Evaluation of Trace Mineral Supplementation in Neonatal and Nursery Swine

by

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(Under the Direction of C. Robert Dove)

ABSTRACT

The objective of these experiments was to evaluate trace mineral supplementation in neonatal and nursery swine. The first experiment determined there was a linear response to the increasing levels of supplementary manganese in nursery pig diets. Manganese superoxide dismutase activity tended to be lower in treatment groups supplemented with less selenium and manganese. The second experiment evaluated the use of a multi-element trace mineral supplementation product on piglets. There was no significant effect of the product on average daily gain or survivability, but there were significant effects of birthweight on average daily gain and sire on survivability.

INDEX WORDS: Trace minerals, injectable trace minerals, manganese, selenium, manganese superoxide dismutase, neonatal swine, nursery swine, survivability, growth performance

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TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

Page

CHAPTER 1

Introduction

Feed costs are the primary expense in swine production. The end goal of the swine industry is to grow swine as quickly and efficiently as possible. Nutrient requirements for swine have been established for most classes of nutrients (NRC, 2012). However, there is always room for improvement in many areas. Trace minerals are an interesting subclass of nutrients. The requirements of trace minerals are less understood than those of protein or energy. Requirements were first established by the minimum amount of a mineral that was needed to overcome a deficiency symptom. Deficiency and toxicity symptoms in swine can vary in severity depending on the mineral and the degree to which it has been supplemented.

The goal of these experiments was to evaluate trace mineral supplementation in nursery and nursing swine. Growth performance was the main parameter of interest in both experiments because the impact of altering trace mineral supplementation on growth performance has the greatest implication in the scope of the swine industry. The effect of weaning on the levels of oxidative stress in swine have also been established (Cao et al., 2018). Antioxidants are an important defense mechanism against reactive oxygen species. Manganese and selenium are both cofactors (or activators) for two different antioxidant enzymes: manganese superoxide dismutase and glutathione peroxidase (Haikarainen et al., 2014; Reese & Hill, 2006; Rotruck et al., 1973). Manganese superoxide dismutase activity was an additional parameter of interest in the nursery study to determine the impact of manganese and selenium supplementation on the antioxidant enzyme activity.

Injectable trace mineral supplements are often used in the beef and dairy cattle industries. There has been limited work done to determine if these supplements have any effect on the growth performance of swine. The second experiment evaluated the effect of an injectable multi-element trace mineral supplement on the growth performance and survivability of nursing piglets. This product has the potential to be utilized in the industry if the results of this experiment are positive.

CHAPTER 2

Literature Review

Mineral Supplementation in Swine Nutrition

Mineral supplementation plays an important role in the proper growth and development of not only swine, but all livestock species. Over decades of animal research, there have been a handful of minerals that have been marked as being essential to include in the diets of animals for a variety of reasons (McDowell, 2003). Generally speaking, there are two classes of minerals: macrominerals and microminerals (or trace minerals). The prefixes associated with these two classes (macro- and micro-) have less to do with the physical size of mineral itself but the inclusion level of the mineral in the diet (McDowell, 2003; Reese & Hill, 2006). Macrominerals such as calcium and phosphorus are included as a percentage of the diet (g/kg in the diet, whereas microminerals such as copper, manganese, and iron are included as parts per million (mg/kg in the diet) (Reese & Hill, 2006).

Minerals have highly specific physiological roles in the organism. For example, in eukaryotic cells, iron-sulfur clusters are found in several enzymes such as ribonucleotide reductases which are involved with DNA repair and replication, and hemoglobin which is involved in oxygen transport (Zhang, 2014). There are many other enzymes and transport proteins that require the presence of a metal ion as a cofactor or activator in order to function (McDowell, 2003). Minerals are involved with structural functions in some tissues, while they serve in a regulatory function in others (McDowell, 2003). Calcium makes up a large portion of bone mineral composition along with phosphorus, but calcium also plays a role in the synaptic transmission of signals between neurons (Arruda & Hotamisligil, 2015; McDowell, 2003). The activation of enzymes by the presence of the appropriate mineral cofactor is critical for normal physiological function.

Ingredients that are commonly used in swine diets such as cereal grains and soybean meal are often deficient in required minerals or the minerals that are in the ingredients are not considered to be available to the animal (Reese & Hill, 2006). Depending on the part of the country ingredients are grown, certain minerals can have higher levels than others, such as selenium (McDowell, 2003). For example, selenium in the northeastern part of the country is less than 0.05 ppm in approximately 80% of the forage and grain, but in areas like Texas and Oklahoma, 80% of the forages and grain contain more than 0.1 ppm selenium (McDowell, 2003).

Meeting the physiological mineral requirement of an animal is dependent on the bioavailability of the mineral itself. Some minerals are supplemented in an organic form (e.g. chelated to an amino acid or peptide) or in an inorganic form (e.g. a mineral salt). Organic, inorganic, and no trace mineral supplementation in the grow-finish phase of swine production has been studied to determine the effects on growth performance and carcass characteristics (Burkett et al., 2006). Not supplementing trace minerals has an adverse effect on growth performance at this stage of production (Burkett et al., 2006). There were not significant differences in growth performance or carcass characteristics observed when comparing organic and inorganic sources of trace minerals (Burkett et al., 2006). The use of organic minerals has the potential to reduce mineral excretions in swine, since these can be more bioavailable to the organism (Burkett et al., 2006). A study performed in broilers came to the conclusion that organic trace minerals can be fed at lower amounts and can still maintain broiler performance under commercial conditions (Tavares et al., 2014). A case could be made for using either source exclusively or in part, but it is important to know the difference between the sources and the impact it can have on the animal and the surrounding environment.

There are publications released every few years or so that highlight the specific nutrient requirements of certain species, and swine are no different (NRC, 2012). Mineral requirements are less understood than requirements for protein or energy (López-Alonso, 2012). When too much or too little of a mineral included in a diet, clinical toxicity and deficiency signs are noted (respectively) and studied closely (López-Alonso, 2012; Reese & Hill, 2006). Each mineral is unique in its physiological role and properties, and when in short supply or in excess, the organism is not able to function properly. Requirements for minerals are initially based on the minimum amount of a mineral needed to overcome a deficiency symptom (López-Alonso, 2012). Signs of deficiencies and toxicity can range from visible signs to behavioral signs (clinical) to signs that can only be noticed when bloodwork is taken (subclinical) (McDowell, 2003). Livestock industries will only profit from animals who are performing properly, and mineral nutrition plays a large part in the wellbeing and productivity of livestock species (López-Alonso, 2012). Although minerals are included in the diet in such small amounts, they can have a large economical and biological impact on these livestock industries, including swine production (López-Alonso, 2012).

Weaning Stress

The process of weaning for any species of mammal can be a difficult and stressful time for the offspring. Stress can be the result of a situation that increases the anxiety of an individual. The newly weaned animal is going through a state of forced adaptation and is subjected to a variety of new stressors: nutritional, immunological, social, and even psychological (Cao et al., 2018). The animal is immersed into a new environment, where exposure to new pathogens and other sources of disease becomes more prevalent.

With all of these new stresses that the newly weaned animal is exposed to, one that seems to play a huge role in the developing animal is nutritional stressors. In swine, these piglets are switching from a liquid diet that is high in fat and to a solid diet that is low in fat and higher in protein and carbohydrate content (DeRouchey et al., 2010). At this point in the piglets emerging digestive system, digestive enzymes have not yet fully developed and so during the first week to week and a half the piglet does not eat much feed or gain much weight (Owsley et al., 1986).

There has also been evidence to suggest that a piglet's intestinal barrier function is significantly decreased after weaning (Cao et al., 2018; Hu et al., 2013). Part of this impaired intestinal barrier is due to the production of ROS species in the intestinal mucosal cells, which have a high energy requirement, and therefore are metabolizing oxygen at a higher rate (Holley, Bakthavatchalu, Velez-Roman, & St. Clair, 2011). These accumulating ROS repress the expression of tight junction proteins, whose sole purpose is to minimize paracellular space in the intestinal mucosa (Anderson & Van Itallie, 2009). When tight junctions are compromised, pathogens are allowed to leak through these junctions and are able to enter into the organism's bloodstream, which can lead to subsequent infection and inflammation (Anderson & Van Itallie, 2009).

A newly weaned piglet's antioxidant system is immature and cannot process and eliminate ROS efficiently. Antioxidant enzymes like SOD and GPX have significantly decreased activity and mRNA expression days three and seven post-weaning (Cao et al., 2018). Explanations for this decrease in activity in the intestine could be due to an overproduction of ROS with a limited amount of antioxidant enzymes being produced and subsequently eliminating these ROS. In piglets with an immature antioxidant and immune system, and a compromised intestinal barrier, these newly weaned animals are susceptible to new disease and physiological stresses, which in turn can have a large impact on the development of the animal (Wijtten et al., 2011).

Reactive Oxygen Species

Reactive oxygen species (ROS) are the by-products of oxygen metabolism and have been shown to be crucial mediators in a variety of cellular processes such as cell adhesion, apoptosis, and immune response in the host (Holley et al., 2011). There must be a delicate balance between ROS production and destruction, and when that balance is interrupted an accumulation of ROS can occur (Holley et al., 2011). If left unchecked by antioxidant enzymes such as SODs and GSH-Px, ROS can cause oxidative damage to membrane lipids, proteins, and DNA (Lubos et al., 2011) This accumulation can lead to a number of diseases, including certain types of cancer and neurological disorders (Brière et al., 2006; Waris & Ahsan, 2006).

The mitochondrion is the major oxygen-metabolizing organelle of the cell and is therefore a major source of ROS in the cell, with the superoxide radical ($O_2^{\bullet -}$), as the first mitochondrial ROS produced (Holley et al., 2011). The superoxide radical is then able to mediate the production of other reactive species, including reactive nitrogen species such as peroxynitrate (Huie et al., 1993). ROS is able to alter cellular function by affecting the activity of proteins such as serine/threonine kinases and tyrosine and serine/threonine phosphatases, and multiple transcription factors like HIF-1 and p53 (Fojta et al., 1999; Galanis et al., 2008).

