EVALUATION OF TRACE MINERAL SUPPLEMENTATION ON THE REPRODUCTIVE PERFORMANCE OF SOWS AND THE GUT HEALTH AND GROWTH PERFORMANCE OF NURSERY PIGS

by

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(Under the Direction of C. Robert Dove)

ABSTRACT

Mineral supplementation is a requirement in the diets of all food animal species, including pigs. Trace minerals are included in mg/kg concentrations in the complete diet and although the concentration is low, a lack of these minerals can be detrimental to the growth and physiology of the animal. Manganese is a trace mineral supplemented to promote growth and reproductive efficiency in swine. Manganese plays a role in the structure and physiological function of the pig. Establishing optimal manganese requirements in pigs is of great importance to the swine industry as a whole, because existing literature is variable in its conclusions surrounding the subject. Iodine is another trace mineral that is known to be required, but an ideal dietary requirement promoting growth has yet to be determined. Iodine is a cofactor required for the synthesis of thyroid hormones, which promote the growth of the animal. The research presented in this dissertation will evaluate these mentioned trace minerals on various reproductive, growth, and physiological parameters in swine. In addition, there is preliminary evidence presented in this dissertation investigating the effects of manganese on the fecal microbial populations present in pigs. Data presented here can serve as a foundation for

additional research surrounding the supplementation of these minerals to the diets of pigs and have a positive impact on the global swine industry.

INDEX WORDS: iodine, manganese, nursery pigs, sows, trace minerals

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CHAPTER 1

Introduction

Swine production and pork products are integral links to the food animal industry. The main goal of swine production systems is to increase the size of litters per sow while minimizing labor costs (Knox, 2014; Knox et al., 2005). As a result, reproductive efficiency of the sow is key to optimizing piglet production. Sows have multiple offspring per pregnancy, short gestation length, and can be rebred quickly after weaning (Kyriazakis and Whittemore, 2006). There are multiple components that contribute to reproductive proficiency: genetics, nutrition, and the environment (Kyriazakis and Whittemore, 2006). Nutrition is the easiest of these factors for producers to control, as feed costs comprise 50- 85% of operation costs (McGlone and Pond, 2003). Proper nutrition of the gestating and lactating sow is a delicate balance of energy expenditure and investment as her body and metabolism shifts to accommodate the developing offspring (Kyriazakis and Whittemore, 2006). Levels of required nutrients need to be altered depending on the stage of development and production of the animal.

Trace minerals are an important nutrient to swine diets and must be included to support the developing animal. Trace minerals provide a variety of physiological functions to the animal and without supplementation can lead to a number of deficiency issues that stunt growth, cause digestive issues, and decrease intake and growth performance (Burkett et al., 2006; McDowell, 2003; Reese and Hill, 2006). NRC recommendations for trace mineral inclusion rates in swine diets have been well researched over the decades, but determination of optimal concentrations

for animal growth and performance are of utmost importance and are lesser known for some of the essential trace minerals (NRC, 2012).

This series of experiments was performed to determine the effect of trace mineral supplementation on reproductive performance, growth performance, and gut health of swine. The first experiment was performed to determine the effect of increasing dietary manganese on the reproductive performance of sows. The ideal concentration of manganese to optimize reproductive performance of sows has yet to be determined. Deficiencies in manganese can result in reproductive failures and birth of small and weak piglets (Plumlee et al., 1956). The second and third experiments were used to determine the effects of altering dietary concentrations of manganese and selenium on the growth performance, antioxidant status, gut microbiome, and fecal volatile fatty acid concentrations of nursery pigs. Manganese and selenium are mineral cofactors that are integrated into the antioxidant enzymes, superoxide dismutase and glutathione peroxidase, respectively (Haikarainen et al., 2014; Reese and Hill, 2006; Rotruck et al., 1973). The fourth experiment was performed to determine the optimal dietary concentration of iodine for growth performance and biomarker concentrations of triiodothyronine (T3) and thyroxine (T4); these are indicators of iodine status of the animal (Flachowsky, 2007; Franke et al., 2008). These experiments provide a basis for future research in the area of trace mineral nutrition and supplementation in the diets of swine and optimizing their effects on characteristics of animal growth, reproductive performance, and physiological processes.

CHAPTER 2

Literature Review

Motivation

Global populations are estimated to reach around 9.2 billion people by the year 2050 (Bongaarts, 2009). Drivers of these population increases include: increased fertility, decreased mortality rates in southern hemisphere African, Asian, and Latin American countries (Bongaarts, 2009). Food security and availability will be the focus of agricultural research in the years to come based on current population trends. With the majority of people living in towns and cities, feeding the globe with require a 70-100% increase in the food currently produced (Burney et al., 2010). Food demands in rapidly emerging nations will need to include more animal protein based foods (meat, eggs, milk, etc) as the standards of living increase (Smith et al., 2013). Animal-sourced food consumption will increase more than other agricultural sectors (Herrero et al., 2011), but ultimately allocation of scarce and limited natural resources (land and water resources) depends on policy and international distribution set forth by national, regional, and local communities (Smith et al., 2013).

Current and future agricultural livestock research should focus on the improvement of growth efficiency, longevity, and animal health as the global population continues to increase. Livestock producers play important roles in the livelihood and productivity of their animals. Adequate animal nutrition is the simplest way for producers to affect growth and feed efficiency along with improved management strategies (Bongaarts, 2009). Proper education of the public and raising awareness of the impending calorie shortage looming in the future are tools we as

animal scientists can use in order to further our research interests in the areas of improving feed efficiency, animal health, and reproductive longevity.

Mineral Supplementation in Swine Nutrition

Swine diets are formulated with to meet the ideal concentrations of necessary classes of nutrients. While it is important to include the major classes of nutrients like energy, fat, and protein, the dietary components that we add in much smaller concentrations (micronutrients) have just as much of an impact as those included in larger concentrations. Mineral supplementation plays a pivotal role in the proper growth and development of swine, cattle, and other livestock species. These minerals are present in most traditional feed ingredients, but the concentrations vary greatly and bioavailability to the animal is unknown (Acda and Chae, 2002). There are two classes of minerals: macrominerals and microminerals (or trace minerals). These two prefixes (macro- and micro-) have nothing to do with the physical size of the mineral but with the inclusion level in the diet (McDowell, 2003; Reese and Hill, 2006). Macrominerals like phosphorus and calcium, are included as a percentage of the diet formulation (g/kg of diet), while microminerals, like manganese, copper, and iron, are included as parts per million (mg/kg of diet) (Reese and Hill, 2006). Both macrominerals and microminerals are traditionally supplemented to swine diets in the form of inorganic salts, however, in more recent years, minerals bound to organic complexes have been of interest due to increased bioavailability to the animal and decreased mineral excretions (Acda and Chae, 2002; Burkett et al., 2006).

Minerals have diverse physiological roles in the animal. For example, in the eukaryotic cell, iron-sulfur clusters are found in enzymes like ribonucleotide reductases which are responsible for DNA replication and repair, and hemoglobin which is involved in oxygen transport (Zhang, 2014). While some minerals provide structural roles in tissues, others provide

regulatory roles in the animal (McDowell, 2003). Calcium plays a role in bone mineral composition, bone resorption, and in the synaptic transmission of signals between neurons (Arruda and Hotamisligil, 2015; McDowell, 2003). Still other minerals are involved with the function of certain enzymes as a cofactor or activator like selenium with glutathione peroxidase (Hilal et al., 2016; Lauridsen, 2019). With such diverse roles, it becomes necessary to ensure that minerals are properly supplemented to the animal at the appropriate concentrations and are available to the animal.

Microminerals in particular are important for the development of a strong immune system. Microminerals like copper and manganese are cofactors for enzymes that serve in a defensive role against oxidative stress and cellular damage (NRC, 2012; Underwood and Suttle, 1999). Microminerals are typically supplemented in excess which results in increased mineral excretion (Carlson et al., 1999; Hill et al., 2000). Optimizing micromineral supplementation to meet but not exceed physiological requirements has the potential to reduce mineral excretion and introduce more economical feeding strategies to the industry (Liu et al., 2014). Organic mineral supplementation has the potential to reduce mineral excretion from swine and has piqued interest in many researchers in recent years (Burkett et al., 2009; Veum et al., 2004).

Publications highlighting nutrient requirements for various livestock species are released every decade or so and the latest release for swine was in 2012 (NRC, 2012). Some nutrient recommendations from this most recent publication are even considered out of date based on recent research in determining what nutrient concentrations are promoting growth and animal health. Trace mineral requirements are not as well understood as the requirements for energy or protein in practical swine diets (López-Alonso, 2012). When inclusion rates of trace minerals are too low or too high, clinical and subclinical deficiency or toxicity symptoms (respectively) are

noted in research settings (López-Alonso, 2012; NRC, 2012). The classical definition of a nutrient requirement was defined to be the minimum amount of a particular nutrient that is supplemented that is needed to overcome a deficiency symptom (López-Alonso, 2012; Reese and Hill, 2006). It is now known that feeding concentrations of trace minerals above the published requirements can have growth promoting effects (Cromwell et al., 1989; Dove, 1993; Hill et al., 2000). Food animal production systems profit from efficiently producing animals, and mineral nutrition plays a pivotal role in the health, wellbeing, and productivity of livestock species. Though trace mineral inclusion is a small part of a complete swine diet, it can have a large economical and biological impact on the swine industry (López-Alonso, 2012).

Manganese

Manganese is an essential inorganic micronutrient that is classified as a trace mineral. Manganese is often supplemented to swine diets because corn grain and soybeans are relatively low in basal manganese content (5-6 mg/kg and appx 30 mg/kg, respectively) (Berta et al., 2004). Manganese, along with zinc, copper, and iron, are often supplemented in inorganic oxide or sulfate salts because they are more cost effective than organic alternatives (Burkett et al., 2009; Liu et al., 2014; Veum et al., 2004). However, trace minerals that have been chelated to an amino acid are effective and can be more available to the animal for physiological use (Berta et al., 2004). Hydrolyzed peptides and amino acids are the most common forms of commercially complexed manganese; the advantages to its increased bioavailability are attributed include increased solubility and improved compound structure (Berta et al., 2004).

Inorganic Mn supplements (sulfates, chlorides, or carbonates) will more easily or completely dissociate in the highly acidic and volatile gastric stomach environment due to the presence of ionic bonds in the structure of the compound (Chen et al., 2018). Manganese is

absorbed in the small intestinal tract and regulation of tissue levels is achieved via bile and intestinal signals (NRC, 1980). The mineral will enter the intestinal mucosal cell via passive diffusion or active transport in a similar manner as other divalent cations like calcium or iron (Chen et al., 2018). Passive diffusion does not require energy for transport in the form of adenosine triphosphate, while active transport does. The divalent transporter 1 (DMT1) is one of the primary transporters for cellular Mn influx (Chen et al., 2018). In the presence of additional divalent cations, Mn must compete with other metals for DMT1. By altering levels of other minerals, the absorption of Mn can be affected. Feeding excess levels of dietary calcium, phosphorus, and iron, the absorption of Mn is decreased due to transporter competition (Hansen et al., 2009; NRC, 1980). DMT1, along with other transporters like transferrin, albumin, and β-globulins, transport Mn to the liver. The oxidation state of Mn will dictate the type of transporter that is used (Nielsen, 2012). Excretion of Mn occurs by the liver, through bile; collectively, the net absorption of Mn is low due to a high turnover and loss in bile (Chen et al., 2018; Li and Yang, 2018; Nielsen, 2012).

One of the physiological reactions that involve manganese is mitochondrial superoxide dismutase (SOD); whose primary function is the protection of the mitochondria from oxidative damage (Haikarainen et al., 2014). Manganese is an obligatory cofactor of mitochondrial SOD (NRC, 2012). The mitochondria are the most metabolically active organelle in the eukaryotic cell and produce high concentrations of reactive oxygen species (ROS) relative to the rest of the cell (Holley et al., 2011). Increased concentrations of ROS in the cell can drastically alter the metabolic function of the cell, thus it becomes necessary to ensure that manganese is supplemented to the animal to prevent cellular damage.

Manganese is the obligatory cofactor of glucosyl transferase, whose function is integral to the synthesis of chondroitin sulfate (NRC, 1980). Chondroitin sulfate plays an important role in forming the mucopolysaccharides in the organic matrix of bone (Leach & Muenster, 1962). Several laboratory species demonstrated that a dietary deficiency in manganese led to bone growth abnormalities like perosis (Wilgus et al., 1939; Plumlee et al., 1960; Chen et al., 2018). These experiments provide further evidence for the inclusion of manganese in the diets of animals.

In a series of experiments regarding manganese deficiency in swine, several conclusions were made in relation to the swine manganese requirement (Plumlee et al., 1960). When prolonged feeding of a manganese-deficient diet (0.5 ppm Mn), gilts experienced a variety of deficiency symptoms including: reduction of bone growth, skeletal muscle weakness, irregular estrus cycles, absence of estrus signals, fetal resorption, and birth of small or weak piglets (Plumlee et al., 1960). This demonstrates that decreased levels of dietary manganese can result in negative reproductive consequences. Manganese has been suggested as a cofactor for mevalonate kinase and farnesyl pyrophosphate synthase (Curran, 1961; Xie et al., 2014). These enzymes are intermediate steps in the synthesis of squalene, a precursor for cholesterol, and thus progesterone the "pro-gestation" hormone in females (Xie et al., 2014). As a result of manganese deficiency, the downstream effect of decreased progesterone can have a negative impact on the reproductive cycle of a female (of any species). Hansen et al. demonstrated the effects of feeding varying concentrations of supplemental manganese to heifers on reproductive performance through gestation and parturition (Hansen et al., 2006b, 2006a). There was not an impact on reproductive performance; however, in the study regarding these heifers' calves, lower birth weights and reduced serum Mn concentrations were observed in calves from heifers fed basal levels of Mn

(Hansen et al., 2006a). Physical signs of manganese deficiency such as swollen joints and unsteadiness, were observed in calves born to heifers fed control levels of Mn (Hansen et al., 2006a).

Excess Mn supplementation can have a negative effect on the animal. When excess Mn is present, the target tissue of Mn is the brain (Chen et al., 2018). Toxicity effects of manganese in humans can include reduction in response to stimuli, changes in mood or hormonal synthesis and regulation (Chen et al., 2018). In animals and humans, Mn toxicity can lead to reproductive and developmental issues in addition to neurological defects (Chen et al., 2018; Milatovic, 2018). Dietary Mn levels must be properly maintained to ensure proper growth and development of any species. Concentrations exceeding or falling below the requirement of the animal results in negative physiological consequences (Chen et al., 2018; Milatovic, 2018).

As mentioned previously, Mn is included in a swine diet via trace mineral premix along with other essential trace minerals like iron, copper, and zinc. It is critical for Mn (and other trace minerals to be supplemented to meet but not significantly exceed the physiological requirement of the animal (McDowell, 2003). Research is important in this area, because in the case of most trace minerals, it is not known what concentration best benefits the animal physiologically. Excess dietary trace minerals that are not utilized by the animal are excreted, which is a huge environmental concern (Liu et al., 2014). Form of mineral supplementation is an important aspect to consider when formulating mineral premixes. Zinc, copper, manganese, and iron are most often included in formulations in their inorganic salt form, like sulfates and oxides (Thomaz et al., 2015). A low gastric pH has the ability to completely dissociate these inorganic trace mineral salts due to the presence of ionic bonds. This can result in the formation of insoluble antagonisms with phytic acid ahead of the small intestine, which can impair and

prevent the absorption of other nutrients (Liu et al., 2014). Organic minerals may be more bioavailable than inorganic sources when fed to pigs or poultry (Liu et al., 2014). Organic minerals are sources where a trace mineral has been chelated to an amino acid or other organic molecule. Organic mineral complexes will remain intact through the harsh acid environment of the stomach due to the presence of covalent bonds in the complex (Berta et al., 2004; Thomaz et al., 2015). Remaining intact from the stomach allows for better trace mineral absorption in the small intestine. Organic mineral complexes will be absorbed by amino acid or peptide transporters on the apical membrane of the intestinal epithelia (Berta et al., 2004). Following absorptions, the mineral will be cleaved and subsequently transported in the blood to the liver, metabolized, and excreted just like any other trace mineral source. This ensures that more mineral is absorbed and utilized and less is excreted into the environment.

