	88.00
Limestone	28.90	28.20	26.60	24.30	23.60
Biofos 16/21P	21.70	19.70	17.30	13.80	12.60
Salt	8.75	8.20	7.15	6.25	4.15
Sodium Bicarb	--	0.80	2.25	3.55	6.80
Tamu Vitamins[2]	2.50	2.50	2.50	2.50	2.50
Mn Custom Trace Min[3]	2.00	2.00	2.00	2.00	2.00
Corn	1106	1224	1269	1300	1386
SBM	651	540	489	453	366
DDGS	100	100	100	100	100
Phytase[4]	0.25	0.25	0.25	0.25	0.25

[1] Target Mn inclusions for each treatment were 0, 40, 80, 120, & 160 ppm. Analyzed dietary Mn levels in the starter were 45, 72, 101, 145, and 177 ppm respectively. Grower levels were 40, 69, 117, 159, and 190 ppm respectively. Finisher levels were 39, 83, 104, 166, and 179 ppm respectively. Finisher II levels were 34, 72, 127, 149, and 214 ppm respectively. Finisher III levels were 38, 79, 126, 177, and 192 ppm respectively.

[2]Vitamin premix added at this rate (0.5 lbs per ton) yields 7700 IU vitamin A, 5500 ICU vitamin D_3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B_{12}, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet

[3]Mineral Premix added at this rate (1.0 kg per ton) yields 80ppm Zn, 30 ppm iron, 8 ppm Cu, 0.3 ppm selenium, and 3.0 ppm iodine

[4]OptiPhos® - Huvepharma – Peachtree City, Georgia

Table 12. Continued

Ingredient	Starter	Grower	Finisher	Finisher II	Finisher III
Calculated Nutrient Content (%)					
Protein	21.85	19.59	18.47	17.70	15.93
Calcium	0.95	0.90	0.84	0.76	0.72
Available Phosphorus	0.48	0.45	0.42	0.38	0.36
AME Poultry (kcal/lb)	1380	1400	1425	1450	1470
Dig Methionine	0.60	0.53	0.49	0.46	0.40
Dig Lysine	1.20	1.05	0.95	0.90	0.80
Dig TSAA	0.89	0.80	0.74	0.70	0.63
Dig Tryptophan	0.22	0.19	0.18	0.17	0.15
Dig Threonine	0.78	0.69	0.65	0.61	0.54
Dig Arginine	1.26	1.10	1.03	0.97	0.85
Sodium	0.19	0.19	0.19	0.19	0.20
Analyzed Nutrient Content (%)					
Protein	22.10	19.20	18.60	18.30	14.80
Crude Fat	5.99	5.82	6.42	6.81	7.03
Crude Fiber	3.10	2.60	2.60	2.40	2.40
Calcium	0.98	1.18	1.16	0.88	0.92
Phosphorus	0.69	0.69	0.69	0.58	0.56
Sodium	0.22	0.24	0.24	0.24	0.25
Mn (ppm)	45	40	39	34	38
Cu (ppm)	15	15	14	14	15
Zn (ppm)	125	139	143	133	144

Birds and feed were weighed on d 14, 28, 42, 49, and 55 to determine average BW; FCR, and FI. Mortality was observed and reported daily. On d 42 and 55, 5 broilers were randomly selected from each replicate pen and euthanized via carbon dioxide asphyxiation. Following asphyxiation right tibias were excised from each bird for determination of bone breaking strength, ash concentration, and tibia Mn concentration. All animal husbandry procedures were conducted in accordance with an approved Texas A&M University animal use protocol (IACUC).

Bone Analysis

On d 42 and 55, 5 right tibias from each replicate pen were excised and cleaned of all tissue prior to storage at -4°C. After termination of Experiment 2, bones were removed from freezer and allowed to completely thaw to room temperature prior to bone strength analysis. Prior to breaking, tibias were placed in the exact center of the 3 point bend test (4.0 cm gap) on a TA-XT2 Texture Analyzer (Texture Technologies) using a test speed of 1.67 mm/s and a breaking sensitivity of 1000g. Data were expressed as breaking strength of the bone in kg.

After strength analysis for each bone was determined, bone remains were pooled by pen and then dried at 100°C for 24 h. Once dried, bones were defatted by Soxhlet extraction apparatus using diethyl ether and then allowed to air dry for 12 h. Samples were then re-dried at 100°C for 24 h, weighed and then ashed at 600°C for 24 h and re-weighed for determination of inorganic matter (ash %). Ash material was then further analyzed for Mn content.

Statistical Analysis

All data were subject to an Analysis of Variance (ANOVA) using the General Linear Model Procedure (SPSS V18.0). Means were deemed significantly different at $P \leq 0.05$ and separated using Duncan's Multiple Range Test. Linear and quadratic regressions were conducted to determine impacts of increasing levels of MnHCl on all evaluated criteria.

Results

Inclusion of MnHCl at all levels did not statistically impact BW **(Table 13)**. On d 28, 42, 49, and 55 birds consuming treatments supplemented with 80 and 160 ppm Mn produced numerically heavier BW, though not statistically significant.

Table 13. Average BW (kg) of male broilers fed increasing levels of MnHCl in Experiment 2.

Treatment	d 14	d 28	d 42	d 49	d 55
Mn (ppm)					
0	0.455	1.507	2.967	3.683	4.236
40	0.461	1.511	2.971	3.647	4.188
80	0.460	1.525	3.007	3.716	4.297
120	0.460	1.517	2.971	3.650	4.217
160	0.463	1.524	2.998	3.728	4.293
ANOVA					
Pooled SEM	0.001	0.006	0.009	0.014	0.018
P-value	0.351	0.710	0.294	0.086	0.059
Regression					
Linear	0.095	0.334	0.359	0.383	0.286
Quadratic	0.221	0.595	0.620	0.515	0.532

Mortality corrected FCR was more sensitive to MnHCl inclusion compared to observed BW. Initially, Mn supplementation seemed to have minimal effects on FCR, as no differences amongst treatments were observed through d 28 **(Table 14)**. However, beginning in the finisher phase, increasing the level of MnHCl improved ($P<0.05$) FCR. Broilers fed diets absent of supplemental MnHCl exhibited the highest FCR while supplementations above 40 ppm reduced ($P<0.05$) FCR. Cumulatively, through d 42, all diets supplemented with MnHCl performed at a higher rate than non-supplemented broilers. Cumulative FCR through 49 and 55 d exhibited a similar trend. Significant improvements in FCR were observed in broilers supplemented with 80 ppm and above of MnHCl as compared to non-supplemented broilers. Regression analysis indicated increasing levels of Mn linearly improved ($p < 0.05$) FCR during the starter phase and cumulatively through d 28, 42, 49, and 55 **(Table 14)**. Similarly, quadratic improvements in FCR were observed with increasing Mn level through d 28, 42, 49, and 55. However, no linear or quadratic correlations were observed when evaluating BW or FI.

Table 14. Mortality corrected feed conversion ratio by phase of male broilers fed increasing levels of MnHCl in Experiment 2.

Mn (ppm)	d 14	Grower	d 1-28	Finisher	d 1-42	Finisher II	d 1-49	Finisher III	d 1-55
0	1.229	1.514	1.433	1.778[a]	1.603[a]	2.075	1.682[a]	2.446	1.767[a]
40	1.214	1.504	1.421	1.757[ab]	1.586[b]	2.125	1.671[ab]	2.453	1.755[ab]
80	1.212	1.499	1.417	1.744[b]	1.578[b]	2.101	1.663[b]	2.391	1.742[b]
120	1.202	1.498	1.414	1.743[b]	1.576[b]	2.143	1.665[b]	2.42	1.750[b]
160	1.208	1.498	1.415	1.751[b]	1.580[b]	2.075	1.662[b]	2.467	1.749[b]
					ANOVA				
SEM	0.003	0.003	0.002	0.005	0.003	0.014	0.003	0.021	0.003
P-value	0.248	0.339	0.077	0.036	<0.001	0.312	0.037	0.594	0.013
					Regression				
Lin.	0.039	0.063	0.012	0.065	0.005	0.824	0.015	0.986	0.032
Quad.	0.062	0.105	0.017	0.051	0.003	0.358	0.030	0.627	0.015

[a-b] Means within a column with different superscripts differ at $p < 0.05$

Interestingly, no differences in FI were observed amongst treatments at any point **(Table 15)**. The absence of differences is interesting as only numerical differences in BW were observed. With the differences observed in FCR at multiple stages, multiple differences in BW or consumption were to be expected, however was not the case. The lack of separation is attributed to the higher variability observed in FI and BW data as compared to FCR. Similarly, Mn concentration did not affect mortality in Experiment 2.

Table 15. Feed intake (g/b/d) by phase of male broilers fed increasing levels of MnHCl in Experiment 2.

Mn (ppm)	Starter	Grower	d 28	Finisher	d 42	Finisher II	d 49	Finisher III	d 55
0	35.8	113.5	74.4	183.8	110.5	211.2	122.7	221.3	131.8
40	36.0	112.6	74.2	180.5	109.3	204.6	120.8	214.4	129.4
80	35.7	112.7	73.9	182.7	109.4	211.4	121.6	222.1	130.7
120	35.4	112.8	73.9	179.5	108.9	204.2	120.3	217.3	129.2
160	35.8	112.9	74.1	182.1	109.6	213.0	121.9	225.8	131.1
ANOVA									
SEM	0.116	0.474	0.277	0.910	0.467	1.562	0.565	1.905	0.660
P-value	0.576	0.921	0.923	0.334	0.636	0.092	0.393	0.287	0.417
Regression									
Linear	0.577	0.766	0.643	0.469	0.500	0.820	0.578	0.659	0.510
Quadratic	0.800	0.845	0.807	0.555	0.593	0.447	0.518	0.683	0.607

Bone measurements did not appear to be extremely sensitive to Mn supplementation, with the exception of tibia Mn. Manganese supplementation had no impact on bone breaking strength, ash percentage, or ash weight of bone at 42 d of age **(Table 16)**. On d 55 the broilers consuming the diet supplemented with 160 ppm increased ($p < 0.05$) tibia breaking strength compared to broilers supplemented with 80 and 120 ppm Mn. Similar to d 42, Mn level had no impact on ash % or weight. Tibia Mn concentration was highly correlated with increasing levels of dietary Mn, observing stair-step increases with each dietary elevation at both d 42 and 55. Linear and quadratic regression analysis further indicated increases in dietary Mn significantly increased tibia Mn concentration.