The superoxide radical ($O_2^{\bullet -}$) is short-lived, however it is able to spontaneously or enzymatically transformed into hydrogen peroxide and molecular oxygen (Lubos et al., 2010) . The regulation of hydrogen peroxide can prevent the formation of the hydroxyl radical, which is highly reactive and destructive. The hydroxyl is formed by the reaction of hydrogen peroxide and Fe^{2+}, known as the Fenton reaction (Liddell et al., 2006). Under oxidative stress conditions where hydrogen peroxide may accumulate within the cell and Fe^{2+} is released from intracellular storage sites, the Fenton reaction plays a more prominent role in oxidative damage (Perron et al., 2009). Low levels of hydrogen peroxide serve two functions: maintaining the essential modifications of protein thiols and acting as a second messenger in signal transduction by monitoring the oxidation state of redox-sensitive cysteines (Cys) in order to allow for proper kinase function (Forman et al., 2010; Paulsen et al., 2010).

Basal levels of ROS can be maintained by either increasing the production of ROS from internal sources, introducing exogenous ROS-generating agents, or diminishing cellular antioxidant capacity, or a combination of the three (Holley et al., 2011). Altering the function or expression of MnSOD can have dramatic consequences on mitochondrial function and overall health of the cells due to oxidative damage to various key metabolic enzymes localized to this organelle and can lead to the development of different diseases (Miao & St. Clair, 2009; Oberley & Buettner, 1979). The role of MnSOD is in scavenging mitochondrial ROS and maintaining them at the proper levels, which protects the mitochondria in turn from the adverse effects of those mitochondria-derived ROS (Holley et al., 2011).

Manganese

Manganese (Mn) is a crucial inorganic element and a part of a group of minerals known as trace minerals. This group of minerals is found in small concentrations in many feedstuffs that are fed to livestock species. Corn grain is typically poor in manganese (5-6 mg/kg dry matter) and soybeans only slightly exceed 30 mg/kg dry matter (Berta et al. 2004).

Manganese is absorbed via the intestinal tract and tissue levels are regulated by excretion via bile and the intestine (Mineral Tolerance of Domestic Animals 1980). Manganese enters cells via passive diffusion or active transport and is transported in a similar manner to other divalent cations like calcium or iron (Chen et al., 2018). Passive diffusion does not require energy in the form of adenosine tri-phosphate, whereas active transport does. The divalent transporter 1 (DMT1) is one of the main transporters for Mn influx into the cell, however in the presence of other divalent cations, Mn must compete with other metals for the transporter (Chen et al., 2018). Absorption of manganese can be decreased by feeding excessive levels of calcium, phosphorus and iron (Mineral Tolerance 1980; Hansen et al. 2009).

One of the known physiological reactions that involve manganese is that of mitochondrial superoxide dismutase (SOD); whose primary function is to protect the mitochondria from oxidative damage caused by reactive oxygen species (Haikarainen et al., 2014). Manganese is an obligatory component of mitochondrial SOD. It is also the preferred cofactor of glucosyl transferases, whose function is associated with the synthesis of chondroitin sulfate (Mineral Tolerance 1980, NRC 2012). Chondroitin sulfate is an essential component of mucopolysaccharides in the organic matrix of bone (Leach & Muenster, 1962). It has been shown in several laboratory species such as mice, rats, and rabbits that a deficiency in manganese can result in a variety of abnormalities in bone growth like perosis as well as reproductive failures (Chen et al., 2018; Wilgus et al., 1939). In a series of experiments dealing with manganese deficiency in swine, several important conclusions were made regarding the manganese requirement of this species (Plumlee et al. 1960). When there was prolonged feeding of a diet that was manganese-deficient (0.5 ppm Mn), gilts began to express a variety of deficiency symptoms including: reduced bone growth and skeletal muscle weakness, increased fatness, irregular estrus cycles, complete absence of estrus or absence of signs of estrus, and fetus resorption or birth of small, weak pigs (Plumlee et al., 1960). Other Mn deficiency signs noted were poor udder development and absence of milk production. In a related study, diets with 75, 225, 675 and 2,025 ppm Mn were fed to nursery pigs and effects of supplementation were analyzed (Leibholz et al., 1962). Piglets supplemented with lower levels showed no adverse effects, but those supplemented levels 675 ppm and higher exhibited decreased growth and hemoglobin levels and increased bone Mn levels (Leibholz et al., 1962).

In addition to impairing proper bone development, manganese deficiencies have also been linked to reproductive abnormalities in multiple species. Manganese has been suggested as being a cofactor for both mevalonate kinase and farnesyl pyrophosphate synthase (Curran, 1961; Xie et al., 2014). Both of these enzymes are involved in the production of squalene, a precursor for cholesterol, and subsequently progesterone (Xie et al., 2014). If manganese is deficient, progesterone levels would ultimately be decreased. Therefore, the estrous cycle of an animal (of any species) can be potentially altered. Hansen et al. performed a study that looked at feeding varying levels of supplemental manganese to heifers and its impact on reproductive performance, and another study following the same animals through gestation and parturition (Hansen et al., 2006b, 2006a). There did not seem to be a significant impact on reproductive performance, however, in the study involving these heifers' calves, it was noted that calves born to heifers fed basal levels of manganese had significantly lower birth weights and serum Mn concentrations (Hansen et al., 2006a). Physical signs of manganese-deficiency were also significant in calves from heifers fed control levels of Mn (Hansen et al., 2006a). Calves from this group experienced significant unsteadiness, swollen joints, and disproportionate dwarfism (Hansen et al., 2006a).

Accumulation of excess Mn can also cause issues in the animal. When in excess, the target tissue of Mn is the brain (Chen et al., 2018). Symptoms associated with accumulation of

Mn in humans have been reduced response time to stimuli, mood changes, and hormonal synthesis and regulation can be altered (Chen et al., 2018). In animals and humans both, Mn toxicity can lead to developmental and reproductive issues in addition to neurological issues (Chen et al., 2018; Milatovic et al., 2018).

As described above, dietary Mn levels must be properly maintained in order to ensure proper growth and development of any species. Concentrations that exceed or fall below the animal's Mn requirement can have negative physiological consequences (Chen et al., 2018; Milatovic et al., 2018).

Forms of Manganese

Typically, manganese is included into a diet through a trace mineral premix along with other minerals like zinc, copper, and iron. It is important for Mn (as well as other trace minerals) to be supplemented in order to meet but not exceed the physiological requirement of the animal (McDowell, 2003). When trace minerals are supplemented in excess, there are increased excretions of minerals (Liu et al., 2014). As a result, many diets today are formulated to optimize the appropriate levels of trace minerals like Mn while minimizing the amount of mineral excretions from the animal.

Other than meeting the trace mineral requirements of an animal, the form in which they are supplemented (organic versus inorganic) can also vary and may reduce mineral excretions (Burkett et al., 2009; Veum et al., 2004). Zinc, copper, manganese, and iron are most often included in pig diets in the form of inorganic salts, like sulfates or oxides. There is a working theory that may elucidate why inorganic mineral sources may not be as efficacious as organic sources (Liu et al., 2014). The lower gastric pH has the potential to dissociate the trace mineral salts, resulting in the formation of "antagonisms" among trace minerals and phytic acid, which could impair mineral absorption and bioavailability of these minerals (Liu et al., 2014). The inorganic form of manganese that is typically supplemented in swine species as well as poultry species is manganese sulfate ($MnSO_4$) and manganese (II) oxide (MnO). As mentioned previously, these forms must be fed to meet the physiological requirements of the animal.

Organic trace minerals may be more bioavailable than inorganic sources when fed to pigs or poultry (Liu et al., 2014). Organic trace minerals are minerals that have been chelated to organic molecules such as amino acids. Peptides or amino acids from proteins that have been hydrolyzed are some of the most common forms of commercially complexed manganese (Berta et al., 2004). The supposed advantages in bioavailability of manganese from these supplements have primarily been attributed to better solubility or to the structure of the compound (Berta et al., 2004). Studies that center on the bioavailability of manganese are quite controversial in their results; some studies report that Mn chelated with protein or methionine have an equal (Baker & Halpin, 1987) or greater (Henry et al., 1989) effect when compared to inorganic sources.

In a study performed by Berta et al, the effect of inorganic and organic manganese was on tissue manganese content in broiler chicks was evaluated (Berta et al., 2004). Two sources of manganese were used in this study: organic Mn (Mn fumarate) and inorganic Mn (MnO). A basal soybean diet was supplemented with levels of 0, 30, 60, and 240 ppm Mn from both sources (Berta et al., 2004). There were no significant effects of treatment on body weight, efficiency (F:G), or mortality rates. Necropsy results showed no significant growth impairments among treatments and average (and absolute) organ weights were not significantly different. At the same levels of supplementation of the two sources, there were no significant differences between the Mn concentrations of tissues and organs. The Mn found in droppings reflected the intake, however the feathers and blood plasma showed only extreme Mn loading, which was to be expected (Berta et al., 2004). This is an example of a study whose results showed no significant differences between organic and inorganic sources of manganese.

In a study performed by Liu et.al, the effect of inorganic and organic sources of trace minerals (Zn, Cu, Mn, and Fe) on the apparent total tract digestibility and retention rate in pigs (Liu et al., 2014). There were two types of diets (corn-grits based and corn-soybean meal based) and three micromineral premixes (basal micromineral premix, inorganic micromineral premix, and organic micromineral premix) that were fed. Organic Zn, Cu, and Mn had greater digestibilitiy and retention rates than their inorganic sulfate counterparts, when included in the corn-soybean meal diet (Liu et al., 2014).

Manganese Superoxide Dismutase (MnSOD)

Manganese superoxide dismutase is a metalloenzyme that is crucial for the survival of all aerobic organisms from bacteria to humans and even is needed in anaerobes when they are exposed to aerobic conditions (Haikarainen et al., 2014). As mentioned previously, manganese (Mn) is a critical dietary component of animals and humans, because it is an essential component of MnSOD (Holley et al., 2011). In mitochondria, MnSOD is the main antioxidant and provides much of the much of protection against radical oxygen species and oxidative stress in the form of the superoxide anion (Haikarainen et al., 2014). The mitochondrion is one of the most metabolically active organelles in the cell; it houses many pathways and contributes most if not all important enzymes for diverse functions such as the urea cycle, the citric acid cycle, β-oxidation of fatty acids, and ATP synthesis (Holley et al., 2011). In addition to being so metabolically active, the mitochondrion is also an organelle that produces a relatively high amount of reactive oxygen species (ROS) in the cell (Holley et al., 2011). These ROS produced in such a high amount can alter the cell's function drastically by altering key metabolic enzymes and proteins that are localized within the mitochondrion. MnSOD is highly expressed in organs that contain a large amount of mitochondria such as liver, heart, and kidneys (Li et al., 2011). Understanding how MnSOD processes and manages the overproduction of ROS has a number of human and animal health implications and could lead to novel treatments for various diseases that involve ROS (Holley et al., 2011). MnSOD has sometimes been referred to the guardian of the powerhouse [mitochondrion] because it will mediate the function and protection of the mitochondrion; without a properly functioning mitochondrion, the cell cannot perform to its maximum capacity, which in turn affects the metabolism of the organism (Holley et al., 2011).