Bioavailability studies of Mn have determined that Mn chelated to methionine or a protein has an equal (Baker and Halpin, 1987) or greater (Henry et al., 1989) impact on availability when compared to inorganic sources. In poultry, two sources of Mn (Mn fumarate and MnO) were supplemented at increasing levels (0, 30, 60, and 240 ppm) to determine if there was any impact on broiler chick growth or broiler chick tissue composition (Berta et al., 2004). There were no significant effects on body weight, feed efficiency (F:G), or mortality rates ($P > 0.10$). In addition, no difference were observed in chick structure, organ weights, or Mn tissue concentration (Berta et al., 2004). In this instance, source of Mn (organic vs. inorganic) had no impact on growth in broiler chicks. A study evaluating partial substitution of inorganic mineral sources with chelated organic mineral sources for sows from four weeks prior to parturition through lactation determined that there was no impact ($P > 0.10$) on birthweight, growth, or weaning weights (Papadopoulos et al., 2009). Additionally, there were no significant ($P > 0.10$)

differences in total body mineral concentrations at birth or at weaning for the piglets of this study when comparing the two sources of trace minerals (Papadopoulos et al., 2009). In a related digestibility study, Liu et al. (2014) determined that organic Zn, Cu, and Mn had increased digestibility and retention rates than their inorganic sulfate counterparts. With such variability in results, there is no "right" answer in utilizing organic minerals over inorganic minerals or vice versa. Organic minerals are typically more expensive due to increased production cost, but as new sources appear in the coming years, it may become more cost effective and commonplace to include organic minerals in typical livestock diets if it is beneficial to the growing animal.

Mitochondria, Reactive Oxygen Species (ROS), and Antioxidants

Mitochondria are one of the most important organelles in the eukaryotic cell as they produce energy for all of the metabolic processes that are required by the organism (Holley et al., 2011). Mitochondria produce the majority of the component of diverse metabolic pathways like the tri-carboxylic acid (TCA) cycle (Brière et al., 2006), heme synthesis (Ajioka et al., 2006), amino acid metabolism (Guda et al., 2007; Hutson et al., 1988), the urea cycle (Walker, 2009), and oxidative phosphorylation to synthesize ATP (Lemarie and Grimm, 2011). Because the mitochondria metabolizes molecular oxygen (O_2) to produce energy, there are often temporary reactive oxygen species (ROS) like the superoxide anion ($O_2^{\cdot-}$), that are formed as a byproduct of this mechanism (Lemarie and Grimm, 2011). ROS have a metabolic role, but a balance must be maintained to prevent harmful accumulation. ROS accumulation can lead to the development of a number of diseases, including certain types of cancer and neurological disorders (Brière et al., 2006; Waris and Ahsan, 2006).

Organisms produce antioxidant enzymes to eliminate ROS in order to keep the balance of ROS in check (Holley et al., 2011). An accumulation of the superoxide radical can accelerate the

production of other ROS, but enzymes like superoxide dismutase can enzymatically alter the structure of the superoxide anion into hydrogen peroxide and molecular oxygen (Lubos et al., 2010; Perry et al., 2010). Regulation of hydrogen peroxide concentration prevents the formation of the hydroxyl radical, which is highly reactive and destructive to the cell (Liddell et al., 2006; Lubos et al., 2010). Low concentrations of hydrogen peroxide serve two functions: a secondary messenger in signal transduction monitoring the oxidation state of redox-sensitive cysteine for kinase function and maintenance of protein thiols (Forman et al., 2010; Paulsen and Carroll, 2010). Hydrogen peroxide can be metabolized and eliminated by other antioxidant enzymes like catalase and glutathione-peroxidase (Paulsen and Carroll, 2010). In the context of swine production, ROS are of most concern during periods of increased stress, notably at weaning. Weaning is a period of increased environmental, nutritional, immunological, and social stressors on a piglet that is three weeks old (Cao et al., 2018). The nursery period immediately following weaning is a critical stage of growth for the piglet. Intestinal barrier function is significantly decreased at weaning (Cao et al., 2018). Production of ROS in the mucosal cells is increased due to the high energy requirement and oxygen metabolism of these particular cells (Holley et al., 2011). Accumulating ROS repress the expression of tight junction proteins, which function to minimize paracellular space in the intestinal mucosa (Anderson and Van Itallie, 2009). When tight junctions are compromised, harmful pathogens can leak through these junctions and enter into the animal's bloodstream, resulting in infection and inflammation (Anderson and Van Itallie, 2009). With an immature immune and antioxidant system, piglets are susceptible to new disease and physiological stresses if appropriate nutritional strategies are not put in place (Wijtten et al., 2011). Ensuring that the piglet can overcome and mitigate the physiological effects of weaning is important for the piglet as it grows until it reaches market weight.

The first line of defense for ROS that are produced in the mitochondria as a result of oxidative phosphorylation is manganese-superoxide dismutase (Haikarainen et al., 2014). Manganese superoxide dismutase (MnSOD) is a metalloenzyme that is necessary for the survival of all aerobic organisms and anaerobic organisms when they are exposed to aerobic conditions (Haikarainen et al., 2014). Manganese (Mn) is an essential dietary component and cofactor for MnSOD (Holley et al., 2011). As mentioned previously, the mitochondria are the most metabolically active organelle that house diverse metabolic pathways, producing excessive ROS that must be eliminated by the organism (Holley et al., 2011). Altering the expression of MnSOD can have dramatic consequences on mitochondrial function and overall cellular health due to oxidative damage to key enzymes; this damage can lead to the development of different diseases (Miao and St. Clair, 2009; Oberley and Buettner, 1979). MnSOD is highly expressed in tissues containing a large number of mitochondria like the liver, heart, and kidneys (Li et al., 2011). These tissue types have an increased energy requirement compared to other tissues in the body. There is little evidence supporting dietary Mn supplementation having a significant impact on tissue MnSOD status. In weaning pigs fed increasing concentrations of Mn (0.24 to 32 ppm), hepatic MnSOD concentrations averaged 4.89 IU/mg and cardiac concentrations averaged 10.0 IU/mg regardless of Mn dose (Pallauf et al., 2012). In a related study, hepatic and muscle concentrations of MnSOD were measured in grower pigs fed a high Mn (150 ppm) diet; values were much lower than weaning pig values: 2.62 and 0.96 IU/mg, respectively (Schwarz et al., 2017). The reason for this difference may be due to improved antioxidant abilities of older animals compared to weanling pigs.

Reactive oxygen species are produced in other intracellular locations. Other superoxide dismutase enzymes exist in different organisms with different metal cofactors (Haikarainen et al.,

2014). Copper-zinc superoxide dismutase (CuZnSOD) is typically found in the cytosol of eukaryotic cells and functions just as MnSOD to rearrange the superoxide radical into hydrogen peroxide (Edwards et al., 1998; Haikarainen et al., 2014). In addition, there are nickel-SOD and iron-SOD enzymes, but they are typically found in prokaryotic organisms (Haikarainen et al., 2014). Each of these SODs are grouped together due to shared antioxidant function and structure and they differ primarily by metal cofactor.

Another important antioxidant enzyme is glutathione peroxidase (GPX). This enzyme functions to detoxify lipid peroxides and provides necessary cellular and subcellular protection from lipid peroxide damage (Dalto et al., 2018; Rotruck et al., 1973). Hydrogen peroxide is a signaling molecule and reacts with GPX to initiate and regulate a variety of signaling cascades (Brigelius-Flohé and Flohé, 2011). GPX is a cytosolic antioxidant that converts hydrogen peroxide into water; unlike SODs or catalase, GPX require secondary enzymes to perform at a higher efficiency (Li et al., 2000). The majority of GPX enzymes have a seleno-cysteine at their active site, the activity of the enzymes is inextricably linked to the availability of selenium in the organism (Bermingham et al., 2014; Han et al., 2011). Selenium is another trace mineral that is imperative to include in a livestock diet (Biswal et al., 2016; Mahan, D. C., 1978; Mahan et al., 2014; Rotruck et al., 1973). There is relationship between selenium and vitamin E (Reese and Hill, 2006). They both serve as anti-peroxidants, attacking and eliminating ROS; increasing vitamin E in the diet does not eliminate the need for selenium inclusion in the diet (Ewan et al., 1969; Lubos et al., 2011). Though selenium is necessary to include in the diet, it has a narrow tolerance range in livestock species, so dietary concentrations are strictly regulated (Mahan et al., 2014). Although GPX does not work in the same manner as SOD enzymes, both enzymes are

necessary for the organism to have an efficient antioxidant defense system (Li et al., 2000; Lubos et al., 2011, 2010).

Immune Markers and Cytokines

Immunity and proper immune function in the context of swine production is an important component to the health and wellbeing of individual animals as well as the health of the herd (Mair et al., 2014). There are many facets to immunology and each component plays a small, yet vitale role in a complex physiological system that monitors inflammation and antigen recognition and elimination. Cytokines are small proteins secreted by certain types of cells to have a specific effect on intercellular communication and interactions (Zhang and An, 2007). Depending on the metabolic state and age of the animal and the extent of injury or infection, cytokine concentrations can fluctuate widely (Zhang and An, 2007). Maternal immunity is essential for piglets that are suckling because there is no placental transfer of immunoglobulins to the developing offspring. As is the case for the majority of mammals, immunity must be passed from the dam to the neonate via colostrum in order for the newborn piglet to combat pathogenic organisms (Salmon et al., 2009; Sinkora et al., 2002; Šinkora and Butler, 2009). Sow colostrum and milk contain a number of immunomodulatory agents and hormones: pro-immunological nucleotides, cytokines, T-helper cells, and prolactin (Salmon et al., 2009). There are a handful of research studies that look at various immune markers around parturition but there are no definitive concentrations of any of these markers that a sow should have at this time of her reproductive cycle. As mentioned previously, immune function and cytokine expression can be affected by a number of physiological factors. Cytokine profiles have been investigated and reported in postpartum women (Bränn et al., 2019), and these concentrations may simulate the profiles observed in other mammalian species like swine. Pigs serve as an excellent translational

model for a number of human physiology research, especially in the areas of the nervous and immune systems (Kaiser and West, 2020; Mair et al., 2014).

Pro-inflammatory cytokines are produced primarily by activated macrophages are involved in the upregulation of inflammatory responses (Zhang and An, 2007). Following parturition, sows experience metabolic stress and it has been shown that pro-inflammatory cytokines like IL-1α and IL-4 increase after farrowing, while concentrations of IFN-γ were reduced (Brigadirov et al., 2018). In addition, it is known that weaning induces the expression of pro-inflammatory cytokines in piglets, with peak expression occurring 1 d post-weaning (Cao et al., 2018; Pié et al., 2004). IL-1α plays an important role in the regulation of immune responses and is an intermediary cellular signal in the pathway activating the pro-inflammatory cytokine, tumor necrosis factor- α (TNF-α) (Boka et al., 1994; Salmon et al., 2009; Zhang and An, 2007). IL-4, an anti-inflammatory that induces the differentiation of naïve helper T-cells, while IL-2 is a signaling molecule that regulates the activities of white blood cells that are responsible for immune status (Salmon et al., 2009; Zhang and An, 2007). IL-1β is a potent pro-inflammatory cytokine that is important for host-defense responses to injury and pathogen infection and is the most studied cytokine of the IL-1 family members (Lopez-Castejon and Brough, 2011). IL-6 is a pro-inflammatory cytokine that has been shown to play a central role in neuronal reaction to nerve energy, suppression of feed intake, and the stimulation of the acute phase immune response (Klein et al., 1997; Maier et al., 1993). IL-18 facilitates type 1 immune responses along with IL-12, for the induction of cell-mediated immunity following infection (Biet et al., 2002; Trinchieri, 2003). Pro-inflammatory responses are necessary to produce inflammation in areas of injury or pathogen infection.

Anti-inflammatory cytokines (as the name might suggest) are responsible for the opposite action of pro-inflammatory cytokines. Anti-inflammatory cytokines are a series of immunoregulatory molecules that control and regulate the pro-inflammatory response; they do this by acting in concert with specific inhibitors and soluble receptors of cytokines to regulate immune responses (Zhang and An, 2007). IL-10 is an anti-inflammatory cytokine with multiple, pleiotropic effects in immune regulation and inflammation (Iyer and Cheng, 2012). IL-10 possesses potent anti-inflammatory properties that play a central role in limiting host immune response to pathogens; preventing damage to the host and maintaining normal tissue homeostasis. Regulation of IL-10 can be disrupted in the event of infection and extended disruption can lead to the development of several autoimmune disorders (Iyer and Cheng, 2012). IL-10 primarily functions to repress the expression of inflammatory cytokines like TNF-α, IL-6, and IL-1 via activated macrophages (Iyer and Cheng, 2012; Zhang and An, 2007).

There is a link that exists between the immune system/markers and the gut health and microbiome of the animal; this link will be described in detail in a later section of the review. There are many interlocked pathways and processes in the host that contribute to the overall health and wellbeing of an animal and gut immunity is one of those processes that a healthy animal possesses.

Progesterone

The growth and development of the mammalian conceptus, which encompasses the embryo/fetus and associated membranes, requires progesterone and placental hormonal influences on the uterus that regulates endometrial differentiation, signaling of pregnancy recognition, uterine receptivity for implantation, and conceptus-uterine interactions (Carson et al., 2000; Gray et al., 2001; Spencer et al., 2004; Spencer and Bazer, 2004). Domesticated

animals are spontaneous ovulators that will go through uterine dependent estrous cycles until pregnancy is established (Bazer et al., 1998; McCracken et al., 1999; Spencer and Bazer, 2002, 1996; Wathes, 1995). Maintenance of pregnancy requires interactions between the conceptus and the uterine endometrium; hormones from the placenta act directly on the endometrium to regulate cell function and differentiation (Spencer and Bazer, 2004).

The estrous cycle is dependent on the uterus, as it is the source of prostaglandin F2 alpha ($PGF_{2\alpha}$), the luteolysin (Spencer et al., 2004). During the estrous cycle, the endometrium will release pulses of PGF that results in structural and functional regression of the corpus luteum (CL); this process is known as luteolysis (Spencer et al., 2004). At estrus, estrogen levels peak from an ovulatory Graafian follicle and stimulate increased expression of uterine estrogen receptor alpha (ERα), progesterone receptor (PR), and oxytocin receptors (OTR). In association with luteolysis of the CL, progesterone concentrations decline 3-4 days prior to parturition, while $PGF_{2\alpha}$ increases (Akers, 2016). During early diestrus, the period of sexual inactivity between recurring estrus cycles, progesterone from the newly formed CL stimulates the accumulation of phospholipids in the endometrial luminal epithelia (LE) and superficial ductal glandular epithelium (sGE) that can free arachidonic acid for the synthesis and secretion of $PGF_{2\alpha}$ (McCracken, 1980; Spencer et al., 2004). The last stages of follicular maturation under the influence of luteinizing hormone (LH), the cells of the ovulatory follicle undergo luteinization and differentiate into luteal cells (Ziecik et al., 2018). After the formation of new CLs and luteinization, these follicular cells utilize cholesterol for steroidogenesis and the production of progesterone, the main functional steroid and product of CLs (LaVoie, 2017). Progesterone is involved first in the suppression and then development of the induction of endometrial luteolysis mechanism during the estrous cycle.

