2022

Trace Mineral Source and Inclusion Level on Wean-to-Finish Pig Production

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TRACE MINERAL SOURCE AND INCLUSION LEVEL ON WEAN-TO-FINISH PIG PRODUCTION

BY

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A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Animal Science

South Dakota State University

2022

THESIS ACCEPTANCE PAGE

Marissa LaRosae

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ACKNOWLEDGEMENTS

I would like to start by thanking my advisor Dr. Ryan Samuel for the opportunity to complete my Master's program under his guidance at South Dakota State University. In addition to Dr. Samuel's guidance, I am appreciative of the mentorship of Drs. Robert Thaler, Crystal Levesque, and Eric Weaver as they played a critical role in my education at SDSU. Drs. Robert Thaler and Laura Hasselquist, thank you for serving on my advisory committee and providing guidance in your areas of expertise.

Thank you to all of my fellow graduate students who helped with data collections, laboratory analysis, and statistical analysis. A special thanks to Saulo Zoca who spent countless hours in the computer lab discussing statistics and Talia Everding who helped re-run and trouble shoot my lab analysis. I am forever grateful and indebted to David Clizer, who started as my lab mate but quickly turned into a mentor and friend always providing me help and guidance when he could. To my undergraduate students Alyssa Streich and Emily Walton, thank you for all of your help with weigh days, data collection, labeling tubes, etc. you played a bigger role in the completion of my research than I can express to you. I would also like to thank Analicia Swanson, Hannah Miller, and Jordan Jansen for their friendship both inside and outside of school. I wouldn't have made it to the finish line without all of their words of encouragement and all of our crazy adventures that kept me sane during the hard times.

I am ever grateful for Dr. Kara Stewart for taking me under her wing during my time at Purdue University. Her mentorship and passion for the swine industry inspired me to continue my education. Additionally, I would like to thank Dr. Casey Bradley of the Sunswine Group for providing me with endless opportunities throughout my Master's

program to develop my soft skills and grow my professional network. To all the others who have played a mentorship role during my educational career- from the bottom of my heart, thank you for seeing my potential and believing in me.

Saving the biggest thank you for last, I cannot express my gratitude for the support and encouragement that my family and friends have provided throughout my education. The text messages, phone calls, and visits to South Dakota did not go unrecognized- thank you.

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ABBREVIATIONS

AA	Amino Acid
ADF	Acid detergent fiber
ADG	Average daily gain
ADFI	Average daily feed intake
BF	Backfat
BW	Body weight
CP	Crude protein
Ca	Calcium
Cl	Chloride
Co	Cobalt
Cr	Chromium
Cu	Copper
$CuSO_4$	Copper sulfate
d	Days(s)
DDGS	Dried distillers' grains with solubles
Fe	Iron
g	gram
ga	gauge
G:F	Gain to feed ratio
HCW	Hot carcass weight
I	Iodine
Isc	Short-circuit current

K	Potassium
kg	Kilograms
LD	loin depth
L:M	Lactulose to mannitol ratio
m	Meter(s)
Mg	Magnesium
Mn	Manganese
Na	Sodium
NDF	Neutral detergent fiber
P	Phosphorus
ppm	Parts per million
PH	Phase(s)
S	Sulfur
SAS	Statistical analysis system
Se	Selenium
TBCC	Tribasic copper chloride
TEER	Transepithelial resistance
Zn	Zinc
ZnO	Zinc oxide

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ABSTRACT

TRACE MINERAL SOURCE AND INCLUSION LEVEL ON WEAN-TO-FINISH PIG PRODUCTION

MARISSA N. LAROSAE

2022

Trace mineral requirements to optimize production for swine are not well defined, leading to excess trace mineral supplementation in swine diets and ultimately to excess trace minerals excreted in swine manure. Potential strategies to reduce the waste of excess trace minerals in diets include utilizing organic sources with higher bioavailability or reducing the overall trace mineral supplementation in the diet. Two studies were conducted in an effort to determine if utilizing organic trace minerals or reducing trace mineral inclusion into diets has an impact on overall performance measurements for nursery and grow-finish pigs, respectively. The first experiment utilized 1,144 weaned pigs (21-d of age, 5.8 ± 0.1 kg) in a 42-d nursery trial to evaluate the effect of zinc oxide (ZnO) and organic zinc (Zn) on growth performance, gut permeability, and fecal consistency. Dietary treatments were applied in a factorial arrangement and consisted of a complex commercial nursery diet with Zn at nutritional requirement (NRC, 2012) with: 1) no additional Zn, 2) addition of Zn from ZnO, 3) addition of Zn from organic Zn, or 4) a combination of ZnO and organic Zn. Zinc from ZnO was provided at 3000 ppm for PH1-2, 1500 ppm for PH3, and removed at PH4 and Zn from organic Zn was provided at 100 ppm for PH1-4. Dietary treatments were replicated eleven times and each pen contained 26 pigs balanced for sex. Data was analyzed as a 2 x 2 factorial design

comparing factors of Zn source (ZnO vs. organic Zn) and Zn inclusion (with or without the addition of ZnO or organic Zn) and the interaction between factors as the main effects with pen as the experimental unit. The addition of organic Zn increased (P<0.01) ADFI from d 0 to d 7 (0.175 vs. 0.149 kg/d), but decreased (P<0.01) G:F (0.763 vs. 0.902) and then improved (P<0.01) G:F from d 7 to d 14 (0.821 vs. 0.754). The addition of Zn from ZnO increased (P<0.05) ADG (0.312 vs. 0.290 kg/d) and ADFI (0.428 vs. 0.401 kg/d) for the initial 28 d and improved (P<0.05) G:F (0.776 vs. 0.738) for the initial 21 d post-weaning, but not for the latter 14 d (28 to 42 d) of the nursery phase or overall (0 to 42 d). There were no differences in measured gut permeability with addition of Zn. However, supplementation from either Zn source resulted in some improvements in fecal consistency.

In the second experiment, 442 growing pigs in two groups (group 1: n=195; 34.8±0.9 kg initial BW; group 2: n=247; 39.1±2.6 kg initial BW) were followed through phase 6 to 9 of a 9 phase feeding program to evaluate the effect of trace mineral inclusion level on growing-finishing pig performance, trace mineral fecal, manure, and serum concentrations, and carcass characteristics. Each group utilized one room with 20 pens, two feeder types (dry and wet-dry), and four pits. Shallow manure pit location of each room and feeder type dictated dietary treatment as either 1) corn-soybean meal diet with full trace mineral supplementation or 2) corn-soybean meal with 50% trace mineral supplementation. Full and reduced trace mineral supplementation from a commercial trace mineral premix both exceed NRC (2012) recommendations. Overall, there were no differences (P>0.05) observed in ADG, ADFI, or G:F between pigs fed full or 50% trace

mineral supplementation. This supports previous observations that reducing or eliminating dietary trace mineral supplementation does not impact pig performance. In contrast, the accumulated concentration of Fe (230 vs. 136 ppm) and Mn (63 vs. 32 ppm) in the pit manure samples were reduced ($P<0.01$) by feeding 50% trace minerals. Similarly, trace mineral concentrations in the fecal samples were reduced ($P<0.01$) for Cu, Fe, Mn, and Zn when pigs were fed 50% trace minerals. However, there were no differences ($P>0.05$) in carcass characteristics between pigs supplemented with the different rates of dietary trace mineral.

In overall conclusion, supplementation of Zn early in the nursery period provided benefits to growth performance and improved fecal consistency, with no measured impact on gut permeability. Reducing trace mineral inclusion level for grow-finish pigs did not impact growth performance or carcass characteristics, but did decrease the trace mineral concentrations found in the feces and pit manure. These results suggest that supplementation of organic Zn at lower concentrations than pharmacological levels of ZnO has the potential to produce similar benefits in weaned pigs and reducing trace mineral supplementation in diets for grow-finish pigs can reduce trace mineral excretion in the manure without any impact on productivity.

CHAPTER 1

LITERATURE REVIEW

1.1 THE WEANED PIG

Weaning is a critical time as the pig experiences significant physical, environmental and social challenges that can predispose the pig to subsequent diseases, post-wean diarrhea and additional production losses. In commercial pig production, weaning occurs abruptly between 14 to 30 days of age with industry average around 21 days of age. In nature, the process of weaning happens gradually, approaching completion around 10 to 12 weeks of age (Moeser et al., 2017). It has been demonstrated that increasing wean age from 12 to 21.5 days of age resulted in linear improvements in growth rate and feed efficiency, reductions in mortality and increased weight sold per pig weaned (Main et al., 2004). Moreover, recently (Faccin et al., 2020) reported growth performance of weaned pigs increases linearly with increasing wean age to 25 days of age, which appears to be the optimal weaning age. When pigs are weaned, they undergo the stress of an abrupt removal from the sow, transportation to a new facility, a different physical environment, co-mingling with pigs from other litters, and dietary stress (Campbell et al., 2013). Weaned pigs must rapidly adapt and overcome these stressors in order to be productive and efficient; therefore, many factors need to be considered when feeding the weaned pig. This part of the review will focus on nutrient absorption and digestive capabilities, gut permeability, and growth performance of the weaned pig.

1.1.1 Nutrient absorption and digestive capabilities

The digestive process of feed starts in the mouth where particle size is reduced and surface area is increased through manual grinding due to chewing and saliva is mixed with feed particles to begin the chemical breakdown. Next, the feed travels through the pharynx and the esophagus to enter into the stomach. In the stomach, the breakdown of nutrients is initiated and the digesta is then passed into the small intestine. The small intestine is divided into three sections consisting of the duodenum, jejunum, and ileum. The primary function of the duodenum is digestion where the breakdown of macromolecules (carbohydrates, proteins, and lipids) into their specific monomers continues, meanwhile the jejunum and ileum play a major role in absorbing those macromolecules across the intestine to enter into the blood stream (Feher, 2017). The intestinal wall is a single layer of epithelial cells, or enterocytes, that compose the finger-like villi which are primarily responsible for the transport of nutrients into the bloodstream. The finger-like villi are projections into the lumen that allow for increased surface area of the intestine on the apical surface of the enterocytes; microvilli continue to amplify the surface area of the enterocytes lining the intestine (Lewis and Southern, 2000). These villi and microvilli comprise the intestinal mucosa, also known as the brush border membrane. The brush border membrane is also responsible for the production and release of digestive enzymes. In between each villus, there are crypts of Lieberkühn, which produce new epithelial cells to constantly mature as they migrate up the villus and then will be sloughed off at the tip of the villus within a few days of being produced (Feher, 2017).

The gastrointestinal tract of the pig is responsible for a multitude of different functions, including digestion and absorption of nutrients, serving as a barrier between the host animal and outside pathogens and antigens, and supplying necessary digestive enzymes, mucin, and other components needed in the digestion and absorption process (Campbell et al., 2013). As a result of weaning stress, young pigs produce a high concentration of free radicals which destroys the redox equilibrium (Yin et al., 2013). This leads to physiological and mechanical changes to the structure and function of the intestine in the weaned pig. These physiological changes affect the absorptive capacity of the small intestine after weaning by inducing both acute and long-lasting structural changes including shortened villi (villous atrophy) and increased crypt depth (crypt elongation) (Pluske et al., 1997; Boudry et al. 2004). Additionally, weaned pigs experience a reduction in brush-border enzyme production and activity when a new diet is introduced, which can interrupt digestion and absorption of feeds (Lallès et al., 2004; Campbell et al., 2013). More specifically, lactase (responsible for lactose digestion) activity decreases after weaning, which may be directly related to the change in dietary composition away from sow milk (Pluske et al., 1997; Pié et al., 2004). Alkaline phosphatase, an enzyme that plays a role in detoxification of pathogenic bacterial lipopolysaccharide endotoxin and impacts intestinal inflammation (Lallès, 2010), is also reduced in weaned pigs compared to suckling pigs (Lackeyram et al., 2010). In contrast, aminopeptidase and dipeptidylpeptidase, proteolytic enzymes responsible for protein digestion, are shown to increase within 5 to 9 days post-weaning (Hedemann et al., 2003). Lallès et al. (2004) demonstrated that maltase activity increased day 8 to 15 after weaning and pancreatic enzymes trypsin and amylase activity began to increase 15 days

after weaning. For quite some time, the activity of brush–border enzymes have been a marker of cell maturation and digestive capabilities (Henning, 1985; Hampson and Kidder, 1986). These alterations to digestive and absorptive capacity of the intestine can ultimately impact the intestinal barrier function. Maximizing feed intake after weaning can help sustain an adequate gut structure for nutrient absorption (Pluske et al., 1996) and to reduce the occurrence of post-wean diarrhea in weaned pigs (Madec et al., 1998).

1.1.2 Gut permeability

The epithelial layer of the intestinal lumen has three primary functions. The first function is the secretion and absorption of water and electrolytes to maintain proper viscosity of the luminal contents and to flush out any harmful components (Wijtten et al., 2011). Secondly, to prevent an overwhelming immune activation by serving as a barrier to the pathogenic and antigenic components in the lumen, which is critical for host survival (Moeser et al., 2017). Lastly, the digestion of food and absorption of essential nutrients, such as water, sugar, peptides, and lipids (Kong et al., 2018), via brush border enzymes and an array of nutrient transporters (Moeser et al., 2017). Particles can move through the epithelial cells via transcellular or paracellular transport. Transcellular transport is directional, energy dependent, and controlled by cell-specific transporters and channels, whereas paracellular transport is passive and results from diffusion or osmosis down the concentration gradients created by transcellular mechanisms (Anderson, 2001). Thus, the paracellular pathway complements the transcellular mechanisms by determining the degree and selectivity of back leak for ions and solutes (Bazzoni et al., 2000; Powell, 1981). The tight junctions exist between cells and serve as the primary

physical structure defining the specific properties of the paracellular barrier (Anderson, 2001). Tight junctions are made up of several proteins which are involved in maintaining cell polarity, in establishing organ-specific apical domains, and, lastly, in recruiting signaling proteins involved the regulation of multiple cellular functions such as proliferation, differentiation, and migration. (Bhat et al., 2019). Thus, tight junctions create a major barrier, which is variable, regulating paracellular movement of water and solutes across the epithelia (Powell, 1981; Mitic et al., 2000). Ultimately, the responsibility of tight junctions is to maintain the integrity and permeability of the intestine (Blikslager et al., 2007). As a critical part of the epithelial barrier, tight junctions are under a constant threat from pathogenic viruses and bacteria, proinflammatory mediators, and the development of disease (Bhat et al., 2019). Tight junctions only allow predetermined particles to pass through and are important to regulate the epithelium to prevent “leaky gut”, which can lead to enteric diseases (Moeser et al., 2017). Leaky gut may be the result of tight junction dysfunction, otherwise known as increased intestinal permeability, allowing various unwanted digesta, pathogens, and toxins to leak through the gut lining into the blood stream initiating an inflammatory response in the body. After weaning, pigs are highly susceptible to infections (Lallès et al., 2004) due to weaning stress being a crucial factor related to the compromised paracellular barrier function (Wijtten et al., 2011). The impact of weaning on intestinal permeability in pigs has been widely researched and there is evidence to show that there is decreased intestinal barrier function at weaning, when compared to pre-weaning levels (Boudry et al., 2004; Moeser et al, 2007; Wijtten et al., 2011; Moeser et al., 2017). However, adequate feed intake

levels after weaning (Wijtten et al., 2011) and weaning at 28 days, compared to weaning at 19 days, prevent the loss of the intestinal barrier function (Moeser et al., 2007).

Intestinal permeability can be measured to determine the effects of weaning stress, using *in-vivo* or *ex-vivo* methods. The *ex-vivo* method typically includes utilizing Ussing chambers, where a section of intestinal mucosa is mounted between two chambers and marker probes, such as mannitol, fluoresceine isothiocyanate dextran (FD4), and horseradish peroxide (HRP) (Lallès et al., 2004) are added to the solution in the chamber at the mucosal site. The appearance of these marker probes at the serosal side of the chamber indicates the permeability of the intestinal tissue to these probes (Wijtten et al., 2011). Mannitol is a small molecule that can pass through the epithelium via paracellular transport when tight junctions are fully functional, therefore measuring paracellular permeability (Galipeau and Verdu, 2016). In contrast, FD4 and HRP are larger molecules that cannot pass through the cell paracellularly unless there is an interruption in the tight junction barrier (Wijtten et al., 2011). Three of the electrophysical parameters that can be determined in Ussing chambers are transepithelial electrical resistance (TEER), transepithelial electrical conductance, and short-circuit current (Isc). First, TEER is a measurement that reflects the opening of tight junctions between epithelial cells and how well the tight junctions are inhibiting the movement of charged molecules (Li et al., 2004). An increased TEER represents decreased paracellular permeability; likewise, a decreased TEER represents increased paracellular permeability (Wijtten et al., 2011). Secondly, the transepithelial electrical conductance is the inverse of TEER. Lastly, Isc which Li et al. (2004), defined as ‘the charge per unit time when the tissue is short-

circuited', measures the active electrogenic ion transport across the epithelium. An increased Isc can reflect two things: 1) increased electrogenic anion secretion (e.g. Cl^- and HCO_3^-) and 2) increased electrogenic cation absorption (Wijtten et al., 2011).