MnSOD Structure

Although it has not specifically been characterized and analyzed in porcine species, researchers have been able to elucidate the crystal structure of MnSOD in other microscopic species such as *C. thermophilum* (Haikarainen et al., 2014), *E. coli* (Edwards et al., 1998), and *T. thermophilus* (Ludwig et al., 1991). MnSOD belongs to a family of metalloenzymes, and is one of four main classes of SODs; the remaining three are: CuZnSOD, FeSOD, and NiSOD (Haikarainen et al., 2014). These three classes are grouped together based on their active metal requirements and protein folds (Haikarainen et al., 2014). CuZnSODs are typically found in eukaryotes whereas FeSODs are more common in prokaryotes, and MnSODs can be found in both eukaryotic and prokaryotic species (Edwards et al., 1998). NiSODs were characterized more recently in the species *Streptomyces* (Youn et al., 1996). The enzymatic reaction in each of these enzymes proceeds via the cyclical reduction and reoxidation of its respective bound metal ion (Haikarainen et al., 2014). The MnSOD enzyme exhibits a three-dimensional structure that consists of a general fold composed of an α-hairpin N-terminus domain and an α/β C-terminus domain (Haikarainen et al., 2014). These components of the enzyme would be the tertiary structure of the enzyme where the domains interact with one another. The enzyme has been found in both dimeric and tetrameric forms; the difference between the two being the α-hairpin N-terminus domain (Haikarainen et al., 2014). The tetrameric form of MnSOD can be formed from a pair of dimeric MnSODs because the “extended conformation and lack of an additional helix” allows for the favorable interaction of dimeric subunits (Haikarainen et al., 2014).

The specific active site of MnSOD and what amino acids interact with the metal ion is species-dependent. In *C. thermophilum*, the protein ligands for the Mn^{2+} ions are His26 and His74 from the N-terminus domain and Asp160 and His164 from the C-terminus domain (Haikarainen et al., 2014). However in *E. coli*, the N-terminus domain provides His26 and His81 and the C-terminus domain provides Asp167 and His171 (Edwards et al., 1998). These amino acids are found in approximately the same location within each subunit (Edwards et al., 1998). The Mn^{2+} -binding site, surrounded by a cluster of conserved and mostly hydrophobic residues (Edwards et al., 1998). The active site is terminated at His30 and Tyr34 which is conserved among MnSODs (Haikarainen et al., 2014). The substrate-binding site for the superoxide anion ($O_2^{\bullet-}$) is found adjacent to the Mn-binding site (Haikarainen et al., 2014). In general, there are not many structural changes to MnSOD across species and so understanding the common structure of this enzyme is useful information (Haikarainen et al., 2014).

MnSOD and Mitochondrial Integrity

Mitochondrial ROS can interact with enzymes that are important in several metabolic pathways and MnSOD is needed to mediate and control the levels of ROS. Several of these pathways are mentioned briefly below.

In the electron transport chain (ETC) complexes (I, II, III), MnSOD is important in the scavenging of superoxides generated there; it may also be central in preventing ROS-induced inactivation of these electron transport complexes (Holley et al., 2011). The TCA cycle produces reducing equivalents, such as NADH and $FADH_{2}$, that are used in the ETC for ATP production and generation of intermediate substrates used in several other metabolic pathways (Mailloux et al., 2007). One of the enzymes susceptible to deactivation by superoxide is aconitase, which catalyzes the conversion of citrate to isocitrate (Hausladen & Fridovich, 1994). With aconitase inactivated, the TCA cycle cannot be completed and the concentration of citrate would increase (Mailloux et al., 2007). This enzyme contains iron-sulfur centers and when it is deactivated, the Fe (II) is released from the complex [aconitase] (Gardner et al., 1995).

Mitochondria are also involved in proper iron handling and utilization; it is the site for two iron-consuming pathways: iron-sulfur center synthesis and heme synthesis (Ajioka et al., 2006; Levi & Rovida, 2009). Iron-sulfur centers are often used as components of enzymes and the presence of heme allows for myoglobin and hemoglobin to bind oxygen for transport in the blood. When iron is not sequestered or used properly, iron-induced oxidative damage occurs and can lead to several disorders involving poor oxygen transport (anemias and ataxias) (Napier et al., 2005; Ye & Rouault, 2010). Altered expression of MnSOD can have an effect on the cell's iron handling capabilities (Holley et al., 2011). *SOD2* is a heme-responsive gene in yeast that encodes the MnSOD enzyme (Pinkham et al., 1997). The heme-dependent regulation of *SOD2* occurs through the presence of three cis elements involved that are bound by a heme-binding transcription factor, Hap1p (Pinkham et al., 1997). Heme oxygenase-1 (HO-1) is a mitochondrial enzyme that plays an important part in cleaving heme into biliverdin-IX, carbon monoxide and free iron (Converso et al., 2006). HO-1 monitors mitochondrial heme content and the expression of several related genes: cytochrome c oxidase subunit I and mitochondrial nitric oxide synthase (Converso et al., 2006). It was determined that the transfection of rat astroglial cells with HO-1 induces the expression of MnSOD (Frankel et al., 2000). This suggests a role for oxidative stress in MnSOD activity stimulated by the enzyme HO-1 (Frankel et al., 2000). In human patients with a neurodegenerative disease (Friedreich ataxia), iron-induced expression of MnSOD was impaired in fibroblast cells in comparison to normal patients (Jiralerspong et al., 2001). Only high levels of iron could induce the expression of MnSOD in Freidreich ataxia fibroblast cells (Jiralerspong et al., 2001). These studies reveal that iron metabolism and MnSOD expression are regulated tightly. *SOD2* (the MnSOD gene) is a heme-responsive gene, therefore the amount of iron (in the form of heme) can control the expression of MnSOD enzyme (Pinkham et al., 1997). This feedback mechanism is present to carefully balance cellular iron metabolism and oxidative stress.

Apoptosis is a highly regulated and coordinated type of cell death that targets specific cells or groups of cells (Elmore, 2007). Cells will undergo a condensation of the cytoplasm and nucleus and will subsequently form apoptotic bodies which are small membrane bound fragments that contain cellular parts (Elmore, 2007). Surrounding healthy cells will then phagocytose or devour the apoptotic bodies (Afford & Randhawa, 2000; Majno & Joris, 1995). Apoptosis is also associated with many types of cancer; it may be inactivated and so unhealthy cells that are supposed to be destroyed by the body will continue to live and grow (Elmore, 2007). Apoptosis can occur by two pathways: via receiving an extracellular stimulus (extrinsic) or internal stimulus (intrinsic) (Basu et al., 2006; Reed, 2000; Roy & Nicholson, 2002). The mitochondria are key for the initiation and progression of apoptosis (Spierings et al., 2005). Changes to the mitochondrial membrane potential, permeabilization of the membrane, and ROS production can all trigger apoptosis (Green & Reed, 1998). MnSOD can mediate the suppression of apoptosis by monitoring ROS levels and ROS-related damage; it can also affect apoptosis that is stimulated by inflammatory cytokines such as tumor necrosis factor (TNF) (Goossens et al., 2006). TNF induces apoptosis by stimulating ROS production; therefore MnSOD scavenges the ROS that TNF induced the production of in the mitochondria (Wong et al., 1989).

MnSOD plays a pivotal part in maintaining mitochondrial integrity and function. Several metabolic pathways produce ROS and MnSOD must handle these and keep their concentrations to a basal level to allow the cell or mitochondria to properly function (Holley et al., 2011).

Selenium

Selenium, another key trace mineral, has a very narrow tolerance range in most species, but it is imperative to include in the dietary trace mineral mix of swine (NRC 2012). Selenium is a component of the enzyme, glutathione peroxidase (GPX). This enzyme functions to detoxify lipid peroxides and provides cellular and subcellular membrane protection from peroxide damage (NRC 2012, and Rotruck et al., 1973). There is a crucial interrelationship that has been discovered between selenium and vitamin E (Reese & Hill, 2006). They both serve as antiperoxidants, attacking and eliminating reactive oxygen species (Lubos et al., 2011). However, increasing vitamin E in the diet does not eliminate the need for including selenium in the diet (Ewan et al., 1969). Selenium has also been shown to play a role in thyroid metabolism (Arthur 1994). Iodothyronine 5'-deiodinase has been identified as a selenoprotein; meaning selenium is a required cofactor for proper function of this enzyme, that is involved with the activation and deactivation of the thyroid hormones T_4 and T_3 (Arthur, 1994).

Selenium requirements for swine can vary based on the growth stage of the animal. In weanling pigs, 0.3 ppm is acceptable and in finishing pigs and sows the requirement is cut in half to 0.15 ppm; this has been well established by a variety of studies over several decades, the most recent being Lei et al. (1998). It was not until 1974 that the U.S. Food and Drug Administration approved the addition of selenium to the diet of all swine (0.1 ppm) and subsequently approved the addition of 0.3 ppm to diets of pigs up to 20 kg in size in 1982 (Mahan et al., 2014). This was due to the fact that it was discovered 0.1 ppm Se was not sufficient to prevent deficiencies in weanling pigs. The current FDA regulation of Se allows up to 0.3 ppm in the diet for all pigs, although efforts have been made over the years to reduce this regulation due to environmental concerns (NRC 2012).

Throughout the United States, there are regions with soil deficient in Se. When swine diets comprise of ingredients solely grown in such regions, supplementary Se is required (Mahan et al., 2014). Even with supplementary Se included in the diet, tissue levels of Se will be more based on the levels of Se of the ingredients grown on those soils. Environmental stresses may potentially increase the incidence and degree of selenium deficiency (NRC 2012).