Table 16. Bone characteristics of male broilers fed increasing levels of MnHCl during later stages of production (d 42 & 55) in Experiment 2.

Treatment	d 42				d 55			
Mn (ppm)	**Break (kg)**	**Ash (%)**	**Ash wt (g)**	**Mn (ppm)**	**Break (kg)**	**Ash (%)**	**Ash wt (g)**	**Mn (ppm)**
0	39.78	51.57	3.19	6.29[b]	57.04[ab]	53.11	4.50	4.36[c]
40	39.75	53.71	3.13	7.33[ab]	55.68[ab]	53.52	4.27	5.17[c]
80	39.15	50.76	3.21	7.85[ab]	53.55[bc]	51.93	4.53	6.01[b]
120	41.87	51.83	3.25	8.12[a]	49.91[c]	54.85	4.51	6.15[ab]
160	38.77	51.28	3.17	9.05[a]	60.02[a]	52.99	4.48	6.89[a]
ANOVA								
SEM	0.641	0.570	0.025	0.265	0.849	0.450	0.041	0.196
CV	12.5	8.5	6.2	26.6	11.9	6.5	7.1	26.7
P-value	0.519	0.502	0.668	0.026	0.001	0.325	0.280	<0.001
Regression								
Linear	0.982	0.544	0.694	<0.001	0.974	0.736	0.484	<0.001
Quadratic	0.875	0.800	0.867	0.002	0.008	0.945	0.742	<0.001

[a-c] Means within a column with different superscripts differ at $p < 0.05$

Discussion

Broiler Mn requirement is reported to be 60 ppm based on the NRC (1994). However, the genetic potential of the current market broiler has dramatically changed since the publication of the NRC (1994). Changes in birds genetic potential, force nutritionists to consider mineral requirements may be changed as well. Thus, common inclusion rates of Mn in industry diets exceeds 100 ppm in most cases. In addition to changing genetics, more mineral types are also available to poultry companies for use in diet formulation aside from traditional inorganic sources. M'Sadeq et al. (2018) noted possible reductions in dietary mineral concentration with the use of hydroxychloride or organically chelated sources without negatively affecting performance as compared to

traditional inorganic sources. A reduction in the inclusion level without failing performance indicates improved bioavailability of these minerals.

In a comparison of hydroxychloride and organic mineral sources, the supplementation of Mn at 22.5 to 30 ppm had no impact on BW through 25 d of age, however at 38 d, there was a noticeable improvement in BW, compared to inorganic Mn source supplemented at 120 ppm (M'Sadeq et al., 2018). Lu et al. (2007) reported no differences in daily gain when comparing inorganic and organic amino acid complexed Mn compared to a basal diet absent of Mn supplementation through 21 and 42 d. Similarly, Collins and Moran (1999) observed no differences in BW at 21, 42, and 49 d when evaluating diets absent in added Mn compared to diets supplemented with 180 ppm. These data correspond with Experiment 2 as no differences in BW were observed for the duration of trial. However, on d 55 elevated levels of Mn (80 & 160 ppm) produced heavier BW in general compared to diets containing 0 and 40 ppm added Mn. Though each study evaluates slightly different parameters, in all cases a trend was observed as Mn level did not statistically impact BW. The absence of differences observed in BW with increasing inclusions of Mn may indicate; a) the requirement for weight gain is relatively low and can be achieved with the Mn concentration in dietary ingredients, b) the enzymes affected by Mn do not correlate directly to an increase in body mass, or c) a combination of the two. The reason for the late impact on growth is not well understood, but could be related to increases in broiler size and maintenance requirements for Mn. At this point muscle accretion, bone growth, and maintenance require elevated levels of Mn compared to younger birds.

Similar to BW, FI was not affected by increases in dietary Mn concentration. The current experiment corresponds with prior research and suggests broilers do not regulate FI based on Mn concentration nor does Mn have the ability to influence broiler FI. Brooks et al. (2012) evaluated a diet absent of Mn inclusion, Mn sulfate, and an organic form of Mn at 20, 100, and 500 ppm (for sulfate and organic), and observed no differences in d 21 FI. When evaluating Mn from a chloride and sulfate source at inclusion levels of 3,600, 4,500, and 5,400 ppm and a control diet containing approximately 100 ppm Mn, Conly et al. (2012) did not observe differences in FI from d 6 to 20 and cumulatively through d 35. However, Bao et al. (2007) observed an increase in consumption with the inclusion of Cu, Zn, Fe, and Mn regardless of source (inorganic and organic) compared to a control with no added mineral inclusion. The response is likely due to the addition of other minerals especially Zn, as Zn is known to regulate and alter appetite. Although, M'Sadeq et al. (2018) observed no differences in consumption through d 38 with the inclusion of inorganic, organic, or hydroxychloride minerals (Cu, Fe, I, Se, Mn, Zn, and Cr) which does not agree with reported results of Bao et al. (2007).

Interestingly, though only numerical differences were observed in BW and no differences in consumption; elevated Mn supplementation improved cumulative FCR during later stages of production which is associated with a reduction in data variability. Similar to Experiment 2, M'Sadeq et al. (2018) observed improvements in FCR cumulatively through 38 d with the use of minerals including Mn from organic and hydroxychloride sources compared to inorganic, however, no differences were observed

early on at d 10, 16, and 25. Bao et al. (2007), also observed improvements in FCR with the inclusion of minerals from inorganic and organic sources compared to broilers fed a diet absent in Cu, Fe, Zn, and Mn supplementation. Contrary to Experiment 2 many studies have reported Mn inability to impact FCR. In a requirement study conducted by Li et al. (2011) evaluating increasing levels on Mn sulfate from 0 to 140 ppm (20 ppm increments) in broilers, no differences in FCR were observed during the duration of the study in 21 d broilers. The lack of differences reported by Li et al. (2011) could be due to the short duration of the experiment, as differences in FCR were not observed until the finisher phase in the current experiment. Mondal et al. (2010) compared diets absent of supplemental Cu, Zn, and Mn to those containing inorganic and organic (decreased levels compared to inorganic) sources. Through d 21, no differences in FCR were observed, however, the inclusion of either mineral source improved FCR from d 20 to 39. Cumulatively, only the organic mineral source was able to improve FCR (Mondal et al., 2010). Sunder et al. (2011) evaluated Mn and Zn supplementation at increasing levels from 60 to 240 and 40 to 160 ppm, respectively, no differences in FCR were observed during the 35 d assay period. Lu et al. (2007) studied the effects on Mn from sulfate and chelated to an amino acid at 100 and 200 ppm and observed no differences in gain:feed ratio at 21 or 42 d, even when compared to a control diet containing approximately 20 ppm Mn. The lack of differences in FCR associated with changes in dietary Mn concentration is not well understood, but may imply Mn effect on feed efficiency is minimal based on prior research. However, Experiment 2 as well as results observed by M'Sadeq et al. (2018) indicate possible improvements in FCR can be

achieved with increasing levels of Mn. Experiment 2 further supports this idea with significant linear relationships correlating the increase in Mn supplementation to an improvement in FCR. Improvements in FCR were primarily observed during the later stages of broiler production. However, research is needed to determine if the elevated level in the early stages of growth are necessary to observe the benefit of increased Mn on FCR during the later stages of growth.

As expected, increasing levels of dietary Mn increased tibia Mn concentration. Multiple studies have a reported increases in tissue Mn concentration in liver (Sunder et al., 2011), tibia (Wong-Valle et al., 1989; Smith et al., 1995; Sunder et al., 2011; Brooks et al., 2012; Conly et al., 2012), kidney (Wong-Valle et al., 1989), and spleen (M'Sadeq et al., 2018) with increases in dietary Mn. Others have also reported elevated levels of Mn (98.1 ppm and greater) reduce the incidence of leg abnormalities in broilers 15 to 21 d old (Li et al., 2011), which were classified by visual swelling of the tibia metatarsal joints. Similarly, Lu et al. (2007) observed reductions in leg abnormalities through 21 and 42 d of age when including 200 ppm compared to 100 ppm Mn. The increases in Mn concentration associated with elevated dietary levels may indicate an increase in ultrastructure and strengthening of the bone. However, research evaluating bone breaking strength with respect to Mn concentration is not well documented. In Experiment 2, elevated Mn level only had an impact on breaking strength at termination (d 55), and seemed to provide vague results as the two highest inclusion rates (120 and 160 ppm) of Mn produced the lowest and highest breaking strength values. Sunder et al. (2011) reported no differences in tibia strength in 35 d old broilers fed varying

concentrations on Mn (60 to 240 ppm) and Zn (40 to 160 ppm). Similarly, M'Sadeq et al. (2018) observed differences in Mn content of the tibia with no differences in strength in 38 d old broilers. The lack of differences in Experiment 2 as well as others when evaluating Mn effects on bone strength may indicate the levels of Mn measured were not enough to influence strength or that a larger number of birds need to be sampled to observe a potential benefit. In Experiment 2, increasing MnHCl concentration in broiler diets improved FCR in the later stages of production along with increased tibia deposition at d 42 and 55.