The *in-vivo* method of measuring intestinal permeability utilizes orally administered test probes that are not metabolized in the body, can pass through the intestinal epithelium, and then are excreted in the urine within a short time of administering (Wijtten et al., 2011). Commonly, both a disaccharide and monosaccharide are used, which are both transported through the epithelium via passive diffusion. Lactulose, a disaccharide, does not undergo carbohydrate digestion like other sugars and can pass through the epithelial membrane via paracellular transport when tight junctions are not fully functional. On the other hand, mannitol can pass through epithelial cells via both paracellular and transcellular transport. These two sugars have similar transit and uptake processes (Camilleri et al., 2010). Both sugars are not metabolized by the pig and are absorbed into the bloodstream as whole molecules, transported to the kidney, and then excreted in the urine. Therefore, a ratio of lactulose:mannitol in the urine can be used as a predictor of gut permeability. An increase in the lactulose:mannitol ratio represents a decrease in the intestinal barrier function, whereas, when the ratio decreases, this indicates an increase in the intestinal barrier function (Wijtten et al., 2011). Nguyen et al. (2014), observed that pigs exhibiting intestinal inflammation, which can occur after weaning, have been found to have higher lactulose:mannitol ratios in their urine in comparison to pigs without inflammation. In addition, Zhang and Guo (2009) and Li et al

(2018) observed that there was an increase in the ratio of lactulose:mannitol following weaning.

1.1.3 Growth Rate

Several factors contribute to the growth rate of weaned pigs including age and weight at weaning, feed intake, and health status of the pig. Weaning pigs at 21 days or later is beneficial to ameliorate the stressful effects of weaning and improve growth performance in the nursery period (Main et al., 2004; Moeser et al., 2007). In fact, Colson et al. (2006) demonstrated that weaning at 21 days of age when compared to weaning at 28 days of age, has more negative consequences on growth rate. Furthermore, because feed intake contributes directly to the growth rate and health status of weanling pigs, any increase in feed intake improves growth rate and lean tissue deposition (Menegat et al., 2019). In addition, maximizing feed intake after weaning can help sustain an adequate gut structure for nutrient absorption (Pluske et al., 1996) and to reduce the occurrence of post-wean diarrhea in weaned pigs (Madec et al., 1998). As discussed previously, adequate feed intake after weaning and a later wean age can both lead to improved gut barrier function. Research has shown that additional body weight gained during the nursery period translates to fewer days to market (DeRouchey et al., 2007). Therefore, improving feed intake and gut barrier function are critical to getting the wean pig started and maximizing gain during the nursery period.

1.2 WEAN-TO-FINISH FEEDING PROGRAM

Diet formulation is the process of selecting ingredients and amounts of the ingredients to be used in the production of a diet. A balanced swine diet provides the

necessary nutrients in the appropriate proportions based upon available information such as the NRC (2012) recommendations to adequately meet the animals' nutritional requirements. Nutrients required by the pig can be grouped into five main groups: energy, protein, minerals, vitamins and water. Water is supplemented in addition to the diet, whereas the rest of the nutrients are met through feed ingredients included in the diet. For swine, feed cost represents 55 to 70 % of the total cost of production. Therefore, in addition to meeting the pigs' nutrient requirements, ingredients should be evaluated for cost, nutrient composition, nutrient availability, and functional value. Nutritionists can use these considerations alongside their production goals to formulate economically sound diets. This part of the review will focus on the different nutrient requirements, feed ingredients, and considerations when formulating a feeding program.

1.2.1. Phase feeding

Dietary nutrient requirements of the pig change as feed intake increases and growth occurs. Therefore, the strategy of phase feeding is commonly practiced in the swine industry. Phase feeding is a term used to describe the feeding of several diets for short periods of time in order to more closely meet the nutrient requirements of the pigs (In K. Han et al, 2000). Phases may be based on feed budgets or weight ranges and are a way to improve a feeding program both economically and environmentally. It is a common industry practice to utilize a seven to eight phase feeding program from wean-to-finish, with a four phase nursery program and three to four phase grow-finish program.

1.2.2. Wean-to-finish diet formulation consistent factors

Factors to consider when formulating nursery diets versus grow-finish diets include: the ability of the pigs to utilize nutrients from different feedstuffs, nutrient requirement differences, different feeding strategies, and feed intake. However, when looking across nursery and grow-finish diet formulations, there are some consistencies in the indispensable amino acids, use of synthetic amino acids, maintaining a calcium:phosphorus ratio, regulating sodium and potassium intake, and the inclusion of vitamins and trace minerals above the defined NRC (2012) requirements.

1.2.2.1. Use of synthetic amino acids

Synthetic amino acids such as L-Lysine HCL, DL-Methionine, L-Threonine, L-tryptophan, L-valine, and L-isoleucine may be added to supplement each phase of a wean-to-finish feeding program to ensure that dietary amino acid requirements are met. Soybean meal is recognized as the “gold standard” of supplemental protein sources due to the high protein content and well balanced and digestible amino acid profile. Additionally, the amino acid profile of soybean meal complements the amino acids deficient in corn. Corn is the most common cereal grain used in swine diets in the US as it contains a greater energy level compared to other cereal grains due to its high concentration of starch and oil. The lysine content in corn is limited compared to the requirement of the pig. Therefore, in traditional corn-soybean meal diets, the inclusion of soybean meal is based on providing sufficient dietary lysine, resulting in high crude protein levels. Alternatively, increasing the supplementation of synthetic amino acids has been demonstrated to increase nitrogen use, reduce feed costs, and promote gut health

without impacting growth performance (Kerr et al, 2003; Nyachoti et al, 2006). Thus, beginning in 1950, inclusion of feed-grade amino acids in diets to supplement dietary protein sources has become an adopted practice in animal agriculture (Kidd et al., 2013).

1.2.2.2. Calcium and phosphorus

Calcium and phosphorus are the most abundant minerals in the pig as they play an important role in development and maintenance of the skeletal system and many other regulatory functions. Requirements of both calcium and phosphorus must be met through diet formulation. Feedstuffs of animal and plant origin may provide calcium, however calcium from feed ingredients is not always fully digested and absorbed by the pig. Additionally, previous data indicated that calcium digestibility was reduced by increasing dietary phytate (Almaguer et al., 2014) and phytate can chelate calcium ions and form calcium-phytate compounds (Selle et al., 2009). Phytate is the primary form of phosphorus storage in feedstuffs of plant origin, however because pigs lack the secretion of phytase in the body, they cannot fully utilize the phytate-bound phosphorus. Phytase is an enzyme that directly acts on phytate, releasing phytate-bound phosphorus in a form more available to the animal (Jacela et al., 2010). Supplemental phytase has been shown to increase the digestibility of both calcium and phosphorus (Gonzáles- Vega et al., 2015). Calcium is often supplied to the diet through limestone and supplemental calcium phosphate sources like mono- and di-calcium phosphate. The digestibility of calcium from mono- and di-calcium phosphate appears not to be affected by the use of exogenous phytase as the calcium is already bound to phosphoric acid (Walk, 2016). When formulating diets an adequate ratio of calcium:phosphorus must be maintained due to

excess or deficiency of one mineral affecting the utilization of the other (Crenshaw, 2001). The NRC (2012) suggests a total calcium to total phosphorus ratio is 1:1 or 1.25:1 or a total calcium: digestible phosphorus ratio of 2.15:1.

1.2.2.3. Sodium and chloride

Sodium and chloride are involved in nutrient absorption, osmotic regulation, and regulation of pH. Salt or sodium chloride is commonly supplemented to swine diets, to offset the low concentrations of sodium and chloride in cereal grains. However, some commonly used ingredients in nursery diets contain high levels of sodium such as dried whey and spray-dried plasma protein. Shawk et al. (2018a,b) determined that the estimated NRC (2012) requirements for sodium and chloride for nursey pigs are accurate with as much as 0.5 to 0.6% added salt needed to meet requirements. For nursery pigs, the requirements for sodium are higher and significantly decrease for grow-finish pigs.

1.2.2.4. Vitamin and trace mineral premixes

Vitamins are required for normal metabolic processes in the body. The pig is able to produce some vitamins in sufficient quantities to meet its needs, while others may be present in adequate amounts in feed ingredients commonly used in swine diets. Vitamin requirements that are not met by the pig or through other feed ingredients are supplemented to the diet, commonly through pre-mixes. A pre-mix is a complex mixture of micronutrients, in this case vitamins, that are typically added to swine diets in micro amounts. Pre-mixes help simplify the weighing process and improve the accuracy of mixing and distributing the micronutrients throughout the feed. Most commercial swine

diets are formulated to exceed the NRC (2012) vitamin and trace-mineral requirement estimates due to bioavailability of sources and the limited amount of knowledge on the requirements. Both vitamins and trace-minerals can be supplied in the form of a pre-mix to optimize performance and avoid deficiency, while minimizing unnecessary cost. Trace minerals requirements, functions, and sources will be discussed later in this review.

1.2.3 Nursery feeding program

Common industry practices are to feed a four-phase nursery feeding program to best match nutrient requirements and digestive capabilities of the weaned pig with the most economical diet possible, while still maintaining maximum performance in the nursery (DeRouchey et al., 2007). This leads to more ingredients in the first few phases of nursery diets, when compared to the later phases in the nursery and growing-finishing diets. Complex diets (i.e. multiple feedstuffs) improve feed intake for the first few weeks following weaning, however, complexity should be reduced rapidly as its impact on feed intake reduces rapidly (DeRouchey et al., 2007).

The phase one transition diet is often fed in pellet form, while phases two, three, and four are often fed in meal form. The advantages of feeding phase one as pellet form include, but are not limited to: improved feed efficiency and growth performance, less feed wastage, and improved flow-ability characteristics of the diet (DeRouchey et al., 2007). When curating a nursery feeding program, it is crucial to formulate to the young pig's high protein deposition, low feed intake, high lactase activity, and low amylase, maltase, sucrose and lipase activities (Menegat et al., 2019). This means that newly weaned pigs can easily digest lactose and specialty proteins sources, however, they have

a limited ability to digest plant protein sources, sugars, and have limited utilization of fat. Dietary lactose, commonly found as dried whey, eases the transition from milk to solid feed by playing a critical role in the growth performance of newly weaned pigs due to being palatable and an easily digestible energy source (Zhao et al., 2021). As the weaned pig develops and transitions, dried whey is removed, and should be replaced by another high-quality protein source.

Dietary protein can be provided from either plant or animal sources. Common animal protein sources include rendered animal by-products such as blood meal, meat meal, meat and bone meal, poultry by-products, and fish meal (Meeker and Meisinger, 2015). Animal-based proteins are typically highly palatable and can serve as high-concentrated sources of amino acids, B-vitamins, and minerals. The use of plant-based protein sources from soybean meal becomes a concern for young pigs due to a soy allergen. Li et. al (1990) found that pigs had a depressed weight gain from week three to four of age when fed a diet containing soybean meal. However, similar to humans, these allergies tend to decrease with age (Radcliffe et al., 2019), which allows for the addition of soybean meal as the pig develops. Soybean meal as a protein source is a standard industry practice, therefore the ultimate goal is to strategically remove specialty high-cost ingredients from the nursery diets as quickly as possible and replace them with typical lower-cost ingredients (Menegat et al., 2019).

Corn, wheat, milo, oat products, and other grains can serve as the main grain source in nursery diets (DeRouchey et al., 2007) contributing to the energy requirement of the pig. High quality dietary fat sources, such as choice white grease, may be added to

the diet to contribute to the energy requirement of the pig, as well as aid as lubrication in the pelleting process. Trace minerals such as zinc and copper may be added to the nursery feeding program above the requirements to provide additional growth benefits. Pharmacological levels of zinc as zinc oxide between 2,000 and 4,000 ppm is a common recommendation, for phase one and two of nursery diets, to improve growth performance and reduce post-wean diarrhea (Hill et al., 2000; Shelton et al., 2011). In addition, pharmacological levels of copper between 150 and 250 ppm may be used in phases three and four as a replacement for zinc oxide to improve growth performance (DeRouchey et al., 2007).

1.2.4 Growing-finishing feeding program

In the past, a five to six phase program was recommended for the growing-finishing period to closely meet the nutrient requirements of the pig and reduce excretion in waste. However, recently it has been shown that the five to six phase programs may not provide greater economic return than a three to four phase program (Menegat et al., 2019). Additionally, decreasing the number of diets fed can provide benefits to the feed manufacturing process by improving feed mill efficiency and simplifying mill logistics (Moore et al., 2013).

In order to design a successful grow-finish nutritional program, there needs to be an understanding of the relationship between nutrition and growth. First, optimal energy level must be determined. Nutrient requirements are often established utilizing a growth curve, due to the relationship of changes in protein deposition and feed intake in the growing-finishing period. In growing pigs, feed intake is generally determined by the

energy density of the diet. The growing-finishing pig will go through different phases of growth as age and body weight increase. Early in the growing period, there is a linear relationship between dietary energy intake and protein deposition (Campbell and Taverner, 1988; Bikker et al., 1994). Blikker et al. (1996) found that body protein increases linearly with energy intake until maximum protein deposition is reached. This indicates that pigs are in an energy dependent state of growth in the early growing period. The rate of lipid accretion increases rapidly as pigs enter the late finishing period while lean tissue accretion rates stay relatively constant (Wagner et al., 1999). This indicates that the pigs are in a protein dependent phase in the late finishing period because energy intake is no longer limiting protein deposition. Therefore, increasing dietary energy intake in the late finishing period can lead to increases in lipid deposition, instead of protein deposition (Blikker et al., 1996).

Once the optimum energy level is determined, a lysine:calorie ratio may be established according to the dietary lysine requirement. Lysine is the essential amino acid to become first deficient in most swine diets, otherwise known as the first-limiting amino acid. Voluntary feed intake of growing pigs given *ad libitum* access to feed is related to the dietary energy content of the diet. Therefore, amino acid levels of a diet should correlate to its dietary energy concentration (Chiba et al., 1991). Thus, if dietary energy density or feed intake is altered, it is appropriate to change the proportion of dietary amino acids. This is why lysine requirement is expressed as a ratio instead of a dietary percentage. Understanding lysine requirements has become a crucial component of economically sound grow-finish feeding strategies, as cost of energy and amino acid

sources have increased. This has led to the need to optimize the lysine:calorie ratio in order to maximize growth in young and rapidly growing pigs (Schneider et al., 2010). After the dietary lysine percentage is determined, levels of the other essential amino acids may be determined. These levels are determined by using a ratio for each amino acid relative to lysine. Lastly calcium, phosphorus, salt, vitamins, mineral and other ingredient levels can be set.

By the time that pigs enter the growing-finishing feeding program, all specialty feed ingredients are removed and they will be transitioned to the industry standard corn-soybean meal diets. Alternative feedstuffs such as dried distillers' grains with solubles (DDGS), wheat middlings, and bakery meal can be used in the diet formulation for replacements for corn and soybean meal. The addition of alternative feedstuffs to the diet is based on a few factors including availability of ingredients, nutrient availability, amino acid profile, and palatability, with the biggest factor being cost effectiveness. Due to the variability of the nutrient composition of these products it is important to understand the nutrient composition of these products prior to considering implementation in diet formulation (Woyengo et al., 2014). Nutrient composition of ingredients can influence pig performance and carcass quality, therefore nutrient composition of ingredients should be well understood before incorporating into the diet formulation. The addition of alternative feedstuffs to the diet is based on a few factors including availability of ingredients, nutrient availability, amino acid profile, and palatability, with the biggest factor being cost effectiveness. For example, the fat in DDGS has a relatively high concentration of unsaturated fatty acids, which may cause an increase the softness of

bellies of pigs fed a diet containing more than 20% DDGS (Whitney et al., 2006). Additionally, DDGS from corn are relatively low in lysine and, potentially tryptophan depending on dietary inclusion level, and have a higher concentration and digestibility of phosphorus in comparison to corn and soybean meal (Stein, 2007). This means that additional crystalline L-lysine and crystalline L-tryptophan need to be included in the diet and monocalcium phosphate can be reduced, but an increase in limestone is needed to maintain a proper concentration of calcium.

1.3 TRACE MINERALS

Trace minerals, also referred to as micro minerals, are minerals that are required in quantities less than 100 mg/kg in the diet. Trace minerals include chromium, cobalt, copper, iodine, iron, manganese, selenium, and zinc. Over the last 75 years, the importance of trace minerals in swine diets have become well recognized (Miller and Kornegay, 1983). The primary functions of trace minerals are to serve as components of enzymes, coenzymes, and hormones (Goff, 2015). In this part of the review, focus will be on trace mineral functions, requirements, sources, antagonistic relationships, and the environmental impact.

1.3.1 Organic vs. Inorganic Trace Minerals

Trace minerals have traditionally been supplemented in swine diets as inorganic oxides and sulfates. More recently, there has been considerable interest in the use of chelated or organic trace minerals in swine diets. This interest has stemmed from reports of improved bioavailability, performance and health responses. Specific trace mineral sources and examples will be provided later in this review.

Commercially available organic trace mineral supplements vary in regard to the type of ligand(s) used to form the metal complex or chelate. The Association of American Feed Control Officials provides definitions for the various types of organic products: metal amino acid complex is the product resulting from complexing of a soluble metal salt with an amino acid(s), metal amino acid chelate is the product resulting from the reaction of a metal ion from soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three moles of amino acids to form coordinate covalent bonds, metal proteinate is the product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein, and metal polysaccharide complex is the product results from complexing of a soluble salt with a polysaccharide solution. It is important to note that not all metal complexes are chelates, as chelation refers to a special type of complex formed between a ligand and a metal ion. In order to be classified as a chelate the ligand or chelating agent must: 1) contain a minimum of two functional groups each capable or donating a pair of electrons to covalently bond with a metal and 2) form a heterocyclic ring structure with the metal (Kratzer and Vohra, 1986).