Prolactin, Mammogenesis, Lactogenesis, and Milk

Prolactin is a polypeptide hormone that exists in all vertebrates and is well known for its versatility of numerous biological functions (Freeman et al., 2006). It is known most commonly for its involvement in mammalian lactation but it also plays roles in immune system regulation, metabolism, and behavior (Farmer, 2016; Freeman et al., 2006). Prolactin is produced primarily by the anterior pituitary gland and is uniquely inhibited by the hypothalamus rather than stimulated, like all other pituitary hormones (Grattan and Kokay, 2008). The main target organ of prolactin is not an endocrine organ, resulting in an absence of the typical hormone-mediated feedback loops that control other pituitary hormones (Freeman et al., 2006; Grattan and Kokay, 2008). Prolactin acts on a variety of cell types rather than being restricted to a defined endocrine target (Freeman et al., 2006). Prolactin is also unique because it can be produced in the brain, and as a result function as a neuropeptide in addition to a neuroendocrine hormone (Grattan and Kokay, 2008).

In the context of swine, the key role of prolactin for lactation is necessary because sow milk yield is a critical determinant of piglet performance in the farrowing house (Farmer, 2016). Prolactin was clearly demonstrated as being an effector of sow milk yield by playing a critical role in mammary development during the late gestation phase of reproduction (Farmer et al., 2000; Farmer and Petitclerc, 2003). Prolactin binds to cell surface receptors in target tissues like the mammary gland (VanKlompenberg et al., 2013). Concentrations of endocrine prolactin in pigs is generally low, but a prolactin surge around parturition preceded increased concentrations during the lactation period (DeHoff et al., 1986). Mammogenesis or mammary tissue growth and development, is an important process in swine because the amount of tissue that is present is determinant of the sow's milking capacity (Farmer, 2001; Hurley, 2019). The majority of

mammary DNA accumulation in the gestating gilt occurs the last third of pregnancy (Hurley, 2019). Mammary gland development in pregnant gilts appears to be stimulated predominantly by estrogen production from the fetus, and declining progesterone concentrations after d 90 of gestation (DeHoff et al., 1986; Kensinger, 1982). Injections of porcine prolactin have been studied to determine possible effects on mammogenesis in growing gilts; an early indication of this effect was suggested by McLaughlin et al. (1997). Enhanced mammary development was observed after gilts were injected for 28 d with a recombinant porcine prolactin at 75 kg (McLaughlin et al., 1997). Manipulation of prolactin secretion or mammary development can be an important application to the swine industry due to the potential of increasing sow milk yield.

Control of prolactin secretion is primarily under the negative regulation of the neurotransmitter, dopamine (Farmer et al., 2000, 1998; Farmer and Petitclerc, 2003; Kraeling et al., 1982). Because of this, dopamine agonists can be given to inhibit prolactin secretion, while antagonists of dopamine can promote prolactin secretion (Kendall et al., 1983; VanKlompenberg et al., 2013). Knowing how prolactin secretion is controlled is an important concept from an industry standpoint because inhibition of prolactin at the wrong time can ultimately have an impact on piglet growth.

As mentioned previously, Mn is an essential trace mineral in livestock diets. Mn concentrations in organs are kept at optimal levels and blood Mn concentrations are maintained by gastrointestinal absorption and biliary excretion (Au et al., 2008; Michalke et al., 2007). In the context of humans, low levels of Mn exposure occur through water, air, and food regularly. Manganese accumulation can occur in certain parts of the brain following increased exposure (Marreilha dos Santos et al., 2011). Over exposure in Mn most commonly occurs in occupational environments; Mn neurotoxicity, known as manganism, has been reported in miners, smelters,

and workers in the alloy industry due to inhalation of Mn-dust (Aschner et al., 2007; Marreilha dos Santos et al., 2011). Symptoms are typically minimal and subtle early on, but after establishment, Mn intoxication becomes irreversible, leading to permanent neurological damage (Aschner et al., 2007; Jiang et al., 2006). Accumulation of Mn in the brain is not common, compared to other substances or minerals. Though mechanisms are not fully understood, Mn is able to cross the blood-brain-barrier (BBB) in adults and developing conceptus (Aschner and Aschner, 1991). Iron is another trace mineral that is able to cross the BBB. It is believed that uptake of these minerals (Mn and Fe) may be related to their shared transport molecule, divalent metal-transporter 1 (Marreilha dos Santos et al., 2011). In the brain, Mn interacts negatively with cellular dopamine; it promotes the autoxidation of dopamine and dopaminergic cell death (Marreilha dos Santos et al., 2011). As mentioned previously, dopamine is a negative regulator of prolactin secretion (Farmer et al., 2000, 1998; Farmer and Petitclerc, 2003). Due to this, Mn acts as an indirect regulator of prolactin secretion in humans, and prolactin may be used as a peripheral marker of Mn neurotoxicity (Marreilha dos Santos et al., 2011). Though the previously mentioned paper relates to rats and humans, pigs share a remarkably similar brain to humans in terms of white and gray matter composition and shape (Kaiser et al., 2020; Kaiser and West, 2020). With such similarity to human brains, pigs are used in research to model strokes and stroke therapy methods (Kaiser et al., 2020; Kaiser and West, 2020). It is conceivable that prolactin secretion in sows could provide information on Mn status to an extent, but it may only be in situations of dietary Mn toxicity, which is not likely to occur in a research or industry setting. Regardless, the existence of the link between Mn and prolactin secretion is important to know.

Sow milk composition and sow milk yield are critical components to a piglet's early growth and development in the first few weeks of life (Salmon et al., 2009). Colostrum, or the sow's "first milk," is nutrient-rich and is necessary for the establishment of an immune system in the neonate (Salmon et al., 2009; Sinkora et al., 2002; Šinkora and Butler, 2009). There is no placental transfer of immunoglobulins, thus displaying the need to obtain them from milk. Colostrum contains a number of immunomodulatory agents: cytokines, T-helper cells, macrophages, prolactin, and nucleotides that enhance the activity of natural killer (NK) cells (Salmon et al., 2009).

Due to an immature thermoregulatory system, piglets require fat in order to survive; this fat must come from the sow's milk (Zhang et al., 2018). Sow colostrum and milk are notably higher in fat than in other livestock species (Kim, 2013). Sow milk averages 5-7% fat, while cow milk is typically around 4% fat; the differentiation exists due to thermoregulatory requirements by the neonates (Kim, 2013; Zhang et al., 2018). In addition to fat, colostrum and milk contains the necessary carbohydrates and protein for piglet energy and growth (Zhang et al., 2018). Transfer of trace minerals through colostrum or milk is minimal in swine (Ma et al., 2020). Minerals are present in the milk but at very low concentrations (µg/mL). Minerals like iron oxidize readily when exposed to oxygen, so iron concentrations in milk are very low to prevent milk oxidation (Ma et al., 2020; Zhang et al., 2018). Because of low concentrations of iron in milk and no placental transfer, we must give piglets supplemental iron to ensure adequate growth and hemoglobin production (Szudzik et al., 2018).

Copper

Copper is an additional essential trace mineral which is included in swine diets to prevent certain undesirable physiological defects in the animal. The essentiality of copper in the diet was first determined when researchers were working with anemic, milk-fed rodents (Hart et al., 1928). Copper is important to include due to the copper-dependent enzymes involved in iron-metabolism, primarily ceruloplasmin (NRC, 1980). Like iron, copper is bound to amino acids and proteins to prevent oxidation/reduction reactions from occurring spontaneously (Chiba, 2012). In addition, copper plays a role in the formation of melanin, elastin, collagen, and is needed for proper central nervous system function (Chiba, 2012). Dietary requirements for copper, like many other elements, depend highly on species, age, and purpose of the animal. The amount recommended for swine by the NRC is 5-6 ppm for a neonatal pig, while suggestions for sow supplementation are almost 20 ppm (NRC, 2012). Regardless of NRC recommended levels, actual requirements and recommendations for copper are ill-defined and highly debated.

Absorption of copper for the majority of species occurs in the ileum and jejunum and is affected by the particular form of copper that is supplemented (NRC, 1980). Copper salts, like copper sulfate and copper chloride, are known to be the most biologically available copper salts to supplement to swine; these forms of copper are better used by the pig and promotes increased efficiency and growth (Cromwell et al., 1998). Copper sulfide and copper oxide are less available according to a nursery pig study performed to determine the effect of source and level of copper on performance and liver copper stores (Cromwell et al., 1989; Cromwell and Hays, 1978). Organic copper complexes have been shown to be equally as biologically available to the animal according to several studies (Apgar et al., 1995; Apgar and Kornegay, 1996; Coffey et al., 1994). Copper in a copper-lysine complex supplemented to pigs had increased growth

performance when compared to pigs fed copper sulfate as the source of mineral (Coffey et al., 1994). Proteases and stomach acids release copper from whatever protein or amino acid it is bound to which allows it to be absorbed by the ileum/jejunum via active and passive transport (Chiba, 2012).

The primary copper transporter is Ctr1, but divalent metal transporter 1 (DMT1) may transport copper to an extent, due to its common valence form Cu^{2+} (Hill and Link, 2009). Like iron, changes in the valency of copper can be important for transport of copper and the functionality of associated enzymes (Chiba, 2012). Once absorbed, copper appears first in the plasma as the cupric ion bound to albumin. When the liver is synthesizing ceruloplasmin, a metalloprotein that carries over half of the copper in the blood, copper is bound and released to general circulation (NRC, 1980). Excretion of copper occurs via the biliary system, sweating, lactation, and urination (NRC, 1980).

Deficiencies in copper can result in decreased iron mobilization, abnormal cell production, and poor production of collagen, elastin, and melanin (NRC, 2012). Clinical copper deficiency symptoms include: anemia, cardiac disorders, spontaneous bone fractures, and abnormal pigmentation of the skin. Copper toxicity can occur in swine when excess amounts are fed over an extended period of time; toxicity symptoms can include reduced hemoglobin levels and jaundice (NRC, 2012). Jaundice is a result of copper accumulating in the liver and other organs (NRC, 2012). When reduced dietary levels of zinc and iron are present, copper toxicity symptoms can be amplified and more readily observed. When zinc concentrations are increased, ultimately copper deficiency occurs in the animal (Chiba, 2012). Increased iron intake leads to reduced copper absorption due to competition for absorption within the gastrointestinal tract for utilization in metabolic function. CuZn superoxide dismutase activity in the blood will be

diminished due to increased iron supplementation (Barclay et al., 1991). Since copper interacts with iron and zinc due to similar valence characteristics, the levels supplemented to the diet must be closely monitored (Hill and Link, 2009).

Iodine, Thyroid Gland, and Thyroid Hormones

Iodine (I) is an essential trace mineral for humans and animals; it is required for the synthesis of thyroid hormones triiodothyronine (T3) and thyroxine (T4) that are regulators of metabolic processes and metabolic rate (Flachowsky, 2007; Franke et al., 2008; Stepien and Huttner, 2019). Thyroid hormones regulate brain development, reproduction, and growth of the organism (Franke et al., 2008). A severe deficiency of iodine can lead to incidence of hypothyroidism and related symptoms like goiter, diminished growth, and mental and physical deformations (Andrews et al., 1948; Delange, 1994; Flachowsky, 2007; Kaufmann and Rambeck, 1998). In the early 20th century, it was noted by several research stations in the Great Lakes regions of the United States that there were high concentrations of pigs with hypothyroidism due to iodine-deficient feedstuffs grown in iodine-deficient soils (Hart and Steenbock, 1918; Kalkus, 1920; Welch, 1928). The dietary requirement for iodine is not well established; in addition, the requirement can be increased in the presence of goitrogens, which are found in certain feedstuffs like peanuts, rapeseed, and soybeans (Kaufmann and Rambeck, 1998; McCarrison, 1933; Schöne, 1999). A concentration of 0.14 ppm iodine in a corn-soybean meal diet is sufficient to prevent thyroid hypertrophy in grower pigs (Cromwell et al., 1975), while 0.35 ppm of added iodine prevented deficiency in sows (Andrews et al., 1948). Sows fed iodine-deficient diets farrowed weak or dead hairless piglets, myxedema symptoms, and an enlarged thyroid (Devilat and Skoknic, 1971; Hart and Steenbock, 1918; Slatter, 1955). In growing pigs, a dietary iodine concentration of 800 ppm decreased growth, hemoglobin, and

liver iron concentrations (Newton and Clawson, 1974). As much as 1,500-2,500 ppm of iodine had no harmful effects on sows during the last 30 days of gestation and during lactation (Arrington et al., 1965). The range between an iodine concentration low enough to prevent a hypertrophic thyroid gland (0.14 ppm) and preventing a depression of growth (800 ppm) is quite wide so optimizing the dietary concentration with growth promotion would be ideal from an industry perspective.

Mammalian thyroid hormones (THs) are produced in two distinct forms, T3 or T4; T4 is the main product of thyroid gland secretion (Stepien and Huttner, 2019). Thyroxine (T4) has low affinity for nuclear TH receptors and is thought to act primarily as a biologically inactive prohormone (Stepien and Huttner, 2019; Wassen et al., 2004). The main biologically active form of thyroid hormone is T3 (Wassen et al., 2004). In humans, about 20% of T3 is secreted by the thyroid gland; most of the circulating is derived from the de-iodination of T4 in peripheral tissues (Chanoine et al., 1993; Van Doorn et al., 1983). Three iodothyronine deiodinases (D1, D2, and D3) are responsible for the regulation of systemic and local availability of thyroid hormone (Wassen et al., 2004). Expression and localization (or lack thereof) of these three enzymes are important in the animal from a physiological point of view. Wassen et al. (2004) were able to determine and characterize deiodinase enzymes in swine, a more pertinent translational animal model than rodents. Because iodine is necessary for thyroid hormone function in the animal, T3 and T4 hormones are great indicators of iodine status. There has been little current research evaluating the current NRC recommendation of 0.14 ppm iodine in nursery pigs (NRC, 2012). As has been determined in other trace minerals, like copper and manganese, that supplementation of concentrations exceeding the NRC requirement may have growth promoting effects for the animal (Grummer et al., 1950; Luo and Dove, 1996).

Iron

The majority of the iron (Fe) in the body is contained within hemoglobin for the purpose of oxygen transport (Levi and Rovida, 2009). Smaller concentrations can be found in myoglobin (muscle cells) and enzymes. There are two valence forms of iron found in the body: ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron (Levi and Rovida, 2009; Theil, 2011). These two forms are typically the only forms present in swine feed ingredients (Chiba, 2012). Heme iron is a form of iron present in feed ingredients that are produced from blood or muscle. Non-heme iron is released in the gastrointestinal tract due to the acidic physiological conditions of the stomach and small intestine. Ferrous iron (and other divalent metal ions like Zn, Cu, and Mn) use DMT1 and other transporter proteins to cross the brush border of the intestine and enter the intestinal mucosal cells (Hill and Link, 2009). Once in the mucosal cell, iron is able to move into the bloodstream for circulation, remain within the intestinal cell for later use, or be sloughed off (Chiba, 2012). Non-circulating iron can bind to apoferritin for short term storage within the cell; long term storage occurs within the liver, bone marrow, and spleen (Chiba, 2012). Iron is contained within ferritin, which is degraded and synthesized as a continual source of iron to the body (Bakker and Boyer, 1986).