CHAPTER IV

EVALUATION OF MANGANESE HYDROXYCHLORIDE REQUIREMENT IN 45 WEEK OLD WHITE LEGHORN LAYERS AS DETERMINED USING YOLK AND SHELL MN CONTENT

Introduction

Manganese (Mn) is an important trace mineral for structural development (Leach et al., 1969), reproduction, egg production, and shell strength (Caskey et al., 1939; Lyons, 1939) in laying hens. Additionally, Mn is vital for proper prenatal embryonic development and chick quality at hatch (Richards et al., 2010). Metalloenzymes including arginase, pyruvate carboxylase, glutamine synthetase, and Mn superoxide dismutase, rely on Mn for activation (Organization, 1996). Manganese is also required for the glycosylation of the protein core in the proteoglycan matrix, which provides the base structure for bone development. In addition, Mn promotes calcite crystal formation and assists in eggshell formation. Deficiencies in Mn are usually associated with inhibitory effects on the formation of the extracellular shell matrix (Leach and Gross, 1983). Reductions in egg production (20%) and shell thickness have been associated with Mn deficiencies in laying hens; along with abnormal eggshell ultrastructure formation, morphology, and quantity of mammillary knobs on the shell (Leach and Gross, 1983; Gheisari et al., 2011). Similarly, Lyons (1938) reported a deficient diet containing 7 ppm Mn fed for 10 to 14 d increased embryo mortality (d 1 to

7 and 14 to 21) and abnormal chick development (shortened appendages, protruding abdomen, disproportional bone growth, and edema)

Manganese requirements for white-egg laying strains is 20 ppm during egg production as stated by the NRC (1994). Since the establishment of these requirements, other Mn sources, in addition to traditional inorganic mineral sources, have been researched and implemented in commercial production. Newer sources of Mn used in poultry production have resulted in improvements in availability and production performance with reduced inclusion rates as compared to inorganic minerals. As the goals for egg producing poultry may differ based on product, poultry breeders remain concerned with shell quality (Jackson et al., 1987; Roberts, 2004), production rate, and nutrients available to the developing embryo during incubation. Novel feed ingredients and additives may offer innovative methods to improve dietary formulation to benefit the animal, producer, and consumer. The implication of these new sources of Mn have raised attention regarding bioavailability, impact on performance, and dietary inclusion level.

As previously discussed, Mn is important for a myriad of pathways and functions in developing and advanced stages of growth. In poultry, severe reductions in egg production and hatchability were observed by Gallup and Norris (1939a) with diets containing 13 ppm Mn reducing lay rate (approximately 32 & 20%), egg weight (approximately 3 & 4 g), fertility (7 and 8%), embryo mortality (43 & 45%), and hatch of fertile (approximately 43 & 45%) compared to hens fed diets containing 200 and 53 ppm Mn respectively. These reductions in performance and reproduction signify the

importance of Mn for early development and sustainability. The absorption of trace minerals in the egg is mediated by the yolk sac membrane which is responsible for the regulation of trace minerals from the liver of the hen to the developing embryo (Richards, 1997). Thus, impairment and malformation of embryonic growth and development is controlled by the developing yolk, and may be able to be used as an indicator of potential deficiency in Mn. The increasing use and growing interest in alternative mineral sources led to the objective of Experiment 3, which was to evaluate the effect of increasing levels of hydroxychloride Mn in White Leghorn hens on yolk and shell Mn content in determination of a requirement.

Materials and Methods

Experimental Design

The experimental design consisted of 5 experimental treatments containing increasing levels of Mn in the form on MnHCl at 0, 15, 30, 60, or 90 ppm. A reference diet containing Mn at 70 ppm was fed to 14 replicate pens, the reference diet represented the 6th treatment. Prior to feeding the experimental treatments containing MnHCl, 70 layers (5 experimental treatments) were fed a diet absent of supplemental Mn for a 21 d period. This period was defined as the depletion phase. During the depletion phase, reference diet layers remained on the reference diet for the 21 d period. All treatments contained 14 replicates per treatment, each containing 1, 45-wk-old Hyline W-36 white-leghorn layer placed in battery cages for a 21 d depletion period followed by a 35 d experimental period (56 total d).

Experimental Diets

Diets were corn and SBM based and contained DDGS at 5.0% during the entirety of Experiment 3 **(Table 17)**. During the 35 d evaluation period, post depletion, experimental treatments contained Mn in the form of hydroxychloride at 0, 15, 30, 60, or 90 ppm. The control diet contained 70 ppm Mn in the form of Mn oxide during the depletion and experimental phase of the experiment to provide a reference for Mn inclusion. Diets were manufactured from one large basal, and a custom premix containing Mn was added to achieve target Mn level. All diets were fed as a mash.

Animals and Management Practice

In Experiment 3, a total of 84 45-wk-old Hy-line White Leghorn laying hens were placed in battery style layer cages at initiation of the experiment. Cages were 1,587 cm^2 (246 in^2) and equipped with one nipple drinker and a trough style feeder. Water was available *ad libitum*, however, feed allocation was restricted to no more than 115 g/d to ensure compensative eating to meet Mn requirement was avoided. Layers were provided age appropriate environmental temperature of approximately 70 to 75 ˚F. The lighting program consisted of 16 h of continuous light (2 foot candles) and 8 h of dark for the duration of Experiment 3.

Table 17. Ingredient profile and nutrient content for basal diet fed 45-wk-old White Leghorn hens in Experiment 3.

Ingredient	Experimental
Corn	55.44
SBM	21.48
DL-Methionine	0.19
Lysine HCl	0.09
L-Threonine	0.02
Soy Oil	4.25
Fine Limestone	7.85
Coarse Limestone	3.00
Monocalcium Phosphate	1.90
Salt	0.21
Sodium Bicarb	0.30
Vitamins[2]	0.25
Mineral Premix[3]	0.05
DDGS	5.00
Meat and Bone Meal	--

[1] Target Mn inclusions for each treatment during the experimental phase were 0, 15, 30, 60, 90, and 70 ppm. Analyzed dietary Mn levels were 65, 73, 93, 129, 151, and 117 ppm respectively.
[2] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.
[3]Mineral Premix added at this rate (1.0 lbs. per ton) yields 60 ppm Zn, 30 ppm iron, 8 ppm Cu, 0.3 ppm selenium, and 0.8 ppm iodine

Table 17. Continued

Ingredient	Experimental
Calculated Nutrient Content (%)	
Protein	16.80
Calcium	4.50
Available Phosphorus	0.51
AME Poultry (kcal/lb)	1305
Dig Methionine	0.43
Dig Lysine	0.79
Dig TSAA	0.66
Dig Tryptophan	0.16
Dig Threonine	0.55
Dig Arginine	0.93
Sodium	0.20
Analyzed Nutrient Content (%)	
Protein	15.2
Crude Fat	6.8
Crude Fiber	3.3
Calcium	4.58
Phosphorus	0.78
Sodium	0.21
Manganese (ppm)	65
Cu (ppm)	15
Zn (ppm)	90

During Experiment 3, feed was weighed every 10 d (d 10, 20, 30, and 35) for the determination of FI during the experimental phase. Egg production was recorded daily for determination of lay rate. On d 5, 10, 15, 25, and 35 eggs were collected for the determination of weight, breaking strength, and Mn content of the yolk and shell. The yolk portion of the egg was separated from the albumen and freeze-dried using a Labconco free-zone freeze dryer (Kansas City, MO). Manganese content of yolk and shell were determined using inductively coupled plasma – optical emission spectrometry (ICP-OES). Shell and yolk Mn concentration were selected to evaluate multiple parameters in layer production, without the destruction of birds used from the previous time point. The sampling method practiced, allowed for the evaluation of Mn deposition over the duration of Experiment 3. All animal husbandry procedures were conducted in accordance with a Texas A&M University approved animal use protocol (IACUC).

Statistical Analysis

All data were subject to a one-way Analysis of Variance (ANOVA) using the GLM model (SPSS software). Means were deemed significantly different at $p \leq 0.05$ and were further separated by Duncan's multiple range test. Linear and quadratic regression analysis were conducted on MnHCl treatments to determine the correlation between increasing levels of hydroxychloride Mn on performance.

Results

Of the parameters evaluated in Experiment 3, yolk Mn concentration was most sensitive to changes in dietary Mn inclusion. At the termination of the depletion phase, yolks recovered from layers fed the Mn absent diet had significantly less Mn recovered

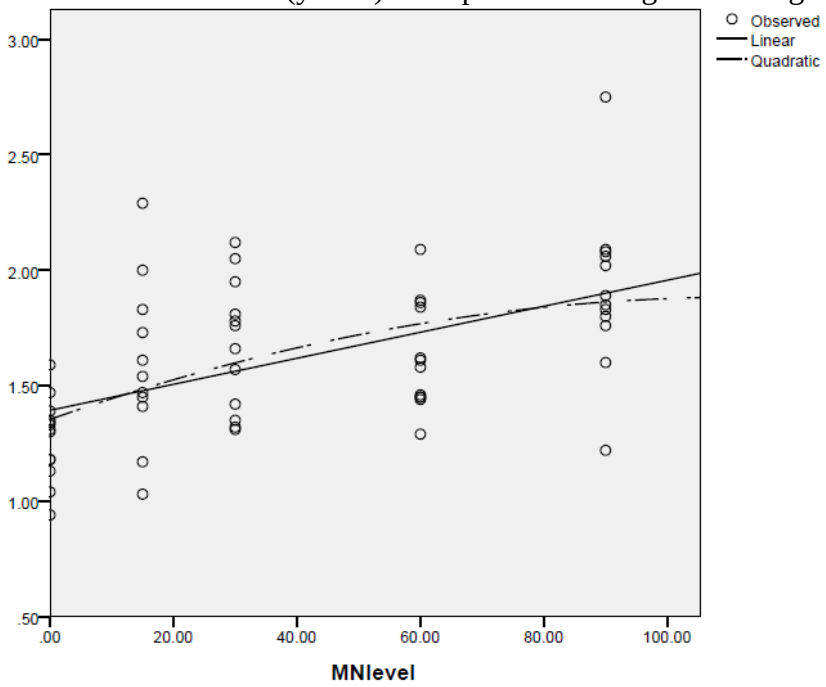
as compared to hens that remained on the reference diet **(Table 18)**. Yolk Mn depletion results were as anticipated, and provided a baseline for the experimental phase of the study. After 5 and 10 d consuming experimental diets, reference fed layers still had elevated levels of Mn present in the yolk regardless of MnHCl level in the experimental diets. Through d 5 and 10, layers fed 0 ppm Mn produced the lowest concentration of yolk Mn, with each elevation in dietary Mn directly increasing Mn recovery. Layers consuming 90 ppm MnHCl for 15 d produced the highest yolk Mn concentration compared to all other treatments, with a similar stair-step elevation in yolk Mn with increases in dietary Mn. On d 25 and 35 a similar correlation was observed for yolk Mn concentration, with layers fed 90 ppm Mn depositing the most yolk Mn and the reference diet producing similar concentrations. Linear and quadratic correlations were analyzed for the increasing levels of MnHCl (0 to 90 ppm) on d 35 **(Figure 1)**. Both analysis produced significant correlations for Mn yolk concentration, indicating a direct relationship between dietary Mn and yolk Mn deposition **(Table 18 & Figure 1)**.