One of the main biochemical changes seen in Se deficiencies is a decrease in glutathione peroxidase activity; therefore, the level of glutathione peroxidase in plasma is relatively indicative of the Se status in pigs (Adkins & Ewan, 1984). In the study just referenced, pigs from sows fed a Se-deficient diet were studied. Growth performance data and blood samples were collected (Adkins & Ewan, 1984). Five dietary treatments were fed based on supplemental levels of selenium ranging from 0-0.1 ppm. Increasing levels of supplemental Se had no significant effect on average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) (Adkins & Ewan, 1984). However, serum Se concentration and serum GPX results indicated a significant linear increase with increasing supplemental Se in both serum Se level and GPX activity. There was also a significant quadratic trend seen in serum GPX activity (Adkins & Ewan, 1984). Based on a multitude of studies, selenium deficiency syndrome (a general deficiency in Se) has a variety of symptoms including: sudden death, hepatic necrosis, edema of the spinal column, subcutaneous tissues, and the lungs; bilateral paleness and dystrophy of the skeletal muscles; reduced milk production and immune responses (NRC 2012). In the study performed by Adkins & Ewan, death loss occurred in pigs that received no supplemental Se at around five weeks of age (1984). Autopsy reports on these individuals indicated liver and heart lesions which are typical in Se and vitamin E deficiencies (Adkins & Ewan, 1984). In general, the symptoms and lesions caused by selenium and vitamin E deficiencies are identical in many regards (NRC 2012), which is, in part, due to their similar and interwoven biochemical functions.

Glutathione Peroxidase (GPX or GPSH-Px)

Glutathione peroxidases are known as antioxidants. They contribute to the detoxification of hydrogen peroxides as well as organic hydroperoxides (Dalto et al., 2018). Just like the SOD enzymes, this class of enzymes also contributes to the control of reactive oxygen species (Dalto et al., 2018). Hydrogen peroxide is a signaling molecule and the reaction with GPX that is catalyzed is thought to regulate a variety of signaling cascades (Brigelius-Flohé & Flohé, 2011). GPX works in the cytosol and functions by converting hydrogen peroxide into water (Li et al., 2000). Unlike SODs or catalase, GPXs require secondary enzymes (glucose-6-phosphate DH) and cofactors (GSH, glucose-6-phosphate, and NADPH) to perform at a higher efficiency (Li et al., 2000). Since the majority of GPXs have a selenocysteine (abbreviated “Sec”) in their catalytic site, the activity of these enzymes is highly related to the availability of selenium (Dalto et al., 2018). Therefore, the measuring of SeGPX in the blood is indicative of Se status in an animal (Bermingham et al., 2014; Han et al., 2011). GPX was described to “protect hemoglobin from oxidative breakdown” and was identified as a selenoprotein by Mills in 1957 (Mills, 1957).

GPx-1 is one of the most widely found members of the GPx family of enzymes (Lubos et al., 2011). GPx-1 is an important antioxidant enzyme that maintains the proper concentrations of intracellular hydrogen peroxide, while preventing the harmful accumulation (Li et al., 2000). Present in the cytosol and mitochondria of all cells, GPx-1 has been found to be more effective than catalase in its removal of harmful ROS (Antunes et al., 2002). GPx-1 has also been known to regulate and reduce lipid hydroperoxides and other soluble hydroperoxides when they are released from the lipid membrane (Martinez et al., 1982; Miwa et al., 1983).

The enzyme detoxifies peroxides in the form of a "bi-substrate ping-pong" mechanism (Lubos et al., 2011). The mechanism involves the production of stable intermediate alterations to the selenocysteine in the GPx-1 active site (Takebe et al., 2002).. By the mechanism of enzymatic detoxification, GPx-1 monitors cellular oxidant status via the oxidation of GSH, which directly eliminates hydroperoxides. Although GPX does not work in the exact manner as MnSOD, both enzymes must work in concert for the organism to have an efficient antioxidant system (Li et al., 2000).

Zinc

Zinc (Zn) is another important trace mineral element because it is an important cofactor and component of several metalloenzymes including: RNA/DNA synthetases and transferases and digestive enzymes (NRC 2012). It is also associated with the hormone insulin (Emdin et al., 2004; Jayawardena et al., 2012). This element essential in carbohydrate, lipid, and protein synthesis. Zinc fingers are an enzymatic control element for DNA and play a crucial role in cellular transcription as well as assisting in the transduction of inter- and intra- cellular signals (Laity et al., 2001). Zinc is important to include into swine diets, but the dietary inclusion must be limited due to environmental concerns (Nitrayova et al., 2012).

Zinc is absorbed in the intestinal tract based upon physiological need and is subsequently excreted in the feces. Small amounts of zinc are lost in the urine and in shed integumental tissues in the body (Mineral Tolerance 1980). Zinc is transported in the body in a highly regulated manner with respect to intake within the appropriate tolerance range. It can bind to sulfhydryl, amino, and imidazole groups, meaning it can attach to nucleic acids, proteins, and other organic compounds under normal conditions (Mineral Tolerance 1980).

Bioavailability is an issue with many trace minerals and zinc is no different. Many of the zinc salts like zinc chloride, zinc sulfate, and zinc carbonate are highly available (NRC 2012). However, the amount of Zn that is actually absorbed and retained is less than 50% of the intake, and depending on the source can be even less available if supplemented in the form of zinc oxide or zinc sulfide (Miller, 1991). Evaluation of organic zinc sources have been the subject of quite a few research studies. In one study that focused on the effectiveness of a zinc-lysine complex versus zinc sulfate on growth performance in nursery piglets, it was determined that the two zinc sources were equally effective in promoting growth performance and in the absorption of Zn (Cheng et al., 1998). When 500 weaned pigs were supplemented 0, 25, 50, 75, 100 mg/kg of Zn supplied as either organic or inorganic Zn or 50 mg/kg with 50% organic and 50% inorganic Zn, it was determined that 75 mg/kg organic Zn in a complex nursery diet best benefits today’s growing pigs with a high lean tissue composition (Hill et al., 2014). Growth performance was not affected significantly by Zn, but those diets with supplemental zinc performed better ($P < 0.05$) than those fed a basal diet (Hill et al., 2014). In a growing pig study evaluating organic sources of zinc, it was determined organically bound zinc, in particular Zn from Zn-Methionine 1:2 and Zn-yeast, could replace higher doses of ZnO due to better bioavailability (Nitrayova et al., 2012). This type of chelated Zn is important for Zn retention, and consequently may reduce Zn excretion and environmental concerns as well (Nitrayova et al., 2012). This study supplemented 10 or 100 Zn (from ZnO) mg/kg feed or 10 mg/kg of organic Zn (from four different organic sources) (Nitrayova et al., 2012). ADG from pigs supplemented 10 mg/kg Zn (ZnO) was significantly lower ($P < 0.05$) than the other treatment groups, and pigs supplemented organic Zn sources performed just as well as those supplemented 100 Zn mg/kg feed (Nitrayova et al., 2012).

Deficiencies in zinc in swine are characterized by a variety of clinical signs, primarily the hyperkeratinization of the animals skin, which is known as parakeratosis (Tucker & Salmon, 1955). Hyperkeratinization results in skin lesions and can be painful for the animal; other deficiency symptoms include reduced growth efficiency and reduced serum Zn, albumin, and alkaline phosphatase (Miller et al., 1968). In sows fed Zn-deficient diets it can lead to issues with farrowing, lactation, and a variety of issues with the offspring including smaller size piglets with lower tissue and serum levels of Zn (Prasad et al., 1969). Conversely, pigs fed higher-than-normal levels of zinc exhibit signs of zinc toxicities. Clinical signs of Zn toxicity include arthritis, lethargy, and in some severe cases (with Zn levels reaching 2,000 ppm) internal hemorrhaging (Mineral Tolerance 1980). It is important to understand how much Zn should be supplemented to an animal, and Zn requirements will vary based on age and sex of the animal. However, based on the most recent NRC recommendation, Zn is typically not provided in concentrations exceeding 80 ppm in the diet (NRC 2012). The need for zinc supplemented in the diet is essential for proper growth and development of swine regardless of the reported recommended values in the NRC. Supplementation prevents the development of deficiency symptoms, which ultimately leads to better growth efficiency and productivity (Miller et al., 1968).

Zinc is a divalent metal ion (2+). There are several elements with similar charges that will compete with zinc for a certain active site on a particular enzyme. One such enzyme is the copper zinc superoxide dismutase (CuZnSOD). Like MnSOD, CuZnSOD works to reduce the harmful effects of reactive oxygen species in the cell. Unlike MnSOD, CuZnSOD exists as a homodimer and is found in the cytoplasm (Perry et al., 2010). There are other enzymes that require a cofactor like Zn that has a (2+) charge in order to function properly. The proposed transporter of Zn^{2+} in the plasma is serum albumin or transferrin (Mertz, 1986). Serum albumin can also transport a variety of other organic and inorganic molecules in the blood (Larsen et al., 2016). Albumin is also an option for the delivery of certain types of drugs, which could have a large impact in human medicine (Larsen et al., 2016). There can, therefore, be competition for serum transporter proteins depending on the availability of the possible cofactors that can bind to albumin, due to there being several hydrophobic active sites and an overall negatively charged enzymes (Larsen et al., 2016).

Copper

Copper (Cu) is an essential trace mineral that must be included into the diet of swine. Initially, the essentiality of copper in the diet was recognized when working with anemic, milk-fed rats (Hart et al., 1928). Copper is primarily important to include due to the copper-dependent enzymes involved in iron (Fe) metabolism, specifically ceruloplasmin (Mineral Tolerance, 1980). Like Fe, copper is found bound to amino acids and proteins to prevent it from being involved in oxidation/reduction reactions (Chiba, 2012). There are several other processes that copper is involved with include: the formation of melanin, collagen, elastin, and proper function of the central nervous system. Requirements for copper vary based on species and several other factors. In swine, the amount recommended by the NRC is 5-6 ppm for a neonatal pig, while suggestions for sow supplementation are almost 20 ppm (NRC 2012). The copper requirements and recommendations for sows in the NRC are ill-defined and debated among many.