There is little to no placental transfer of iron to the developing piglets *in utero* (Starzyński et al., 2013). Newborn piglets rely on the sow's milk for their required nutrients, with a few exceptions. Iron, unlike other minerals, is not obtained via sow milk or colostrum. Iron rapidly oxidizes when exposed to oxygen and moisture. Therefore, neonates often become anemic (iron deficient) due to limited iron stores and using up what they have very quickly; it becomes necessary to provide piglets with supplemental iron to combat pre-weaning piglet mortality (Starzyński et al., 2013). In modern production systems, iron is provided to neonates

via 100-200 mg intramuscular injection of iron dextran, a polysaccharide (Starzyński et al., 2013). Iron dextran is typically injected within the first few days of birth (NRC, 2012). When there is excessive non-heme iron present in the diet, iron is able to reduce the absorption of Zn due to competition for binding sites and transport molecules (Chiba, 2012). A deficiency in vitamin A can result in the accumulation of iron in tissues (Chiba, 2012; Mendes et al., 2016).

In a study regarding the regulation of iron transporters via supplemental dietary iron of young pigs, a relationship between iron and manganese was observed (Hansen et al., 2009). Increased concentrations of dietary iron has been linked to negative effects related to increased oxidative stress in addition to interference with competing absorption of other essential trace minerals like copper and manganese (Hansen et al., 2009). Interference of absorption is due to competition of certain mineral transporters in the gastrointestinal tract (Hansen et al., 2009). Gene expression of iron-transporters such as DMT1 and ZIP14 in the duodenum and several additional transporters were affected by the concentration of supplemental dietary iron (Hansen et al., 2009). Nursery pigs fed increased dietary iron had decreased tissue levels of Mn in the liver and duodenum (Hansen et al., 2009). DMT1 plays a role in absorption of both iron and manganese into the small intestine of the nursery pig (Au et al., 2008; Hansen et al., 2009). It is important to understand the interrelationship between the concentrations of iron and manganese in the swine diet (Chiba, 2012). As mentioned previously, supplementation of phytase can improve iron absorption (and other positively charged nutrients) due to the prevention of insoluble phytate complexes from forming (Humer et al., 2015; Schlegel et al., 2013; Selle et al., 2009).

Zinc

Zinc is another essential dietary trace mineral that is a cofactor for DNA/RNA synthetases, transferases, and digestive enzymes (NRC, 2012). In addition, it is associated with the hormone, insulin (Emdin et al., 1980; Jayawardena et al., 2012). Zinc plays a role in lipid, carbohydrate, and protein synthesis. Zinc finger proteins are an enzymatic control mechanism for DNA, controlling cellular transcription, and assisting in the transduction of inter- and intra-cellular signals (Laity et al., 2001). Dietary inclusion of zinc must be carefully monitored and limited in swine diets due to environmental concerns (Nitrayova et al., 2012). The intestinal absorption of zinc is based on physiological need and will eventually be excreted in feces following physiological utilization (NRC, 1980). Small concentrations of zinc are lost in the urine and integumental tissue shed in the body (Brugger and Windisch, 2019; NRC, 1980). Transportation of zinc in the body following absorption across the brush border of the intestinal mucosa occurs is highly regulated with respect to intake (NRC, 1980). Under normal conditions zinc can attach to nucleic acids, transporter proteins, and other organic compounds via sulfhydryl, amino, and imidazole functional groups (Hill and Link, 2009).

Bioavailability is an issue with zinc as well. Zinc salts like zinc chloride, zinc sulfate, and zinc carbonate are highly available to the animal (NRC, 2012). The true amount of Zn that is actually absorbed and retained is less than 50% of the intake (Miller et al., 1991). Depending on the source, zinc may be less available if supplemented in the form of zinc oxide or zinc sulfide (Miller et al., 1991). Over the years, organic zinc sources have been evaluated by several research studies to determine if bioavailability of organic sources was improved. Two zinc sources, zinc sulfate and a zinc-lysine complex, were equally effectively in promoting growth performance and Zn absorption (Cheng et al., 1998). Weaned piglets (N = 500) were

supplemented 0, 25, 50, 75, and 100 mg/kg of Zn as either organic or inorganic or 50 mg/kg with 50% organic and 50% inorganic Zn, and it was determined that 75 mg/kg organic Zn complex best supplemented todays growing pigs with a high lean tissue composition (Hill et al., 2014).

Zinc deficiencies in swine can be identified by a number of clinical signs, primarily the hyperkeratinization of the skin known as parakeratosis (Tucker and Salmon, 1955). This skin condition includes skin lesions that are painful for the animal; other symptoms may include reduced growth efficiency and reduced serum Zn, albumin, and alkaline phosphatase (Miller et al., 1968). In sows fed zinc-deficient diets, the dams experienced issues with farrowing, lactation, and reproductive performance (Prasad et al., 1969). There are also issues with feeding too much zinc in the diet, as zinc toxicity symptoms can start to be observed: arthritis, lethargy, and in severe cases (2000+ ppm Zn) internal hemorrhaging can occur (NRC, 1980). Proper supplementation of zinc ultimately results in more productive and efficient animals.

As one of several divalent (2+) metal ions, zinc will compete with other metal ions for the active site on an enzyme or transport molecule. Copper-zinc superoxide dismutase (CuZnSOD) is an enzyme that metal ions compete for. This SOD works to reduce the detrimental effects of reactive oxygen species in the cell. Unlike the MnSOD, the CuZnSOD enzyme exists as a homodimer and is located in the cytoplasm (Perry et al., 2010). The proposed transporter of Zn^{2+} in the plasma is serum albumin or transferrin (Mertz, 1986). Serum albumin transports other organic and inorganic molecules via the bloodstream, and is an option for a delivery system for certain types of drugs (Larsen et al., 2016). There can be competition between zinc and other molecules in the context of human and animal medicine when particular drugs with this method of delivery are utilized. Competition depends on availability of

hydrophobic active sites on overall negatively-charged serum transporter proteins and availability of possible cofactor substrates present (Larsen et al., 2016).

Another consideration that must be taken into account when talking about zinc supplementation in swine (and poultry) diets is the presence of phytic acid, also known as phytate. Approximately 2/3 of the available phosphorus in primary feedstuffs, like corn and soybean meal, are nutritionally unavailable to the animal because they are in the form of phytate (Humer et al., 2015; Schlegel et al., 2013). Phytate is a compound found in plants and is a primary phosphorus store in the seeds of plants (Humer et al., 2015). Phytate will make its way through the digestive tract of the animal, then bind and create insoluble phytate complexes with any nutrient or other organic molecule with a positive charge (Schlegel et al., 2013). Zinc is one of the nutrients that phytate will bind, in addition to iron, calcium, and others (Humer et al., 2015; Selle et al., 2009). This insoluble complex simultaneously reduces the utilization of other nutrients and increases phosphorus excretion, which leads to environmental concerns. Improvements to zinc utilization can be made through the use of phytase, an enzyme that cleaves phytate phosphorus from its ring structure (Humer et al., 2015; Selle et al., 2009). Phytase improves growth performance and nutrient digestibility in both poultry and pigs (Hanni et al., 2005; Walk and Olukosi, 2019)

Gut Health, Intestinal Microbiome, and Volatile Fatty Acids

Gastrointestinal tract (GIT) health, also called "gut health," is a term that has gained a lot of traction in the areas of animal agriculture but also in human medicine in recent years (Pluske et al., 2018). Our traditional definition of health has been understood as "a body without ailment;" whether that ailment is an injury, infection, or otherwise. These definitions applied to the gut would associate pathogens that cause either clinical or subclinical illness, mortality, or

morbidity to animals or humans (Bischoff, 2011). The gut health of pigs, however, can be compromised even in the absence of diseases. Low intake occurs post-weaning in pigs due to the introduction of several stressors (Cao et al., 2018), but also the induction of structural and functional changes to the GIT (Celi et al., 2017; Jayaraman and Nyachoti, 2017; Kim et al., 2012). Bischoff (2011) defined five characteristics of adequate gut health: 1) the effective digestion and absorption of food, 2) absence of GIT illness, 3) normal and stable intestinal microbiome, 4) effective immune status, and 5) status of well-being. A more recent review was in general agreement in the components that made up gut health and GIT function were: the diet itself, host interaction with the gut microbial populations, efficient digestion and absorption and feed, and immune status of the animal (Celi et al., 2017). Something that is not well defined in this area of research is: what is an ideal healthy microbiome and what are the components of that gut microbial population(s)? Researchers today in the field of human and food animal nutrition are trying to answer that question.

Pigs experience a wide range of stressors at the time of weaning, most notably, nutritional stress. Following weaning, there is typically an increased incidence of diarrhea and GIT issues due to decreased feed intake, and immature digestive and immune systems (Spreeuwenberg et al., 2001; Vente-Spreeuwenberg et al., 2003). The GIT and underlying lamina propria are continually exposed to a harsh luminal environment, of which include: toxins, pathogens, and antigens (Moeser et al., 2017, 2007). Weaning transition activates stress and inflammation signaling physiological pathways and results in the abnormal expression of various intestinal genes and proteins in pigs (Cao et al., 2018; Pluske et al., 2018). The gut has a complicated role in the context of the organism; it must act as a barrier to these aforementioned microbes but also provide efficient transport of nutrients, electrolytes, and water across the various GIT membranes

(Moeser et al., 2017). These nutrient components are critical for normal physiological maintenance and growth; the gut does sort and take in specific dietary and microbial antigens to oversee the development of the mucosal immune system of the GIT (Moeser et al., 2017, 2007). The GIT is made selectively permeable through a number of intracellular and apical intercellular membranes including: zonula occludens, occludin, and claudins (Edelblum and Turner, 2009; Groschwitz and Hogan, 2009; Marchiando et al., 2010). These proteins regulate the "leakiness" of the apical membrane by sifting through ions selectively and adjusting pore sizes accordingly; these proteins are aptly named tight junction proteins (Edelblum and Turner, 2009). Maintaining this physiological barrier in the GIT is key in providing a first line of defense for the host immune system and sustaining a "healthy" gut.

The gut microbiota benefits the host in a plethora of ways including: digesting and fermenting carbohydrates, maintenance of intestinal villi, production of volatile short-chain fatty acids (SCFAs), immune response regulation, and protection from pathogenic bacteria (Gresse et al., 2017; Levy et al., 2017). In the ruminant animal, the rumen microbiome is critical in digesting forages that the animal could not digest with host digestive enzymes alone (Welch et al., 2020). Ruminant animals source the majority of their energy from volatile fatty acids (VFAs; also known as SCFAs), which are derived from ruminal microbial fermentation (Welch et al., 2020). While monogastric diets and digestive systems are not the same as ruminant animals, there is still some microbial fermentation and VFA production that occurs, but to a much lesser extent (Meng et al., 2020).

Volatile fatty acids are produced primarily from undigested carbohydrates (CHO) in the diet of the animal by anaerobic bacterial fermentation and have 1 to 6 carbons in a chain (Yang et al., 2021). The primary VFAs of interest include: acetate, propionate, butyrate, isobutyrate,

valerate, and isovalerate; these are the main products of dietary fiber fermentation by intestinal bacteria (Corrêa-Oliveira et al., 2016). Research over the years demonstrated that acetate is produced mainly by colonic anaerobes fermenting the undigested and unabsorbed CHO in the foregut, propionate is the primary product of *Bacteroides* (and other genera) fermentation and butyrate is produced by *Firmicutes* fermentation (Bianchi et al., 2011; Marquet et al., 2009; Nakamura et al., 1998). Acetate and propionate are involved in energy metabolism in the liver, and butyrate can provide the energy for absorption process by intestinal epithelia (Duncan et al., 2004; Tremaroli and Bäckhed, 2012). Butyrate plays additional roles in host protection from pathogens (Guilloteau et al., 2010; Raqib et al., 2006) and reduction of chyme flow rate in the GIT to oversee nutrient absorption by the intestinal epithelial cells (Le Gall et al., 2009; Woliński et al., 2003). In addition, there are physiological roles in immunity, obesity, and the regulation of blood sugar (Polyviou et al., 2016).

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CHAPTER 3

THE EFFECT OF INCREASING DIETARY MANGANESE FROM AN ORGANIC SOURCE ON THE REPRODUCTIVE PERFORMANCE OF SOWS

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Abstract

The objective of this study was to determine the effect of dietary manganese on the reproductive performance of sows. Sows (N = 39; 231 ± 8 kg) were randomly assigned to one of three dietary levels of supplemented Mn (CON: 0 ppm Mn; PRO20: 20 ppm Mn; PRO40: 40 ppm Mn). Experimental treatments were initiated at breeding and continued through two parities. Sows were blocked by parity within each farrowing group and dietary treatments were represented within each block. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS with diet as a fixed effect and block as a random effect. Dietary treatment did not affect sow body weights (P > 0.10). Lactation feed intake was increased in PRO20 sows compared to CON and PRO40 sows (P < 0.05). PRO20 and PRO40 sows farrowed heavier piglets (CON 1.23 kg; PRO20 1.57 kg; PRO40 1.40 kg; P = 0.001) with improved average daily gain to weaning (CON 213 g/day; PRO20 237 g/day; 220 g/day; P < 0.05), compared to CON sows. Milk fat content (average from d 7 and 14 of lactation) was lowered in PRO20 (5.5%) and PRO40 sows (6.1%; P < 0.05) compared to CON sows (7.8%), possibly due to increased milk demand from the piglets. There were no differences in milk mineral concentrations during lactation or piglet tissue mineral concentrations at weaning (P > 0.10). On day 3 of lactation, prolactin concentrations were similar across treatments (P > 0.10), while progesterone concentrations tended to differ in response to Mn level (CON 23.70 ng/mL; PRO20 26.15 ng/mL; PRO40 22.10 ng/ml; P = 0.09). Supplementary dietary Mn throughout two gestation and lactation cycles led to improved birth weights and pre-weaning growth of piglets.

Key Words: Lactation; manganese; reproductive performance, sows

3.1: Introduction

Reproductive efficiency is an important aspect of the swine industry. Genetics, nutrition, and the environment are contributing factors to a sow's reproductive efficiency (Kyriazakis and Whittemore, 2006), yet diet is one of the easiest factors for producers to control. Adequate nutrition for the gestating and lactating sow ensures a balance between energy expenditure and investment as her body and metabolism shift to accommodate the developing offspring and mammary tissue (Kyriazakis and Whittemore, 2006; Schoknecht, 1997). Neonates experience innate nutritional deficiencies that must be corrected via the formulation of the sow's diet, because suckling piglets obtain the majority of their required nutrients from the sow via colostrum and milk until weaning (Knox, 2014; Knox et al., 2005). Maximizing the performance of sows and their litters during the lactation period of reproduction is therefore a major focus for swine nutritionists (Derouchey et al., 2003).

Manganese (**Mn**) is an important inorganic dietary component found in low concentrations in most feedstuffs (Berta et al., 2004). Basal levels of Mn in feedstuffs alone are not sufficient for optimal growth and are of unknown availability to the animal (Berta et al., 2004); therefore, Mn must be supplemented in the diets of pigs. It is well established that Mn plays a role in development, digestion, reproduction, antioxidant defense and immune function in multiple species (Chen et al., 2018; Hansen et al., 2006; Holley et al., 2011; Leach and Muenster, 1962; Leibholz et al., 1962; McDowell, 2003). Plumlee et al. (1956) showed that feeding a Mn-deficient diet to gilts results in bone growth abnormalities, irregular estrous cycles, fetal resorption, and birth of small and weak neonates. Supplementary Mn in the diets of swine, poultry, and other species is necessary to prevent growth abnormalities, reproductive failure, and overall negative health concerns (Berta et al., 2004; Hansen et al., 2006; Plumlee et al., 1956).