When conventional inorganic trace mineral oxides and sulfates breakdown in the stomach, the released ions are free to interact with ligands, which will allow them to either remain soluble or bind to insoluble compounds, such as phytic acid, and form low solubility salts which the animal cannot absorb. On the other hand, organic trace mineral chelates or complexes can be protected from forming complexes with other dietary components, remaining electrically neutral at the acidic pH conditions in the digestive tract, thus allowing them to be absorbed and circulated to target positions efficiently

(Acda and Chae, 2002; Burkett et al., 2009). Additionally, metal chelates and complexes are more similar than inorganic forms that occur naturally in the body and feedstuffs.

1.3.2 Chromium and Cobalt

Chromium (Cr) is involved in carbohydrate, lipid, protein, and nucleic acid metabolism (Nielsen, 1994). The primary role of Cr is to potentiate the action of insulin, facilitating the bond between insulin and insulin receptors on the cell membrane (NRC, 1997). There is no quantitative estimate of the Cr requirement established for pigs (NRC, 2012). Additionally, there is no dietary requirement for cobalt (Co) for swine. Cobalt functions only as a component of vitamin B_{12} (Rickes et al., 1948). However, pigs are unable to synthesize vitamin B_{12} efficiently. Therefore, vitamin B_{12} must be supplemented and there is no dietary requirement for Co.

1.3.3 Copper

Copper (Cu) is essential for the activation of several oxidative enzymes and therefore plays a role in oxidation-reduction reactions, transport of oxygen and electrons, and protection against oxidative stress (Manto, 2014; Hill, 2013). Additionally, Cu is involved in metabolic reactions including cellular respiration, tissue pigmentation, hemoglobin formation, and connective tissue development (Turnlund, 1998). Historically, copper sulfate ($CuSO_4$) has been the most commonly utilized inorganic source of supplemental Cu in swine diets due to its bioavailability and relatively low cost compared to other Cu sources (Shelton et al., 2011). However, more recently another inorganic Cu source called tribasic copper chloride (TBCC) has been introduced into

swine diets. TBCC is insoluble in water due to covalent bonding of Cu to hydroxyl groups, but is highly soluble in acidic conditions. Furthermore, TBCC has less oxidative instability during storage compared to $CuSO_4$, which may offer benefits when combined with vitamins in base mixes, premixes, and diets (Luo et al., 2005; Lu, et al., 2010).

The NRC (2012) suggests that requirement for Cu is 6 ppm for the weaned pig and decreases to 3 ppm for the finishing pig. However, supplementing Cu between 100 to 250 ppm has been utilized for the growth promoting effect. For weanling pigs, pharmacological levels of Cu improve ADG and ADFI (Pérez et al., 2010, Cromwell et al., 1998, Hill et al., 2000). Additionally, Espinosa et al. (2019) observed that feeding high concentrations of Cu reduced the frequency of diarrhea in weanling pigs. Previous research suggests that feeding pharmacological levels of Cu improves growth performance during the early and late finishing period (Davis et al., 2002; Coble et al., 2017). However, others have reported no growth performance benefits during the late finishing period (Feldpausch et al., 2015).

With the use of high levels of Cu in swine diets, comes the potential environmental impact. Pigs only absorb about 5 to 10 % of dietary Cu or even less (Jondreville et al., 2003). Therefore, high levels of Cu in diets leads to increased Cu excreted through the feces and thus results in excessive accumulation of Cu in the soil where the manure is applied. However, Payne et al. (1988) indicated that when manure from pigs fed 250 ppm Cu was spread for 8 years on three different types of soil, there was not a decrease in corn yield and plant tissue Cu concentrations remained in normal ranges.

1.3.4 Iodine

In swine, iodine (I) is primarily present in the thyroid gland (80%), with small amounts found within the innards and blood (14%), muscle and fat (5%), and bones (1%) (Franke et al., 2008). The only known roles of iodine are its incorporation into the thyroid hormones, mono-, di-, and tri-, and tetraiodothyronine, which are important in the regulation of metabolic rate (NRC, 2012). The dietary iodine requirement is not well established. In a corn-soybean meal diet, a level of 0.14 ppm is adequate to prevent thyroid hypertrophy in growing pigs (Cromwell et al., 1975). Nutritionally available forms of iodine include calcium iodate, potassium iodate, and pentacalcium orthoperiodate and are more stable in salt mixtures than are sodium iodide or potassium iodide (Kuhajek and Andelfinger, 1970). Incorporating iodized salt (0.007% iodine) at a level of 0.2% of the diet in grain-soybean meal diets, provides a sufficient iodine to meet the needs of growing pigs (NRC, 2012).

1.3.5 Iron

Iron (Fe) is a vital component of hemoglobin in red blood cells. Additionally, Fe is also found in muscle as myoglobin, in serum as transferrin, in the placenta as uteroferrin, in milk as lactoferrin and in the liver as ferritin and hemosiderin (Zimmerman, 1980; Ducsay et al., 1984), as well as plays an important role in the body as a component of several metabolic enzymes (Hill and Spears, 2001). Bioavailability of Fe varies greatly between sources (Zimmerman, 1980). Ferrous sulfate, ferric chloride, ferric citrate, and ferric ammonium have been proven effective in preventing Fe deficiency (Harmon et al., 1967; Ammerman and Miller, 1972; Miller et al., 1981). Due to its low solubility, ferric

oxide is an ineffective Fe source (Ammerman and Miller, 1972). On the other hand, absorption and activity of inorganic Fe may be impaired by antagonisms present between trace minerals and macrominerals (Umbreit, 2005). Chelated or protein sources of iron have been reported to be up to 185% as bioavailable as ferrous sulfate (Henry and Miller, 1995). The level of Fe in soybean meal is 175-200 ppm with the bioavailability of Fe estimated to be 38% (Biehl et al, 1997).

The young pig is born with a limited supply of Fe in their body stores and sow's milk is low in supplemental iron, thus Fe deficiency is prevalent in young pigs (Svoboda and Drabek, 2005). To meet the demands of the fast growing young pig, supplemental Fe is provided often through injectable or oral Fe supplements in the first three days of life. The post-weaning dietary Fe requirement is 100 ppm and decreases to 40 ppm in the late-finishing phase as the rate of increase in blood volume slows (NRC, 2012). Post-weaning Fe requirements are often met through the Fe levels in natural feed ingredients and dicalcium phosphate, which supplies a substantial amount of Fe (NRC, 2012).

1.3.6 Manganese

Manganese (Mn) is involved in the synthesis and activation of several enzymes involved in the regulation of the metabolism of carbohydrates, lipids, and protein (Li and Yang, 2018). Additionally, Mn plays a role in mitochondrial superoxide dismutase activity and helps comprise the bone matrix (Suttle, 2010; Leach and Muenster, 1962). The dietary Mn requirements for swine are not well established; however, according to the NRC (2012) the quantitative requirement for Mn ranges from 2 ppm to 4 ppm for nursey and growing-finishing pigs. Many swine diets today meet the NRC (2012) from

the major feed ingredients, although the bioavailability of innate Mn in ingredients is not well known. Thus, swine diets typically contain a trace mineral pre-mix with added Mn in the form of Mn sulfate. Flohr et al. (2016) conducted a survey in which Mn supplementation rates were reported to be between 3.3 ppm and 40 ppm, with the average being 9.3 times the requirement estimate, throughout the entire finishing period, which may contribute to excess trace mineral excretion in the manure. Meanwhile, Kerkaert et al. (2021) found no additional benefit to Mn supplementation above 8 ppm on growth performance or carcass characteristics.

1.3.7 Selenium

Selenium (Se) is a fundamental part of selenoproteins which play a part in a variety of physiological processes in production animals. The discovery of the first selenoprotein glutathione peroxidase (Rotruck et al., 1973), which detoxifies lipid peroxidases and can metabolize hydrogen peroxide, made the role of Se more transparent. It is clear that selenoproteins are involved in cell antioxidant systems (Arthur, 2000). Additionally, iodothyronine 5'-deiodinase has been identified as a selenoprotein, which means Se has a function in thyroid metabolism. The NRC (2012) recommended dietary Se requirement ranges from 0.3 ppm in weanling pigs and 0.15 ppm in for finishing pigs. This requirement can be effectively met through supplemental Se sources including organic (Se-enriched yeast) and inorganic (sodium selenite and sodium selenate) (Mahan and Magee, 1991; Mahan and Parrett, 1996). Mahan et al. (1999) reported no performance or carcass quality benefits between inorganic and organic Se sources or increasing the dietary Se levels from 0.05 to 0.30 ppm.

1.3.8 Zinc

The first biochemical role for zinc (Zn) was established in 1939 when Zn was recognized as an integral component of the enzyme carbonic anhydrase (Keilin and Mann, 1939). Subsequently, Zn has been found to serve as a component of a variety of enzymes involved in transcription, intra- and intercellular signals to the nucleus, DNA and RNA transferase and synthetases, digestive enzymes, and is associated with the hormone insulin (Miller et al., 1979; NRC, 2012). Thus, Zn plays an integral role in protein, lipid, and carbohydrate metabolism.

The importance of Zn in swine diets was recognized in 1955 when Tucker and Salmon (1955) reported that Zn in growing pig diets prevented or cured parakeratosis. Parakeratotic lesions on the skin was widely observed in the U.S. when corn-vegetable diets were fed (Tucker and Salmon, 1955). Since this discovery, Zn has been commercially added to grain-plant protein swine diets. The NRC (2012) estimated minimum dietary requirement for nursery pigs is 100 ppm and decreases to 50 ppm for finishing pigs. However, many factors influence the dietary Zn requirement including Ca (Luecke et al., 1956), phytate (Oberleas et al.1962), Cu, Fe, and Co, as well as source (Miller et al., 1979; Hill and Shannon, 2019). In swine diets, Zn is often supplemented as an inorganic form such as Zn oxide (ZnO) and Zn sulfates or Zn from organic complexes such as Zn-methionine. Absorbed and retained Zn as a percentage of intake is typically much less than 50% of intake, however Zn from organic Zn sources is more available than Zn from ZnO (NRC, 2012).

Similar to Cu, the supplementation of pharmacological levels of dietary Zn between 2,000-4,000ppm has shown to have growth performance benefits, as well as reduce post-wean diarrhea and improve intestinal health. Interest in the use of pharmacological use of Zn was sparked when Poulsen (1989) reported supplementing the starting diet with 3,000 ppm of Zn from ZnO for 14 days reduced post-weaning scouring and increased weight gain. These effects with Zn as ZnO have been consistently demonstrated (Heo et al., 2010; Hill et al., 2001; Hollis et al., 2005; Shelton et al., 2011; Walk et al., 2015; Zhang and Guo, 2009). Specifically, ZnO has demonstrated to have positive impact on gut barrier integrity (Hu et al., 2013; Roselli et al., 2003) and intestinal health (Carlson et al., 1999). Results from studies providing supplementation of organic Zn sources added at lower levels than that of ZnO are inconsistent. Several studies have demonstrated the same benefits as ZnO (Ward et al., 1996; Mavromichalis et al., 2001; Case and Carlson, 2002) while results from other studies do not support that conclusion (Hahn and Baker, 1993; Carlson et al., 2004; Hollis et al., 2005). Rising concerns of environmental impact (Jonderville et al., 2003) and implication of pharmacological levels causing an increase in antimicrobial resistance (Yazdankhah et al., 2014) have led to regulations in some countries restricting or prohibiting the use of Zn at pharmacological levels for its growth promoting affect. In fact, the EU has pending regulations on the use of ZnO as a veterinary medicinal product, which can be defined as levels above 150 ppm.

1.3.9 Environmental impact

As stated previously, trace minerals are often supplemented to swine diets above NRC (2012) recommendations due to varying bioavailability of sources and limited

amount of knowledge of requirements. However, supplementing swine diets with excess trace minerals may lead to exceeding the pigs' physiological requirements (Carlson et al., 1999; Hill et al., 2000). Since trace mineral concentrations are heavily regulated by homeostatic mechanisms in the tissue, large amounts of these mineral are then excreted in the feces (Spears, 1996). Excreted trace minerals may lead to bioaccumulation in the soil and potentially threaten water sources due to runoff (Besser, 2001). Regarding environmental impact, alternatives have been investigated to reduce the waste of excess trace minerals in swine manure. Formulating diets with trace mineral concentrations close to the pigs' requirement would be an appropriate means to reduce mineral concentrations in waste. However, trace mineral requirements of swine are not easily established. Yet, it has been demonstrated that reducing or eliminating trace mineral supplementation for growing-finishing pigs does not affect overall pig performance (Patience and Gillis, 1995; Mavromichalis et al., 1999; Shelton et al., 2004; Gowanlock et al., 2013) and may reduce excretion of trace minerals in the manure (Thomaz et al., 2014). Another strategy for reducing trace mineral concentrations in the diet is inclusion of organic mineral sources based on the higher bioavailability than the alternative inorganic sources (Wedekind et al., 1992; Wedekind et al., 1994). Reducing trace mineral concentrations in swine manure is beneficial to contribute to good environmental stewardship.

CHAPTER 2

EFFECT OF ZINC OXIDE AND ORGANIC ZINC ON WEANLING PIG PERFORMANCE, GUT PERMEABILITY, AND FECAL CONSISTENCY

2.1 ABSTRACT

This experiment investigated the effects of source and concentration of zinc (Zn) on weanling pig growth performance, gut permeability, and fecal consistency. A total of 1,144 newly weaned pigs (21-d of age, 5.8 ± 0.1 kg) were used in a 2 x 2 factorial design comparing main effects of Zn source (Zn oxide (ZnO) vs. organic Zn) and Zn inclusion (with or without the addition of ZnO or organic Zn). Pens were stocked with 26 pigs of equal sex and randomly allotted one of four dietary treatments consisting of complex commercial nursey diets with basal Zn requirement met (NRC, 2012): 1) with no additional Zn, 2) addition of Zn from ZnO, 3) Zn from organic Zn or 4) a combination of ZnO and organic Zn. Zinc from ZnO was provided at 3000 ppm for PH1-2, 1500 ppm for PH3, and removed at PH4 and Zn from organic Zn was provided at 100 ppm for PH1-4. Each dietary treatment was replicated eleven times utilizing a total of 44 pens. Data was analyzed as a 2 x 2 factorial arrangement with factor and factor interaction as the main effects and pen as the experimental unit. The addition of organic Zn increased (P<0.01) ADFI from d 0 to d 7 (0.175 vs. 0.149 kg/d), but decreased (P<0.01) G:F (0.763 vs. 0.902) and then improved (P<0.01) G:F from d 7 to d 14 (0.821 vs. 0.754). The addition of Zn from ZnO increased (P<0.05) ADG (0.312 vs. 0.290 kg/d) and ADFI (0.428 vs. 0.401 kg/d) for the initial 28-d and improved (P<0.05) G:F (0.776 vs. 0.738) for the initial 21-d post-weaning, but not for the latter 14 d (28 to 42-d) of the nursery phase or

overall (0 to 42 d). There were no improvements in measured gut permeability with addition of Zn. However, supplementation from either Zn source resulted in some improvements in fecal consistency. These results suggest that supplementation of organic Zn at lower concentrations than pharmacological levels of ZnO has the potential to produce similar benefits.

2.2 INTRODUCTION

At the time of weaning, pigs undergo many stressors such as removal from the sow, transportation to a new facility, a new physical environment, co-mingling with pigs from other litters, and a dietary transition from a liquid diet of sows' milk to a solid, dry diet (Campbell et al., 2013). In turn, these stressors may result in reduced feed intake, reduced intestinal barrier function (Wijtten et al., 2011), and structural and functional changes in the small intestine including shortening of the villi, increased crypt depth, and reduced brush border enzyme activity (Pluske et al., 1997; Boudry et al., 2004; Lallès et al., 2004). All of these factors have the potential to contribute to reduced growth rate and feed efficiency, inflammation and increased gut permeability, and post-wean diarrhea.

A common industry strategy utilized to mitigate the negative impacts associated with weaning is to supplement post-wean diets with pharmacological levels (2,000 to 4,000 ppm) of zinc oxide (ZnO). The use of pharmacological levels of ZnO has shown to increase growth performance and feed efficiency, while reducing gut permeability and the incidence of post-wean diarrhea (Poulsen et al., 1989; Hill et al., 2001; Walk et al., 2015). On the other hand, the use of pharmacological levels of zinc (Zn) has raised some concerns such as environmental pollution from Zn-rich manure applications on fields and

acceleration of antimicrobial gene spread (Jonderville et al., 2003; Yazdankhah et al., 2014), which has led to regulations in some countries restricting or prohibiting the use of Zn at pharmacological levels for its growth promoting effect.

In an effort to prepare for implementation of bans, and to avoid the overuse of ZnO, there has been investigation into ZnO alternatives. A potential alternative to pharmacological levels of ZnO is the use of organic Zn at lower concentrations than ZnO, as organic sources have shown to be more bioavailable (Wedekind et al., 1994; Ammerman et al., 1995). Additionally, supplementation of organic Zn sources added at lower levels than that of pharmacological levels of ZnO have shown to have similar benefits to ZnO (Mavromichalis et al., 2001; Case and Carlson, 2002). However, these results are inconsistent (Carlson et al., 2004; Hollis et al., 2005). Therefore, the objective of this study was to evaluate the effect of ZnO and organic Zn on weanling pig performance, gut permeability, and fecal consistency.

2.3 MATERIALS AND METHODS

South Dakota State University's Institutional Animal Care and Use Committee approved the protocol (IACUC #2010-051E) used in this study. The experiment was conducted at the South Dakota State University commercial wean-to-finish research facility.