Cu absorption for most species takes place in the ileum and jejunum and is affected mainly by the form ingested by the animal (Mineral Tolerance 1980). The highest biologically available salts in swine are copper sulfate and copper chloride, meaning that these forms of copper are better used by the animal and promote better growth and efficiency (Cromwell et al., 1998). The Cu in copper sulfide and copper oxide are less available according to Cromwell et al. (1978, 1989b). Organic forms and complexes of copper have been shown to be equally as biologically available according to a number of studies (Apgar et al., 1995; Apgar & Kornegay, 1996; Coffey et al., 1994). However, there are studies that have reported that pigs supplemented with Cu in a Cu-lysine complex had greater growth performance than those fed Cu sulfate as a supplement (Coffey et al., 1994). Stomach acids and proteases will release Cu from whatever protein or amino acid it is bound to and allow it to be absorbed by the ileum/jejunum via active and passive transport (Chiba, 2012).

The major Cu transporter is Ctr1, but the divalent metal transporter 1 (DMT1) may transport Cu to an extent, due to its more common valence form Cu^{2+} (cupric form) (Hill & Link, 2009). Like Fe, changes in valence of Cu (from Cu^{1+} to Cu^{2+} or vice versa) can be important for transport of Cu and functionality of associated enzymes (Chiba, 2012). Cu that has been absorbed appears first in the plasma as the cupric ion bound to albumin. When the liver is synthesizing ceruloplasmin, a metalloprotein that carries well over half the copper in the blood, copper is tightly bound and is then released to general circulation (Mineral Tolerance 1980). Generally speaking, copper exits the organism via the biliary system, but can also be excreted through sweating, lactation, and urination (Mineral Tolerance 1980).

Deficiencies in copper can lead to poor iron mobilization, abnormal blood cell production, and poor production of collagen, melanin, and elastin (NRC 2012). Deficiency signs can include anemia, spontaneous bone fractures, cardiac disorders, as well as abnormal pigmentation of the skin. Cu may be toxic in swine when fed in excess amounts for long periods of time. Signs of toxicity can include reduced hemoglobin levels and jaundice. The jaundice is a result of excess copper in the liver and other organs (NRC 2012). When reduced dietary levels of iron and zinc are present, the signs of copper toxicity are amplified and more easily observed; when Zn concentrations are high, ultimately copper deficiency occurs (Chiba, 2012). Higher levels of iron intake lead to reduced copper absorption due to competition for absorption within the gastrointestinal tract or for use in metabolic function. Cu/Zn SOD activity in the blood will hence be diminished due to higher Fe supplementation (Barclay et al., 1991). Because copper interacts with iron and zinc due to similar valence characteristics, the levels supplemented must be monitored carefully (Hill & Link, 2009).

Iron

Most of the iron (Fe) found in the body is contained in the hemoglobin for the purpose of oxygen transport throughout the organism (Levi & Rovida, 2009). Smaller amounts are found in myoglobin (in muscle cells), enzymes, and storage. The only forms of iron found in the body are the ferric (3+) and ferrous (2+) forms (Levi & Rovida, 2009; Theil, 2011). These are also typically the only forms found in feed ingredients (Chiba, 2012). Heme Fe is the form found in the ingredients that are produced from blood or muscle. Non heme iron is released in the GI tract due to the acidic environment of the stomach/small intestine and enzymes. Ferrous iron, as well as other divalent metal ions (Zn, Cu, and Mn) use DMT1 and potentially other transporter proteins to cross the brush border of the intestine and enter into the intestinal mucosal cells (Hill & Link, 2009). Once in the mucosal cell the iron is able to move into the bloodstream for circulation, remain within the intestinal cell for later use, or be sloughed off (Chiba, 2012). The iron that does not enter into circulation can bind to a protein called apoferritin for short term storage within the cell; long term iron storage occurs in the liver, bone marrow, and the spleen. In these sites, iron is contained within ferritin, which is continuously degraded and synthesized to in order to use as a continual source of Fe to be used as the body needs it (Bakker & Boyer, 1986).

Newborn piglets rely on the sow's milk for nutrients. They often become anemic (or Fe deficient) due to limited iron stores, therefore it becomes necessary to provide piglets with supplemental iron in order to combat pre-weaning mortality of piglets (Starzyński et al., 2013). In production systems today, Fe is provided to the piglet via a 100-200 mg intramuscular injection of iron dextran, a polysaccharide (Starzyński et al., 2013). This injection is typically given within the first few days of birth (NRC 2012). When there is excessive non-heme Fe present in the diet, Fe is able to reduce the absorption of Zn (Chiba, 2012). An accumulation of Fe leads to vitamin A deficiency (Chiba, 2012). Fe deficiency has also been associated with repressed Se concentrations and as a result, reduced glutathione peroxidase (GPX) activity (Chiba, 2012).

In a study regarding the regulation of iron transporters by supplemental iron in the diets of young pigs a relationship between iron and manganese was noted (Hansen et al., 2009). High amounts of dietary iron has be thought of to have harmful effects such related to increased oxidative stress and in addition to interference with the absorption of other essential trace minerals such as copper and manganese (Hansen et al., 2009). This interference is due to competition of particular mineral transporters in the intestines (Hansen et al., 2009). Expression of Fe-transporter genes such as DMT1 and ZIP14 in the duodenum and several others in the liver were affected by the concentration of supplemental iron included in the diet (Hansen et al., 2009). It was also noted that nursery pigs fed the high dietary iron had decreased tissue levels of Mn in the liver and duodenum. These results support the idea that DMT1 plays a role in the absorption of both Fe and Mn into the small intestine of the nursery pig. Because Mn plays such an important part of bone growth and development in the young pig, it is imperative to understand the interrelationship between the concentrations of Fe and Mn in the diet of nursery pigs (Chiba, 2012).

Injectable Trace Minerals

As mentioned previously, trace minerals are primarily supplemented to an organism through the feed they consume. There are other methods to supplement trace minerals to an animal such as injectable trace mineral products. These products are mostly used in the beef and dairy industries though, in order to combat mineral deficiencies. Cattle experience increased oxidative stress at weaning just as most species, so it is important for antioxidant enzymes to function properly and eliminate reactive oxygen species. In addition, minerals play important roles in the structure of an organism as well as cofactors for a wide array of enzymes (McDowell, 2003). Minerals play an important role in the immune function of an organism as well. Injectable trace minerals have an advantage in that you can know the precise amount of trace mineral that is being supplemented to the animal (Genther and Hansen, 2014). When minerals are supplemented in the diet, one must make the assumption that the animal is consuming the nutrients provided to them.

One of the most widely used trace mineral injectable product is Multimin90®, which supplements zinc, selenium, manganese, and copper. This product has been shown to provide an adequate antioxidant response under an aflatoxin challenge in dairy cattle (Pate et al., 2018). It has also shown to improve rib eye area and increase growth in feedlot steers who were mineral deficient (Genther and Hansen, 2014). Repeated Multimin90® injections were given to beef heifers in order to observe the effect the product has on reproductive performance; the repeated supplementation resulted in an increase in copper and selenium status (Stokes et al., 2018). The product did cause a decrease in the attainment in cyclicity of estrus, but did not affect artificial insemination pregnancy rates of beef heifers (Stokes et al., 2018). This may suggest that injectable trace minerals may contribute to an increase in trace mineral status, but there was no improvement in heifer performance or reproductive success in beef heifers (Stokes et al., 2018).

Limited work has been done to show if an injectable trace mineral source has any effect on performance in swine. Neonatal swine are supplemented with 100-200 mg of iron in the form of iron dextran within the first week of life (NRC 2012; Reese and Hill, 2006). Other trace minerals are not typically supplemented to piglets directly. Repeated selenium and vitamin E injection treatments have been given to pregnant sows to observe the impact on immunity and performance of their offspring (Mavromatis et al., 1999). Piglets that were born to sows supplemented both selenium and vitamin E had higher immunoglobulin concentrations and greater birthweights and weaning weights. It is established that vitamin E and selenium work together, so these conclusions support this statement (Mavromatis et al., 1999). Multi-element trace mineral injections have not been utilized in piglets directly.

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CHAPTER 3

The Effect of Varying Dietary Manganese and Selenium Levels on the Growth Performance and Manganese-superoxide Dismutase Activity of Nursery Pigs

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Abstract

The nursery phase of swine production can be one of the most stressful times experienced by the pigs. Reactive oxygen species (ROS) rapidly accumulate during the weaning process and the pig must start producing antioxidant enzymes as a defense against these ROS. In this study, 216 weaned piglets (5.21 ± 1.17 kg, 21 ± 3 days old) were used in three 35-day nursery trials. Six dietary treatments were fed in three phases and differed only by levels of selenium (0.1 and 0.3 ppm Se) and manganese (0, 12, and 24 ppm Mn). Pigs and orts were weighed weekly and were bled on days that dietary treatments were changed (d 0, 7, 21, 35) to look at manganese superoxide dismutase activity. There was a linear response ($P < 0.05$) in average daily gain across the levels of manganese supplemented in the diet. There was a linear trend ($P < 0.1$) in average daily feed intake across the levels of manganese as well. There was a significant linear response ($P < 0.05$) in MnSOD activity across the levels of manganese supplemented on bleeding day 7. Levels of MnSOD activity were significantly lower ($P < 0.05$) on bleeding day 35 in the group supplemented with 0.1 ppm Se. MnSOD typically peaked on bleeding day 7 and subsequently decreased for the remainder of the study. The dietary treatment that allowed for the best maintenance of MnSOD activity was the diet that supplemented 0.3 ppm Se and 12 ppm Mn.

Keywords: trace minerals, manganese, selenium, nursery swine, manganese superoxide dismutase

Introduction

Pigs experience a variety of new stressors during the process of weaning including: nutritional, immunological, social and even psychological (Cao et al., 2018). Oxidative stress is another source of stress that affects the animal after they have been weaned (Cao et al., 2018; Lu et al., 2014). Oxidative stress occurs when reactive oxygen species (ROS), like the superoxide anion ($O_2^{\bullet -}$), accumulate the cell and impair normal cellular function and can lead to the development of certain types of cancer and neurological disorders (Brière et al., 2006; Lu et al., 2014; Valko et al., 2006; Waris & Ahsan, 2006). Antioxidant enzymes are produced by the animal to combat these harmful ROS (Mates et al., 1999).