Table 18. Yolk Mn composition (ppm) in 45-wk-old White Leghorn hens fed MnHCl, at the end of the depletion phase and throughout the experimental phase in Experiment 3.

Mn	d 0	d 5	d 10	d 15	d 25	d 35
0	1.10^{b}	0.97^{d}	1.16^{d}	1.25^{d}	1.30^{b}	1.27^{c}
15		1.06^{cd}	1.21^{cd}	1.55^{c}	1.57^{a}	1.59^{b}
30		1.16^{bcd}	1.35^{cd}	1.86^{b}	1.64^{a}	1.68^{ab}
60		1.31^{bc}	1.46^{bc}	1.89^{b}	1.65^{a}	1.63^{b}
90		1.38^{b}	1.63^{ab}	2.14^{a}	1.81^{a}	1.91^{a}
Ref.	1.59^{a}	1.83^{a}	1.83^{a}	1.87^{b}	1.80^{a}	1.78^{ab}
			ANOVA			
SEM	0.085	0.069	0.058	0.064	0.055	0.050
P-value	0.036	<0.001	<0.001	<0.001	0.003	<0.001
			Regression			
Linear						<0.001
Quadratic						<0.001

[a-d] Means within a column with different superscripts differ at $p < 0.05$

Figure 1. Linear and quadratic correlations for elevating level of dietary MnHCl (x-axis) on yolk Mn concentration (y-axis) in Experiment 3. Figure 1 was generated using SPSS.



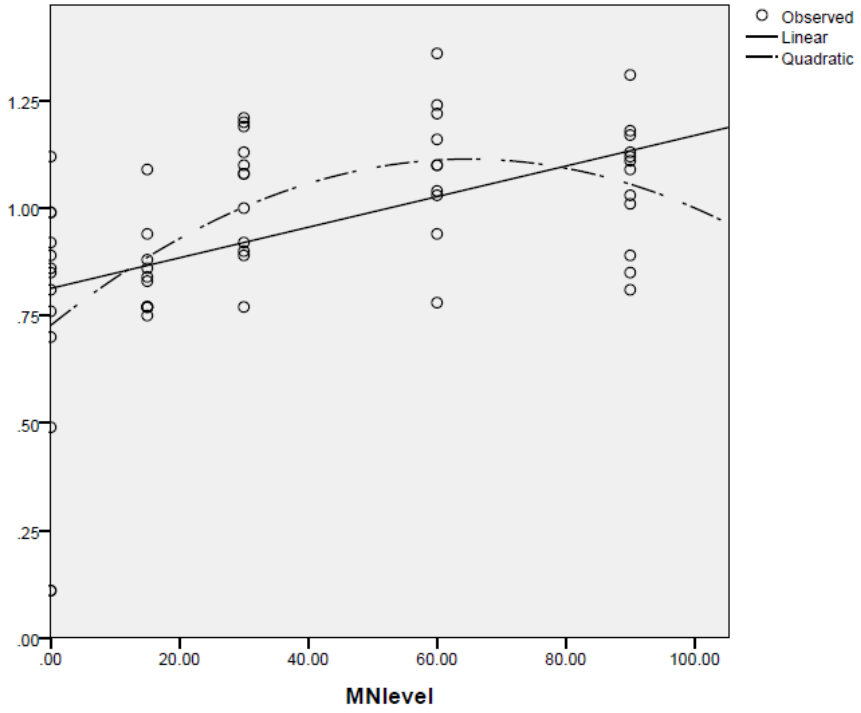
Shell Mn concentration was affected by MnHCl, however egg shell material was not as sensitive to dietary changes in Mn and was present at a lower concentration as compared to yolk material **(Table 19)**. At termination of the depletion phase no differences in shell Mn were observed between egg shells of layers fed the depleted Mn diet and the reference diet. On d 5, 10, 15, and 25 Mn shell deposition was sporadic between treatments. However, after consuming dietary treatments for 35 d, shell Mn levels seemed to resemble that of dietary Mn, layers consuming 0 and 15 ppm reduced Mn shell deposition compared to eggs from hens consuming 30 ppm Mn or greater. On d 35, linear and quadratic relationships between Mn level and shell Mn concentration were observed **(Table 19 and Figure 2)**.

Table 19. Shell Mn composition (ppm) in 45-wk-old White Leghorn hens fed MnHCl, at the end of the depletion phase and throughout the experimental phase in Experiment 3.

Mn	d 0	d 5	d 10	d 15	d 25	d 35
0	0.13	0.61^{c}	0.90^{c}	1.31	1.17^{ab}	0.74^{b}
15		0.51^{c}	1.08^{bc}	1.17	1.15^{ab}	0.84^{b}
30		1.26^{a}	1.13^{abc}	1.14	1.07^{b}	1.04^{a}
60		1.02^{b}	1.23^{abc}	1.10	1.08^{b}	1.10^{a}
90		0.67^{c}	1.28^{ab}	1.16	1.25^{a}	1.06^{a}
Ref.	0.12	0.98^{b}	1.46^{a}	1.10	0.93^{c}	1.06^{a}
			ANOVA			
SEM	0.006	0.055	0.063	0.027	0.027	0.031
P-value	0.532	<0.001	0.027	0.06	0.002	<0.001
			Regression			
Linear						<0.001
Quadratic						<0.001

[a-c] Means within a column with different superscripts differ at $p < 0.05$

Figure 2. Linear and quadratic correlations for elevating level of dietary MnHCl (x-axis) on shell Mn concentration (y-axis) in Experiment 3. Figure 2 was generated using SPSS.



Throughout the experimental phase, no differences in consumption were observed between treatments. The lack of differences ensured layers were not altering FI to compensate for Mn intake **(Table 20)**. Similarly, no statistical differences in lay rate were observed at the end of the depletion phase or during the experimental phases of Experiment 3 between treatments **(Table 20)**. No differences in egg weight were observed at the end of the depletion phase between the reference and depletion diets **(Table 20)**. On d 5, after introducing experimental diets, egg weights of layers consuming the reference diet increased compared to all other diets indicating that the deletion of Mn does impact egg weight. Throughout the duration of Experiment 3, hens consuming the reference diet produced heavier eggs compared to those produced from once depleted hens. Though Mn has been associated with improvements in shell formation, contributing to increases in shell thickness and breaking strength, no differences in egg shell force were observed between any treatments at any time point in Experiment 3 **(Table 20)**.

Table 20. Feed intake (g/b/d), weekly lay rate (%), egg weight (g), and egg force (kg) of 45-wk-old White Leghorn hens from the end of the depletion phase to termination of the experimental phase in Experiment 3.

	Mn Supplementation (ppm)						P-value		
Phase	**0**	**15**	**30**	**60**	**90**	**Ref.**	**ANOVA**	**Lin.[1]**	**Quad.[2]**
Feed Intake (g/b/d)									
d 1-10	111.7	109.8	111.1	112.8	111.2	115.0	0.791	--	--
d 11-20	103.6	100.6	106.2	107.6	103.5	106.6	0.076	--	--
d 21-30	101.6	101.7	100.4	103.1	100.9	105.2	0.298	--	--
d 31-35	107.0	104.0	104.1	105.6	105.0	104.7	0.791	0.758	0.713
Weekly Lay Rate (%)									
wk 0	91.72	--	--	--	--	93.65	0.731	--	--
wk 1	94.89	85.71	89.80	87.76	87.91	94.22	0.899	--	--
wk 2	91.84	75.51	86.74	94.90	94.51	100.00	0.213	--	--
wk 3	88.78	83.67	86.73	89.80	93.41	82.86	0.900	--	--
wk 4	88.78	81.63	84.69	91.84	85.71	82.86	0.934	--	--
wk 5	91.84	74.49	85.71	89.80	92.31	78.57	0.503	0.408	0.534
Egg Weight (g)									
d 0	65.39	--	--	--	--	69.49	0.148	--	--
d 5	63.49^{c}	67.57^{ab}	62.55^{c}	64.54^{bc}	63.53^{c}	68.27^{a}	0.006	--	--
d 10	62.34	63.58	62.22	63.08	64.43	67.50	0.374	--	--
d 15	65.70	66.18	63.37	65.55	66.17	68.24	0.289	--	--
d 25	67.50^{ab}	66.82^{ab}	64.05^{b}	64.69^{b}	64.56^{b}	70.12^{a}	0.023	--	--
d 35	64.15	65.88	64.66	64.63	65.57	68.51	0.269	0.637	0.893
Egg Force (kg)									
d 0	4.45	--	--	--	--	4.15	0.101	--	--
d 5	4.75	4.57	4.57	4.48	4.61	4.75	0.909	--	--
d 10	4.64	4.92	4.98	4.66	4.70	4.17	0.125	--	--
d 15	4.60	4.53	4.66	4.68	4.62	4.86	0.876	--	--
d 25	4.39	4.58	4.48	4.30	4.22	4.44	0.795	--	--
d 35	4.52	4.84	4.85	4.88	4.60	4.73	0.639	0.884	0.204