2.3.1 Animal housing, diets, and experimental design

Approximately 1,200 newly weaned pigs were sorted by sex upon arrival to the SDSU off-site commercial wean-to-finish barn. Any injured, sick, or small pigs were

removed and housed in “off-test” pens. Sequentially, a total of 1,144 weaned pigs of mixed genetics consisting of 60% PIC 359 and 40% DNA 610 sired and all progeny of the same DNA sow line (21-d of age, 5.8 ± 0.1 kg initial BW) were randomly allotted to pens with a stocking density of 26 pigs (equal number of barrows and gilts) and blocked by BW and barn location for a 42-d nursery trial. Pens within block were randomly allotted to one of four dietary treatments in a 2 x 2 factorial arrangement (two dietary Zn sources x two Zn levels). Each dietary treatment was replicated eleven times utilizing 44 total pens. Pens were 3.1m x 6.9m and contained a 5-slot stainless steel dry feeder (SDI, Inc., Alexandra, SD) and two cup waterers, which provided *ad libitum* access to feed and water. The facility is equipped with a single M-series FEEDPro (Feedlogic ComDel Innovation, Wilmar, MN) which delivered daily feed allowances to each pen.

Experimental diets were applied over a four-phase nursery feeding program and phases (PH) were based on a feed budget of 0.9, 3.4, 4.5, and 24 kg/pig (or until end of the trial) for phases 1, 2, 3, and 4 respectively. Dietary treatments included a complex commercial nursery diet with dietary basal Zn requirement met (NRC, 2012) as a control and diets with the addition of ZnO, organic Zn, and a combination of ZnO and organic Zn. The ZnO provided Zn at 3000 ppm in PH1-2, 1500 ppm in PH3, and was removed in PH4 and the organic Zn source was provided at 100 ppm in PH1-4. Samples were collected from each batch of feed delivered for all four phases and stored in a freezer (-20°C) until subsamples were pooled together and sent for analysis. Experimental diets were analyzed for moisture, dry matter, crude protein (CP), soluble protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, and full mineral panel (calcium

(Ca), phosphorus (P), magnesium (Mg), potassium (K), chloride (Cl), sulfur (S), sodium (Na), zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn)) at a commercial laboratory (Analab, Agri-King, Fulton, IL) where all analysis followed AOAC methods.

2.3.2 Growth performance

For the 42-d duration of the trial, feed disappearance and pen weights were measured every 7 days, starting on the day of arrival, to calculate ADG, ADFI, and G:F. Feed delivery data reported by FEEDPro and the amount of feed remaining in each feeder on weigh days was used to determine feed intake. Weight of feed remaining in feeders was calculated using a pre-determined feed density equation utilizing the density of the feed and measurement of the empty space in the feeder.

2.3.3 Intestinal health measurements

Fecal scoring was completed every three days from day 3 to day 21 by one independent trained scorer per day to categorize the consistency of feces per pen. Fecal consistency was categorized as a numerical scale from 1 to 5 as follows: 1 = hard, dry pellet-like feces, 2 = firm, formed feces, 3 = soft, moist feces that retains its shape, 4 = soft, unformed feces that doesn’t keep its shape, 5 = watery, liquid feces.

On day 7 of the trial, one barrow per pen, representing the average BW of the pen, was selected for measuring gut permeability utilizing the dual sugar absorption test (DSAT). This process continued over the course of three days (7, 8, and 9-d after the start of the trial). A bolus that contained 5% lactulose and 5% mannitol was orally administered at 15 mL/kg (Nyguyen et al., 2014) via a syringe and fluid feeder probe.

Pigs were housed in individual creates (0.56 x 0.64 x 0.89 m^2) with access to feed and water for 6-h for a total urine collection. A drop pan with a small hole was placed under each individual crate at an angle titling downwards, therefore all of the urine drained towards the hole and into a container. Sequentially, pigs were transferred back to their original pen. After homogenization, a urine subsample was collected and stored at -80°C and later analyzed using EnzyChrom™ intestinal permeability assay kits (BioAssay Systems, Hayward, CA) following manufacturer's instructions. Concentrations were calculated to provide a ratio of μM lactulose to μM mannitol (lactulose:mannitol) in the urine.

2.3.4 Statistical analysis

The PROC REG procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC) was used to confirm homogeneity of variance and analyze for outliers. Performance and DSAT data were analyzed using the PROC GLIMMIX procedure of SAS in a randomized complete block design with pen as the experimental unit. The model included factor and factor interaction as the main effects and block as the random effect. Fecal score data were analyzed by repeated-measures analysis using the PROC MIXED procedure of SAS and the compound symmetry (CS) covariance structure determined best fit by the smallest Bayesian Information Criterion (BIC) value. The model included the effects of ZnO and organic Zn inclusion and time (day of study) and the associated two- and three-way interactions. Significant differences were reported at $P < 0.05$ and tendencies for significance were reported when $0.05 \leq P \leq 0.10$.

2.4 RESULTS

The analysis of dietary treatments verified that CP, ADF, NDF, fat, starch, ash, and macrominerals were similar between dietary treatments within each phase (Tables 2.1, 2.2, 2.3, & 2.4). Analyzed concentrations of the Zn were reflective of dietary supplementation in PH1-2; however, were not reflective of dietary treatment in PH3-4 (Tables 2.1, 2.2, 2.3, & 2.4).

In the first week post-weaning (d 0 to d 7), pigs supplemented with Zn from ZnO had an increased ADG ($P=0.01$) and AFDI ($P<0.01$). Additionally, pigs fed Zn from organic Zn had an increased ADFI ($P<0.01$), but a decrease in G:F ($P<0.01$). There was also a significant interaction for G:F ($P<0.01$) between ZnO and organic Zn.

For week two (d 7 to d 14), the addition of Zn from ZnO resulted in an increased BW ($P=0.01$), ADG ($P<0.01$), ADFI ($P<0.01$), and G:F ($P<0.01$). Meanwhile, pigs supplemented with Zn from organic Zn had an increased G:F ($P=0.01$). In addition, there was a significant interaction between ZnO and organic Zn for ADFI ($P<0.01$) and G:F ($P<0.01$). Cumulative d 0 to d 14, there was an increase in ADG ($P<0.01$), ADFI ($P<0.01$), and G:F ($P<0.01$) for pigs fed diets with the addition of Zn from ZnO.

From d 14 to d 21, the pigs supplemented with Zn from ZnO had a higher BW ($P=0.01$) in comparison to the pigs not supplemented with ZnO. In the first half of the nursery phase (d 0 to d 21), the addition of Zn from ZnO resulted in an increased ADG ($P<0.01$), ADFI, ($P<0.01$), and G:F ($P<0.01$). No interactions or differences with addition of Zn from organic Zn were observed.

From d 21 to 28 the only observed difference was an increase in G:F ($P < 0.01$) with the addition of Zn from ZnO. Cumulative d 0 to d 28, the addition of Zn from ZnO resulted in an increased ADG ($P=0.02$) and ADFI ($P=0.02$). There were no observed differences in BW, ADG, ADFI, or G:F in the later 14-d (d 28 to d 42) of the study for the addition of Zn from ZnO or organic Zn. Additionally, overall (d 0 to 42) the addition of Zn did not impact BW, ADG, ADFI, or G:F.

Lactulose ($P \geq 0.81$) and mannitol ($P \geq 0.16$) concentrations did not differ with the addition of Zn from ZnO or organic Zn. Lactulose:mannitol ratios did not differ ($P \geq 0.18$) with the addition of Zn, but were numerically different compared to pigs not supplemented with Zn from organic Zn. There was no interaction observed between ZnO and organic Zn.

On day 3, organic Zn supplementation negatively impacted ($P < 0.05$) fecal consistency, however improved ($P < 0.05$) fecal score on day 15. On days 9, 12, and 15 the addition of Zn from ZnO resulted in improved ($P < 0.05$) fecal score. There was an effect of ZnO by day ($P=0.05$) on fecal score and organic Zn by day tended to have an effect on fecal score ($P=0.07$). Additionally, there was an effect ($P \leq 0.05$) of ZnO, day, and ZnO*organic Zn on fecal score.

2.5 DISCUSSION

Analyzed concentrations of Zn for PH3-4 were not as expected in regards to dietary treatment. Potential explanations include: an error at the feed mill in the mixing or delivery process or a mix-up that occurred between the sample being sent for analysis and the analysis occurring. The latter is more likely, factoring in the use of feed dye to

visually distinguish the difference between diets and to ensure no mix ups occurred at the mill. In order to confirm these analyzed Zn concentrations, additional diet samples need to be sent for another analysis.

In order to be productive and efficient, weaned pigs must rapidly overcome the stressors associated with weaning, such as reduced growth rate. Since the findings of Poulsen (1989), the use of pharmacological levels of ZnO have proved to help mitigate the negative impacts related to weaning stressors. The suggested concentration and length of use for supplementing pharmacological levels of ZnO varies. Carlson et al. (1999) suggests supplementing Zn from ZnO at 3,000 ppm for at least two weeks following weaning and up to four weeks following weaning. Simultaneously, Hill et al. (2001) concluded that supplementing ZnO between 1,500-2,000 ppm for 28-d post-weaning maximized performance benefits. There are numerous factors to take into consideration to explain the variation in benefits as a result of the use of Zn from ZnO or Zn from organic Zn sources. Factors to consider are age and health status of the pig, the concentration of Zn supplemented in the diet, length of time supplemented, housing environment, and dietary composition.

Poulsen (1995) and Smith et al. (1997) reported increased growth rate for weaned pigs when fed pharmacological levels of ZnO, with minimal to no improvements in feed intake. Meanwhile, Hill et al. (2000) demonstrated improvements in both gain and feed intake in various phases of the 28-d study when feeding pharmacological levels of ZnO. In addition, Hahn and Baker (1993) found that supplementing 3,000 ppm of ZnO increased daily gain by 17% and daily feed intake by 14% across a 21-d post-wean

period. Hill et al. (2001b) reported improvements in gain, feed intake, and feed efficiency when ZnO was supplemented to early weaned and traditionally weaned pigs over a 28-d post-wean period. On the other hand, Shelton et al. (2011) observed gain and intake benefits for the entire 28-d post-wean period with feed efficiency benefits only occurring in the first 14-d post-weaning, supporting the results of the current trial of impact on gain and feed intake occurring for a longer period of time than feed efficiency benefits (Table 2.5). Significant interactions observed between ZnO and organic Zn indicate that the effects were not additive, suggesting that organic Zn did not display the benefits to the same level as ZnO.

Ward et al. (1996) reported equal improvements in performance when supplementing starter diets with 250 ppm Zn from an organic Zn source and 2,000 ppm Zn from ZnO. Furthermore, Case and Carlson (2002) observed an increase in gain when pigs were offered organic Zn at lower concentrations than pharmacological levels of ZnO in addition to the basal Zn requirement being met. However, Carlson et al. (2004) reported the supplementation of organic Zn had no impact on growth performance. In addition, when feeding complex nursey diets, Hahn and Baker (1993) found that only ZnO resulted in increased weight gain and feed intake, while there was no response from supplementation from organic Zn sources. Moreover, Hollis et al. (2005) demonstrated that pigs fed organic Zn gained faster and consumed more feed than pigs fed the control diet, however pigs fed pharmacological levels of ZnO had superior weight gain and feed intake over pigs supplemented with organic Zn. The improvements in feed intake and feed efficiency early in the post-weaning period in the current trial (Table 2.5) are

supported by the evidence that organic Zn provides performance benefits at lower concentrations than ZnO. However, lack of improvements past 14-d may support the results that ZnO offers superior improvements when compared to lower concentrations of organic Zn sources.

Reduction in intestinal barrier function has shown to be a result of the stressors that occur at the time of weaning (Wijtten et al., 2011). Use of measuring intestinal permeability via both *in-vivo* and *ex-vivo* methods have been reported. Utilizing *ex-vivo* methodology Roselli et al. (2003) reported a high transepithelial electrical resistance (TER) suggesting ZnO may prevent the increase in tight junction permeability. In addition, the results from Hu et al. (2013) show an increase in TER and a decrease in fluoresceine isothiocyanate dextran (FD4) flux indicating intestinal mucosal barrier was improved with the supplementation of ZnO. Zhang and Guo (2009) employed *in-vivo* methodology measuring the urinary excretion of lactulose and mannitol, in which a reduced lactulose:mannitol ratio was observed with ZnO supplementation. Reports from Roselli et al. (2003), Hu et al. (2013) and Zhang and Guo (2009) are in agreeance that supplementation from pharmacological levels of ZnO contributes to improved intestinal barrier function through preventing the increase of tight junction permeability. However, results in the current trial do not measure any benefits of reduced intestinal permeability with Zn supplementation (Table 2.6)

Another symptom associated with weaning stressors is the occurrence of diarrhea or scouring. Poulsen et al. (1995) demonstrated that supplementing weaning pigs with pharmacological levels of ZnO proved beneficial in preventing or alleviating diarrhea. In

addition, Heo et al. (2010) found that ZnO supplementation led to reduced fecal consistency in weaned pigs when the pigs were challenged with an enterotoxigenic stain of *Escherichia coli.* On the other hand, for healthy pigs with a low mortality rate, Walk et al. (2015) reported supplementation of ZnO resulted in fecal scores <2.0, utilizing a 4-score fecal consistency scale. Both Zhang and Guo (2009) and Hu et al. (2013) observed reduced incidence of post-wean diarrhea with for pigs offered diets supplemented with ZnO in addition to improved intestinal barrier function, suggesting the effect of pharmacological levels of ZnO alleviating diarrhea may in part be associated with reduced intestinal permeability. Previous reports of a decrease in fecal consistency with the supplementation of ZnO is supported by the results of the current study (Figure 2.1); however, there was no indication that reduced incidence of post-wean diarrhea was associated with improved intestinal barrier function (Table 2.6). Improvements on fecal score with supplementation of organic Zn are few and far between. Nonetheless, Bouwhius et al. (2017) observed reduced fecal score for pigs offered diets supplemented with organic Zn compared to pigs not offered diets with organic Zn (2.51 vs. 2.89). Although the trial only looked at 8-d post-weaning, this may support the results we observed in the current trial (Figure 2.1)

In conclusion, supplementation of Zn from organic Zn resulted in gain and feed efficiency benefits early in the post-weaning period. Meanwhile, supplementation of Zn from ZnO improved gain, feed intake, and feed efficiency for at least the first half of the 42-d nursery period. In addition, Zn from ZnO and Zn from organic Zn demonstrated improved fecal consistency at different time points. Therefore, lower concentrations of

organic Zn has the potential to provide similar benefits associated with supplementation of pharmacological levels of ZnO.

Table 2.1 Ingredient composition and nutrient analysis for phase 1

Item	Control	ZnO	Organic Zn	ZnO + Organic Zn
Ingredient, %				
Corn	21.43	21.43	21.43	21.43
Steam rolled oats	20.00	20.00	20.00	20.00
Whey permeate	20.00	20.00	20.00	20.00
Specialty proteins	18.00	18.00	18.00	18.00
Soybean meal	15.00	15.00	15.00	15.00
Fat	1.86	1.86	1.86	1.86
Limestone	1.20	1.20	1.20	1.20
Monocalcium phosphate	0.52	0.52	0.52	0.52
Salt	0.35	0.35	0.35	0.35
Crystalline AA	1.33	1.33	1.33	1.33
Vitamin premix[1]	0.05	0.05	0.05	0.05
Trace mineral premix[1]	0.08	0.08	0.08	0.08
Sel-Plex®[1]	0.05	0.05	0.05	0.05
Vitamin E[1]	0.05	0.05	0.05	0.05
Choline Chloride	0.05	0.05	0.05	0.05
Quantum Blue®[1]	0.04	0.04	0.04	0.04
Zinc oxide[2]	-	0.83	-	0.83
Organic zinc[2]	-	-	0.06	0.06
Analyzed Composition (%)				
Moisture	8.30	7.62	8.12	7.92
Dry Matter	91.70	92.38	91.88	92.08
Crude Protein	25.74	25.60	25.87	25.26
Soluble Protein	23.12	25.11	21.42	21.57
ADF	8.56	8.48	8.49	8.35
NDF	8.98	8.99	10.23	9.17
Starch	30.39	30.42	29.28	28.78
Ash	3.80	5.83	3.87	4.57
Ca	1.30	1.57	1.19	1.28
P	0.70	0.71	0.65	0.68
Mg	0.19	0.19	0.18	0.19
K	1.26	1.22	1.23	1.25
Cl	0.70	1.09	1.16	0.85
S	0.48	0.49	0.49	0.48
Na	0.68	0.78	0.71	0.64
Zn (ppm)	1078	4994	1428	4838
Cu (ppm)	97	112	106	106
Fe (ppm)	779	1077	865	977
Mn (ppm)	235	312	272	269

[1] Provided per kg of diet: 1,998 FTU Quantum Blue® phytase, 9.9 TIU vitamin A, 1.8 TIU vitamin D3, 77 IU vitamin E, 220 mg choline, 10 mg Cu from copper sulfate, 100 mg Fe from ferrous sulfide 0.5 mg I from ethylenediamine dihydroiodide, 30 mg Mn from manganese sulfate, 0.3 mg Se from Sel-Plex® PPM, 120 mg Zn from zinc oxide.

[2] Not included into diet formulation, added in addition to the complete diet.