The form in which trace minerals, such as Mn, are supplemented (organic v. inorganic) can affect their efficacy in improving growth, utilization, and decreasing mineral excretion (Burkett et al., 2009; Veum et al., 2004). The objective of this research project was to determine the effect of increasing dietary Mn from an organic source on the reproductive performance of sows and antioxidant status of their offspring.

3.2: Materials and methods

Animal care, handling, and processing procedures were approved by the University of Georgia Institutional Animal Care and Use Committee (AUP #A2018 11-014-R1).

3.2.1: Sow Management

Sows (Choice Genetics Line CG32; N = 39; 231 ± 8 kg) were blocked by parity within farrowing group, with all dietary treatments being represented within each block. Sow diets were formulated with three levels of supplemental Mn (ProPath® Mn, Zinpro Corp, Eden Prairie, MN): Parity 1 (N = 39): Control (n = 13; CON; no supplemental Mn, Table 3-1), 20 ppm Mn (n = 13; PRO20), or 40 ppm Mn (n = 13; PRO40); Parity 2 (N = 35): CON (n = 11), PRO20 (n = 11), PRO40 (n = 13). The current NRC (2012) requirement for Mn in gestating and lactating sows is 25 ppm. Sows began their respective dietary treatments on the day of breeding and remained on dietary treatment until pregnancy determination after the third breeding cycle. All sows were on a common diet for at least 90 d prior to the start of this study. During gestation, the experimental diet was mixed to contain 320 ppm Mn and then fed at 1/16 (20 ppm) or 1/8 (40 ppm) of daily intake with the remainder being control diet. This allowed the use of the dual hopper system in the electronic sow feeder (AP®, AGCO; Duluth, GA). Sows were fed to maintain a body condition score of three during gestation (Knauer and Baitinger, 2015). The low Mn gestation diet and CON lactation diet were both 42 ppm Mn which met the NRC requirement

and was not Mn-deficient (Table 3-1). During breeding and gestation sows were housed in a temperature-controlled (21.1 ± 2.8°C) barn at the University of Georgia Double Bridges Swine Unit (Oglethorpe County, GA).

Pregnant sows were transported to an environmentally controlled (21.1 ± 2.8°C) farrowing room (LARU; University of Georgia, Athens, GA) several days prior to their due date. Sows were restricted on lactation feed upon arrival at the LARU until 1 d post-farrowing with *ad libitum* access to water. Sows consumed lactation feed and water *ad libitum* after 1 d post-farrowing until weaning (Table 3-1). Sows remained on the same dietary treatment during gestation and lactation phases. Sows were weighed upon entering the farrowing unit, within 24 h of farrowing, and at weaning (Mosdal Scale Systems; Broadview, MT). Weekly feed intake was recorded during the lactation period for sows housed at the LARU. Data were collected through two full breeding cycles and lactations. Only those sows that were brought to the LARU for parity 1 were eligible to be brought to the LARU for parity 2 data collection.

3.2.2: Piglet handling and care

Piglets were processed and weighed (Ohaus Corporation; Parsippany, NJ) within 24 h of birth and at 21 ± 3 d of age (weaning). Pre-weaning mortality, number of live piglets, number of stillborn piglets, and number of mummies were recorded. Males were castrated at 7 - 10 d of age. Pre-weaning survivability was calculated on a per litter basis. Number of litters on treatment from the LARU were: Parity 1 (N = 39): CON (n = 13), PRO20 (n = 13), PRO40 (n = 13); Parity 2 (N = 35): CON (n = 11), PRO20 (n = 11), PRO40 (n = 13). Piglets were not cross-fostered or allowed access to creep feed during the course of this study.

3.2.3: Blood collection and storage

Sow blood samples were obtained 10 d ± 1 post-breeding and at 3 d ± 1 of lactation via syringe using the jugular vein. Blood samples were transferred into heparinized blood tubes (BD Vacutainer®, Franklin Lakes, NJ), inverted several times, and were placed on ice until arriving at the laboratory within an hour. Samples were centrifuged (2000 x *g*, 10 min, 4°C) and plasma was aliquoted and stored at -80°C for subsequent analyses. Samples obtained 10 d post-breeding were analyzed for progesterone concentrations. Samples obtained 3 d into lactation were analyzed for prolactin and immune marker concentrations.

Piglets were chosen for blood sampling by selecting an average sized piglet from each litter, based on the average weaning weight for that litter. During the second parity of the study, an average sized piglet was chosen from the remaining piglets in the litter. Piglet blood samples were obtained 5 d ± 1 post-weaning via the orbital sinus (Dove and Alworth, 2015) and collected into heparinized tubes (BD Vacutainer®, Franklin Lakes, NJ). Samples were inverted several times after collection, and placed on ice until arriving at the laboratory within an hour. Samples were then centrifuged (2000 x *g*, 10 min, 4°C) and plasma was aliquoted and stored at -80°C for subsequent analysis of immune marker concentrations. All piglets were on a common phase 1 nursery diet containing 12 ppm Mn post-weaning.

3.2.4: Prolactin and progesterone assays

A previously described radioimmunoassay (**RIA**) was used to determine concentrations of prolactin (Robert et al., 1989) with the modification that 100 µL of plasma sample was used. The radioinert prolactin and the first antibody to porcine prolactin were purchased from A.F. Parlow (U.S. National Hormone and Peptide Program, Harbor UCLA Medical Centre, Torrance, CA). Parallelism of a pooled sample from lactating sows was 98.4%. Average recovery

calculated by addition of various doses of radioinert prolactin to 50 μL of a pooled sample was 96.3%. Sensitivity of the assay was 1.5 ng/mL. The intra- and interassay CV were 1.57% and 3.16%, respectively. Progesterone was measured with a RIA commercial kit (Progesterone CT, ICN Pharmaceuticals Inc., Costa Mesa, CA). Validation showed a parallelism of 105.3% and an average recovery of 94.4%. Intra- and interassay CV were 2.96% and 0.65%, respectively.

3.2.5: Cytokine analyses

Cytokines were measured using Luminex xMAP technology for multiplexed quantification of 13 porcine cytokines, chemokines, and growth factors. The multiplexing analysis was performed using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). Thirteen markers were measured simultaneously using Eve Technologies' Porcine Cytokine 13-Plex Discovery Assay® (MilliporeSigma, Burlington, Massachusetts, USA) according to the manufacturer's protocol. The 13-plex consisted of GM-CSF (granulocyte-macrophage colony-stimulating factor), IFNγ, IL-1α, IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF-α. Assay sensitivities of these markers ranged from 5 to 42 pg/mL for the 13-plex. Individual analyte sensitivity values are available in the MilliporeSigma MILLIPLEX® MAP protocol.

3.2.6: Tissue collection and storage

Piglet tissue samples were collected at weaning (21 ± 3 d of age) during the second parity. An average sized female piglet was chosen at weaning for tissue sampling based on the average weaning weight of each litter. Piglets were euthanized with CO_2 and the ileum, heart, and liver were removed. The ileum was flushed with 1X phosphate buffered saline solution and heart and liver were rinsed with the same solution. The left ventricle of the heart and the right medial lobe of the liver were removed. The gall bladder was removed from the liver before tissue

was homogenized and frozen. Tissues (1.0 ± 0.05 g) were homogenized in 4 mL of homogenizing buffer according to Marklund and Marklund (1974). The resulting tissue homogenates were frozen at -80°C for subsequent analysis of MnSOD activity. Additional ileum, heart, and liver tissues (5.0 ± 0.1 g) were sent to the University of Georgia Feed, Water, and Soil Laboratory (Athens, GA) to determine tissue mineral concentrations via inductively coupled plasma (ICP) analysis. Samples were kept at -20°C until analysis.

3.2.7: MnSOD analysis

Tissue MnSOD specific activity (EC 1.15.1.1) was determined according to the protocol outlined by Marklund and Marklund (1974) with slight modifications. Tissue samples were collected and homogenized as previously stated. Tissue homogenates were then kept at -80°C until analysis according to the cited protocol.

In order to inactivate the Cu/Zn-dependent SOD, 1 mmol potassium cyanide (KCN) was added to the reaction buffer (50 mM Tris-HCl, 1.0 mM diethylenetriamine pentaacetic acid, pH 8.2) and was used to measure relative MnSOD activity (Marklund and Marklund, 1974). The tissue homogenate (0.5 mL) was added to 1.1 mL of reaction buffer and centrifuged (2000 x *g*, 15 min, 4°C). The resulting supernatant was diluted in reaction buffer (1:10). An assay control with no added sample (900 µL reaction buffer + 50 µL of 10 mM sodium azide (NaN_3)), 200 µL (of diluted homogenate + 700 µL reaction buffer + 50 µL NaN_3), and 400 µL (of diluted homogenate + 500 µL reaction buffer + 50 µL NaN_3) were plated in triplicate on a 12-well microcuvette plate (VWR® Tissue Culture Plates, Radnor, PA), with each well having a volume of 950 µL. The reaction was initiated when 50 µL of 4 mM pyrogallol in 10 mM HCl was added to each well and the plate was quickly mixed. The reaction was monitored at 320 nm for 3 min using the kinetic reading program of a spectrophotometer (Biotek® µQuant, 2006). Deionized

water (1.0 mL) was used to blank the spectrophotometer. The amount of supernatant that resulted in the 50% inhibition of the autooxidation of pyrogallol was the equivalent of one unit of MnSOD activity (IU). Lowry protein determination (Lowry et al., 1951) was performed on the tissue homogenate to determine mg of soluble protein. Specific activity was defined by the internationally recognized unit, IU mg/soluble protein.

3.2.8: Milk collection and component analysis

Milk samples were obtained from sows on d 1, 7, and 14 of their individual lactation. Success of adequate milk collection was dependent on sow temperament and safety of collection. Piglets were removed 45 min to one hour prior to milking. The sow's udder was manually stimulated in order to induce milk letdown. In cases where milk letdown would not occur after manual stimulation, an injection of 0.25 mL of oxytocin (20 USP oxytocin/ml) was administered to accelerate milk letdown. Several minutes post-injection, milk was collected from the sow into labelled 15-mL conical tubes. Milk was collected from as many of the sow's functional teats as possible to ensure a representative sample. Samples were frozen at -20°C until milk component analysis.

Protein concentration of colostrum and milk was determined with the Lowry method (Lowry et al., 1951). Total fat percent of colostrum and milk was determined using a modification of the method described by Folch et al., (1957). Milk samples were thawed, mixed thoroughly, and one mL of sample was placed in a 25 mL glass screw top tube. Ten milliliters of chloroform:methanol (1:2) were added to the tube, which was then capped and vortexed, and left at room temperature for at least 10 min. Then, 5 mL of chloroform and 5 mL of saline (9.0 g/L) were added, tubes were capped, vortexed, and phases were allowed to separate overnight. The next morning, samples were centrifuged (800 x *g*; 37°C; 10 min) to further separate the phases.

The upper methanol and water layer was aspirated and the protein precipitate at the interface was discarded. Aluminum weigh pans were accurately weighed using gloves and forceps and 6 mL of chloroform were then transferred from the tube to its corresponding weigh pan. Weigh pans with chloroform were left under the fume hood to allow chloroform to evaporate (1 hr minimum) and were placed in a 100°C drying oven for an hour to allow any additional water to evaporate. Pans with dried lipid were weighed.

Milk samples were submitted to the University of Georgia Feed, Water, and Soil Laboratory (Athens, GA) for determination of mineral concentration via method of ICP analysis.

3.2.9: Statistical analysis

All analyses were performed using sow or litter as the experimental unit and farrowing group, based on farrowing dates, as block. There were five groups of 8, 8, 8, 8, and 7 sows, respectively. Sow performance data were analyzed as a randomized complete block design. Dietary treatment served as a fixed effect and block served as a random effect. All models were analyzed using the MIXED procedure of SAS 9.4 (SAS Enterprise, Cary, NC). Dietary treatment within study parity (first or second) was included to detect differences between diets within each parity. Lactation length was utilized as a covariate for: sow body weights and relative weight changes, sow feed intake, total number born, number of stillborn, number of mummies, total number weaned, and survivability. Piglet birthweights, weaning weights, and piglet average daily gain were analyzed on an individual piglet basis to allow for more statistical power. For total litter weaning weight, the number piglets weaned was used as a covariate in addition to lactation length. Cytokine data was not normally distributed and thus a $\log_{10}$ transformation of the cytokine concentrations were analyzed with the MIXED procedure of SAS as described above. Pairwise comparisons between the least squares means of the Mn level comparisons were

computed using the PDIFF option of the LSMEANS statement. Statistical significance was declared at $P < 0.05$ and tendencies were considered at $0.05 < P < 0.10$.

3.3: Results

3.3.1: Sow performance

Sow body weights during lactation were not affected by treatment ($P > 0.10$; Table 3-2). There was a dietary treatment within parity effect on relative weight change between d 110 of gestation and d 1 of lactation ($P = 0.019$). The PRO20 sows lost less weight from d 110 until d 1 of lactation in the second parity compared to their first ($P < 0.05$). There were no differences between treatments in sow body weight loss during lactation or from d 110 until weaning ($P > 0.10$). Gestation length was not affected by dietary treatment ($P > 0.10$). Weekly sow feed intake was affected by dietary treatment during all three weeks of lactation ($P < 0.05$). In either parity, feed intake during weeks 1 of lactation was increased for PRO20 sows from CON sows ($P < 0.05$) and did not differ from that of PRO40 sows ($P > 0.10$). Feed intake during week 2 of the second parity was increased by 0.5-1.0 kg when compared to the first parity across all dietary treatments ($P < 0.01$). In either parity, feed intake during week 3 of lactation was increased for PRO20 sows from CON sows ($P < 0.05$) and did not differ from that of PRO40 sows ($P > 0.10$). Overall average daily feed intake was affected by dietary treatment. In the first parity, PRO20 sows ate significantly more feed ($P < 0.05$) when compared to either CON or PRO40 sows. During parity 2, PRO20 sows ate numerically more feed than CON sows ($P > 0.10$), while they ate significantly more feed than PRO40 sows ($P < 0.05$).

3.3.2: Litter performance

The total number of piglets born, total number of piglets born alive, number of stillborn piglets, and number of mummies were not affected by dietary treatment ($P > 0.10$; Table 3-2).

There was a tendency for Mn to affect total number of piglets born ($P = 0.06$) and total number of piglets born alive ($P = 0.10$). The numbers of total piglets and total live piglets were numerically greater for CON sows compared to PRO20 or PRO40 sows, while values were lowest for PRO20 sows. PRO40 sows were intermediate between CON and PRO20 sows. The number of stillborn piglets between Mn treatments within parity tended to be different ($P = 0.10$). PRO20 sows tended to have lower numbers of stillborn piglets in the first parity. There was no effect Mn and parity on the previously listed variables. Total number of piglets weaned and litter survivability were not affected by dietary treatment ($P > 0.23$). Average piglet birthweight increased linearly in response to increasing concentration of dietary Mn ($P < 0.01$). PRO20 piglets weighed more at birth (1.57 kg; $P < 0.05$) than CON piglets (1.23 kg) and PRO40 piglets (1.40 kg), and PRO40 piglets weighed more at birth than CON piglets ($P < 0.05$). Weaning weights differed in response to dietary treatment ($P < 0.01$). PRO20 and PRO40 piglets weighed more at weaning than CON piglets ($P < 0.05$), PRO20 piglets (5.6 kg) had similar weights to PRO40 piglets (5.5 kg) at weaning ($P > 0.10$). Dietary treatment did have a significant effect on litter weaning weight ($P < 0.05$; Table 3-2). Looking at parity 1 and parity 2 separately, there were no significant differences in litter weaning weight ($P > 0.10$). However, there was a significant difference ($P < 0.05$) in weaning weights of PRO20 litters from parity 1 (59.08 kg) and PRO40 litters from parity 2 (51.40 kg). It does not appear that litter weaning weights followed any particular biological pattern in response to maternal dietary treatment. Piglet average daily gain (**ADG**) was affected by dietary treatment ($P < 0.01$). PRO20 piglets gained, on average, 23 grams more per day (237 g/pig/day) than CON piglets (214 g/pig/day; $P < 0.05$), while it was similar for PRO20 and PRO40 (220 g/pig/day; $P > 0.10$). In the second parity, PRO40 piglets had decreased ADG compared to that in the first parity.