The most active antioxidant enzymes are mitochondrial manganese-superoxide dismutase (MnSOD), glutathione peroxidase (GPX), and catalase (Valko et al., 2006). As the name suggests, MnSOD uses Mn as a cofactor for catalyzing the rearrangement of the superoxide radical into hydrogen peroxide or molecular oxygen, which are subsequently utilized or eliminated by the body (Haikarainen et al., 2014; Holley et al., 2011). Glutathione peroxidase is a selenoprotein located in the cytosol that utilizes the trace mineral selenium to convert hydrogen peroxide into water (Dalto et al., 2018; Li et al., 2000). Altering levels of manganese and selenium could have an impact on the antioxidant status of nursery pigs.

The purpose of this experiment was to determine if altering dietary levels of selenium and manganese had an effect on the growth performance and MnSOD activity levels nursery pigs.

Materials and Methods:

Animal Care and Experimental Design:

Care, handling, and sampling procedures were approved by the University of Georgia Animal Care and Use Committee prior to the start of this study (AUP#: A2018 08-012-A1).

Two-hundred and sixteen piglets averaging 21 ± 3 days of age and 5.21 ± 1.17 kg from the University of Georgia Swine Unit were used between three 35-day trials. Pre-weights were recorded several days prior to the start of the trial and animals were blocked by weight and sex. Six dietary treatments were randomly assigned within each weight block. Trace mineral premixes were designed in a 2 x 3 factorial: two levels of selenium (0.1 and 0.3 ppm) and three levels of manganese (0, 12, 24 ppm) (Table 3-1). The resulting 6 dietary treatments were randomly assigned within each weight block.

Pig Feeding, Management, and Sampling

Pigs were housed in pens of four (9 replicate pens per dietary treatment) in an environmentally controlled nursery room and consumed feed and water ad libitum. Nursery diets were fed in three phases (Table 2): Phase I from Day 0 to Day 7 post-weaning, Phase II from Day 7 to Day 21 post-weaning, and Phase III from Day 21 to Day 35 post-weaning. Pig body weights were recorded weekly (Day 0, 7, 14, 21, 28, and 35 post weaning). Feed intake was recorded weekly starting on Day 7 (Day 7, 14, 21, 28, and 35 post-weaning). Animals were bled via orbital sinus (Dove & Alworth, 2015) on d 0, 7, 21, and 35 post weaning for analysis of MnSOD levels. Pigs were not food-deprived prior to bleeding. Blood for MnSOD analysis was collected in heparinized vacuum tubes and transported on ice to the laboratory. Fecal samples were also collected for microbiome analysis on Day 0 and 35 post weaning.

Laboratory Analysis

Determination of Mn superoxide dismutase (MnSOD) activity (EC 1.15.1.1) was accomplished by our modification of the protocol (Hill et al., 1999; Johnson & Murphy, 1988; Marklund & Marklund, 1974; Percival & Layden-Patrice, 1992; Prohaska, 1983). In our method, blood was collected from the pig via the orbital sinus into heparinized tubes (Fischer Scientific) and centrifuged (2000 x g, 10 min, 4°C) to separate plasma and red blood cells (RBC). RBC were washed three times with 1X phosphate buffered saline solution. Washed cells were lysed with an equal volume of ice-cold deionized water and subsequently frozen (-80°C) until analyzed.

In order to inactivate the Cu/Zn-dependent SOD, 1 mmol potassium cyanide (0.065 g/L) was added to the reaction buffer (50 mM Tris-HCl, 1.0 mM diethylenetriamine pentaacetic acid, pH 8.2) and was used to measure MnSOD activity. RBC hemolysate was diluted into the reaction buffer and was centrifuged (5,000 x g, 15 min, 4°C). The resulting supernatant was diluted 1:10 and incubated at 25°C for 5 minutes with 50uL of 10 mM sodium azide and the reaction buffer. A sample control with no added sample, 200 uL, and 400uL (in triplicate) were plated on a 12-well microcuvette plate. The reaction was initiated when 4 mM pyrogallol in 10 mM hydrochloric acid was added to each well followed by rapid mixing. The reaction was monitored at 320 nm for 3 min using the kinetic reading program of the spectrophotometer (Biotek® µQuant, 2006). The amount of supernatant that results in the 50% autooxidation of pyrogallol is the equivalent of one unit of MnSOD activity (IU). Units will be expressed in IU/mg protein after a Lowry protein determination assay is performed on the supernatant.

Statistical Analysis

Statistical analysis of efficiency data were performed via the PROC GLM procedure of SAS. Each pen was considered an experimental unit and initial body weight was employed as a covariate. Statistical analysis of MnSOD activity was performed via the PROC GLM procedure of SAS as a 2 x 3 repeated measure. Statistical significance was declared at $P < 0.05$ and trends were declared at $0.05 < P < 0.1$.

Results

There were no significant selenium effect on ADG, ADFI, or G:F (Table 3-3). There was a significant effect ($P < 0.01$, Table 3-4) of selenium level on MnSOD activity on day 35.

There was a significant linear response ($P < 0.05$, Table 3-3) in overall ADG across the levels of manganese. There was a significant linear response ($P < 0.05$, Table 3-3) in ADFI across levels of manganese in the diet for days 0-7, and a linear trend ($P < 0.1$, Table 3-3) in overall ADFI (d 0-35). There were significant linear manganese trends ($P < 0.1$, Table 3-3) in G:F for days 0-7, and 7-21. There was a significant linear response ($P < 0.01$, Table 3-4) in MnSOD activity across the levels of manganese on day 7. There was a significant difference ($P < 0.05$, Table 3-4) across manganese levels in MnSOD activity on day 35.

There were no significant manganese-selenium interaction effects for ADG, ADFI, or G:F (Table 3). There was a significant ($P < 0.05$, Table 3-4) manganese-selenium interaction on MnSOD activity on day 35.

Discussion

There were no significant differences observed in growth performance of pigs fed the differing levels of selenium. The current NRC recommendation is 0.3 ppm Se , and it was surprising that the pigs fed the lower level of selenium did not perform lower than those fed the NRC recommended amount of Se (NRC, 2012). There was a significant difference observed between the two levels of selenium when looking at MnSOD activity. The groups supplemented 0.3 ppm Se had a significantly higher number of units of MnSOD activity. Average daily gain did respond linearly to the increasing amounts of manganese that was added to the diet (Table 3-3). This suggests that innate levels of manganese found in corn and soybean meal ingredients tend to make pigs perform less efficiently than those supplemented with manganese, at least during the first week after weaning (Table 3-3).

MnSOD activity changed over the 35 days of the study. Immediately after weaning, MnSOD levels across all treatment groups differed numerically but were not significantly different from one another. There tended to be an increase and peak in MnSOD activity on day 7 and subsequently a decrease on days 21 and 35 across all treatment groups (Table 3-4). On day 35, MnSOD activity in some treatment groups (0.3 ppm Se, 12 ppm Mn) returned to around the same level as they were on day 0 (Table 4). In other treatment groups (all 0.1 ppm Se groups), the day 35 level of MnSOD activity dropped much lower than on day 0 (Table 3-4). This trend of MnSOD activity suggests that at day 7 post-weaning, antioxidant activity is at its highest level and more ROS are needing to be eliminated. MnSOD activity decreases the further away from weaning the pigs get. The pigs supplemented 0.3 ppm Se and 12 ppm Mn displayed the best return to initial MnSOD activity levels on day 35 (Table 3-4). These pigs also had the highest peak in MnSOD activity on day 7 (Table 3-4). This could imply that in order to maintain antioxidant levels, at least 12 ppm Mn would be the best recommendation based on the results of this study.

Conclusion

In conclusion, there was a significant linear response in average daily gain to the increasing levels of manganese supplemented in the diets of nursery pigs. There was also a decreasing linear response in levels of MnSOD activity across the levels of manganese. MnSOD activity levels peaked on day 7 across most of the treatment groups and subsequently decreased. Supplementing 12 ppm Mn and 0.3 ppm Se in a nursery pig diet caused the day 35 level of MnSOD activity to return to around the same level of MnSOD activity present on day 0. This particular mineral combination may be the key to maintaining MnSOD levels during the nursery phase of swine production.

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Table 3-1: Dietary Mineral Premix Composition							
% of Premix	**% Element**	**Amount Added in Grams**					
Mineral Source		**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Copper Citrate	58%	0.45	0.45	0.45	0.45	0.45	0.45
Iron Sulfate	30%	4.17	4.17	4.17	4.17	4.17	4.17
Zinc Oxide	72%	1.39	1.39	1.39	1.39	1.39	1.39
Potassium Iodate	59.30%	0.02	0.02	0.02	0.02	0.02	0.02
Selenium	2%	0.05	0.05	0.05	0.15	0.15	0.15
Manganese Sulfate	29.50%	0.00	0.41	0.81	0.00	0.41	0.81
Corn		93.93	93.52	93.12	93.83	93.42	93.02
Analysis							
Manganese (ppm)		45.94	41.59	51.72	30.70	33.83	52.96

[1] Mineral analysis was performed by the University of Georgia Feed, Water, and Soil Laboratory. Other minerals were analyzed but were not included in this table for simplicity. Selenium was not analyzed, due to the sensitivity restrictions at the UGA Feed Lab.

Table 3-2. Common Diet Composition and Analysis[1]			
Items			
Ingredients, %	**Phase I**	**Phase II**	**Phase III**
Corn	22.8	40.2	57.3
Soybean Meal 47.5%	15.0	21.0	28.1
Whey	15.0	10.0	7.0
Oats	10.0	5.0	.
Hamlet Protein	10.0	7.5	.
Lactose	10.0	3.0	.
Fish Meal	5.0	3.0	3.0
Blood Plasma	3.0	1.5	.
Fat	5.2	4.4	0.5
L-Lysine	0.3	0.4	0.3
DL Methionine	0.2	0.2	0.1
L-Threonine	0.1	0.2	0.1
Dicalcium Phostphate	1.5	1.7	1.3
Limestone	0.3	0.4	0.6
Salt	0.3	0.3	0.3
UGA Vitamin Mix[2]	0.3	0.3	0.3
Trace Mineral Mix	1.0	1.0	1.0
Analysis			
Crude Protein %	20.4	23.2	21.3
Fat %	7.1	6.9	3.3
Ash %	6.3	6.2	5.6
Crude Fiber %	1.6	16.5	7.7
Phosphorus %	0.81	0.76	0.71
Calcium %	1.01	1.00	0.97

[1] Proximate analysis was performed by the University of Georgia Feed, Water, and Soil Laboratory.