Table 2.2 Ingredient composition and nutrient analysis of phase 2

Item	Control	ZnO	Organic Zn	ZnO + Organic Zn
Ingredient, %				
Corn	33.31	32.84	33.19	32.78
Soybean Meal	12.45	12.45	12.45	12.45
SDSU 800 base[1, 2]	40.00	40.00	40.00	40.00
Steam Rolled Oats	10.00	10.00	10.00	10.00
Fat	0.98	0.98	0.98	0.98
Limestone	1.08	1.08	1.08	1.08
Salt	0.35	0.35	0.35	0.35
Monocalcium phosphate	0.86	0.86	0.86	0.86
Crystalline AA	1.01	1.01	1.01	1.01
VTM Premix	-	-	-	-
Zinc oxide	-	0.42	-	0.42
Organic Zn	-	-	0.06	0.06
Black ferroxide[3]	-	0.05	-	-
Blue ferroxide[3, 4]	-	-	-	0.05
Red iron oxide[3]	-	-	0.05	-
Analyzed Composition (%)				
Moisture	10.86	10.92	10.81	11.02
Dry Matter	89.14	89.08	89.19	88.98
Crude Protein	24.56	23.38	24.72	24.48
Soluble Protein	21.69	23.58	21.57	20.96
ADF	6.90	9.13	7.39	6.73
NDF	8.91	13.39	8.47	8.71
Starch	34.65	31.65	33.23	37.25
Ash	4.36	6.76	5.54	4.82
Ca	0.81	0.97	0.87	0.91
P	0.65	0.72	0.72	0.66
Mg	0.19	0.19	0.20	0.19
K	1.28	1.25	1.29	1.19
Cl	0.85	0.76	0.74	0.64
S	0.31	0.32	0.33	0.33
Na	0.44	0.42	0.39	0.36
Zn (ppm)	528	2872	700	3381
Cu (ppm)	38	38	28	26
Fe (ppm)	280	637	390	350
Mn (ppm)	92	83	77	84

[1] Composed of: whey permeate, soybean meal, specialty proteins, steam rolled oats, fat, L-Valine, trace mineral premix, vitamin premix, organic selenium, phytase, and choline chloride.

[2] When added to the diet at 363.2 kg, SDSU 800 base provided per kilogram of the diet: 1998 FTU phytase (Quantum Blue® 5G), 12 TIU vitamin A, 2.2 TIU vitamin D3, 66 IU vitamin E, 110 mg choline, 10 mg Cu from copper sulfate, 100 mg Fe from ferrous sulfide, 0.5 mg I from ethylenediamine dihydroiodide, 30 mg Mn from manganese sulfate, 0.3 mg Se from Sel-Plex® 600 PPM, 120 mg Zn from zinc oxide.

[3] Added to provide color to the diet to differentiate between diets and may contribute to Fe level in the diet.

[4] Included in the diet formulation, however product was not included into the diet as fed.

Table 2.3 Ingredient composition and nutrient analysis for phase 3

Item	Control	ZnO	Organic Zn	ZnO + Organic Zn
Ingredient, %				
Corn	57.32	57.06	57.20	56.99
Soybean Meal	21.30	21.30	21.30	21.30
SDSU 800 base[1, 2, 4]	17.50	17.50	17.50	17.50
Fat	0.80	0.80	0.80	0.80
Limestone	1.00	1.00	1.00	1.00
Salt	0.40	0.40	0.40	0.40
Monocalcium phosphate	0.55	0.55	0.55	0.55
Crystalline AA	0.99	0.99	0.99	0.99
VTM Premix[3, 4]	0.10	0.10	0.10	0.10
Zinc oxide	-	0.21	-	0.21
Organic Zn	-	-	0.06	0.06
Black ferroxide[5]	-	0.05	-	-
Blue ferroxide[5, 6]	-	-	-	0.05
Red iron oxide[5]	-	-	0.05	-
Analyzed Composition (%)				
Moisture	11.72	10.84	14.09	12.39
Dry Matter	88.28	89.16	85.91	87.61
Crude Protein	18.44	17.91	19.36	18.36
Soluble Protein	22.17	26.91	18.25	19.40
ADF	3.28	5.24	4.26	2.63
NDF	5.54	13.79	8.53	5.23
Starch	55.93	53.35	55.15	58.59
Ash	5.38	7.47	4.16	4.46
Ca	2.36	1.75	1.20	2.04
P	0.55	0.62	0.51	0.56
Mg	0.14	0.15	0.15	0.14
K	0.78	0.81	0.81	0.76
Cl	0.69	0.90	0.61	0.57
S	0.26	0.25	0.26	0.28
Na	0.31	0.28	0.22	0.27
Zn (ppm)	1110	1784	564	2163
Cu (ppm)	40	18	20	23
Fe (ppm)	650	824	584	405
Mn (ppm)	70	57	53	60

[1] Composed of: whey permeate, soybean meal, specialty protein sources, steam rolled oats, fat, L-Valine, trace mineral premix, vitamin premix, organic selenium, phytase, and choline chloride.

[2] When added to the diet at 159 kg provided per kilogram of the diet: 874 FTU phytase (Quantum Blue® 5G), 5.3 TIU vitamin A, 1 TIU vitamin D3, 29 IU vitamin E, 48 mg choline, 4.4 mg Cu from copper sulfate, 44 mg Fe from ferrous sulfide, 0.2 mg I from ethylenediamine dihydroiodide, 13.1 mg Mn from manganese sulfate, 0.13 mg Se from Sel-Plex® 600 PPM, 52.5 mg Zn from zinc oxide.

[3] Provided per kilogram of the diet: 1,332 FTU phytase, 2,348 IU vitamin A, 734 IU vitamin D3, 14.5 IU vitamin E, 2.0 mg vitamin K3, 17.6 mg niacin, 11.7 mg pantothenic acid, 3.5 mg riboflavin, 5.9 ug vitamin B12, 8 mg Cu from copper chloride, 53 mg Fe from ferrous sulfate, 0.3 mg I from ethylenediamine dihydroiodide, 20 mg Mn from manganous oxide, 0.2 mg Se from sodium selenite, 67 mg Zn from zinc hydroxychloride.

[4] Combined total per kg of diet: 2,206 FTU phytase, 2,353 IU vitamin A, 735 IU vitamin D3, 43.5 IU vitamin E, 48 mg choline, 2.0 mg vitamin K3, 17.6 mg niacin, 11.7 mg pantothenic acid, 3.5 mg riboflavin, 5.9 ug vitamin B12, 12.4 mg Cu, 97 mg Fe, 0.5 mg I, 33 mg Mn, 0.33 mg Se, 120 mg Zn.

[5] Added to provide color to the diet to differentiate between diets and may contribute to Fe level in the diet.

[6] Included in the diet formulation, however product not included into the diet as fed.

Table 2.4 Ingredient composition and nutrient analysis of phase 4

Item	Control	ZnO	Organic Zn	ZnO + Organic Zn
Ingredient, %				
Corn	66.84	66.76	66.70	66.70
Soybean Meal	28.90	28.90	28.90	28.90
Fat	0.90	0.90	0.90	0.90
Limestone	0.73	0.73	0.73	0.73
Salt	0.60	0.60	0.60	0.60
Monocalcium phosphate	0.86	0.86	0.86	0.86
Crystalline AA	0.99	0.99	0.99	0.99
VTM Premix[1]	0.15	0.15	0.15	0.15
Zinc oxide	-	-	-	-
Organic Zn	-	-	0.06	0.06
Black ferroxide[2]	-	0.075	-	-
Blue ferroxide[2, 3]	-	-	-	0.075
Red iron oxide[2]	-	-	0.075	-
Analyzed Composition (%)				
Moisture	12.85	12.95	12.97	12.63
Dry Matter	87.15	87.05	87.03	87.37
Crude Protein	21.54	20.69	20.30	19.25
Soluble Protein	16.26	19.42	15.28	13.37
ADF	4.15	6.01	3.67	2.87
NDF	9.30	16.44	8.78	8.44
Starch	53.23	50.54	55.23	58.90
Ash	4.65	6.42	4.12	3.07
Ca	0.59	0.59	0.52	0.50
P	0.60	0.58	0.55	0.48
Mg	0.17	0.17	0.16	0.16
K	0.95	0.95	0.91	0.80
Cl	0.65	0.44	0.57	0.58
S	0.23	0.23	0.24	0.22
Na	0.33	0.24	0.26	0.25
Zn (ppm)	1529	440	359	247
Cu (ppm)	28	20	40	90
Fe (ppm)	263	675	367	195
Mn (ppm)	61	56	46	44

[1] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 12 mg Cu from copper chloride, 80 mg Fe from ferrous sulfate, 0.4 mg I from ethylenediamine dihydroiodide, 30 mg Mn from manganous oxide, 0.3 mg Se from sodium selenite, 100 mg Zn from zinc hydroxychloride.

[2] Added to provide color to the diet to differentiate between diets and may contribute to Fe level in the diet.

[3] Included in the diet formulation, however product not included into the diet as fed.

Table 2.5 Growth performance of nursery pigs fed diets containing no additional zinc, addition of zinc from zinc oxide or organic zinc, or a combination of zinc oxide and organic zinc

	ZnO		Organic Zn			*P*-value		
Items	With	Without	With	Without	SEM	ZnO	Zn	ZnO x Zn
BW, kg								
Initial	5.77	5.76	5.76	5.77	0.161	0.96	0.96	0.96
d 7	6.77	6.62	6.68	6.71	0.188	0.43	0.83	0.59
d 14	8.88*	8.24	8.55	8.57	0.273	0.01	0.93	0.52
d 21	11.90*	11.13	11.48	11.55	0.337	0.03	0.84	0.71
d 28	14.53	13.96	14.35	14.14	0.380	0.14	0.60	0.81
d 35	18.00	17.60	17.67	17.93	0.436	0.36	0.54	0.85
d 42	22.78	22.61	22.53	22.86	0.535	0.75	0.55	0.97
d 0 to d 7								
ADG, kg	0.143*	0.122	0.123	0.135	0.008	0.01	0.51	0.09
ADFI, kg	0.180*	0.145	0.175*	0.149	0.008	<0.01	<0.01	0.09
G:F	0.820	0.845	0.763	0.902*	0.031	0.59	<0.01	0.01
d 7 to d 14								
ADG, kg	0.301*	0.232	0.268	0.265	0.010	<0.01	0.71	0.49
ADFI, kg	0.351*	0.327	0.329	0.349	0.010	<0.01	0.06	<0.01
G:F	0.864*	0.711	0.821*	0.754	0.017	<0.01	0.01	<0.01
d 14 to d 21								
ADG, kg	0.430	0.411	0.417	0.424	0.017	0.29	0.70	0.94
ADFI, kg	0.593	0.562	0.574	0.581	0.017	0.08	0.67	0.88
G:F	0.723	0.728	0.725	0.726	0.012	0.74	0.93	0.99
d 21 to d 28								
ADG, kg	0.375	0.397	0.378	0.378	0.011	0.06	0.15	0.76
ADFI, kg	0.588	0.574	0.571	0.591	0.016	0.40	0.22	0.98
G:F	0.640	0.693*	0.669	0.664	0.011	<0.01	0.75	0.72
d 28 to d 35								
ADG, kg	0.495	0.517	0.499	0.512	0.013	0.12	0.34	0.70
ADFI, kg	0.762	0.781	0.761	0.782	0.018	0.30	0.24	0.83
G:F	0.651	0.662	0.657	0.656	0.008	0.32	0.92	0.37
d 35 to d 42								
ADG, kg	0.596	0.627	0.607	0.616	0.017	0.08	0.62	0.70
ADFI, kg	0.971	1.016	0.994	0.992	0.047	0.35	0.97	0.30
G:F	0.758	0.620	0.613	0.765	0.118	0.41	0.37	0.31
d 0 to d 14								
ADG, kg	0.222*	0.178	0.199	0.200	0.007	<0.01	0.92	0.17

ADFI, kg	0.265*	0.236	0.252	0.249	0.006	<0.01	0.66	0.08
G:F	0.835*	0.796	0.788	0.796	0.009	<0.01	0.56	0.88
d 0 to d 21								
ADG, kg	0.291*	0.255	0.272	0.274	0.009	<0.01	0.77	0.52
ADFI, kg	0.374*	0.344	0.359	0.360	0.009	<0.01	0.96	0.45
G:F	0.776*	0.738	0.759	0.755	0.008	<0.01	0.67	0.88
d 0 to d 28								
ADG, kg	0.312*	0.290	0.298	0.304	0.009	0.02	0.51	0.68
ADFI, kg	0.428*	0.401	0.412	0.417	0.010	0.02	0.63	0.60
G:F	0.730	0.723	0.725	0.728	0.006	0.43	0.77	0.95
d 0 to d 35								
ADG, kg	0.349	0.335	0.339	0.345	0.009	0.15	0.46	0.77
ADFI, kg	0.494	0.476	0.481	0.489	0.012	0.14	0.50	0.65
G:F	0.705	0.704	0.704	0.704	0.004	0.73	0.85	0.62
d 0 to d 42								
ADG, kg	0.394	0.389	0.388	0.395	0.010	0.58	0.48	0.91
ADFI, kg	0.582	0.576	0.576	0.582	0.014	0.66	0.68	0.74
G:F	0.676	0.680	0.675	0.681	0.009	0.76	0.62	0.45

*Within main effect, means lacking a common superscript differ ($P < 0.05$)

Table 2.6 Lactulose and mannitol concentrations and lactulose to mannitol ratio in urine from nursery pigs fed diets containing no additional zinc above nutritional levels, additional zinc from zinc oxide or organic zinc, or a combination of zinc oxide and organic zinc[1]

	ZnO		Organic Zn			*P*-value	
Item	With	Without	With	Without	SEM	ZnO	Zn
Lactulose, mM	0.041	0.040	0.039	0.042	0.010	0.93	0.81
Mannitol, mM	0.219	0.213	0.257	0.174	0.057	0.92	0.16
Lactulose:mannitol	0.248	0.232	0.190	0.291	0.073	0.83	0.18

[1] ZnO x Organic Zn interaction removed from model, no significant interactions found.

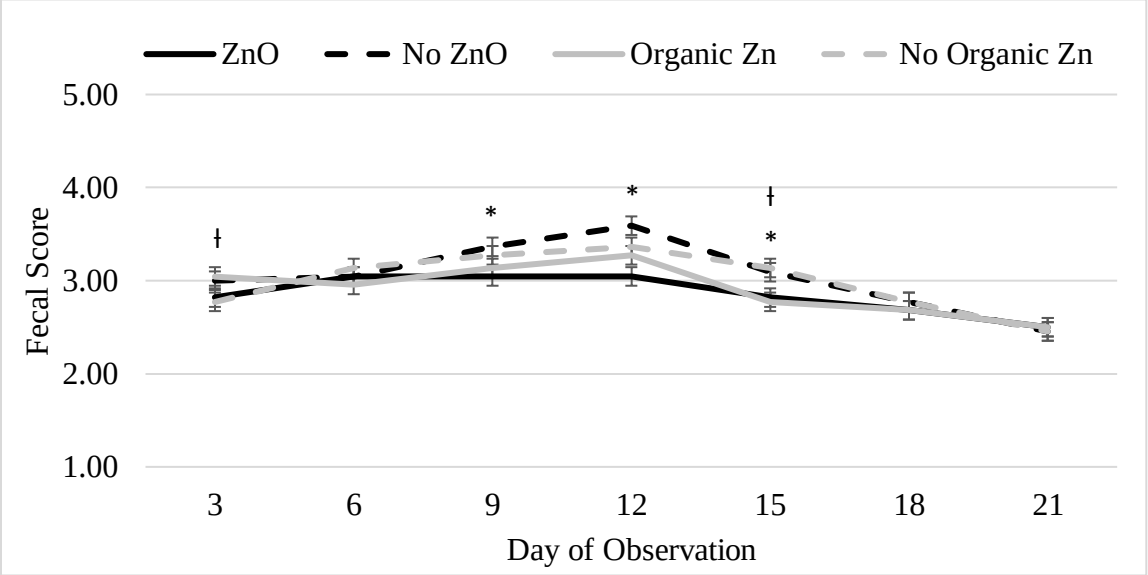


Figure 2.1 Fecal score observations from nursery pigs fed diets containing no additional zinc above nutritional levels, additional zinc from zinc oxide or organic zinc, or a combination of zinc oxide and organic zinc. Fecal consistency was categorized as a numerical scale from 1 to 5 as follows: 1 = hard, dry pellet-like feces, 2 = firm, formed feces, 3 = soft, moist feces that retains its shape, 4 = soft, unformed feces that doesn't keep its shape, 5 = watery, liquid feces.

There was an effect of ZnO by day (P = 0.05) on fecal score and Organic Zn by day tended to have an effect of fecal score (P = 0.07). Additionally, there was an effect (P ≤ 0.05) of ZnO, day, and ZnO*Organic Zn on fecal score.