3.3.3: Sow immune marker and plasma hormone concentrations

In sows, log plasma concentrations of GM-CSF, IFN-γ, IL-1β, IL-1rα, IL-6, IL-8, and TNF-α did not differ in response to increasing dietary Mn concentration ($P > 0.10$; Table 3-3) or effect of dietary treatment within parity ($P > 0.10$). There was an effect of dietary treatment on the log concentrations of IL-1α, IL-2, IL-4, IL-10, IL-12, and IL-18 ($P < 0.05$; Table 3-3) as well as a significant linear effect on the log concentrations of these same markers ($P < 0.05$). There was a significant linear effect of Mn on IL-1β and IL-6 ($P < 0.05$), but not a significant Mn effect; however, mean separations for these two markers were still presented in Table 3-3. In the first parity, log concentrations of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, and IL-18 were not significantly different from one another in response to dietary treatment, but a numerical decrease from CON to PRO20 and then to PRO40 was observed. During the second parity, IL-1α, IL-1β, IL-4, IL-6, IL-10, and IL-12 had similar patterns of log concentrations. PRO40 sows had significantly decreased log concentrations compared to CON sows ($P < 0.05$), while PRO20 sows had an intermediate log concentration that did not differ from CON or PRO40 sows ($P > 0.10$). During the second parity, CON sow log concentrations of IL-2 were significantly increased compared to log concentrations of PRO20 and PRO40 sows ($P < 0.05$). In addition, PRO20 sows had a significantly increased log concentration of IL-2 compared to PRO40 sows ($P < 0.05$). During the second parity, CON and PRO20 sow log concentrations of IL-18 were significantly increased compared to PRO40 sows ($P < 0.05$).

Prolactin concentrations in sows did not differ due to increasing dietary Mn ($P > 0.10$; Table 3-3). There was a tendency of progesterone concentration to differ in response to dietary Mn level ($P < 0.10$; Table 3-3), with PRO20 concentrations being numerically increased compared to CON and PRO40 sows.

3.3.4: Piglet immune marker concentrations

In piglets, log plasma concentrations of all tested immune markers did not differ in response to increasing maternal dietary Mn concentrations ($P > 0.10$; Table 3-4).

3.3.5: Tissue MnSOD activity and tissue mineral composition in piglets

Dietary treatment had no effect on MnSOD activity in ileal or cardiac tissue of piglets ($P > 0.10$; Table 3-4). Hepatic MnSOD activity decreased in a linear manner across increasing levels of maternal dietary Mn supplementation ($P = 0.03$; Table 3-4). There was a tendency for Mn to affect cardiac and hepatic zinc concentrations ($P = 0.09$, $P = 0.07$, respectively). None of the other analyzed mineral concentrations differed across dietary treatments ($P > 0.10$; Table 3-5).

3.3.6: Sow milk composition

There was no interaction between Mn and parity for any of the measured milk components, therefore, averages were presented across dietary treatments (Table 3-6). There was no day effect between d 7 and d 14 milk samples for any measured component, therefore, averages were presented and an orthogonal contrast between colostrum (d 1) and milk (average of d 7 and 14) was reported (Table 3-6). Protein percentage decreased ($P < 0.01$) between colostrum and milk, while there was a tendency for milk protein to decrease in response to supplemented dietary Mn ($P = 0.08$). Colostral fat content differed in response to dietary treatment ($P < 0.05$), with PRO20 sows having higher colostral fat than PRO40 sows ($P < 0.05$), but similar values to CON sows ($P > 0.10$). CON sows had the highest percent milk fat when compared to PRO20 and PRO40 sows ($P < 0.05$), whereas PRO20 sows and PRO40 sows had similar values ($P > 0.10$). Increased dietary supplementation of Mn did not affect the mineral

composition of colostrum or milk ($P > 0.10$; Table 3-6). Calcium content increased ($P < 0.01$) from colostrum to milk, while copper and zinc concentrations decreased ($P < 0.01$).

3.4: Discussion

Sow body weight is an important variable to monitor during gestation and lactation as it can affect subsequent reproductive performance and longevity (Kim et al., 2016). Before farrowing, feed intake is restricted to prevent unnecessary weight gain and the onset of constipation in the days leading up to parturition (Kim et al., 2016). In general, sows lose body weight after farrowing and lose weight during lactation until weaning (Close et al., 1984; Kim et al., 2016). Dietary treatment had no effect on sow body weight before farrowing, after farrowing, or after weaning. Though there was an effect of Mn level within parity on relative body weight change from d 110 to birth ($P < 0.02$), there was no clear or logical pattern of change. This statistically significant observation does not have a significant impact in the larger frame of the study, based on the absence of observed differences in the other relative weight change variables.

Lactation feed intake across all treatments approximated the industry mean of 6.5 kg/sow/day (Knauer and Hostetler, 2013). The increased lactation feed intake in sows fed 20 ppm of Mn is in agreement with Tsai et al. (2020) in regards to organic minerals improving sow growth characteristics. Nevertheless, Peters and Mahan (2008) have reported no change in sow feed intake between mineral sources. Trace minerals are involved in many physiological pathways and affect many cellular processes, including hormone synthesis and distribution.

Progesterone is the pregnancy and conceptus maintenance hormone and is necessary for fetal growth (Spencer et al., 2004; Spencer and Bazer, 2004). Manganese is a cofactor for enzymes related to squalene synthesis, a precursor for steroid hormones like progesterone; however, dietary treatment did not impact plasma progesterone concentrations in the current

study (Curran, 1961; Xie et al., 2014). Current findings indicate that the dietary levels of Mn provided sufficient support of progesterone synthesis and did not differ based on Mn supplementation. Prolactin stimulates the production of milk in mammals and has key roles in mammary development (Farmer, 2016). Increasing prolactin secretion in late gestation (VanKlompenberg et al., 2013) or during lactation (Farmer and Palin, 2021) led to greater sow milk yield. Prolactin is known to be a peripheral marker of Mn toxicity in rats and could also serve as a sensitive biomarker of cumulative exposure to Mn (Marreilha dos Santos et al., 2011). More specifically, Mn stimulates dopamine depletion, thereby increasing prolactin secretion and circulating concentrations (Marreilha dos Santos et al., 2011). Current findings indicate that prolactin concentrations are not affected by feeding increasing amounts of Mn from an organic source, even though organic minerals have a better absorption and body retention compared to the inorganic form (Liu et al., 2014).

Efficient reproductive performance is a key component in swine husbandry. Increasing litter size while minimizing labor cost is the ultimate goal in piglet production, but there are many routes that may lead to this goal (Knox, 2014; Knox et al., 2005). Plumlee et al. (1956) demonstrated that sows fed Mn-deficient diets gave birth to weak and poorly structured piglets. These authors determined that sows fed Mn-deficient diets gave birth to weak and poorly structured piglets. Therefore, it was anticipated that sows fed increasing amounts of supplemental Mn would have improved Mn utilization for mineral deposition and bone development in the conceptus during gestation. It was predicted in the current study that more piglets would be born to PRO20 and PRO40 sows and would be heavier in comparison to CON piglets due to improved dam nutrient intake and deposition in fetal piglets. Increased maternal Mn supplementation may lead to improved Mn utilization and as a result lead to more piglets

being born alive by the improvement of embryonic survivability and oxidative defense of the sow.

Sows in the current study had litter size characteristics (total number, total live born, total number weaned) slightly below US industry average. Piglet weights at birth and pre-weaning survivability were similar to industry means. There was no significant effect of dietary treatment on litter size characteristics. There was a numerical increase in pre-weaning survivability when comparing CON to PRO20 litters (79.4% v 85.4% over two parities, respectively). This is likely linked to the greater birthweight of PRO20 piglets (Feldpausch et al., 2019; Fix et al., 2010). Improved piglet birthweights, weaning weights, and pre-weaning average daily rates of gain are important factors to efficient piglet production (Knox, 2014; Knox et al., 2005). Piglets that are born heavier have a reduced risk of pre-weaning mortality and generally will gain more weight during the pre-weaning period, which was the case in the present study. Heavier piglets at weaning also typically result in improved average daily gain during the grow-finish phase of production (Cabrera et al., 2010). PRO20 and PRO40 sows consumed more feed per day than CON sows and as a result may have utilized more efficiently the supplementary Mn during lactation. This is a possible explanation as to why significantly heavier piglets were born to PRO20 and PRO40 sows. In support of the current findings, sows fed 20 ppm of supplementary Mn had increased piglet birthweights compared to sows fed 5 ppm Mn in an earlier series of experiments (Christianson, 1990).

Maternal immunity is essential for suckling piglets because there is no placental transfer of immunoglobulins to the developing offspring in swine. As is the case with most mammals, immunity must be passed from the dam to the neonate via colostrum in order for the newborn piglet to fight off pathogenic organisms (Salmon et al., 2009; Sinkora et al., 2002; Šinkora and

Butler, 2009). Sow colostrum and milk contain a variety of immunomodulatory agents: prolactin, nucleotides that enhance the activity of natural killer (NK) cells, macrophages, T helper cells, and cytokines (Salmon et al., 2009). These immune markers and agents are used by the animal and can usually be found circulating in the plasma (Šinkora and Butler, 2009). It was anticipated that increasing Mn in sow diets may impact immune markers due to improved Mn utilization of the immune system and the reduction of oxidative stress. In a recent study, plasma cytokine profiles of sows during early gestation and the second half of pregnancy were characterized by the increased production of IL-1α and IL-4 and the reduction of the production of IFN-γ (Brigadirov et al., 2018). Following parturition, sows experience metabolic stress and pro-inflammatory cytokine concentrations increase after farrowing (Brigadirov et al., 2018). When looking at the immune status of sows post-farrowing in the present experiment, clear patterns emerge. For markers that did significantly respond to dietary treatment, CON sows generally had increased concentrations compared to PRO20 sows, while PRO40 sows had lower concentrations compared to CON and PRO20 sows. It may be that CON sows had a more primed and capable immune response when faced with the metabolic stress that accompanies parturition. On the other hand, PRO40 sows may have experienced a suppression or deficiency in their immune response based on the lower plasma concentrations of various immune markers. It is also possible that PRO40 sows overcame metabolic stress associated with farrowing more quickly, or had less metabolic stress to begin with and plasma immune marker concentrations had already begun to decrease. It would be of interest to obtain multiple blood samples before and after farrowing as well as throughout lactation, in order to draw meaningful conclusions on the impact of feeding supplementary organic Mn on sow immune status.

Manganese has been linked to nutritional immunity; this is the idea that the body sequesters trace nutrients to impair or prevent the growth of certain pathogens (Haase, 2018). In addition, trace elements can play messenger roles in immune system cascades (Haase, 2018). The reduced log plasma concentrations of IL-1α in PRO40 sows compared to CON and PRO20 sows are of importance because IL-1α is produced by activated macrophages and plays an important role in the regulation of immune responses (Salmon et al., 2009; Zhang and An, 2007). It is an intermediary cellular signal in the pathway activating the pro-inflammatory cytokine, tumor necrosis factor- α (TNF-α). However, log plasma concentrations of TNF-α were not affected by dietary treatment. It has been determined that TNF-α concentrations peak 24-36 h following parturition in sows of differing immune status (Kaiser et al., 2018). Plasma concentrations (log transformed) of the pro-inflammatory cytokines, IL-1β, IL-2, and IL-6 and the anti-inflammatory cytokine, IL-4, were also lower in PRO40 compared with CON and PRO20 sows, indicating altered immune status. IL-1β is released by macrophages and monocytes during cell injury, infection, invasion, and inflammation (Salmon et al., 2009; Zhang and An, 2007). IL-2 is a signaling molecule that regulates the activities of white blood cells that are responsible for immune status, while the anti-inflammatory cytokine IL-4 induces the differentiation of naïve helper T-cells and reduces pro-inflammatory responses (Salmon et al., 2009; Zhang and An, 2007). IL-6 is a pro-inflammatory cytokine that has been shown to suppress feed intake and stimulate the acute phase immune response (Zhang and An, 2007). IL-6 was numerically increased in PRO20 sows but feed intake was increased in those sows, which is counterintuitive. It is possible there were effects outside of the scope of the data that was collected that could be attributed to this observation. Plasma concentrations of IL-10, IL-12, and IL-18 were also lower in PRO40 sows compared with CON and PRO20 sows, especially in

parity 2. IL-1rα is secreted by various cell types (for example: epithelial and adipocytes) and is a natural inhibitor of the pro-inflammatory effects of IL-1α and IL-1β (Zhang and An, 2007). IL-1rα may have suppressed the inflammatory effects of IL-1α and IL-1β in this study. IL-10 is an anti-inflammatory cytokine with multiple, pleiotropic effects in immune regulation and inflammation, while IL-18 is a pro-inflammatory cytokine that facilitates type 1 responses along with IL-12, to induce cell-mediated immunity following infection (Salmon et al., 2009; Zhang and An, 2007). IL-12 stimulates the production of IFN-γ, TNF-α, and NK cells (Zhang and An, 2007). Supplemental Mn concentration exceeding 20 ppm may have a role in the disruption of secondary messenger cascades and as a result a reduction in immune marker expression. Overall, plasma concentrations of immune markers that did change in response to dietary treatment seemed to do so in similar patterns showing that PRO40 sows had reduced immune marker log concentrations compared to CON and PRO20 sows, especially in parity 2. A reduction in log concentrations in response to Mn supplementation is not necessarily a negative result. As previously mentioned, an explanation may be that PRO20 and PRO40 sows overcame the metabolic stress associated with parturition more quickly than CON sows. The significant responses in log concentrations of these markers to dietary Mn occurred in parity 2, it is possible that feeding these diets for a longer period of time could have longer term effects on the sow's immune system. It is important to understand that these are log concentrations at a specific moment in time in the farrowing room; therefore, more definitive conclusions could be made about the immune status of the animal if samples were taken at various points in time. Looking at a variety of acute phase proteins like serum amyloid A, haptoglobin, albumin, and others, could aid in understanding more of what was going on in this study from an immunological perspective. The concentrations and activity of these acute phase proteins can provide a more

complete understanding of the immune system around parturition in sows of differing immune status (Kaiser et al., 2018).

There were no significant differences observed in the log concentrations of immune markers 5 d post-weaning. It is known that weaning induces the expression of pro-inflammatory cytokines in piglets, with peak expression primarily occurring 1 d post-weaning (Cao et al., 2018; Pié et al., 2004). Based on the current data, it can be concluded that there were no long-term effects of maternal Mn supplementation on the immune status of their piglets at 5 d post-weaning.