[a-c] Means within a row with different superscripts differ at $p < 0.05$

[1] Linear P-value

[2] Quadratic P-value

Discussion

The increasing availability associated with hydroxychloride and organic minerals has reverted focus back to the requirement and effect these sources have on layer performance compared to inorganics. In Experiment 3, replenishment of yolk Mn to the reference diet was achieved as quickly as 10 d in hens consuming experimental treatments. Overall, increasing dietary minerals has minimal impact on yolk mineral composition, with the exception of Mn (Naber, 1979). Similar to Experiment 3, Mabe et al. (2003) evaluated the effects of increasing Cu (0, 5, and 10 ppm) and Mn (0, 30, and 60 ppm) from both inorganic and organic sources for a 9-wk period in 64 to 73 wk-old hens depleted of Zn, Mn, and Cu for a 4 wk period. Mabe et al. (2003) reported increases in yolk Mn concentration in relation to elevations in dietary Mn concentration after a 4 wk depletion phase. Venglovska et al. (2014) observed elevations in yolk Mn in layers consuming Mn-glycine chelate compared to the basal diet absent of supplemental Mn. In addition, Li et al. (2017) also reported elevations in yolk Mn content when increasing dietary Mn concentrations, however Mn values in this study seemed to be low (approximately 0.20 ppm) compared to Experiment 3 (approximately 1.00 to 2.15 ppm), Mabe et al. (2003) (0.60 to 0.93 ppm), and Venglovska et al. (2014) (1.65 to 2.40 ppm). The drastic differences in Mn yolk composition between studies could be contributed to the amount of background Mn in the basal diets, or may indicate a Mn depletion phase exacerbates the impact of Mn repletion in yolk. Inconsistent of the previous studies, Inal et al. (2001) did not observe differences in yolk Mn concentration between birds fed a control diet or a diet absent of trace minerals for 10 or 12 wk. The difference in Mn level

between the control (absent of trace mineral) and experimental diet was about 70 ppm, however this difference was not enough to illicit a response (Inal et al., 2001). The availability of the source and the Mn level in the control (approximately 15 ppm) may be the reason Inal et al. (2001) was unable to observe differences in yolk Mn. The results observed in Experiment 3 correlating dietary Mn level directly with yolk Mn level confirms the ability to use the Mn content of the yolk as a marker for dietary Mn level and availability. The current study also indicates a baseline or reference point can be used to compare and contrast between varying levels and sources of Mn.

Shell Mn composition was less sensitive to dietary changes in Mn, however layers consuming higher levels deposited more shell Mn overall. Similarly, Xiao et al. (2015) reported increases in shell Mn composition at dietary inclusion greater than 50 ppm for inorganic and 20 ppm for organic Mn, though no further rise in shell Mn was observed. Mabe et al. (2003) noted dietary mineral level had no impact on shell mineral deposition including Mn. The lack of differences in shell Mn associated with altering mineral levels in the diet may be due to the duration of which this process occurs. The onset of hen maturity allows the yolk to begin to form and mature from the ovaries, however the shell is constructed in the oviduct in less than 24 h. The large variances in time could contribute to the low impact dietary Mn has on shell Mn content.

Similar to Experiment 3 in which Mn concentration had no impact on FI, Abdallah et al. (1994) observed no differences in FI after removing trace minerals from the diet for a 10 wk evaluation period. Similarly, the replacement of inorganic Mn, Zn, or the combination with organic at 0, 50, or 100% from 25 to 70 wk of age, resulted in

no differences in consumption between the use of either source of minerals (Swiatkiewicz and Koreleski, 2008). Similarly, Yang et al. (2012) observed no differences in FI in layers supplemented with Zn and Mn. The lack of differences in consumption between treatments in Experiment 3 was a desired outcome to avoid adjustments in FI of the hens in an effort to meet their requirement. Others have reported differences in consumption when altering mineral levels, Inal et al. (2001) observed a reduction in consumption with the removal of trace minerals from the diet. However this reduction was probably driven by the removal of Zn not Mn, as Zn has been known to regulate appetite (Nollet et al., 2007).

Multiple studies evaluating Mn in egg producing poultry have not observed differences in egg weight associated with variations in dietary Mn (Zamani et al., 2005b; Yang et al., 2012; Xiao et al., 2014; Li et al., 2017). Xiao et al. (2015) reported no differences in egg shell weight in layers 56 to 62 wk-old fed 0 to 200 ppm Mn from inorganic and organic sources. However, birds in Experiment 3 were subject to a depletion phase, which reduced egg weight compared to the reference. Interestingly, egg weights from the depleted hens never seemed to fully recover during the 35 d experimental phase of feeding MnHCl (approximately 3.0 g difference in weight between reference and experimental treatments). Inal et al. (2001) observed no difference in egg weights from young (30 to 40 wk) layers consuming diets absent of supplemental trace minerals and vitamins, however observed reductions in egg weight in older (62 to 74 wk) layers consuming the same diet. Thus, demonstrating the importance

of trace minerals in older birds that tend to be less efficient than their younger counter parts in mineral metabolism and deposition.

In the commercial egg and primary breeding industries, egg quality is of great concern to maintain efficient economic return and reproduction (Zamani et al., 2005a; Swiatkiewicz and Koreleski, 2008). Manganese's ability to improve breaking strength is well documented across multiple aged egg producing hens, including Mn supplementation at 100 ppm in 50 wk-old layers fed for 12 wk (Xiao et al., 2014), full inclusion of organic Zn and Mn compared to inorganic in 62 and 70 wk-old layers (Swiatkiewicz and Koreleski, 2008), and inorganic or organic Mn in layers 32 to 45-wk-old (Zhu et al., 2015). Though, Venglovska et al. (2014) did not evaluate egg breaking strength, reductions in soft shelled and cracked eggs were reported in 20 to 28 wk-old layers consuming 120 ppm Mn compared to a control absent of Mn supplementation. Many studies have confirmed Mn supplementation ability to improve eggshell quality (Abdallah et al., 1994; Mabe et al., 2003; Swiatkiewicz and Koreleski, 2008; Xiao et al., 2014; Xiao et al., 2015). However, in Experiment 3 no differences in breaking strength were observed at the end of the depletion phase or during any time point of the experimental phase. In agreement with Experiment 3, multiple studies have observed no differences in egg breaking strength associated with changes in dietary Mn (Yildiz et al., 2010; Yildiz et al., 2011; Li et al., 2017). It is challenging to understand the correlation between Mn and eggshell strength from these previous studies. The majority of studies reporting differences in egg shell characteristics with variations of dietary Mn, exposed hens to experimental diets for at least 8 wk and were compared to a diet absent of

supplementary Mn in most instances. Contrary to Experiment 3, in which egg shell thickness was not measured, Leach and Gross (1983) reported reductions in egg shell thickness in White Leghorns consuming a Mn deficient diets compared to the control over a 16-wk period. Likewise, Yang et al. (2012) observed thicker shells in eggs from layers consuming increasing levels of Mn supplementation from the control to 55 ppm. However, multiple studies have observed no differences in shell thickness or strength in laying hens consuming 0 to 90 ppm Mn (Zamani et al., 2005b; Li et al., 2017). Prior findings indicate that the absence of dietary Mn may reduce shell thickness, though increasing dietary Mn does not necessarily increase thickness past the deficient diet. Thus, the duration and concentration of dietary Mn could play an integral part on the ability to impact shell quality parameters.

Similar to shell quality, egg production or lay rate is sought after in both commercial layers and breeding poultry. In Experiment 3, neither the depletion nor experimental phase had an impact on lay rate at any time point amongst Mn levels. However, evaluating egg production and characteristics (weight and quality) was not the primary focus of Experiment 3, and the lack of differences in performance and egg quality may be associated with minimal replication and power of the current study (one bird per pen). Likewise, various studies have not been able to correlate dietary Mn level with statistically significant changes in egg production (Leach and Gross, 1983; Abdallah et al., 1994; Zamani et al., 2005a; Swiatkiewicz and Koreleski, 2008; Yildiz et al., 2010; Yildiz et al., 2011; Venglovska et al., 2014). Sazzad et al. (1994) observed elevations in production with increases in Mn, though not significantly different were

linearly correlated. Contrary to the previous experiments, Inal et al. (2001) observed a 8% drop in production in 30 to 40 wk-old hens consuming diets absent of trace minerals and vitamins, however no differences in 62 to 74 wk-old hens. Reductions in production in younger hens may have been associated with a lack of stored minerals necessary for functions contributing to production, however does not indicate a specific mineral or vitamin is responsible for this decline. Zhu et al. (2015), observed no impact on lay rate in layers exposed to a age appropriate environment (21°C) consuming diets containing 0 or 120 ppm supplemental inorganic or organic Mn. In the same experiment birds housed in a heat stressed (32°C) environment consuming 120 ppm organic Mn increased lay rate compared to those consuming no supplemental Mn. The findings of this research support the idea that Mn has minimal impact on lay rate and egg production in a normal production environment.

In Experiment 3, egg yolk demonstrated higher sensitivity to changes in Mn concentration. Inclusion of MnHCl was able to replenish yolk Mn compared to the reference diet after 10 d consuming 90 ppm MnHCl, and 15 d when consuming 30 and 60 ppm MnHCl. Overall, the method utilized in this Experiment to determine yolk Mn could be used in the future as a non-destructive method to determine requirement of different sources over a period of time.

CHAPTER V

CONCLUSIONS

The use of alternative mineral sources has been explored in the poultry industry, with changing bird requirements and available additives, the recommended mineral inclusions for key trace minerals changes as well. While it is well established that the majority of trace minerals are over fed to provide a safety margin, the use of alternative trace minerals may allow producers to improve performance without increasing dietary costs. Furthermore, while though are no restrictions in place limiting dietary trace mineral concentration, increasing concern of toxicity regarding trace minerals could create maximum dietary mineral concentration to reduce the amount of mineral waste and run-off. Hydroxychloride mineral sources could provide producers with an alternative to inorganics for performance or reduce inclusions rates and waste.

When evaluating ZHC on broiler growth performance and processing yield in Chapter II, ZHC level primarily impacted BW and processing yields. In Trail 1, higher levels of dietary ZHC led to improvements in FI, leading to increases in BW, breast weight, and breast meat yield. Interestingly, equivalent levels of ZHC supplementation to $ZnSO_4$ (80 ppm) resulted in elevated early BW, reduced FCR, and increased carcass and breast meat yield. In Trial 2, the combination of ZHC and CuHCl performed similar to the control diet containing inorganic Cu and Zn in conjunction with salinomycin to mimic an antibiotic free program, increasing ZHC led to improvements in breast meat yield as well. The results of these Trials suggest the use of ZHC in broilers can improve

both live performance and processing parameters, as well as outperform inorganic Zn at equal concentrations. The improvements associated with increasing levels of ZHC could be beneficial for commercial producers interested in improving broiler production (live and carcass) as well as reduce the amount of trace minerals used in formulation.