*within main effect ZnO and day of observation means differ ($P < 0.05$)

ł within main effect Organic Zn and day of observation means differ ($P < 0.05$)

CHAPTER 3

EFFECT OF TRACE MINERAL INCLUSION LEVEL ON GROWING-FINISHING PIG PERFORMANCE, CARCASS CHARACTERISTICS, AND TRACE MINERAL FECAL, MANURE, AND SERUM CONCENTRATION

3.1 ABSTRACT

This experiment investigated the effect of trace mineral inclusion level on growing-finishing pig performance, carcass characteristics, and trace mineral fecal, manure, and serum concentrations. A total of 442 growing pigs in two groups were followed through phases 6 to 9 of a 9-phase feeding program. Each group utilized one room with 20 pens, two feeder types, and four manure pits: group 1 (n=195; 34.8 ± 0.9 kg initial BW) with a stocking density of 9 or 10 pigs per pen and group 2 (n=247; 39.1 ± 2.6 kg initial BW) with a stocking density of 12 or 13 pigs per pen. Shallow manure pit location of each room and feeder type dictated dietary treatment allocation as either 1) corn-soybean meal diet with full trace mineral supplementation or 2) corn-soybean meal with 50% trace mineral supplementation. Full and reduced trace mineral supplementation were provided from a commercial trace mineral premix and both exceeded NRC (2012) recommendations. Overall, there were no differences (P>0.05) observed in ADG, ADFI, or G:F between pigs fed full or 50% trace mineral supplementation. Additionally, there were no differences (P>0.05) in carcass characteristics between pigs supplemented with the different rates of dietary trace mineral. This supports previous observations that reducing or eliminating dietary trace mineral supplementation does not impact pig performance or carcass characterics. In contrast, the accumulated concentration of Fe

(230 vs. 136 ppm) and Mn (63 vs. 32 ppm) were reduced in the pit manure samples ($P<0.01$) as well as trace mineral concentration in the fecal samples were reduced ($P<0.01$) for Cu, Fe, Mn, and Zn. These results suggest increasing trace mineral supplementation contributes to excess trace mineral excretion in the manure; however, does not contribute to improved productivity.

3.2 INTRODUCTION

Commercial swine diets are formulated to meet the animals' requirements, while taking into consideration economic efficiency and environmental impact. Traditionally, most commercial swine diets are formulated well above NRC (2012) trace mineral requirement estimates (Flohr et al., 2016). Limited knowledge on trace mineral supplementation required to optimize performance while also minimizing unnecessary cost has made it difficult to define trace mineral requirements for swine. Therefore, excess trace minerals, often supplemented via a premix, are added to the diet at a "margin of safety" to account for variations of trace mineral content and bioavailability of sources. Additionally, contributions from innate dietary trace minerals in feedstuffs are ignored in diet formulation, adding to that "margin of safety".

Trace mineral concentrations are heavily regulated by homeostatic mechanisms in the body. However, excess trace mineral supplementation may lead to exceeding the pigs' physiological requirements (Hill et al., 2000; Spears 1996). Therefore, once the pigs' physiological requirement is met, large amounts of these excess trace minerals fed are then excreted in the feces. With regards to environmental impact, alternatives are being investigated to reduce the amount of nutrients excreted, including trace minerals. A

potential way to reduce the amount of trace minerals excreted in the feces is to reduce the overall trace mineral inclusion in the diet. However, with reducing trace mineral supplementation in the diet comes the concern of losing production efficiency due to the small gap between trace mineral requirements for production and potential risk of deficiency. Conversely, Patience and Gillis (1995), Mavromichalis et al. (1999), Shelton et al. (2005), and Gowanlock et al. (2013) reported that reducing or eliminating dietary trace minerals did not have an effect on pig performance or carcass characteristics. In addition, Creech et al. (2004) and Thomaz et al. (2014) reported that reducing trace mineral supplementation resulted in a decrease in the excretion of trace minerals in the feces. Therefore, the objective of this study was to evaluate the effect of trace mineral inclusion level on growth performance, carcass characteristics, and trace mineral fecal, manure, and serum concentration of the growing-finishing pig.

3.3 MATERIALS AND METHODS

South Dakota State University's Institutional Animal Care and Use Committee approved of the protocol (IACUC #2101-006E) used in this study. The experiment was conducted at the South Dakota State University Swine Research and Education wean-to-finish facility.

3.3.1. Animal housing, diets, and experimental design

A total of 442 growing pigs in two groups were followed through phases 6 to 9 of a 9-phase feeding program at the SDSU on-site wean-to-finish barn. Each group utilized one room with 20 pens, two feeder types (dry and wet-dry), and four shallow pits. Group 1 was on trial from February 2021 to April 2021 (n=195; 34.8 ± 0.9 kg initial BW) with a

stocking density of 9 or 10 pigs per pen (1.25m^2/ pig). Group 2 was on trial from March 2021 to May 2021 (n=247; 39.1 ± 2.6 kg initial BW) with a stocking density of 12 or 13 pigs per pen (0.95m^2/ pig). Pigs from both groups were sourced from the South Dakota State University Sow Teaching and Research facility and were progeny of PIC 1050 (Landrace x Large White) sows and composite Duroc boars. Pens within room were allotted to one of two dietary treatments dictated by feeder type (wet and wet-dry) and shallow manure pit location. Each dietary treatment utilized equal numbers of dry and wet-dry feeders and two shallow manure pits within each room; therefore, feeder type should not have an effect on performance differences between dietary treatments. Pens were 2.44 m x 4.88 m and contained a 4-slot stainless steel dry feeder or wet-dry feeder (SDI, Inc., Alexandra, SD) and two cup waterers, which provided *ad libitum* access to feed and water. Feed was delivered manually and weight of feed delivered to each pen was recorded.

Experimental diets were applied over phases 6 to 9 of a 9-phase feeding program and phases were based on a feed budget of 47.2, 52.7, 58.6, and 82.6 kg/pig (or until end of the trial) for phases 6, 7, 8, and 9 respectively. Dietary treatments included 1) corn-soybean meal diet with full trace mineral supplementation or 2) corn-soybean meal diet with 50% trace mineral supplementation. Trace minerals were supplied from a commercial trace mineral premix (J & R Distributing, Inc., Lake Norden, SD) and both full and reduced trace mineral supplementation exceeded the NRC (2012) recommendations. Samples were collected from all batches delivered for all four phases and stored in a freezer (-20°C) until subsamples were pooled together and sent for

analysis. Experimental diets were analyzed for moisture, dry matter, crude protein (CP), soluble protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), fat, starch, ash, and a full mineral panel (calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), chloride (Cl), sulfur (S), sodium (Na), zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn)) at a commercial laboratory (Analab, Agri-King, Fulton, IL). Trace minerals were analyzed utilizing Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) following AOAC official method 985.01.

3.3.2. Growth performance and carcass characteristics

Feed disappearance and pen weights were measured on day 0, 21, 42, 64, and at load out (final) for market to calculate ADG, ADFI, and G:F. Feed delivery reports and the amount of feed remaining in each feeder on weigh days were used to determine feed intake. Weight of feed remaining in feeders was calculated using a pre-determined feed density equation utilizing the density of the feed and measurement of the empty space in the feeder. Group 1 was marketed in two groups, with the initial cut occurring on day 76 of the study and the remaining pigs marketed on day 83. The initial cut represented two pigs per pen or approximately 21% of the total group inventory. Group 2 was marketed as one group on day 91 of the study. Prior to being shipped, pigs within pen were given a tattoo identification number. Pigs were shipped to a commercial processing facility where hot carcass weight (HCW), back fat (BF), loin depth (LD), and percent lean were measured and recorded for every pig.

3.3.3. Trace mineral concentration measurements

Two days after weigh days, blood, fecal, and pit samples were collected. Two barrows and two gilts representing the average of each pen were selected for blood and fecal sampling. Blood samples were collected from the jugular vein using an 18-ga x 1 ½ inch needle and a royal blue top trace element blood collection tube (BD Vacutainer, Franklin Lakes, NJ). Samples were centrifuged at 28,000 x g for 20 minutes. Serum was harvested, allocated into 1.5 mL microcentrifuge tubes, and 0.5 mL subsamples from all four pigs in each pen were pooled for analysis. Samples representing pen were stored at -20°C until shipped to be analyzed for Ca, cobalt (Co), chromium (Cr), Cu, Fe, K, Mg, Mn, Na, P, selenium (Se), and Zn (Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS). Fecal samples were collected in 50 mL conical tubes and stored at -80°C until freeze dried (Dura-Dry, Fits Systems, Kinetics Thermal Systems) and then finely ground (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany). Subsamples (1 g) from all four pigs in each pen were pooled and analyzed for Ca, P, Mg, K, Cl, S, Na, Zn, Cu, Fe, and Mn at a commercial laboratory (Analab, Agri-King, Fulton, IL). Trace minerals were analyzed utilizing ICP-OES following AOAC official guidelines 985.01. Pit samples were taken utilizing a hand-crafted ladle tool to reach the bottom of the pit. Two scoops were taken in each pen by using the tool to start the scoop as close to the bottom of the pit as possible and collecting manure all the way to the top of the manure. The two scoops per pen were pooled (10 total scoops) to represent manure in each pit. The pooled pit sample per time point for each pit was shipped and analyzed for moisture,

DM, total nitrogen, ammonium nitrogen, organic nitrogen, P, K, Ca, Mg, Na, S, Cu, Fe, Mn, and Zn. (Stearns DHIA Laboratories, Sauk Centre, MN)

3.3.4. Statistical analysis

Performance and carcass data were analyzed using the PROC GLIMMIX procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) considering the effect of dietary treatment where pen was the experimental unit. Fecal, serum, and pit manure data were analyzed by repeated-measures analysis using the PROC MIXED procedure and the compound symmetry covariance structure (CS) determined best fit by the smallest Bayesian Information Criterion (BIC) value. Significant differences were reported at $P < 0.05$ and tendencies for significance were reported when $0.05 \leq P \leq 0.10$.

3.4 RESULTS

The analysis of dietary treatments verified that CP, ADF, NDF, fat, starch, ash, and macrominerals were similar between dietary treatments within each phase (Tables 2.1 & 2.2). Analyzed concentrations of the trace minerals Zn, Cu, Fe, and Mn were greater in the diet with full trace mineral supplementation for all phases, as anticipated (Tables 2.1 & 2.2).

From d 0 to d 63, dietary treatment did not impact ADG ($P \geq 0.261$), ADFI ($P \geq 0.433$), or G:F ($P \geq 0.277$) for group 1, group 2, or for the groups combined. However, from d 63 to final, reduction in dietary trace mineral supplementation tended to decrease ADG (P=0.081) and G:F (P=0.062) for the groups combined. Additionally, overall (d 0 to final) G:F tended to decrease for group 2 (P=0.088) and for the groups combined

(P=0.063) with the reduction in dietary trace mineral supplementation. Dietary treatment did not have an impact on HCW ($P \geq 0.639$), BF ($P \geq 0.403$), LD ($P \geq 0.187$) or percent lean ($P \geq 0.346$) for group 1, group 2, or for the groups combined.

Reducing dietary trace mineral supplementation decreased ($P > 0.05$) Cu, Fe, Mn, and Zn fecal concentration over the entire course of sampling for group 1, group 2, and the groups combined. For the groups combined, pit manure Cu and Zn concentrations were not impacted ($P > 0.05$) by dietary treatment. However, pit manure Fe and Mn concentrations were increased ($P < 0.05$) when pigs were supplemented with full trace mineral. For group 1, dietary treatment only impacted ($P < 0.05$) pit manure Cu and Fe concentrations on day 2 of the trial, but no trace mineral accumulation differences occurred throughout the sampling period. In contrast, for group 2 full trace mineral supplementation increased ($P < 0.05$) Cu, Fe, Mn and Zn concentrations recovered from the shallow manure pits.

Group 2 serum concentrations did not differ ($P > 0.05$) between dietary treatments for Cu, Fe, Se, or Zn (Figure 3.7). However, on day 2 of the trial, pigs fed diets with reduced dietary trace mineral supplementation had increased ($P > 0.05$) serum Co concentration (Figure 3.7(A)). Pigs fed diets with full trace mineral supplementation had increased ($P > 0.05$) serum Cr concentration on day 44 (Figure 3.7(B) and serum Mn concentration on day 65 (Figure 3.7(E)). Group 1 serum samples were lost in transportation for analysis. Consequently, once receiving results from group 2 serum concentration, there was no indications for the need to re-send serum samples from group 1.

3.5 DISCUSSION

Previous research has reported that removing dietary trace mineral supplementation in late finishing swine diets does not affect growth performance (Patience and Gillis, 1995; Mavromichalis et al., 1999). More recently, Shelton et al. (2004) and Gowanlock et al. (2013) reported no adverse effects of removing trace mineral supplementation throughout the entire growing-finishing period. For the studies observing the entire growing-finishing period, limited number of pigs ($n \leq 222$) were utilized with lower stocking density per pen, fewer replicates, and the use of more dietary treatments. Factors such as stocking density may increase the requirement for trace mineral supplementation (Wu et al., 2018). In the current study, there were only tendencies for growth performance and feed efficiency in the last period prior to marketing (d 63 to final) for the groups combined and overall (d 0 to final) for feed efficiency for the groups combined and group 2 (Tables 3.3 & 3.5). However, in group 1, dietary treatment had no negative impact on growth performance (Table 3.4). These results may be explained by the difference in stocking density, feed wastage due to the different feeder types, or could be explained by a type I statistical error. Overall, there was no significant impacts to pig performance due to the reduction of dietary trace mineral supplementation (Tables 3.3, 3.4, and 3.5), which supports previous findings. Moreover, Patience and Gillis (1995), Mavromichalis et al. (1999), Shelton et al. (2004), and Gowanlock et al. (2013) conclusively reported that eliminating dietary trace mineral supplementation had no effect on carcass characteristics; these data agree with the results of our study (Tables 3.6, 3.7, & 3.8).

Excretion in the feces for Cu, Fe, Mn, and Zn were reduced throughout the entire growing-finishing period by reducing trace mineral supplementation to 50% (Figures 3.1,

3.2, and 3.3). Thomaz et al. (2014) reported reducing trace mineral supplementation by 50% for weanling pig diets resulted in a decrease in Cu excretion in the feces, but did not impact Fe, Mn, or Zn excretion. Meanwhile, Creech et al. (2004) reported that reducing trace mineral supplementation for gilts from weaning through growing and development reduced fecal mineral concentrations for Cu, Fe, Mn, and Zn, thus supporting the findings in the current trial. In addition, the increase in fecal mineral concentration contributed to an increase in Fe and Mn accumulation recovered from the shallow manure pits for both groups combined (Figure 3.4). However, when looking at the groups separate; group 1 trace mineral accumulation in the shallow manure pits were not impacted by dietary treatment (Figure 3.5), while in group 2 reducing dietary trace mineral supplementation significantly decreased Cu, Fe, Mn, and Zn concentrations (Figure 3.6). An important note to make is the shallow manure pits in group 2 were partially drained half-way through the trial due to manure levels reaching too close to the slats. If you take into consideration that all pits had an ample amount of manure prior to the trial starting, the results from the trace minerals recovered from the shallow manure pits in group 2 may provide a more accurate display of trace mineral accumulation in pit manure overtime. Manure already in the pits when the trial started may contribute to diluting the trace mineral concentrations recovered from the pits. Therefore, once the pits were drained, the manure that remained in the pits primarily represented the manure accrued during the current trial. To avoid potential differences, all pits should have been drained prior to the start of the trial.

Plasma and tissues are commonly used to reflect body mineral status (Martin et al., 2011). However, the concentration of trace minerals circulating and found in the tissue

necessary to maximize their dependent functions has not been defined. A majority of the emphasis is placed on Zn and Cu concentrations due to the potential dietary interactions. Creech et al. (2004) found that in pigs fed reduced trace mineral diets, plasma Zn concentration was lowered, but there was no impact on plasma Cu concentration. Others such as Gowanlock et al. (2013) analyzed hematological measures including hemoglobin and percent hematocrit when investigating the impact of reducing trace mineral supplementation. However, observed no differences as a result of reducing trace mineral supplementation. With the lack of knowledge on adequate trace mineral serum concentrations and previous research looking at Mn specifically, there is no explanation for the increase in serum Mn concentration observed on day 65 of the current trial (Figure 3.7). However, with the lack of differences observed in trace mineral serum concentrations, this supports the current trial findings of excess fecal excretion as a result of decreased efficiency of utilization of the minerals due to exceeding the animal's physiological requirement.

In conclusion, reduced dietary trace mineral supplementation during the growing-finishing phase decreased fecal trace mineral excretion. Meanwhile trace mineral supplementation near NRC (2012) requirements, did not have an impact on overall pig performance. Therefore, reduced dietary trace mineral supplementation may reduce cost input into the diets, while also decreasing the environmental impact associated with excess nutrient excretion.

Table 3.1 Ingredient composition and nutrient analysis of phase 6 and phase 7

	Phase 6		Phase 7	
Item	Full	Reduced	Full	Reduced
Ingredient, %				
Corn	79.25	79.32	83.30	83.37
Soybean Meal	17.68	17.68	13.83	13.83
L-Lysine HCL	0.40	0.40	0.38	0.38
L-Threonine	0.13	0.13	0.12	0.12
DL-Methionine	0.08	0.08	0.07	0.07
L-Tryptophan	0.01	0.01	0.01	0.01
Monocalcium phosphate	0.98	0.98	0.85	0.85
Limestone	0.97	0.97	0.94	0.94
Salt	0.30	0.30	0.30	0.30
Vitamin premix[1]	0.05	0.05	0.05	0.05
Trace mineral premix[2,3]	0.15	0.08	0.15	0.08
Analyzed Composition (%)				
Moisture	12.40	12.36	13.08	13.33
Dry Matter	87.60	87.64	86.92	86.67
Crude Protein	21.29	20.52	16.02	15.51
Soluble Protein	16.40	15.11	16.02	11.78
ADF	3.43	2.99	2.64	2.60
NDF	9.72	8.78	10.59	9.72
Fat	2.56	2.75	2.95	3.04
Starch	55.18	56.61	63.57	64.34
Ash	4.53	3.97	3.58	2.64
Ca	0.91	0.75	0.73	0.68
P	0.61	0.66	0.53	0.52
Mg	0.16	0.18	0.14	0.15
K	0.85	0.86	0.64	0.68
Cl	0.33	0.35	0.39	0.39
S	0.24	0.22	0.18	0.18
Na	0.16	0.17	0.17	0.15
Zn (ppm)	268	137	240	128
Cu (ppm)	25	10	14	12
Fe (ppm)	371	256	272	181
Mn (ppm)	77	38	53	39

[1] Provided per kilogram of the diet: 11,013 IU vitamin A, 1,652 TIU vitamin D3, 55 IU vitamin E, 4.4 IU vitamin K, 0.17 mg biotin, 1.1 mg folic acid, 55 mg niacin, 61 mg pantothenic acid, 10 mg riboflavin, 3.3 IU thiamin, 3.3 IU vitamin B6, 4.4 µg vitamin B12.