Dietary treatment did not affect tissue mineral concentrations in the present study. In a related study, some tissues, such as the liver, showed similar trends in mineral concentration compared to the present study, independent of dietary treatment (Ma et al., 2018; Martin et al., 2011). There is limited research on the impact of supplementing maternal diets with organic minerals on the mineral status of offspring. It was reported that maternal supplementation of an organic mineral source did not impact whole body tissue analysis in piglets when compared to an inorganic source (Papadopoulos et al., 2009). Furthermore, described by Papadopoulos et al. (2009), there was no difference in tissue concentrations of Mn in response to maternal dietary addition of Mn, the element of interest in this study.

Weaning for piglets is a time of nutritional, immunological, social, and oxidative stress (Cao et al., 2018). Oxidative stress results from the formation of reactive oxygen species (**ROS**), a byproduct of oxygen metabolism (Holley et al., 2011). If left unchecked by antioxidant regulators, the accumulation of ROS can cause damage to membrane lipids, proteins, and DNA (Lubos et al., 2011; Schwarz et al., 2017). It was expected that increasing maternal Mn supplementation would increase MnSOD activity in tissues post-weaning. In weanling pigs fed

increasing dietary levels of Mn (0.24 to 32 ppm Mn), hepatic MnSOD concentrations averaged 4.89 IU/mg and cardiac concentrations averaged 10.0 IU/mg irrespective of Mn dose (Pallauf et al., 2012). MnSOD values in the current study are higher than those previously reported. Differences are likely attributed to supplemental Mn post-weaning rather than *in utero* like the current study. In the current study, the liver showed a reduction from CON piglets in MnSOD activity as maternal Mn supplementation increased. This could be a result of suppressed oxidative stress in PRO20 and PRO40 piglets at weaning. Cardiac tissue MnSOD activity, though not significant different, showed a similar numerical reduction in activity from CON to the PRO20 and PRO40 piglets.

Sow Mn supplementation influenced percent fat of both colostrum and milk and tended to change milk protein content. Mineral composition did not change due to supplemental Mn. PRO20 sows had increased colostrum fat when compared to PRO40 sows ($P < 0.05$), while colostrum fat in PRO20 sows did not differ from CON sows ($P > 0.05$). CON sows had the highest milk fat percentage when compared to PRO20 and PRO40 sows. Decreased percent fat of colostrum in response to increased dietary Mn may be due to increased demand from heavier PRO20 and PRO40 piglets. Piglets from PRO20 and PRO40 sows had significantly increased ADG when compared to CON piglets, suggesting increased milk yield. Therefore, milk fat in PRO20 and PRO40 may have been reduced or diluted, due to presumably higher milk yield. Milk and colostral values from the current study are in line with those previously reported (Csapó et al., 1996; Farmer, 2015; Hu et al., 2018).

3.5: Conclusion

Supplementary dietary Mn over two parities, did not have an impact on sow body weight changes, but did increase lactation feed intake, especially in PRO20 sows. PRO20 piglets were

heavier at birth and gained more weight per day until weaning than CON piglets. Improved ADG and weaning weights in PRO20 and PRO40 piglets could have been due to increased milk production in the first parity. Fat percentages in colostrum and milk fat were decreased in PRO20 sows, which may indicate the dilution of fat content due to increased milk yield. CON sows had increased log concentrations of plasma immune markers compared to PRO20 and PRO40 sows, suggesting a more robust immune response following parturition or PRO20 and PRO40 sows were able to more quickly overcome the metabolic stress following parturition. Maternal dietary Mn supplementation had little impact on plasma immune markers of pigs 5 d post-weaning. Improved birthweight, pre-weaning growth rate, and weaning weights are important factors in piglet growth as they can have compounding effects as pigs continue to grow and progress through the nursery and grow-finish phases of production.

Conflict of interest statement

None of the authors have any conflicts of interest to declare.

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Table 3-1. Dietary composition and analysis

Dietary Treatment	Gestation		Lactation		
	Low Mn	High Mn	CON	PRO20	PRO40
Ingredient, %					
Corn	54.37	54.16	54.41	54.40	54.38
Corn DDGS	40.00	40.00	20.00	20.00	20.00
Soybean meal, 47.5%	1.70	1.70	21.62	21.62	21.62
L-Lysine	0.21	0.21	0.22	0.22	0.22
Dicalcium phosphate	0.87	0.87	1.26	1.26	1.26
Limestone	1.6	1.6	1.24	1.24	1.24
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25
Sow Add Pack- Vit[2]	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.5	0.5	0.5	0.5	0.5
ProPath® Mn[4]	.	0.210	.	0.014	0.027
Analysis					
ME[5,6], Mcal/kg	3303	3303	3297	3297	3297
Crude protein, %	18.4	17.6	20.0	19.9	19.9
Lysine[6], %	0.52	0.52	0.97	0.97	0.97
Crude fat, %	3.7	3.6	3.8	4.1	3.8
Ash, %	5.9	5.9	5.5	5.6	6.0
Crude fiber, %	5.4	5.1	3.7	4.0	3.9
Phosphorus (total), %	0.6	0.6	0.7	0.7	0.7
Phosphorus (avail)[6], %	0.40	0.42	0.39	0.39	0.39
Calcium, %	1.0	0.9	0.9	0.9	0.9
Potassium, %	0.80	0.74	0.92	0.94	0.92
Magnesium, %	0.21	0.18	0.19	0.21	0.21
Sulfur, %	0.07	0.08	0.08	0.08	0.08
Manganese, ppm	42	310	42	73	81
Iron, ppm	243	176	464	576	479
Copper, ppm	34	30	48	40	35
Zinc, ppm	181	113	225	255	251

[1]Vitamin Premix: supplied per kg of diet: vitamin A (4,134 IU); vitamin D (1,653 IU); vitamin E (66 IU); vitamin K (3.3 mg); riboflavin (8.27 mg); niacin (49.6 mg); vitamin B_{12} (0.033 mg); pantothenic acid (27.6 mg); ADM Alliance Nutrition, Quincy, IL 62305.

[2]Sow Add Pack: supplied per kg of diet: vitamin A (4,134 IU); vitamin E (33 IU); pyridoxine (0.992 mg); folic acid (2.205 mg); biotin (0.2205 mg); choline (551.25 mg); carnitine (49.6 mg); ADM Alliance Nutrition, Quincy, IL 62305

[3]Mineral Premix: supplied per kg of diet: Copper (10 ppm Cu as $CuSO_4$; 10 ppm Cu as ProPath® Cu, Zinpro); Zinc (50 ppm Zn as ZnO and 50 ppm Zn as ProPath® Zn, Zinpro); Iron (100 ppm Fe as $FeSO_4$); Iodine (1 ppm iodine as KIO_3); Selenium (0.3 ppm Se as Na_2SeO_3).

[4]Zinpro, Eden Prairie, MN

[5]Metabolizable energy

[6]Calculated value

Table 3-2. The effect of supplemental dietary Mn (0, 20, 40 ppm) on the reproductive performance of sows over two parities

	Parity 1			Parity 2				P –values[1]		
Dietary Treatment	CON	PRO20	PRO40	CON	PRO20	PRO40	SEM	Mn	Lin Mn	Mn (Parity)
Sow body weight, kg (*N*)	13	13	13	11	11	13				
Parity	3.5	3.1	4.8	5.3	4.2	5.3				
d 110 ± 1 Gestation	231.8	231.1	231.4	221.4	225.9	231.7	7.9	0.806	0.517	0.770
d 1 ± 1 Lactation	224.1	220.0	224.6	215.6	223.8	222.0	11.2	0.906	0.658	0.895
d 21 ± 1 Lact	232.2	230.1	229.1	229.6	229.7	234.5	9.7	0.970	0.901	0.957
Relative weight change, kg										
d 110 – d 1 Lactation	-14.5^{a}	-12.8^{a}	-8.5^{ab}	-6.2^{ab}	-2.1^{b}	-15.4^{a}	3.6	0.425	0.619	0.019
d 1 Lactation – d 21 Lact	9.8	11.2	5.8	15.2	9.3	14.0	3.6	0.681	0.410	0.178
d 110 – d 21 Lact	0.6	-1.0	-0.5	8.4	3.3	2.9	4.4	0.890	0.696	0.280
Gestation length, d	114.7	115.2	114.8	115.3	115.1	115.0	0.5	0.878	0.899	0.695
Feed intake, kg/sow/day (*N*)	13	13	13	11	11	13				
Week 1	5.15^{b}	6.26^{a}	5.72^{ab}	5.63^{b}	6.68^{a}	6.20^{ab}	0.43	0.012	0.128	0.726
Week 2	6.38^{c}	7.73^{a}	6.38^{c}	7.87^{ab}	8.01^{ab}	7.50^{ab}	0.38	0.039	0.621	0.003
Week 3	6.36^{bc}	6.58^{bc}	6.04^{cd}	7.63^{a}	8.16^{a}	6.41^{b}	0.56	0.025	0.060	0.131
ADFI	5.92^{d}	7.05^{a}	6.04^{bd}	6.96^{abc}	7.52^{a}	6.73^{bc}	0.35	0.006	0.848	0.150
Lactation length, d	18.5	16.1	18.8	17.9	17.9	18.8	3.0			
Litter performance (*N*)	13	13	13	11	11	13				
Parity	3.5	3.1	4.8	5.3	4.2	5.3				
Total number born	16.1	13.4	15.0	15.2	13.2	14.4	0.9	0.060	0.316	0.871
Total live born	13.5	12.3	13.1	12.6	10.6	12.4	0.7	0.102	0.603	0.383
Stillborn	1.7	0.5	1.3	2.3	2.3	2.1	0.6	0.586	0.585	0.101
Mummies	0.9	0.6	0.6	0.2	0.4	0.1	0.3	0.717	0.417	0.380
Total number weaned	10.7	10.0	10.5	9.8	9.2	9.3	0.7	0.686	0.652	0.355
Survival, %	79.8	82.3	82.3	78.9	88.4	77.0	4.3	0.282	0.937	0.592
Avg piglet birthweight, kg[2]	1.23^{c}	1.59^{a}	1.35^{b}	1.22^{c}	1.55^{a}	1.45^{b}	0.04	0.001	0.001	0.150
Avg piglet weaning wt, kg[2]	5.18^{b}	5.44^{ab}	5.74^{a}	5.11^{b}	5.82^{a}	5.24^{b}	0.26	0.001	0.010	0.023
Avg litter weaning wt, kg	52.58^{ab}	59.08^{a}	58.09^{ab}	51.66^{ab}	58.54^{ab}	51.40^{b}	2.7	0.049	0.310	0.318
ADG, g/pig/day[2]	216^{b}	232^{ab}	236^{a}	211^{b}	241^{a}	204^{b}	11	0.001	0.298	0.014

[a-d]LS Means within a row that do not share a letter superscript differ significantly ($P < 0.05$).

[1]*P*-values reported are for the main effect of manganese (Mn), the preplanned linear orthogonal contrast (Lin Mn), and the manganese treatment within parity (Mn(Parity)).

[2]Variables with a quadratic P-value of $P < 0.01$ as a result of a quadratic orthogonal contrast statement.

Table 3-3. The effect of supplemental dietary Mn (0, 20, 40 ppm) on the log concentration of plasma immune markers in sows 3 d into lactation over two parities

	Parity 1			Parity 2				*P* –values[1]		
Dietary Treatment	CON	PRO20	PRO40	CON	PRO20	PRO40	SEM	Mn	Lin Mn	Mn (Parity)
Log_{10}Conc, pg/mL (*N*)	13	13	13	11	11	13				
GM-CSF	2.38	2.57	2.37	2.33	2.57	2.47	0.15	0.24	0.61	0.94
IFN-γ	2.94	3.35	3.29	3.01	3.22	3.09	0.30	0.38	0.30	0.86
IL-1α	2.03^{ab}	1.95^{ab}	1.89^{b}	2.32^{a}	2.08^{ab}	1.82^{b}	0.15	0.05	0.01	0.45
IL-1β	3.10^{ab}	2.96^{ab}	2.94^{ab}	3.38^{a}	3.18^{ab}	2.90^{b}	0.18	0.08	0.02	0.52
IL-1rα	3.00	3.12	3.03	3.37	3.15	2.99	0.14	0.26	0.12	0.24
IL-2	3.03^{abc}	2.96^{abc}	2.86^{bc}	3.33^{a}	3.11^{b}	2.72^{c}	0.17	0.02	0.01	0.38
IL-4	3.53^{ab}	3.36^{ab}	3.30^{b}	3.84^{a}	3.48^{ab}	3.21^{b}	0.19	0.03	0.01	0.61
IL-6	2.43^{ab}	2.41^{ab}	2.31^{b}	2.79^{a}	2.55^{ab}	2.34^{b}	0.17	0.11	0.04	0.45
IL-8	1.47	1.04	1.52	1.25	1.30	1.17	0.18	0.50	0.93	0.24
IL-10	3.29^{ab}	3.31^{ab}	3.07^{b}	3.62^{a}	3.35^{ab}	2.95^{b}	0.19	0.01	0.01	0.52
IL-12	2.89^{ab}	2.90^{ab}	2.83^{b}	3.07^{a}	2.96^{ab}	2.76^{b}	0.09	0.04	0.02	0.38
IL-18	3.51^{ab}	3.52^{ab}	3.35^{b}	3.82^{a}	3.62^{a}	3.25^{b}	0.16	0.01	0.01	0.43
TNF-α	2.51	1.81	2.21	2.43	2.09	1.96	0.28	0.20	0.10	0.69
Prolactin[2], ng/mL	30.95	28.88	30.52	32.03	30.28	28.23	3.55	0.75	0.47	0.91
(*N*)	13	13	13	11	11	13				
Progesterone[3], ng/mL	25.83	26.86	23.04	21.56	25.45	21.12	2.40	0.09	0.36	0.60
(*N*)	13	13	13	11	11	13				

[a-b]LS Means within a row that do not share a letter superscript differ significantly ($P < 0.05$).

[1]*P*-values reported are for the main effect of manganese (Mn), the preplanned linear orthogonal contrast (Lin Mn), and the effect of Mn within parity (Mn(Parity)).

[2]Prolactin was measured from plasma obtained on d 3 ± 1 of lactation.

[3]Progesterone was measured from plasma obtained on 10 ± 1 d post breeding.

Table 3-4. The effect of maternal supplemental dietary manganese on the log concentration of plasma immune markers in piglets 5 d post-weaning and MnSOD tissue activity at weaning

	Parity 1			Parity 2				*P* –values[1]		
Dietary Treatment	CON	PRO20	PRO40	CON	PRO20	PRO40	SEM	Mn	Lin Mn	Mn (Parity)
$Log_{10}Conc$[2], pg/mL (*N*)	13	13	13	11	11	13				
GM-CSF	1.21	0.97	1.35	1.36	1.67	0.88	0.24	0.57	0.42	0.06
IFN-γ	2.18	2.40	2.13	2.03	2.24	2.34	0.20	0.50	0.45	0.68
IL-1α	0.80	0.62	0.96	0.86	0.89	0.79	0.23	0.83	0.80	0.72
IL-1β	2.38	2.31	2.42	2.31	2.38	2.30	0.14	0.99	0.91	0.87
IL-1rα	3.17	3.02	3.15	3.24	3.18	3.17	0.12	0.58	0.64	0.76
IL-2	1.80	1.76	1.89	1.64	1.87	1.81	0.24	0.78	0.51	0.90
IL-4	2.25	1.81	2.04	2.17	2.06	2.12	0.20	0.28	0.41	0.76
IL-6	1.57	1.33	1.52	1.57	1.44	1.49	0.12	0.13	0.50	0.87
IL-8	1.46	1.30	1.38	1.78	1.42	1.66	0.17	0.12	0.44	0.44
IL-10	2.30	2.17	2.13	2.27	2.42	2.13	0.14	0.23	0.14	0.57
IL-12	3.19	3.15	3.13	3.13	3.18	3.17	0.06	0.97	0.88	0.71
IL-18	2.93	2.83	2.92	2.89	3.07	2.85	0.12	0.69	0.70	0.17
TNF-α	1.74	1.84	1.82	1.85	1.63	1.49	0.15	0.51	0.25	0.16
MnSOD[3,4], IU/mg (*N*)				11	11	13				
Ileum	.	.	.	6.95	6.36	6.89	0.81	0.67	0.93	
Heart	.	.	.	8.79	5.73	6.77	1.25	0.21	0.23	
Liver	.	.	.	10.02[a]	7.46[b]	7.87[ab]	0.71	0.03	0.03	

[a-b]LS Means within a row that do not share a letter superscript differ significantly ($P < 0.05$).