[2] Vitamin Pre-mix: supplied per kg of premix: vitamin A (4,400 IU); vitamin D (660,000 IU); vitamin E (17,600 IU); vitamin K (1,760); riboflavin (3960 mg); niacin (22,00 mg); vitamin B12 (17,600 µg); ADM Alliance Nutrition, Quincy, IL 62305

Table 3-3: The Effect of Varying Dietary Manganese and Selenium Levels on Growth Performance of Nursery Pigs[1]

Selenium (ppm)	**0.1**			**0.3**				**P-Values[2]**			
Manganese (ppm)	**0**	**12**	**24**	**0**	**12**	**24**					
BW (kg)							**SEM**	**Mn**	**Mn (Lin)**	**Se**	**Mn x Se**
Day 0	5.19	5.22	5.18	5.14	5.22	5.29	0.1	0.79	0.51	0.84	0.74
Day 7	5.86	6.07	6.13	5.83	6.08	6.22	0.1	0.05	0.02	0.85	0.89
Day 21	10.81	11.39	11.65	10.82	11.06	11.7	0.3	0.04	0.01	0.74	0.8
Day 35	19.01	20.38	20.46	19.25	19.7	20.66	0.5	0.03	0.01	0.85	0.62
ADG (g/pig/day)											
0--7	96	115	129	97	124	133	15	0.06	0.02	0.68	0.97
7--21	352	386	392	375	356	397	19	0.08	0.03	0.61	0.75
21--35	585^{a}	643^{b}	628^{ab}	590^{ab}	618^{ab}	644^{b}	18	0.04	0.02	0.94	0.46
0--35	394^{a}	433^{ab}	434^{ab}	399^{a}	414^{ab}	443^{b}	14	0.03	0.01	0.90	0.61
ADFI (g/pig/day)											
0--7	141	150	173	143	168	168	12	0.07	0.02	0.62	0.61
7--21	486	514	518	489	490	494	20	0.64	0.37	0.35	0.75
21--35	941	865	981	942	921	956	50	0.32	0.59	0.80	0.72
0--35	559^{ab}	579^{ab}	598^{a}	554^{ab}	542^{b}	586^{ab}	19	0.15	0.08	0.27	0.69
G:F											
0--7	0.59	0.73	0.73	0.65	0.72	0.77	0.069	0.14	0.06	0.57	0.88
7--21	0.73^{ab}	0.74^{ab}	0.75^{ab}	0.70^{a}	0.72^{ab}	0.80	0.033	0.21	0.09	0.90	0.42
21--35	0.63	1.26	0.65	0.66	0.67	0.65	0.240	0.32	0.98	0.34	0.36
0--35	0.71	0.75	0.73	0.71	0.77	0.76	0.024	0.16	0.25	0.42	0.82

[1] Values are least square means. Means within a row with different letter superscripts significantly differ ($P < 0.05$). [2] P-values reported are for the effects of manganese level, linear contrast for manganese level, selenium, and the manganese and selenium interaction.

Table 3-4: The Effect of Supplemental Manganese and Selenium on Manganese Superoxide Dismutase Activity

	Se 0.1 ppm			Se 0.3 ppm				P-Values			
IU MnSOD/mL	Mn 0 ppm	Mn 12 ppm	Mn 24 ppm	Mn 0 ppm	Mn 12 ppm	Mn 24 ppm	SEM	Mn	Mn(Lin)	Se	Mn x Se
Day 0	8.98^{c}	7.97^{ab}	7.55^{a}	9.32^{c}	8.99^{c}	8.77^{bc}	0.35	0.02	0.01	< 0.01	0.42
Day 7	10.64^{b}	10.81^{b}	8.21^{a}	10.97^{b}	11.78^{b}	8.24^{a}	0.46	< 0.01	< 0.01	0.26	0.61
Day 21	9.28^{ab}	9.54^{ab}	10.49^{b}	10.38^{b}	8.78^{a}	9.36^{ab}	0.5	0.27	0.85	0.53	0.07
Day 35	5.30^{a}	5.62^{a}	4.65^{a}	5.36^{a}	8.63^{b}	7.61^{b}	0.61	0.02	0.26	< 0.01	0.04

[1] Values are least square means. Means within a row with different letter superscripts significantly differ ($P < 0.05$). [2] P-values reported are for the effects of manganese level, linear contrast for manganese level, selenium, and the manganese and selenium interaction.

CHAPTER 4

The Effect of Multimin90®, Birthweight, and Sire on the Growth Performance and Survivability of Nursing Piglets

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Abstract

When piglets are born, they are often deficient in trace mineral stores. This can lead to a variety of physiological abnormalities, which is not ideal especially at this stage in life where pre-weaning mortality is a big issue already. Multimin90® is a supplemental trace mineral product that is intended for use in cattle. The purpose of this study was to see the effect of this mineral product on the growth performance and overall survivability of nursing piglets. Over the course of 6 months and 6 farrowing groups, piglets within a litter were paired by similar size and sex and one piglet was given 0.1 mL injection of Multimin90®, while the other was given 0 mL of the product. Body weights and death loss were recorded until weaning (d 21 ± 3). In addition to seeing if there was an effect of Multimin90® on growth performance and survivability, birthweight and sire line were also utilized as main effects in this study. Five hundred and two newborn piglets (1.37 ± 0.37 kg) were used initially, however, only 378 piglets survived to weaning (5.19 ± 0.14 kg) and were used for statistical analysis for overall ADG. Multimin90® did not have an effect on ADG or death loss throughout the nursing period ($P < 0.05$). There was a significant linear increase in ADG ($P < 0.05$) in response to birthweight, those piglets that were born under 1.13 kg, 1.13- 1.59 kg, or born above 1.58 kg. There was also a significant linear increase ($P < 0.05$) in survivability across the birthweight categories and numerical difference between the effects of Multimin90® and birthweight on survivability. Based on the results of this study, Multimin90® would not necessarily be an effective tool to improve growth performance of nursing piglets, but some of these results indicate it could be a means to increase survivability in those pigs who are born under 1.13 kg.

Keywords: trace minerals, swine, nursing piglets, growth performance, death loss, injectable trace mineral

Introduction

It is well established that trace minerals are essential for swine to be efficient and healthy. Trace minerals play a variety of structural, enzymatic, and physiological roles in an organism (McDowell, 2003). Often being born deficient in trace minerals, piglets need to be supplemented with minerals to combat pre-weaning mortality. For example, in the industry setting, piglets are supplemented with 100-200 mg of iron within a few days of being born to avoid becoming anemic (Reese & Hill, 2006; Starzyński et al., 2013). Other trace minerals are typically not supplemented to the animal directly until after weaning. In beef and dairy cattle, minerals can be supplemented to the animal in the diet or via supplemental mineral injections before weaning. One such mineral supplement is Multimin90®, which is a supplemental source of the trace minerals zinc (60 mg/ml), copper (15 mg/ml), manganese (10 mg/ml), and selenium (5mg/ml) (Multimin USA). Each of these trace minerals play an important role in the animal. Trace mineral stores within these animals are usually depleted as calves grow and reach weaning age.

Manganese is necessary for proper bone development and is a cofactor for a powerful antioxidant enzyme, superoxide dismutase (Chen et al., 2018; Haikarainen et al., 2014; Leach & Muenster, 1962; Wilgus et al., 1939). Selenium is a component of glutathione peroxidase, another antioxidant enzyme that protects the cell from oxidative damage (Adkins & Ewan, 1984; Dalto et al., 2018; Rotruck et al., 1973). Copper is important for iron and oxygen metabolism and plays an important role in the synthesis of ceruloplasmin (Mineral Tolerance, 1980; Feng et al., 2007). Zinc acts as a cofactor and stability component for a number of enzymes involving transduction of cellular signals, RNA/DNA control elements, and digestive enzymes (Laity et al., 2001; Nitrayova et al., 2012). Zinc in combination with Cu are components in one of the isoforms of superoxide dismutase (Cao et al., 2018; Perry et al., 2010). In addition, zinc and copper have been identified as effective in-feed alternatives to antibiotics (Zhu et al., 2017). Deficiencies in these minerals can lead to impaired growth development, reproductive issues, and suppressed immunity (McDowell, 2003). The severity of mineral deficiency symptoms is heavily dependent on species and the specific deficiency.

Multimin90® has been shown to improve ADG in beef cattle fed diets that are deficient in zinc, manganese, copper, and selenium (Genther et al., 2014). Multimin90® improved carcass characteristics such as ribeye area and marbling scores (Genther et al., 2014). It has also been shown to increase neutralizing antibody titers against bovine viral diarrhea virus (BVDV-1 and BVDV-2) and bovine herpesvirus-1 (BHV-1) in beef calves (Arthington et al., 2012). Just like cattle, swine are also deficient in trace mineral stores before weaning. The purpose of this experiment was to determine the effect of using Multimin90® on piglet growth performance and death loss before weaning.

Materials and Methods

Animal Care and Experimental Design

Care, handling, and sampling procedures were approved by the University of Georgia Animal Care and Use Committee prior to the start of this study (AUP#: A2018 01-012-Y2-A0). This study lasted over the course of 6 months and 6 farrowing groups and initially used 502 newborn piglets from 35 different litters and two sire lines. These piglets had an average birthweight of 1.37 ± 0.37 kg. Within 36 hours of being born, piglets within a litter were paired by similar size and sex and were randomly given one of two treatments: 0 mL or 0.1 mL of

Multimin90®. If there were piglets that remained due to uneven numbers of sexes or size, those piglets were still used and randomly given a treatment. Sows were provided lactation feed and water ad libitum during the nursing period (Table 4-1).

Data Collection

Body weights were recorded weekly (every Friday) until weaning. The average weaning weight of piglets that survived until weaning (21 ± 3 days) were 5.19 ± 0.14 kg (n=377). After all data was recorded, piglets were ranked based on their birthweight into three categories: those that were born lighter than 1.13 kg, 1.13- 1.59 kg, or born heavier than 1.58 kg. This was done in order to see if there was any impact of birthweight and injection on performance or death loss percentage.

Statistical Analysis

For the purposes of this experiment, statistics were performed as a 2x2x3 factorial design (2 sire lines, 2 levels of Multimin90®, and 3 birthweight categories) via the PROC GLM procedure of SAS 9.4. For the performance parameter, each piglet was considered an experimental unit. Survivability analysis was performed the same way as ADG analysis. Statistical significance was declared at $P \leq 0.05$, and trends or tendencies were considered at $0.05 < P < 0.1$.