[2] Full trace mineral supplementation provided per kg of the diet: 16.5 mg Cu from basic copper chloride, 165 mg Fe from ferrous sulfate, 0.36 mg I from ethylenediamine dihydroiodide, 44 mg Mn from manganese sulfate, 0.30 mg Se from sodium selenite, 165 mg Zn from zinc sulfate.

[3] Reduced trace mineral supplementation provided per kg of the diet: 8.8 mg Cu from basic copper chloride, 88 mg Fe from ferrous sulfate, 0.19 mg I from ethylenediamine dihydroiodide, 24 mg Mn from manganese sulfate, 0.16 mg Se from sodium selenite, 88 mg Zn from zinc sulfate.

Table 3.2 Ingredient composition and nutrient analysis of phase 8 and phase 9

	Phase 8		Phase 9	
Item	Full	Reduced	Full	Reduced
Ingredient, %				
Corn	86.54	86.61	90.15	90.22
Soybean Meal	10.77	10.77	7.29	7.29
L-Lysine HCL	0.36	0.36	0.34	0.34
L-Threonine	0.11	0.11	0.11	0.11
DL-Methionine	0.04	0.04	0.03	0.03
L-Tryptophan	0.01	0.01	0.02	0.02
Monocalcium phosphate	0.76	0.76	0.68	0.68
Limestone	0.91	0.91	0.88	0.88
Salt	0.30	0.30	0.30	0.30
Vitamin premix[1]	0.05	0.05	0.05	0.05
Trace mineral premix[2,3]	0.15	0.08	0.15	0.08
Analyzed Composition (%)				
Moisture	13.58	13.91	13.72	13.59
Dry Matter	86.43	86.09	86.28	86.42
Crude Protein	13.80	15.14	12.57	12.45
Soluble Protein	10.71	9.49	6.77	8.66
ADF	1.73	2.53	2.97	1.51
NDF	8.51	9.62	7.51	8.69
Fat	3.14	3.61	3.08	3.39
Starch	67.43	64.18	71.96	70.20
Ash	2.54	2.65	1.71	2.35
Ca	0.71	0.55	0.56	0.52
P	0.50	0.52	0.44	0.44
Mg	0.14	0.15	0.12	0.13
K	0.58	0.63	0.51	0.50
Cl	0.39	0.30	0.39	0.33
S	0.17	0.16	0.16	0.15
Na	0.16	0.15	0.15	0.12
Zn (ppm)	209	115	182	119
Cu (ppm)	18	10	15	12
Fe (ppm)	257	154	231	157
Mn (ppm)	61	31	51	35

[1] Provided per kilogram of the diet: 11,013 IU vitamin A, 1,652 TIU vitamin D3, 55 IU vitamin E, 4.4 IU vitamin K, 0.17 mg biotin, 1.1 mg folic acid, 55 mg niacin, 61 mg pantothenic acid, 10 mg riboflavin, 3.3 IU thiamin, 3.3 IU vitamin B6, 4.4 µg vitamin B12.

[2] Full trace mineral supplementation provided per kg of the diet: 16.5 mg Cu from basic copper chloride, 165 mg Fe from ferrous sulfate, 0.36 mg I from ethylenediamine dihydroiodide, 44 mg Mn from manganese sulfate, 0.30 mg Se from sodium selenite, 165 mg Zn from zinc sulfate.

[3] Reduced trace mineral supplementation provided per kg of the diet: 8.8 mg Cu from basic copper chloride, 88 mg Fe from ferrous sulfate, 0.19 mg I from ethylenediamine dihydroiodide, 24 mg Mn from manganese sulfate, 0.16 mg Se from sodium selenite, 88 mg Zn from zinc sulfate.

Table 3.3 Combined growth performance of growing-finishing pigs in group 1 and group 2 fed full or reduced trace minerals[1]

Items	Full	Reduced	SEM	*P*-Value
BW, kg				
d 0	36.91	37.01	0.62	0.868
d 21	57.86	57.67	0.95	0.848
d 42	77.85	78.66	1.08	0.456
d 63	100.95	101.66	1.36	0.602
Final[2]	123.61	122.76	1.62	0.600
d 0 to d 21				
ADG, kg	1.00	0.98	0.02	0.382
ADFI, kg	2.31	2.34	0.04	0.433
G:F	0.43	0.42	0.01	0.227
d 21 to d 42				
ADG, kg	0.93	0.96	0.02	0.283
ADFI, kg	2.70	2.75	0.07	0.534
G:F	0.35	0.35	0.01	0.587
d 42 to d 63				
ADG, kg	1.07	1.07	0.03	0.825
ADFI, kg	3.13	3.11	0.06	0.766
G:F	0.34	0.35	0.01	0.886
d 63 to final[2]				
ADG, kg	1.06	0.99	0.03	0.081
ADFI, kg	3.23	3.26	0.11	0.803
G:F	0.33	0.31	0.01	0.062
d 0 to final[2]				
ADG, kg	1.02	1.00	0.01	0.195
ADFI, kg	2.85	2.87	0.04	0.638
G:F	0.36	0.35	0.004	0.063

[1] Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

[2] Final= d 83 for group 1 and d 91 for group 2. In group 1, there were two cuts with two pigs per pen removed on d 76.

Table 3.4 Growth performance of growing-finishing pigs in group 1 fed full or reduced trace minerals[1]

Items	Full	Reduced	SEM	*P*-Value
BW, kg				
d 0	35.09	34.60	0.41	0.246
d 21	55.76	55.08	0.85	0.433
d 42	77.45	78.43	1.23	0.431
d 63	100.57	101.76	1.80	0.513
Final[2]	115.05	114.77	2.06	0.894
d 0 to d 21				
ADG, kg	0.98	0.96	0.03	0.461
ADFI, kg	2.21	2.22	0.03	0.739
G:F	0.45	0.43	0.01	0.409
d 21 to d 42				
ADG, kg	1.05	1.01	0.04	0.261
ADFI, kg	2.70	2.75	0.11	0.619
G:F	0.37	0.38	0.01	0.440
d 42 to d 63				
ADG, kg	1.09	1.08	0.03	0.883
ADFI, kg	3.30	3.28	0.09	0.844
G:F	0.33	0.33	0.01	0.869
d 63 to final[2]				
ADG, kg	0.96	0.92	0.05	0.407
ADFI, kg	2.90	3.01	0.18	0.536
G:F	0.34	0.31	0.02	0.194
d 0 to final[2]				
ADG, kg	1.01	1.01	0.02	0.728
ADFI, kg	2.80	2.76	0.05	0.473
G:F	0.37	0.36	0.01	0.324

[1] Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

[2] Final= d 83. Pigs were marketed in two cuts with two pigs per pen removed on day 76.

Table 3.5 Growth performance of growing-finishing pigs in group 2 fed full or reduced trace minerals[1]

Items	Full	Reduced	SEM	*P*-Value
BW, kg				
d 0	38.72	39.42	1.23	0.581
d 21	59.95	60.27	1.71	0.854
d 42	78.25	78.89	1.84	0.731
d 63	101.33	101.56	2.12	0.915
Final[2]	132.18	130.74	2.56	0.581
d 0 to d 21				
ADG, kg	1.01	0.99	0.04	0.629
ADFI, kg	2.40	2.46	0.08	0.487
G:F	0.42	0.41	0.02	0.397
d 21 to d 42				
ADG, kg	0.86	0.86	0.03	0.800
ADFI, kg	2.71	2.74	0.10	0.727
G:F	0.32	0.32	0.01	0.919
d 42 to d 63				
ADG, kg	1.06	1.05	0.04	0.872
ADFI, kg	2.96	2.94	0.10	0.831
G:F	0.36	0.36	0.02	0.939
d 63 to final[2]				
ADG, kg	1.15	1.07	0.05	0.109
ADFI, kg	3.57	3.51	0.12	0.627
G:F	0.32	0.31	0.01	0.147
d 0 to final[2]				
ADG, kg	1.03	1.00	0.02	0.167
ADFI, kg	2.94	2.94	0.06	0.975
G:F	0.35	0.34	0.01	0.088

[1] Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

[2] Final= d 91.

Table 3.6 Combined carcass characteristics of finishing pigs in group 1 and group 2 fed full or reduced trace minerals[1]

Items	Full	Reduced	SEM	*P*-value
HCW, kg	93.4	92.9	1.39	0.705
BF, mm	18.0	18.3	0.47	0.527
LD, mm	70.3	66.2	3.01	0.187
Lean, %	56.1	56.2	0.59	0.750

[1] Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

Table 3.7 Carcass characteristics of finishing pigs in group 1 fed full or reduced trace minerals[1]

Items	Full	Reduced	SEM	*P*-value
HCW, kg	87.37	86.47	1.88	0.639
BF, mm	16.46	15.93	0.61	0.403
LD, mm	72.50	64.52	5.92	0.195
Lean, %	57.23	58.29	1.09	0.346

[1] Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

Table 3.8 Carcass characteristics of finishing pigs in group 2 fed full or reduced trace minerals[1]

Items	Full	Reduced	SEM	*P*-value
HCW, kg	93.45	92.92	2.45	0.830
BF, mm	17.99	18.30	0.79	0.703
LD, mm	70.27	66.23	3.01	0.187
Lean, %	56.06	56.25	0.78	0.812

[1] Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

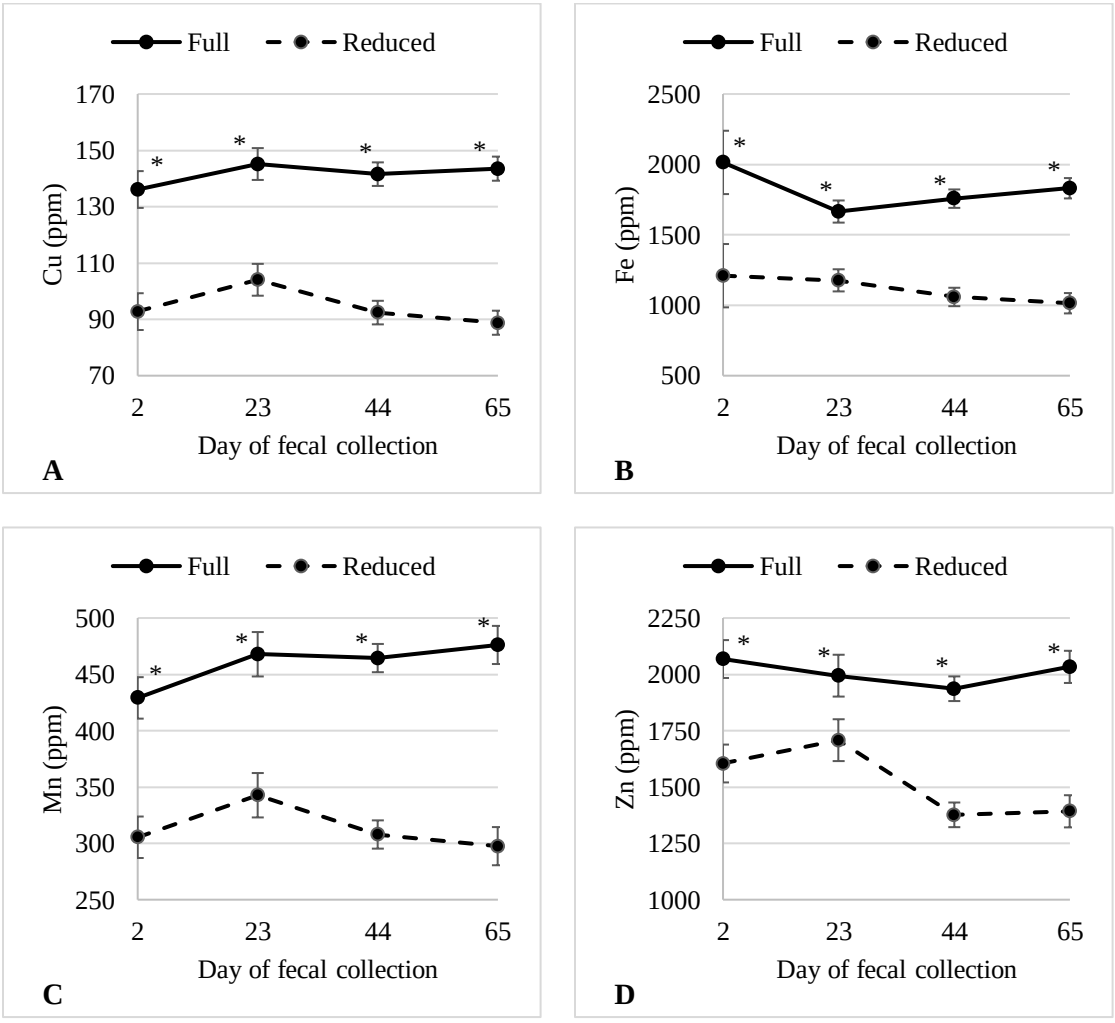


Figure 3.1 Trace mineral fecal concentration [**A**: Copper (Cu). **B**: Iron (Fe). **C**: Manganese (Mn). **D**: Zinc (Zn)] from two groups (group one and group two) over the grow-finish period.

* within day of collection, means lacking a common superscript differ ($P < 0.05$)

Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

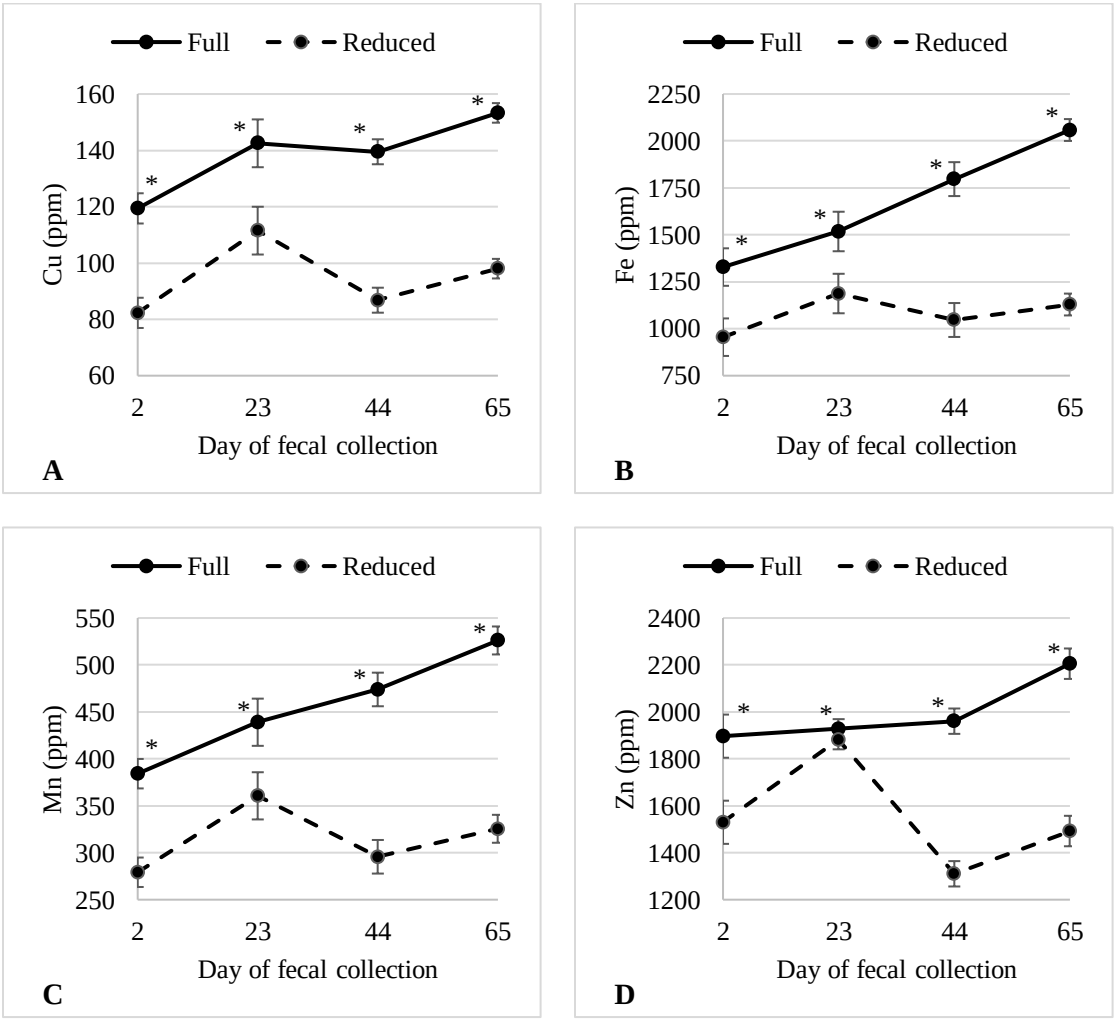


Figure 3.2 Trace mineral fecal concentration [**A**: Copper (Cu). **B**: Iron (Fe). **C**: Manganese (Mn). **D**: Zinc (Zn)] from group 1 over the grow-finish period.