Evaluation of increasing MnHCl level on male broiler growth performance and tibia strength in Chapter III (Experiment 2) indicated the impact of elevated Mn levels on improving FCR. Through d 42, 49, and 55 MnHCl levels at or above 80 ppm improved FCR compared to the control. However, linear and quadratic improvements were observed in FCR with increasing levels of MnHCl through d 28, 42, 49, and 55. Similar to FCR, linear and quadratic improvements in tibia Mn concentration were observed with increasing levels of MnHCl on d 42 and 55. The results from Experiment 2 indicate elevated levels of MnHCl are necessary to improve both growth performance and Mn deposition in bone. Larger broilers grown for longer periods of time may require these elevated levels of Mn (80 ppm and above) to maintain efficient performance and bone strength.

In Chapter IV (Experiment 3), MnHCl evaluation of yolk and shell from White Leghorn hens indicated the yolk to be the more sensitive measurement to alterations in dietary Mn. Hens consuming the 90 ppm MnHCl level returned yolk Mn to the reference diet (70 ppm Mn oxide) after 10 d, and hens consuming 30 and 60 ppm MnHCl returned yolk levels to the reference after 15 d. The sensitivity of the egg yolk to dietary changes in Mn suggests the use of yolk for future Mn evaluation studies evaluating the requirement and availability of alternate sources to adult egg producing poultry as well

as embryos. The data from Experiment 3 suggest supplementing MnHCl at 30 to 60 ppm to be adequate in replenishing yolk Mn levels similar to the reference diet as quickly as 15 d.

In summary, inclusion of hydroxychloride minerals in these Experiments improved broiler growth performance and processing parameters, as well as tissue deposition in broilers (tibia Mn) and layers (yolk Mn). These data implicate improvements in dietary formulation by reducing mineral inclusion without sacrificing performance or maintaining inclusion using an alternate source allowing performance improvements.

REFERENCES

Abdallah, A. G., R. H. Harms, H. R. Wilson, and O. El-Husseiny. 1994. Effect of Removing Trace Minerals from the Diet of Hens Laying Eggs with Heavy or Light Shell Weight. Poult. Sci. 73:295-301. doi 10.3382/ps.0730295

Aksu, D. S., 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 Defense Systems. Asian-Aust. J. Anim. Sci. 23:1066-1072. doi 10.5713/ajas.2010.90534

Aldridge, B., K. Saddoris, and J. Radcliffe. Year. Copper can be absorbed as a Cu-peptide chelate through the PepT1 transporter in the jejunum of weanling pigs. Proc. J. Dairy Sci.

Ammerman, C., P. Henry, and R. Miles. 1998. Supplemental organically-bound mineral compounds in livestock nutrition.67-91.

Ao, T., J. Pierce, R. Power, K. Dawson, A. Pescatore, A. Cantor, and M. Ford. 2006a. Evaluation of Bioplex Zn as an organic zinc source for chicks. International Journal of Poultry Science 5:808-811.

Ao, T., J. L. Pierce, R. Power, A. J. Pescatore, A. H. Cantor, K. A. Dawson, and M. J. Ford. 2006b. Evaluation of Bioplex Zn as an organic zinc source for chicks. Int J Poult Sci. 5:808-811.

Ao, T., J. L. Pierce, R. Power, A. J. Pescatore, A. H. Cantor, K. A. Dawson, and M. J. Ford. 2009. Effects of feeding different forms of zinc and copper on the

performance and tissue mineral content of chicks. Poult. Sci. 88:2171-2175. doi 10.3382/ps.2009-00117

Arias, V. J., and E. A. Koutsos. 2006. Effects of Copper Source and Level on Intestinal Physiology and Growth of Broiler Chickens. Poult. Sci. 85:999-1007. doi 10.1093/ps/85.6.999

Ashmead, H. D., and H. Zunino. 1993. The Roles of Amino Acid Chelates in Animal Nutrition. Noyes Publications, Westwood, New Jersey.

Banks, K. M., K. L. Thompson, J. K. Rush, and T. J. Applegate. 2004. Effects of Copper Source on Phosphorus Retention in Broiler Chicks and Laying Hens Poult. Sci. 83:990-996. doi 10.1093/ps/83.6.990

Bao, Y. M., M. Choct, P. A. Iji, and K. Bruerton. 2007. Effect of Organically Complexed Copper, Iron, Manganese, and Zinc on Broiler Performance, Mineral Excretion, and Accumulation in Tissues. J Appl. Poult. Res. 16:448-455. doi 10.1093/japr/16.3.448

Bartlett, J. R., and M. O. Smith. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult. Sci. 82:1580-1588. doi 10.1093/ps/82.10.1580

Batal, A. B., T. M. Parr, and D. H. Baker. 2001. Zinc Bioavailability in Tetrabasic Zinc Chloride and the Dietary Zinc Requirement of Young Chicks Fed a Soy Concentrate Diet. Poult. Sci. 80:87-90. doi 10.1093/ps/80.1.87

Bodle, B. C., C. Alvarado, R. B. Shirley, Y. Mercier, and J. T. Lee. 2018. Evaluation of different dietary alterations in their ability to mitigate the incidence and severity

of woody breast and white striping in commercial male broilers. Poult. Sci. 97:3298-3310. doi 10.3382/ps/pey166

Brooks, M., J. Grimes, K. Lloyd, S. Verissimo, and J. Speers. 2013. Bioavailability in chicks of zinc from zinc propionate. J Appl. Poult. Res. 22:153-159.

Brooks, M. A., J. L. Grimes, K. E. Lloyd, F. Valdez, and J. W. Spears. 2012. Relative bioavailability in chicks of manganese from manganese propionate. J Appl. Poult. Res. 21:126-130. doi 10.3382/japr.2011-00331

Cao, J., P. Henry, C. Ammerman, R. Miles, and R. Littell. 2000. Relative bioavailability of basic zinc sulfate and basic zinc chloride for chicks. J Appl. Poult. Res. 9:513-517.

Caskey, C. D., W. D. Gallup, and L. C. Norris. 1939. The Need for Manganese in the Bone Development of the ChickOne Figure. The Journal of Nutrition 17:407-417. doi 10.1093/jn/17.5.407

Cervantes, H. M. 2015. Antibiotic-free poultry production: Is it sustainable? J Appl. Poult. Res. 24:91-97. doi 10.3382/japr/pfv006

Collins, N. E., and J. E. T. Moran. 1999. Influence of Supplemental Manganese and Zinc on Live Performance and Carcass Quality of Diverse Broiler Strains. J Appl. Poult. Res. 8:228-235. doi 10.1093/japr/8.2.228

Conly, A. K., R. Poureslami, E. A. Koutsos, A. B. Batal, B. Jung, R. Beckstead, and D. G. Peterson. 2012. Tolerance and efficacy of tribasic manganese chloride in growing broiler chickens. Poult. Sci. 91:1633-1640. doi 10.3382/ps.2011-02056

Cromwell, G. L., M. D. Lindemann, H. J. Monegue, D. D. Hall, and J. D. E. Orr. 1998. Tribasic copper chloride and copper sulfate as copper sources for weanling pigs. J. Anim. Sci. 76:118-123. doi 10.2527/1998.761118x

Dardenne, M., and J. Bach. 1993. Rationale for the mechanism of zinc interaction in the immune system. Marcel Dekker, New York.

Dieck, H. t., F. Döring, H.-P. Roth, and H. Daniel. 2003. Changes in Rat Hepatic Gene Expression in Response to Zinc Deficiency as Assessed by DNA Arrays. The Journal of Nutrition 133:1004-1010. doi 10.1093/jn/133.4.1004

Dozier, W. A., A. J. Davis, M. E. Freeman, and T. L. Ward. 2003. Early growth and environmental implications of dietary zinc and copper concentrations and sources of broiler chicks. Br. Poult. Sci. 44:726-731. doi 10.1080/00071660310001643714

Espinosa, C. D., R. S. Fry, J. L. Usry, and H. H. Stein. 2017. Copper hydroxychloride improves growth performance and reduces diarrhea frequency of weanling pigs fed a corn–soybean meal diet but does not change apparent total tract digestibility of energy and acid hydrolyzed ether extract. J. Anim. Sci. 95:5447-5454. doi 10.2527/jas2017.1702

Fernandes, J., A. Murakami, M. Sakamoto, L. Souza, A. Malaguido, and E. Martins. 2008. Effects of organic mineral dietary supplementation on production performance and egg quality of white layers. Revista Brasileira de Ciência Avícola 10:59-65.

Fraker, P., M. Gershwin, R. Good, and A. Prasad. 1986. Interrelationships between zinc and immune function. Fed. Proc. 45:1474-1479.

Gallup, W. D., and C. Norris. 1939a. The Effect of a Deficiency of Manganese in the Diet of the Hen. Poult. Sci. 18:83-88. doi 10.3382/ps.0180083

Gallup, W. D., and L. C. Norris. 1939b. The Amount of Manganese Required to Prevent Perosis in the Chick. Poult. Sci. 18:76-82. doi 10.3382/ps.0180076

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 Copper from their Organic or Inorganic Sources on Egg Production and Quality Characteristics in Laying Hens. Biol. Trace Elem. Res. 142:557-571. doi 10.1007/s12011-010-8779-x

Harland, B. F., and G. Narula. 1999. Food phytate and its hydrolysis products. Nutr. Res. 19:947-961. doi https://doi.org/10.1016/S0271-5317(99)00055-X

Henry, P. R. 1995. 11 - Manganese bioavailability. Pages 239-256 in Bioavailability of Nutrients for Animals. C. B. Ammerman, D. H. Baker, and A. J. Lewis eds. Academic Press, San Diego.