Results

Multimin90® did not have a significant effect ($P > 0.1$, Table 4-2) on the overall average daily gain (ADG) of piglets. Birthweight did have a significant effect ($P < 0.05$, Table 4-3) on overall ADG of piglets. In addition, there was a significant linear response in growth performance ($P < 0.05$, Table 4-3 linear P-values) to initial birthweight. There was no significant interaction ($P > 0.1$, Table 4-3) between Multimin90® and birthweight. There was not a significant difference (P > 0.1, Table 4-4) between sires in overall growth performance. The lean yield line (EBX) performed better than the meat quality line (EB5) during the first week of the nursing period (P < 0.05, Table 4-4) and tended to perform better the week leading up to weaning (P < 0.1, Table 4-4). Overall, there was not a significant difference (P > 0.1, Table 4-4) in growth performance between the two sire lines. There was no significant interaction effect (P > 0.1, Table 4-4) of Multmin90® and sire or birthweight and sire on overall growth performance. There was no significant three-way interaction (P > 0.1, Table 4-4) effect of Multimin90®, birthweight, and sire on growth performance of piglets.

There was no significant effect (P > 0.1, Table 4-2) of Multimin90® on survivability. There was a significant linear increase (P < 0.05, Table 4-3) in survivability as birthweight increased. There was a significantly lower percentage (P < 0.05, Table 4-3) of piglets that lived during the nursing period that weighed less than 1.13 kg. There no significant interaction effect (P > 0.1, Table 4-3) between Multimin90® and birthweight on survivability. There was a significant difference (P < 0.05, Table 4-4) in survivability between the two sire lines. The lean yield line, EBX, had a higher survivability percentage (P < 0.05, Table 4-4) than EB5, the meat quality line. There was no significant interaction effect (P > 0.1, Table 4-4) of Multimin90® and sire on death loss percentage. There was a significant interaction effect (P < 0.01, Table 4-4) of birthweight and sire on death loss percentage. Piglets from either sire that were born less than 1.13 kg had higher death percentages than the average and heavier birthweight pigs (P < 0.05, Table 4-4). The EBX sire line had a significantly lower death loss percentage (P < 0.05, Table 4-4) than the EB5 sire line in pigs that were born less than 1.13 kg. There was no significant three-way interaction (P > 0.1, Table 4-4) effect of Multimin90®, birthweight, and sire on the survivability of piglets.

Discussion

The results of this study indicated that Multimin90® may not be an effective tool to improve growth performance in nursing piglets (Table 4-2). It was a relief to see that Multimin90® did not have a negative effect and cause a significant decrease in growth performance or a significant increase in death loss percentage (Table 4-2). If we had seen the performance decrease or increase in death loss in piglets given the Multimin90® injection, that would likely would have attributed to some trace mineral toxicity, likely selenium due to its narrow tolerance range in the animal (Mahan et al., 2014). Although the Multimin90® group tended to have numerically lower ADG values, it was no major concern. Even though Multimin90® did not increase growth performance, it could be used as a potential method to increase survivability percentage in piglets born less than 1.13 kg, since those size piglets had a numerically higher survivability than their non-supplemented counterparts (Table 4-3).

It was expected that ADG would increase as birthweight increased (Beaulieu et al., 2010; Magnabosco et al., 2016), and survivability would increase as birthweight increased, and this data is a confirmation of that expectation (Table 4-3). The EBX sire line grew better during the first week of lactation (Table 4-4). The EBX sire line also had a significantly lower death percentage than EB5 for pigs that were born less than 1.13 kg (Table 4-4). Since the EBX is a lean yield line, it was also expected that those pigs would tend to grow better than the meat quality line (EB5) at some point during the nursing period.

When used in beef cattle that were zinc, copper, selenium, and manganese deficient, Multimin90® improved ADG (Genther et al., 2014). This is not a direct comparison to what was performed in this study. The sow would have to be deprived of these minerals, then piglets would need to be treated and subsequent data would need to be collected. This would provide more of a comparison across species. However, depriving the sow of minerals would not benefit the animal at all and it would lead to more problems than desired (Plumlee et al., 1960). This product has potential for use in the swine industry to reduce death loss in lighter-born piglets, but more data would need to be collected before it could be implemented. Piglets that grow better and faster to market age are more desirable than those who have less of a chance of death during the nursing period. Therefore, it would be recommended that this product not be used as a method to improve growth performance of nursing piglets. In terms of death loss, the cost of the product would outweigh the benefit of implementing the use of the product in a production system.

Conclusion

In conclusion, Multimin90® did not have an effect on growth performance or death loss in piglets. There was a significant linear response of initial birthweight on growth performance through the nursing period. There was a significant linear increase in survivability across the birthweight categories and a significant interaction effect of Multimin90® and birthweight on death loss percentage. The EBX sire line grew better during the first week of lactation and had a lower death loss percentage during the nursing period. The EBX sire line also had a lower death loss percentage in piglets born less than 1.13 kg. Multimin90® may not have improved piglet performance during the nursing period, but it did seem to numerically increase survivability in piglets that were born under 1.13 kg. This product may still have potential in combating pre-weaning mortality in nursing piglets by increasing survivability, but more data would need to be collected before this product could be considered for implementation by the swine industry.

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Table 4-1: Lactation Diet Ingredients and Analysis	
Ingredient	
Corn	38.96
SBM	17.23
DDGS	40.00
Poultry Fat	0.46
Limestone	2.16
Salt	0.35
Vitamins[1]	0.25
Trace Minerals[2]	0.15
Sow Pack[3]	0.25
Lysine	0.20
Calculated Analysis	
Crude Protein, %	22.40
Metabolizable Energy, kcal/kg	3300
Crude Fiber, %	5.10
Ether Extract, %	6.14
Lysine, %	1.10
TSAA, %	0.86
Threonine, %	0.92
Tryptophan, %	0.25
Ca, %	0.90
Total P, %	0.57
Available P, %	0.40

[1] Vitamin Pre-mix: supplied per kg of premix: vitamin A (4,400 IU); vitamin D (660,000 IU); vitamin E (17,600 IU); vitamin K (1,760); riboflavin (3960 mg); niacin (22,00 mg); vitamin B12 (17,600 µg); ADM Alliance Nutrition, Quincy, IL 62305

[2] Trace Minerals: supplied per kg of premix: iron (110,000 mg); copper (11,000 mg); manganese (26,400 mg); zinc (110,000 mg); iodine (198 mg); selenium (198 mg); ADM Alliance Nutrition , Quincy, IL 62305

[3] Sow Pack: supplied per kg premix: Biotin (88 mg); choline (220.5 g); folic acid (661.5 mg); pyridoxine (1.98 g); vitamin E (8,882 IU). ADM Alliance Nutrition, Quincy, IL 62305

Table 4-2: The effect of Multimin90® on the growth performance and survivability of nursing pigs

	Multimin90®			
	0.0 mL	**0.1 mL**		**P-value**
N	242	260	**SEM**	**MM**
ADG 0-7 (g/day)	123	117	5	0.45
ADG 7-14 (g/day)	197	190	8	0.53
ADG 14-21 (g/day)	200	192	11	0.61
ADG 0-21 (g/day)	180	174	5	0.46
Survivability %	71.4	72.0	2.6	0.87

[1] Values reported are least square means. Means within a row that do not share the same letter superscript are significantly different from each other ($P < 0.05$). The value in the far right column is the effect P-value for the effect that is being presented in the table.

[2] The abbreviation MM stands for Multimin90®. This convention will be used in the remaining tables, where it is appropriate.

Table 4-3: The effect of birthweight on the growth performance and survivability of nursing pigs[1]

	Birthweight						
	< 1.13 kg	**1.13 -1.59 kg**	**1.59 kg <**		**P-values[2]**		
N	118	266	118	**SEM**	**BW**	**Linear BW**	**BW x MM**
ADG 0-7 (g/day)	80^{a}	118^{b}	161^{c}	6	< 0.01	< 0.01	0.25
ADG 7-14 (g/day)	159^{a}	189^{b}	231^{c}	9	< 0.01	< 0.01	0.38
ADG 14-21 (g/day)	181	198	208	13	0.48	0.24	0.45
ADG 0-21 (g/day)	156^{a}	172^{b}	202^{c}	7	< 0.01	< 0.01	0.92
Survivability %	41.7^{a}	83.2^{b}	90.3^{c}	3.2	< 0.01	< 0.01	0.16

[1] Values reported are least square means. Means within a row that do not share the same letter superscript are significantly different from each other ($P < 0.05$). The value in the far right column is the effect P-value for the effect that is being presented in the table. The BW linear p-value refers to a preplanned linear orthogonal contrast.

[2] The abbreviation BW stands for birthweight. This convention will be used in the remaining tables, where it is appropriate.

Table 4-4: The effect of sire on the growth performance and survivability of nursing pigs

	Sire						
	EB5	**EBX**		**P-values**			
N	253	249	**SEM**	**Sire**	**Sire x MM**	**Sire x BW**	**Sire x MM x BW**
ADG 0-7 (g/day)	108^a	131^b	5	< 0.01	0.48	0.04	0.5
ADG 7-14 (g/day)	185	201	8	0.16	0.70	0.24	0.70
ADG 14-21 (g/day)	178^a	213^b	11	0.03	0.74	0.42	0.49
ADG 0-21 (g/day)	170	183	5	0.10	0.91	0.58	0.45
Survivability %	67.2^a	76.3^b	2.6	0. 01	0.63	< 0.01	0.38

[1] Values reported are least square means. Means within a row that do not share the same letter superscript are significantly different from each other ($P < 0.05$). The value in the far right column is the effect P-value for the effect that is being presented in the table.

CHAPTER 5

Conclusions

Trace mineral stores are depleted at weaning, so it is imperative to supplement trace minerals in the diets of nursery pigs to ensure proper growth and development. It is important to supplement manganese and selenium into nursery pig diets as it can increase ADG and ADFI in nursery pigs. MnSOD activity in red blood cells can also be affected by the level of manganese and selenium supplemented in the diet. An injectable trace mineral product has been shown to increase survivability in piglets born less than 1.13 kg. This product has the potential to be used in the swine industry, but costs may outweigh the benefits of using this product. Trace minerals that are supplemented to swine diets, like manganese and selenium, play an important physiological role in the animal and must not be forgotten.

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