* within day of collection, means lacking a common superscript differ ($P < 0.05$)

Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

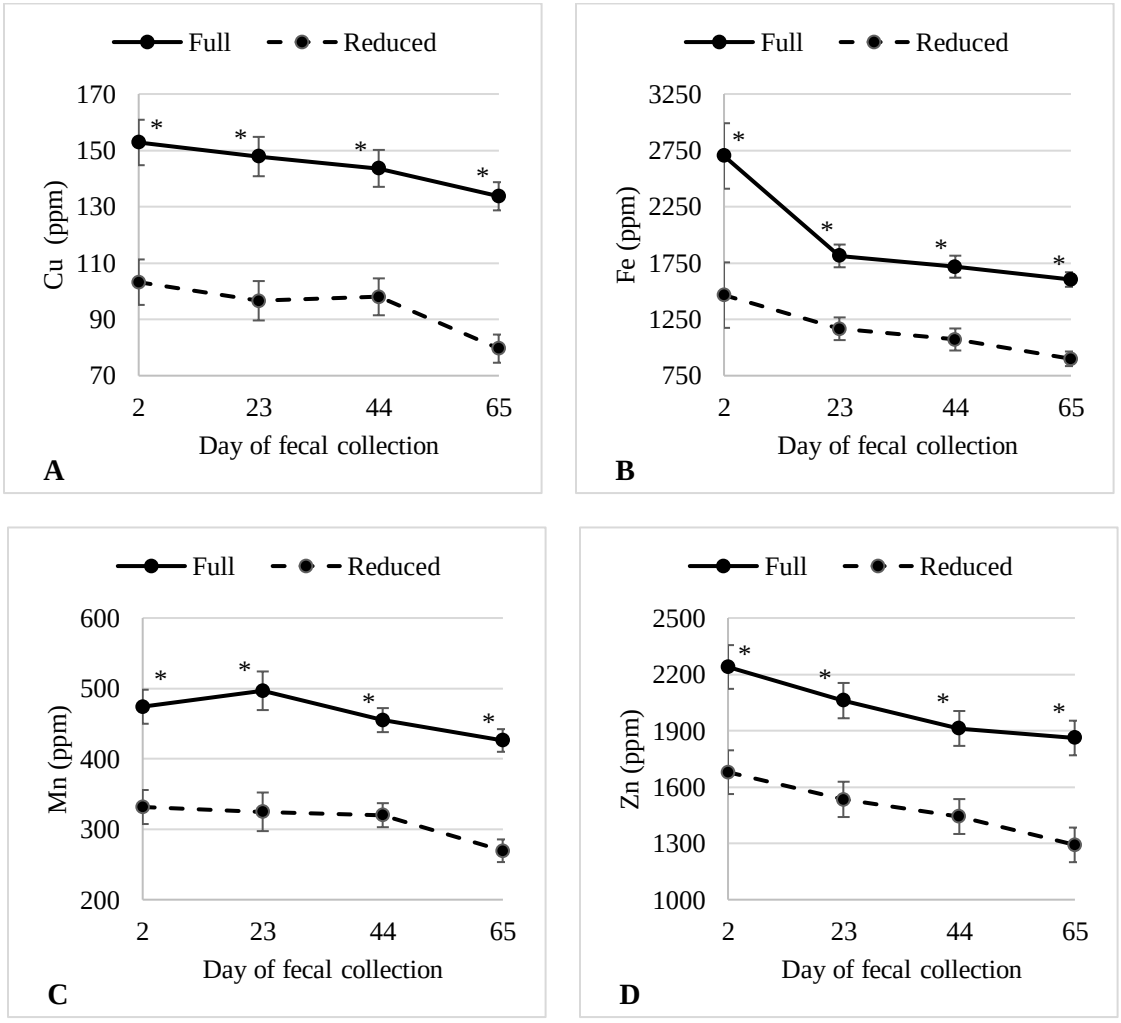
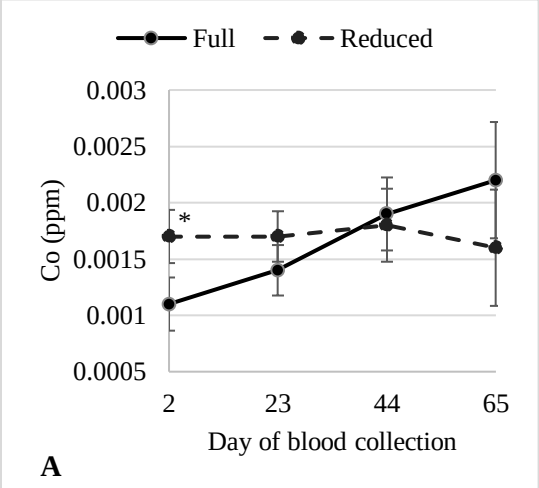


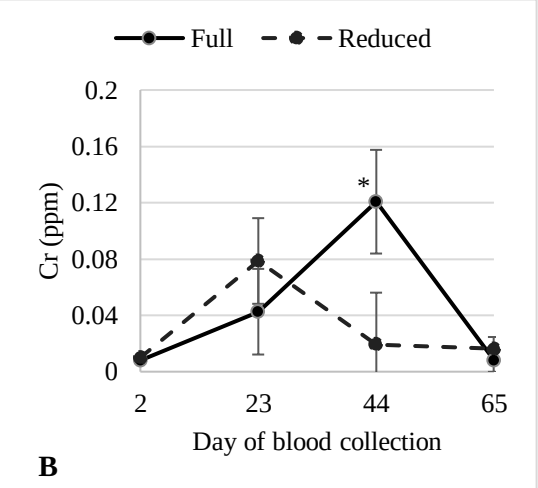
Figure 3.3 Trace mineral fecal concentration [**A**: Copper (Cu). **B**: Iron (Fe). **C**: Manganese (Mn). **D**: Zinc (Zn)] from group 2 over the grow-finish period.

* within day of collection, means lacking a common superscript differ ($P < 0.05$)

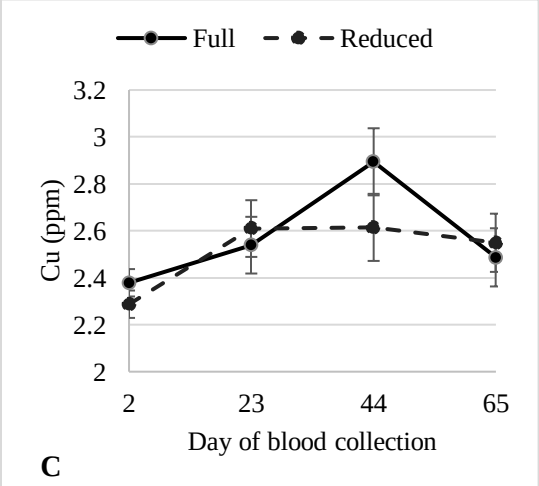
Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.


Full
Reduced
0.003
0.0025
0.002
0.0015
0.001
0.0005
Co (ppm)
*
2
23
44
65
Day of blood collection

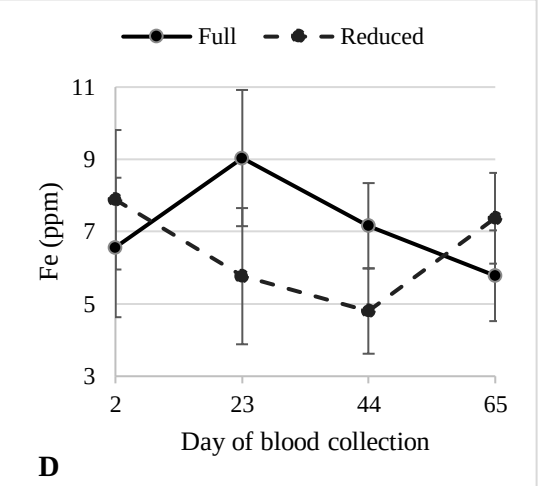
A


Full
Reduced
0.2
0.16
0.12
0.08
0.04
0
Cr (ppm)
*
2
23
44
65
Day of blood collection

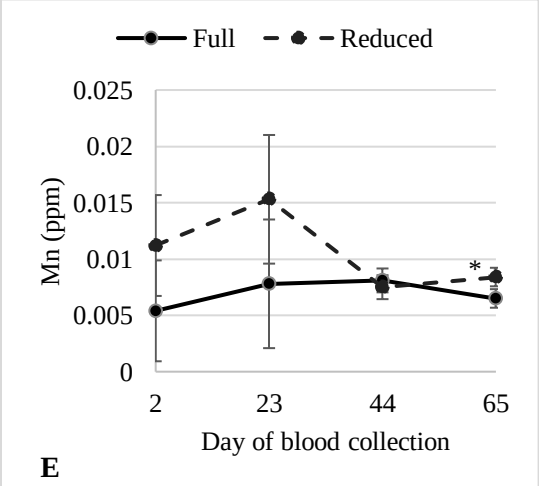
B


Full
Reduced
3.2
3
2.8
2.6
2.4
2.2
2
Cu (ppm)
2
23
44
65
Day of blood collection

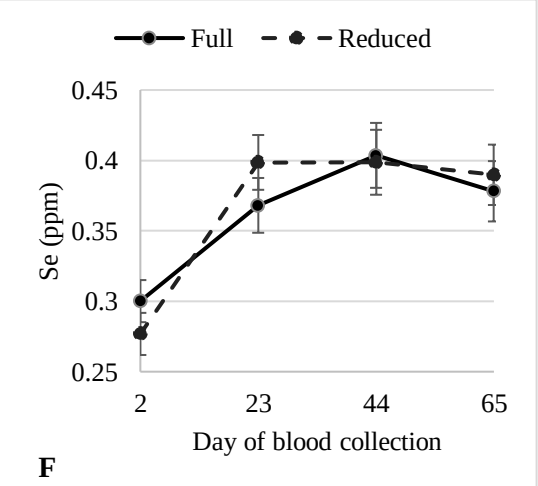
C


Full
Reduced
11
9
7
5
3
Fe (ppm)
2
23
44
65
Day of blood collection

D


Full
Reduced
0.025
0.02
0.015
0.01
0.005
0
Mn (ppm)
*
2
23
44
65
Day of blood collection

E


Full
Reduced
0.45
0.4
0.35
0.3
0.25
Se (ppm)
2
23
44
65
Day of blood collection

F

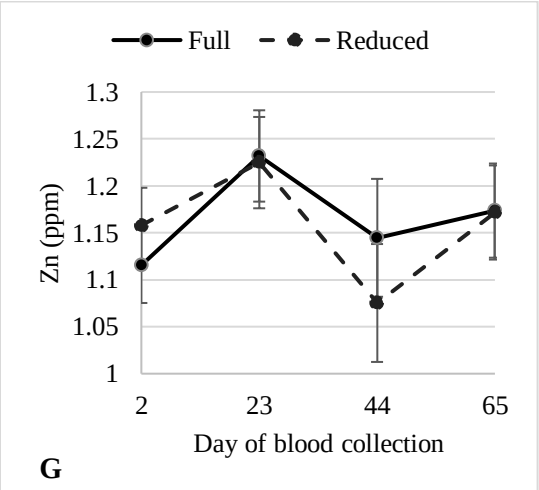


Figure 3.4 Trace mineral serum concentration [**A**: Cobalt (Co). **B**: Chromium (Cr). **C**: Copper (Cu). **D**: Iron (Fe). **E**: Manganese (Mn). **F**: Selenium (Se). **G**: Zinc (Zn)] from group 2 over the grow-finish period. Group 1 samples were lost in transportation.

* within day of collection, means lacking a common superscript differ ($P < 0.05$)

Corn-soybean meal diets with trace mineral supplemented at 100% (Full) and at 50% (Reduced) from a trace mineral premix.

FINAL DISCUSSION

Weaning is often associated with stressors that result in negative impacts to the pig. Zinc oxide (ZnO) fed at pharmacological levels has proven to mitigate these impacts associated with weaning stressors by increasing growth performance and feed efficiency, while reducing gut permeability and the incidence of post-wean diarrhea (Pouslen et al., 1989; Hill et al., 2001; Walk et al., 2015). However, the use of pharmacological levels of ZnO has raised concerns in regards to environmental impact and antimicrobial resistance. Additionally, some countries have even placed regulations on the use of ZnO at pharmacological levels for its growth promoting benefits. In an effort to find an alternative for ZnO, the use of organic zinc (Zn) sources have been investigated due to their higher bioavailability. However, results have been inconsistent. Therefore, it was hypothesized that organic Zn fed at concentrations well below pharmacological levels of ZnO would have similar benefits to growth performance, gut permeability, and fecal consistency for weanling pigs. Organic Zn supplementation proved to have improvements to ADFI for the initial 7-d post-weaning and G:F from d 7 to d 14. While ZnO supplementation resulted in increased ADG and ADFI for the initial 28-d and improved G:F for the initial 21-d post-weaning. Interestingly, in regards to gut permeability, supplementation from either Zn source did not result in any measured improvements in gut permeability. This suggests that the mechanism behind ZnO may not be directly associated with improved intestinal barrier function or it may be beneficial to evaluate gut permeability utilizing *ex-vivo* methodology, to have more measurements that further reflect gut barrier function. Supplementation from either ZnO or organic Zn resulted in improved fecal consistency over different time points during the initial 21-d of

the study. It is thought that reduced incidence of post-wean diarrhea may be associated with improved intestinal barrier function, however our results do not support that conclusion. To continue working towards an alternative for ZnO, there needs to be further investigation on the mechanism of action of ZnO.

Due to varying bioavailability of sources and limited amount of knowledge of requirements to maximize production, trace minerals are often times supplemented to swine diets above NRC (2012) recommendations. Excess trace mineral supplementation may lead to exceeding the pigs' physiological requirement and ultimately lead to large amounts of trace minerals then excreted in the feces. Supplementing trace minerals in excess comes with marginal cost input, but concerns of environmental impact is not something one can put a price tag on. In recent years, Shelton et al. (2004) and Gowanlock et al. (2013) reported no adverse effects on performance or pork quality from eliminating trace mineral supplementation in the growing-finishing period. Meanwhile, Creech et al. (2004) found a decrease in trace minerals excreted with a reduction in trace mineral supplementation in swine diets. Thus, it was hypothesized that reducing trace mineral supplementation by 50% would have no impact on growth performance or carcass characteristics, but decrease the trace mineral concentrations recovered in feces and manure. As observed in Chapter 3, the results are consistent with the null hypothesis.

Based on growth parameters and carcass characteristics, reducing trace mineral inclusion in growing-finishing pig diets is feasible without effecting overall performance. It may be beneficial to test reducing trace mineral supplementation in a commercial facility, to assess growth performance and carcass characteristics with an increased

stocking density. Although previous research reports no negative impacts on pork quality, reducing trace minerals has been shown to decrease trace minerals found in tissue (Shelton et al., 2004). In regards to the effect on human nutrition, it would be beneficial to determine if a reduction in trace minerals found in the tissue has an effect on health of humans consuming pork as a source of these trace minerals. Trace mineral concentrations excreted in the feces decreased with reduced trace mineral supplementation; subsequently trace minerals recovered from the pit manure decreased. The reduction of trace minerals excreted in the feces with feeding pigs diets with reduced trace mineral inclusion is a step towards improving environmental sustainability. However, in an effort to continue to reduce trace mineral inclusion in swine diets and reduce trace mineral excretion in manure, further investigation with the use of organic trace minerals with higher bioavailability may be warranted.

In conclusion, supplementation of Zn early in the post-weaning period provides benefits to growth performance and improved fecal consistency without measured impact on gut permeability. This suggests that supplementation organic Zn at lower concentrations than pharmacological levels of ZnO has potential to produce similar benefits for the weaned pig. Additionally, reducing trace mineral inclusion level did not have an impact on growth performance or carcass characteristics of growing-finishing pigs, but did decrease the trace mineral concentrations found in the feces and pit manure. Therefore, increased trace mineral supplementation contributes to excess trace mineral excretion in the manure; however, does not contribute to improved productivity.

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VITAE

Marissa Noelle LaRosae was born in Peoria, Illinois on July 26, 1999. At a young age her family relocated to Lafayette, Indiana where she spent her youth. She graduated from McCutcheon High School in May of 2016. She was heavily involved in 4-H and FFA which led her pursue an Associates of Science degree in Agriculture at Ivy Tech Community College. While at Ivy Tech Marissa took an animal reproductive physiology course and started working part-time for a commercial sow farm which sparked her passion for reproductive physiology and the swine industry. Following completion of her Associate's degree Marissa decided to transfer to Wilmington College, however only spent one semester there prior to making the choice to transfer again back to her hometown to attend Purdue University. During her time at Purdue University she joined Dr. Kara Stewart's lab group where she had the opportunity to assist in research focused in boar semen quality and her interest in reproductive physiology continued to grow. In addition to research Marissa was also a student employee at the Swine Research and Education Center, farrowing club senior member, and mentored other transfer students. Outside of the university, Marissa spent a summer in Clay Center, Nebraska at the U.S. Meat Animal Research Center (MARC) as a research intern alongside a geneticist investigating sow lameness. Through these experiences she realized that her passion wasn't narrow focused on reproductive physiology, and that her passion was for swine production as a whole. To become more well-rounded in her knowledge of the swine industry she decided that she needed to shift her focus to swine nutrition. Following graduation from Purdue University with a Bachelor's degree in Animal Science in May 2020 her curiosity and passion for the swine industry led her to furthering her education.

Marissa began a Master's degree program at South Dakota State University working in the area of swine nutrition focused on trace mineral source and inclusion level for nursery and growing-finishing pigs under the guidance of Dr. Ryan Samuel. She completed her Master of Animal Science degree on March 23rd, 2022.