Henry, P. R., C. B. Ammerman, and R. D. Miles. 1986. Bioavailability of Manganese Sulfate and Manganese Monoxide in Chicks as Measured by Tissue Uptake of Manganese from Conventional Dietary Levels. Poult. Sci. 65:983-986. doi 10.3382/ps.0650983

Henry, P. R., C. B. Ammerman, and R. D. Miles. 1989. Relative Bioavailability of Manganese in a Manganese-Methionine Complex for Broiler Chicks. Poult. Sci. 68:107-112. doi 10.3382/ps.0680107

Holley, A. K., V. Bakthavatchalu, J. M. Velez-Roman, and D. K. St. Clair. 2011. Manganese Superoxide Dismutase: Guardian of the Powerhouse. Int. J. Mol. Sci. 12:7114-7162. doi 10.3390/ijms12107114

Hopcroft, R. L., A. J. Cowieson, W. I. Muir, and P. J. Groves. 2018. Changes to mineral levels in the yolk of meat chicken embryos during incubation. Poult. Sci.:pey423-pey423. doi 10.3382/ps/pey423

Huang, Y. L., L. Lu, X. G. Luo, and B. Liu. 2007. An Optimal Dietary Zinc Level of Broiler Chicks Fed a Corn-Soybean Meal Diet. Poult. Sci. 86:2582-2589. doi 10.3382/ps.2007-00088

Inal, F., B. Coşkun, N. Gülşen, and V. Kurtoğlu. 2001. The effects of withdrawal of vitamin and trace mineral supplements from layer diets on egg yield and trace mineral composition. Br. Poult. Sci. 42:77-80.

Insko, J. W. M., M. Lyons, and J. H. Martin. 1938. The Effect of Manganese, Zinc, Aluminum, and Iron Salts on the Incidence of Perosis in Chicks. Poult. Sci. 17:264-269. doi 10.3382/ps.0170264

Jackson, M. E., H. M. Hellwig, and P. W. Waldroup. 1987. Shell Quality: Potential for Improvement by Dietary Means and Relationship with Egg Size. Poult. Sci. 66:1702-1713. doi 10.3382/ps.0661702

Ji, F., X. G. Luo, L. Lu, B. Liu, and S. X. Yu. 2006. Effects of manganese source and calcium on manganese uptake by in vitro everted gut sacs of broilers' intestinal segments Poult. Sci. 85:1217-1225. doi 10.1093/ps/85.7.1217

Karimi, K., and M. Zhandi. 2015. The effect of β-mannanase and β-glucanase on small intestine morphology in male broilers fed diets containing various levels of metabolizable energy. J. Appl. Anim. Res. 43:324-329. doi 10.1080/09712119.2014.978770

Kennedy, K. J., T. M. Rains, and N. F. Shay. 1998. Zinc Deficiency Changes Preferred Macronutrient Intake in Subpopulations of Sprague-Dawley Outbred Rats and Reduces Hepatic Pyruvate Kinase Gene Expression. The Journal of Nutrition 128:43-49.

Kidd, M. T., P. R. Ferket, and M. A. Qureshi. 1996. Zinc metabolism with special reference to its role in immunity. Worlds Poult. Sci. J. 52:309-324. doi doi:10.1079/WPS19960022

Kucuk, O., N. Sahin, and K. Sahin. 2003. Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. Biol. Trace Elem. Res. 94:225-235. doi 10.1385/bter:94:3:225

Kwun, I.-S., Y.-E. Cho, R.-A. R. Lomeda, S.-T. Kwon, Y. Kim, and J. H. Beattie. 2007. Marginal zinc deficiency in rats decreases leptin expression independently of food intake and corticotrophin-releasing hormone in relation to food intake. Br. J. Nutr. 98:485-489. doi 10.1017/S0007114507730763

Leach, J. R. M., and J. R. Gross. 1983. The Effect of Manganese Deficiency upon the Ultrastructure of the Eggshell. Poult. Sci. 62:499-504. doi 10.3382/ps.0620499

Leach, R., A.-M. Muenster, and E. M. Wien. 1969. Studies on the role of manganese in bone formation: II. Effect upon chondroitin sulfate synthesis in chick epiphyseal cartilage. Arch. Biochem. Biophys. 133:22-28.

Leach, R. M. 1986. Mn (II) and glycosyltransferases essential for skeletal development. Pages 81 in Manganese in metabolism and enzyme function. Academic Press Orlando.

Li, L. L., N. N. Zhang, Y. J. Gong, M. Y. Zhou, H. Q. Zhan, and X. T. Zou. 2017. Effects of dietary Mn-methionine supplementation on the egg quality of laying hens. Poult. Sci. 97:247-254. doi 10.3382/ps/pex301

Li, S., Y. Lin, L. Lu, L. Xi, Z. Wang, S. Hao, L. Zhang, K. Li, and X. Luo. 2011. An estimation of the manganese requirement for broilers from 1 to 21 days of age. Biol. Trace Elem. Res. 143:939-948.

Lim, H. S., and I. K. Paik. 2003. Effects of Supplementary Mineral Methionine Chelates (Zn, Cu, Mn) on the Performance and Eggshell Quality of Laying Hens. Asian-Aust. J. Anim. Sci. 16:1804-1808. doi 10.5713/ajas.2003.1804

Liu, Z. H., L. Lu, S. F. Li, L. Y. Zhang, L. Xi, K. Y. Zhang, and X. G. Luo. 2011. Effects of supplemental zinc source and level on growth performance, carcass traits, and meat quality of broilers. Poult. Sci. 90:1782-1790. doi 10.3382/ps.2010-01215

Longstaff, M., and R. Hill. 1972. The hexosamine and uronic acid contents of the matrix of shells of eggs from pullets fed on diets of different manganese content. British Poultry Science 13:377-385. doi 10.1080/00071667208415961

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. doi 10.2527/jas.2006-229

Luo, X. G., S. F. Li, L. Lu, B. Liu, X. Kuang, G. Z. Shao, and S. X. Yu. 2007. Gene Expression of Manganese-Containing Superoxide Dismutase as a Biomarker of Manganese Bioavailability for Manganese Sources in Broilers. Poult. Sci. 86:888-894. doi 10.1093/ps/86.5.888

Lyons, M. 1938. Manganese metabolism of the laying hen and of the chick embryo. Pages 56-57 in Arkansas Agricultural Experiment Station University of Arkansas Fayetteville, Arkansas.

Lyons, M. 1939. Some Effects of Manganese on Eggshell Quality. Arkansas Agricultural Experimental Station Bulletin 374.

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.:3176-3182. doi 10.3382/ps/pey197

Mabe, I., C. Rapp, M. M. Bain, and Y. Nys. 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult. Sci. 82:1903-1913. doi 10.1093/ps/82.12.1903

Manangi, M. K., M. Vazques-Añon, J. D. Richards, S. Carter, and C. D. Knight. 2015. The impact of feeding supplemental chelated trace minerals on shell quality, tibia breaking strength, and immune response in laying hens. J Appl. Poult. Res. 24:316-326. doi 10.3382/japr/pfv029

McNaughton, J. L., E. J. Day, B. C. Dilworth, and B. D. Lott. 1974. Iron and Copper Availability from Various Sources. Poult. Sci. 53:1325-1330. doi 10.3382/ps.0531325

Medicine, I. o. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. The National Academies Press, Washington, DC.

Miles, R. D., and P. R. Henry. 2000. Relative trace mineral bioavailability. Ciência Animal Brasileira 1:73-93.

Miles, R. D., S. F. O'Keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. Poult. Sci. 77:416-425. doi 10.1093/ps/77.3.416

Mondal, S., S. Haldar, P. Saha, and T. K. Ghosh. 2010. Metabolism and tissue distribution of trace elements in broiler chickens' fed diets containing deficient and plethoric levels of copper, manganese, and zinc. Biol. Trace Elem. Res. 137:190-205.

Naber, E. C. 1979. The Effect of Nutrition on the Composition of Eggs. Poult. Sci. 58:518-528. doi 10.3382/ps.0580518

Nielsen, F. H. 1999. Ultratrace minerals. Modern Nutrition in Health and Disease. 8.

Nollet, L., G. Huyghebaert, and P. Spring. 2008. Effect of Different Levels of Dietary Organic (Bioplex) Trace Minerals on Live Performance of Broiler Chickens by Growth Phases1. J Appl. Poult. Res. 17:109-115. doi 10.3382/japr.2007-00049

Nollet, L., J. D. van der Klis, M. Lensing, and P. Spring. 2007. The Effect of Replacing Inorganic With Organic Trace Minerals in Broiler Diets on Productive Performance and Mineral Excretion. J Appl. Poult. Res. 16:592-597. doi 10.3382/japr.2006-00115

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. ed. National Academy Press Washington, D.C.

Olukosi, O. A., S. van Kuijk, and Y. Han. 2018. Copper and zinc sources and levels of zinc inclusion influence growth performance, tissue trace mineral content, and carcass yield of broiler chickens. Poult. Sci. 97:3891-3898. doi 10.3382/ps/pey247

Organization, W. H. 1996. Trace elements in human nutrition and health. World Health Organization, Geneva, Switzerland.

Pang, Y., and T. J. Applegate. 2007. Effects of Dietary Copper Supplementation and Copper Source on Digesta pH, Calcium, Zinc, and Copper Complex Size in the Gastrointestinal Tract of the Broiler Chicken. Poult. Sci. 86:531-537. doi 10.1093/ps/86.3.531

Park, S., S. Birkhold, L. Kubena, D. Nisbet, and S. Ricke. 2004. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biol. Trace Elem. Res. 101:147-163.

Perez, V., R. Shanmugasundaram, M. Sifri, T. M. Parr, and R. K. Selvaraj. 2017. Effects of hydroxychloride and sulfate form of zinc and manganese supplementation on superoxide dismutase activity and immune responses post lipopolysaccharide challenge in poultry fed marginally lower doses of zinc and manganese. Poult. Sci. 96:4200-4207. doi 10.3382/ps/pex244

Pesti, G. M., and R. I. Bakalli. 1996. Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poult. Sci. 75:1086-1091.

Rao, S. Year. A Nutritionist's Perspective on the Removal of Antibiotics in Poultry Production. Proc. 2017 Animal Nutrition Conference of Canada, Quebec City, Canada.

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. 1997. Trace mineral metabolism in the avian embryo. Poult. Sci. 76:152-164.

Rimbach, G., J. Pallauf, J. Moehring, K. Kraemer, and A. M. Minihane. 2008. Effect of dietary phytate and microbial phytase on mineral and trace element bioavailability: a literature review. Current Topics in Nutraceutical Research 6:131-144.

Roberts, J. R. 2004. Factors Affecting Egg Internal Quality and Egg Shell Quality in Laying Hens. Poult. Sci. 41:161-177. doi 10.2141/jpsa.41.161

Rossi, P., F. Rutz, M. Anciuti, J. Rech, and N. Zauk. 2007. Influence of graded levels of organic zinc on growth performance and carcass traits of broilers. J. Appl. Poult. Res. 16:219-225.

Rucker, R. B., T. Kosonen, M. S. Clegg, A. E. Mitchell, B. R. Rucker, J. Y. Uriu-Hare, and C. L. Keen. 1998. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. The American Journal of Clinical Nutrition 67:996-1002.

Saenmahayak, B., S. F. Bilgili, J. B. Hess, and M. Singh. 2010. Live and processing performance of broiler chickens fed diets supplemented with complexed zinc. J Appl. Poult. Res. 19:334-340. doi 10.3382/japr.2010-00166

Sahu, A. 2004. Minireview: A Hypothalamic Role in Energy Balance with Special Emphasis on Leptin. Endocrinology 145:2613-2620. doi 10.1210/en.2004-0032

Saleh, A. A., M. M. Ragab, E. A. M. Ahmed, A. M. Abudabos, and T. A. Ebeid. 2018. Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature. J. Appl. Anim. Res. 46:820-827. doi 10.1080/09712119.2017.1407768

Salim, H., C. Jo, and B. Lee. 2008. Zinc in broiler feeding and nutrition. Avian Biology Research 1:5-18.

Santos, T., C. Connolly, and R. Murphy. 2015. Trace Element Inhibition of Phytase Activity. Biol. Trace Elem. Res. 163:255-265. doi 10.1007/s12011-014-0161-y

Sazzad, H. M., A. G. Bertechini, and P. T. C. Nobre. 1994. Egg production, tissue deposition and mineral metabolism in two strains of commercial layers with various levels of manganese in diets. Anim. Feed Sci. Technol. 46:271-275. doi https://doi.org/10.1016/0377-8401(94)90145-7

Sebastian, S., S. P. Touchburn, E. R. Chavez, and P. C. Lague. 1996. The Effects of Supplemental Microbial Phytase on the Performance and Utilization of Dietary Calcium, Phosphorus, Copper, and Zinc in Broiler Chickens Fed Corn-Soybean Diets. Poult. Sci. 75:729-736. doi 10.3382/ps.0750729

Segen's, M. D. 2012. Trace Minerals in Segen's Medical DictionaryFarlex, Inc., Huntingdon Valley, PA.

Shaeffer, G. L., K. E. Lloyd, and J. W. Spears. 2017. Bioavailability of zinc hydroxychloride relative to zinc sulfate in growing cattle fed a corn-cottonseed hull-based diet. Anim. Feed Sci. Technol. 232:1-5. doi https://doi.org/10.1016/j.anifeedsci.2017.07.013

Sihvo, H.-K., K. Immonen, and E. Puolanne. 2014. Myodegeneration With Fibrosis and Regeneration in the Pectoralis Major Muscle of Broilers. Vet. Pathol. 51:619-623. doi 10.1177/0300985813497488

Smith, M. O., I. L. Sherman, L. C. Miller, K. R. Robbins, and J. T. Halley. 1995. Relative Biological Availability of Manganese from Manganese Proteinate, Manganese Sulfate, and Manganese Monoxide in Broilers Reared at Elevated Temperatures. Poult. Sci. 74:702-707. doi 10.3382/ps.0740702

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Anim. Feed Sci. Technol. 58:151-163. doi https://doi.org/10.1016/0377-8401(95)00881-0

SPSS. IBM Corp. *SPSS Version 18.0,* Armonk, NY.

Star, L., J. D. van der Klis, C. Rapp, and T. L. Ward. 2012. Bioavailability of organic and inorganic zinc sources in male broilers. Poult. Sci. 91:3115-3120. doi 10.3382/ps.2012-02314

Sunder, G. S., V. K. Chelasani, A. K. Panda, V. R. Mantena, and R. R. Savaram. 2011. Effect of supplemental inorganic Zn and Mn and their interactions on the performance of broiler chicken, mineral bioavailability, and immune response. Biol. Trace Elem. Res. 139:177-187.

Sunder, G. S., A. K. Panda, N. C. S. Gopinath, S. V. R. Rao, M. V. L. N. Raju, M. R. Reddy, and C. V. Kumar. 2008. Effects of Higher Levels of Zinc Supplementation on Performance, Mineral Availability, and Immune Competence in Broiler Chickens. J Appl. Poult. Res. 17:79-86. doi 10.3382/japr.2007-00029

Suttle, N. F. 2010. Mineral nutrition of livestock. 4th ed. CAB Int., London, UK.

Swiatkiewicz, S., and J. Koreleski. 2008. The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality. Vet. Med. (Praha) 53:555-563.

Titus, H. W. 1932. Perosis, or Deforming Leg Weakness, in the Chicken. Poult. Sci. 11:117-125. doi 10.3382/ps.0110117

Vallee, B. L., and D. S. Auld. 1990. Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry 29:5647-5659. doi 10.1021/bi00476a001

Van der Hoorn, R., H. D. Branion, and W. R. Graham Jr. 1938. Studies in the nutrition of the chick. III. A maintenance factor present in wheat germ and the effect of the addition of a small amount of manganese dioxide to the diet. Poult. Sci. 17:185-192.

Venglovska, K., L. Gresakova, I. Placha, M. Ryzner, and K. Cobanova. 2014. Effects of feed supplementation with manganese from its different sources on performance and egg parameters of laying hens. Czech Journal of Animal Science 59:147-155.

Wedekind, K., G. Collings, J. Hancock, and E. Titgemeyer. 1994. The bioavailability of zinc-methionine relative to zinc sulfate is affected by calcium level. Poultry Sci 73:114.

Wedekind, K., A. Hortin, and D. Baker. 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. J. Anim. Sci. 70:178-187.

Wilgus Jr, H., and A. Patton. 1939. Factors affecting manganese utilization in the chicken. J. Nutr. 18:35-45.

Wong-Valle, J., C. B. Ammerman, P. R. Henry, P. V. Rao, and R. D. Miles. 1989. Bioavailability of Manganese from Feed Grade Manganese Oxides for Broiler Chicks. Poult. Sci. 68:1368-1373. doi 10.3382/ps.0681368

Xiao, J. F., S. G. Wu, H. J. Zhang, H. Y. Yue, J. Wang, F. Ji, and G. H. Qi. 2015. Bioefficacy comparison of organic manganese with inorganic manganese for eggshell quality in Hy-Line Brown laying hens. Poult. Sci. doi 10.3382/ps/pev138

Xiao, J. F., Y. N. Zhang, S. G. Wu, H. J. Zhang, H. Y. Yue, and G. 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. doi 10.3382/ps.2013-03354

Yang, X., L. Zhong, X. An, N. Zhang, L. Zhang, J. Han, J. Yao, C. Cote, and Y. Sun. 2012. Effects of diets supplemented with zinc and manganese on performance and related parameters in laying hens. Animal Science Journal 83:474-481.

Yang, X. J., X. X. Sun, C. Y. Li, X. H. Wu, and J. H. Yao. 2011. Effects of copper, iron, zinc, and manganese supplementation in a corn and soybean meal diet on the growth performance, meat quality, and immune responses of broiler chickens. J Appl. Poult. Res. 20:263-271. doi 10.3382/japr.2010-00204

Yildiz, A., Y. Cufadar, and O. Olgun. 2011. Effects of dietary organic and inorganic manganese supplementation on performance, egg quality and bone mineralisation in laying hens. Rev. Med. Vet. (Toulouse) 162:482-488.

Yildiz, A. O., O. Olgun, and Y. Cufadar. 2010. The effect of manganese and phytase in the diet for laying hens on performance traits and eggshell quality. J. Anim. Vet. Adv. 9:32-36.

Zamani, A., H. Rahmani, and J. Pourreza. 2005a. Effect of different levels of manganese and zinc on performance traits and breaking eggs in laying hens. Pak. J. Biol. Sci 8:1035-1040.

Zamani, A., H. Rahmani, and J. Pourreza. 2005b. Supplementation of a corn-soybean meal diet with manganese and zinc improves eggshell quality in laying hens. Pak. J. Biol. Sci 8:1311-1317.

Zhao, J., R. B. Shirley, J. J. Dibner, K. J. Wedekind, F. Yan, P. Fisher, T. R. Hampton, J. L. Evans, and M. Vazquez-Añon. 2016. Superior growth performance in broiler chicks fed chelated compared to inorganic zinc in presence of elevated dietary copper. Journal of Animal Science and Biotechnology 7:13-21. doi 10.1186/s40104-016-0072-1

Zhao, J., R. B. Shirley, M. Vazquez-Anon, J. J. Dibner, J. D. Richards, P. Fisher, T. Hampton, K. D. Christensen, J. P. Allard, and A. F. Giesen. 2010. Effects of chelated trace minerals on growth performance, breast meat yield, and footpad health in commercial meat broilers. J Appl. Poult. Res. 19:365-372. doi 10.3382/japr.2009-00020

Zhu, Y. W., J. J. Xie, W. X. Li, L. Lu, L. Y. Zhang, C. Ji, X. Lin, H. C. Liu, J. Odle, and X. G. Luo. 2015. Effects of environmental temperature and dietary manganese on egg production performance, egg quality, and some plasma biochemical traits of broiler breeders. J. Anim. Sci. 93:3431-3440. doi 10.2527/jas.2015-8956