[1]*P*-values reported are for the main effect of manganese (Mn), the preplanning linear orthogonal contrast (Lin Mn), and the effect of Mn within parity (Mn(Parity)).

[2]This data is derived from plasma obtained from piglets on d 5±1 post-weaning. One average sized piglet was bled from each litter, based on the average weaning weight for their litter.

[3]MnSOD activity is expressed in the internationally recognized unit for enzymatic activity, IU/mg soluble protein. In addition, tissue samples were only collected from piglets during the second lactation of the study.

[4]*P* – values reported (from left to right) are for the main effect of manganese (Mn), the preplanned linear orthogonal contrast (Lin Mn), and the effect of tissue on MnSOD activity. There was no Mn x Tissue interaction ($P = 0.653$).

Table 3-5. The effect of supplemental maternal dietary manganese on tissue mineral concentrations of piglets at weaning

Dietary Treatment		CON	PRO20	PRO40	SEM	*P* - value
Mineral Concentration[1,2]	*N*	11	11	13		Mn
Ileum						
Phosphorus, %		1.385	1.320	1.427	0.060	0.249
Calcium, %		0.041	0.046	0.051	0.004	0.114
Manganese, ppm		5.316	5.000	5.556	0.235	0.227
Iron, ppm		116	114	118	11	0.937
Copper, ppm		6	15	6	5	0.364
Zinc, ppm		124	118	119	5	0.728
Heart						
Phosphorus, %		1.015	0.970	1.013	0.010	0.117
Calcium, %		0.023	0.024	0.031	0.004	0.226
Manganese, ppm		5.704	5.410	5.670	0.190	0.479
Iron, ppm		238	222	219	16	0.589
Copper, ppm		7	7	16	4	0.215
Zinc, ppm		89	87	91	2	0.091
Liver						
Phosphorus, %		0.908	0.912	0.922	0.060	0.970
Calcium, %		0.022	0.023	0.023	0.002	0.692
Manganese, ppm		6.023	6.441	5.907	0.446	0.591
Iron, ppm		845	1152	863	204	0.478
Copper, ppm		172	159	148	27	0.662
Zinc, ppm		262	265	199	24	0.077

[1]Mineral concentration analysis performed via inductively coupled plasma (ICP) analysis.
[2]Tissue samples were only collected from the second parity of the study.

Table 3-6. The effect of supplemental dietary manganese on the colostrum (d 1) and milk (d 7 and 14) composition of lactating sows

Dietary Treatment	CON	PRO20	PRO40	SEM	*P* – values	
Milk composition (as-received)					Mn	Col v. Milk
Protein[1], %						
Colostrum (*N*)	15.4 (19)	16.0 (18)	13.5 (21)	1.37	0.39	0.01
Milk[2] (*N*)	9.2 (16)	8.7 (15)	7.7 (20)	1.63	0.08	
Fat[1], %						
Colostrum (*N*)	5.9[ab] (19)	6.2[a] (18)	4.4[b] (17)	0.49	0.05	0.02
Milk[2] (*N*)	7.8[a] (15)	5.5[b] (12)	6.1[b] (16)	0.57	0.01	
Mineral Concentration[1,3] (as-received)						
Colostrum (*N*)	19	17	17			
Milk[2] (*N*)	15	11	15			
Phosphorus, %						
Colostrum	0.11	0.12	0.12	0.01	0.21	0.85
Milk[2]	0.12	0.12	0.12	0.01	0.20	
Calcium, %						
Colostrum	0.09	0.11	0.12	0.01	0.22	0.01
Milk[2]	0.16	0.17	0.17	0.01	0.71	
Manganese, ppm						
Colostrum	0.25	0.25	0.26	0.01	0.32	0.13
Milk[2]	0.26	0.25	0.30	0.01	0.12	
Iron, ppm						
Colostrum	1.69	1.90	1.53	0.33	0.68	0.52
Milk[2]	1.68	1.84	2.17	0.41	0.63	
Copper, ppm						
Colostrum	2.59	2.60	2.21	0.22	0.52	0.01
Milk[2]	1.29	1.03	1.10	0.26	0.30	
Zinc, ppm						
Colostrum	9.05	10.34	8.66	0.82	0.48	0.01
Milk[2]	5.34	5.74	5.58	0.98	0.80	

[a-b]LS Means within a row that do not share a letter superscript differ significantly ($P < 0.05$).

[1]There was no Mn x parity effect, so data were averaged over both lactation periods for protein and fat percentages and mineral composition.

[2]There was no day effect ($P > 0.10$) between d 7 and d 14 samples for any component, therefore those samples were combined and an orthogonal contrast between colostrum and milk was reported.

[3]Mineral analysis performed via inductively coupled plasma (ICP) analysis.

CHAPTER 4

THE EFFECT OF VARYING DIETARY MANGANESE AND SELENIUM LEVELS ON THE GROWTH PERFORMANCE AND MANGANESE-SUPEROXIDE DISMUTASE ACTIVITY IN NURSERY PIGS

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Abstract

Antioxidant activity in the weaned piglet is important for the developing animal at a cellular level. This study examined the impact of varying dietary levels of manganese and selenium on piglet growth performance and plasma manganese superoxide-dismutase (MnSOD) specific activity. Weaned piglets were blocked by weight (N = 216; 5.21 ± 1.17 kg; 21 ± 3 d) and sex (2 barrows and 2 gilts/pen). Pens (n=9 pens/treatment) within a block were randomly assigned to dietary treatments in a 2 x 3 factorial design to examine the main effects of Se (0.1 and 0.3 mg/kg added Se) and Mn (0, 12, and 24 mg/kg added Mn). Diets were fed in three phases (P1 = d 1-7, P2 = d 8-21, P3 = d 22-35). Pigs and orts were weighed weekly and blood collected on d 0, 7, 21, and 35 for analysis of specific activity of red blood cell MnSOD. Data were analyzed as a factorial via the GLM procedure of SAS. No Mn x Se interactions were observed. There was a linear response ($P < 0.05$) in overall ADG across dietary Mn levels. There was a linear trend ($P < 0.10$) in overall ADFI across Mn levels. Gain to feed ratio (d 0-35) was not impacted by Mn or Se ($P > 0.10$). MnSOD specific activity in RBC increased from d 0-7, peaked at d 7, and decreased until d 35. On d 7, diets with 12 mg/kg Mn significantly increased ($P < 0.05$) MnSOD specific activity (0.91 IU/mg) compared to diets with 24 mg/kg Mn (0.67 IU/mg) but did not differ from diets with 0 mg/kg Mn (0.87 IU/mg). The dietary treatment that best maintained specific MnSOD activity was 12 mg/kg Mn supplemented, while ADG was maximized in the diet with 24 mg/kg Mn supplemented.

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

1. Introduction

Piglets experience a variety of physiological stressors during weaning attributed to nutritional, social, and immunological challenges (Cao et al., 2018). Oxidative stress is another type of physiological challenge that affects the piglet at weaning (Cao et al., 2018; Lu et al., 2014). Oxidative stress occurs when reactive oxygen species (**ROS**), like the superoxide anion ($O2^{\bullet -}$), accumulate intracellularly and impair normal cellular function in response to a stressor (Holley et al., 2011; Miriyala et al., 2012; Richards et al., 2010; Waris and Ahsan, 2006; Zhu et al., 2012). Reactive oxygen species accumulation can lead to abnormal cellular growth, certain types of cancer, and neurological disorders (Brière et al., 2006; Lu et al., 2014; Valko et al., 2006; Waris and Ahsan, 2006). Antioxidant enzymes are produced by the animal to combat harmful ROS (Mates et al., 1999). Mitochondrial manganese-superoxide dismutase (**MnSOD**), glutathione peroxidase, and catalase are considered three of the most active and important antioxidant enzymes (Valko et al., 2006). MnSOD uses manganese (**Mn**) as a cofactor for catalyzing the rearrangement of the superoxide anion into hydrogen peroxide or molecular oxygen, which are subsequently metabolized and eliminated by the body (Haikarainen et al., 2014; Holley et al., 2011). Altering levels of supplemental Mn and selenium (**Se**) could have an impact on the antioxidant status of weaned piglets and therefore the activity level of MnSOD. The objective of this study was to determine if altering dietary levels of supplemental Mn and Se had an effect on the growth performance and MnSOD activity levels of nursery pigs.

2. Materials and methods

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

2.1 Animal care and experimental design:

Weaned piglets (*N* = 216; 5.21 ± 1.17 kg; 21 ± 3 d old) were blocked by weight into pens of 4 with 2 gilts and 2 barrows per pen. Pigs from this herd were considered of good health status, were vaccinated as per typical commercial vaccine protocols, and were not allowed access to creep feed during the lactation period. Pens (*n* = 9 / treatment) were placed in an environmentally controlled nursery room and piglets consumed feed and water *ad libitum* (Large Animal Research Unit (LARU); University of Georgia, Athens, GA). Trace mineral premixes were designed in a 2 × 3 factorial (Table 4-1): two levels of added selenium (0.1 and 0.3 mg/kg Se from Na_2SeO_3) and three levels of added manganese (0, 12, and 24 mg/kg Mn from $MnSO_4$). Chosen levels of Se and Mn were based on previous research (Cao et al., 2018). The premixes, which were included at 1% of the basal diet, were formulated to meet NRC requirements for all other minerals (NRC, 2012). The resulting pelleted dietary treatments were randomly assigned within each weight block. Nursery diets were fed in three phases (Table 1): Phase 1 from d 0-7, Phase 2 from d 8-21, and Phase 3 from d 22-35. Piglet body weights and feed intake were recorded weekly. Animals were bled via orbital sinus (Dove & Alworth, 2015) on days when dietary changes occurred for analysis of red blood cell (**RBC**) MnSOD levels. Piglets were not food-deprived prior to bleeding. Blood was collected in heparinized blood collection tubes (BD Vacutainer®, Franklin Lakes, NJ) and transported on ice to the laboratory.

2.2 MnSOD analysis

Determination of MnSOD specific activity (EC 1.15.1.1) was accomplished by the modification of the protocol outlined by Marklund and Marklund (1974). Blood was collected from the pig as described above into heparinized blood tubes (BD Vacutainer®, Franklin Lakes, NJ) and centrifuged under refrigeration (2000 x *g*, 10 min, 4°C) to separate RBC. Red blood cells were washed three times with 1X phosphate buffered saline then lysed with an equal volume of ice-cold deionized water and frozen at -80°C until analyzed.

In order to inactivate the Cu/Zn-dependent SOD (Marklund and Marklund, 1974), 1 mmol potassium cyanide was added to the reaction buffer (50 mM Tris-HCl, 1.0 mM diethylenetriamine pentaacetic acid, pH 8.2) and was used to measure relative MnSOD activity. Hemolysate (0.5 mL) was added to 1.1 mL of reaction buffer and centrifuged (2000 x *g*, 15 min, 4°C). The resulting supernatant was diluted in reaction buffer 1:10. An assay control with no added sample (900 µL reaction buffer + 50 µL of 10 mM sodium azide (NaN_3)), 200 µL (of diluted hemolysate + 700 µL reaction buffer + 50 µL NaN_3), and 400 µL (of diluted hemolysate + 500 µL reaction buffer + 50 µL NaN_3; all in triplicate) were plated on a 12-well microcuvette plate (VWR® Tissue Culture Plates, Radnor, PA), with each well having a volume of 950 µL. The reaction was initiated when 50 µL of 4 mM pyrogallol in 10 mM HCl was added to each well and rapidly mixed. The reaction was monitored at 320 nm for 3 min using the kinetic reading program of a spectrophotometer (Biotek® µQuant, 2006). Deionized water (1.0 mL) was used to blank the spectrophotometer. The amount of supernatant that resulted in the 50% inhibition of the autooxidation of pyrogallol was the equivalent of one unit of MnSOD activity

(Marklund and Marklund, 1974). Lowry protein determination (Lowry et al., 1951) was performed on the samples to determine specific activity of MnSOD (IU/mg soluble protein).

2.3 Statistical analysis

All analyses were performed using pen as the experimental unit and weight as the blocking factor. Initial body weight was utilized as a covariate for all growth performance parameters. Growth performance data was analyzed as a factorial design via PROC GLM in SAS 9.4 (SAS Enterprise, Cary, NC). Analysis of MnSOD concentration was performed as a 2 × 3 factorial with repeated measures, which accounts for the interaction of main effects with time. Comparisons between the least square means of the factor level comparisons were computed using the PDIFF option of the LSMEANS statement. Statistical significance was declared at $P < 0.05$ and trends were considered at $0.05 < P < 0.10$.

3. Results

3.1 Growth performance

Two piglets died within the first week after weaning in two different pens independent of treatment. These animals were excluded from analysis. Average daily gain and feed intake calculations and analysis were adjusted accordingly and data reported is from a total of 214 animals.

There was no Mn × Se interaction for any growth performance parameter (Table 4-2). There was no effect of Se on growth performance.

There was a linear increase in piglet body weight with increasing levels of dietary Mn on d 7, 21, and 35 (Table 4-2; $P < 0.05$), and there was a linear increase in average daily gain in all nursery phases ($P < 0.05$) and overall with increasing levels of dietary Mn ($P < 0.01$; 394, 433, 434, 399, 414, 443 g/pig/day for 0.1 mg/kg Se; 0, 12, 24 mg/kg Mn and 0.3 mg/kg Se; 0, 12, 24 mg/kg Mn, respectively). Overall average daily feed intake tended to increase linearly with increasing levels of dietary Mn ($P < 0.10$; 559, 579, 598, 554, 542, 586 g/pig/day for 0.1 mg/kg Se; 0, 12, 24 mg/kg Mn and 0.3 mg/kg Se; 0, 12, 24 mg/kg Mn, respectively). There were linear trends to improve G:F during nursery phase 1 (d 0 – 7; $P < 0.10$) and phase 2 (d 8 – 21; $P < 0.10$) in response to increasing dietary Mn levels; however, Mn had no linear effect on G:F during phase 3 or over the 35 d study ($P > 0.10$). Overall efficiency values were 0.71, 0.75, 0.73, 0.71, 0.77, 0.76 for 0.1 mg/kg Se; 0, 12, 24 mg/kg Mn and 0.3 mg/kg Se; 0, 12, 24 mg/kg Mn, respectively.

3.2 MnSOD concentration

There was no Mn × Se interaction or Se effect for MnSOD concentration on any sample day ($P > 0.10$; Table 4-3). MnSOD concentration was affected by dietary Mn level on d 7 and d 35 ($P < 0.01$; $P < 0.05$, respectively). There was a linear response in MnSOD concentration in relation to increasing level of dietary Mn on d 7 ($P < 0.01$). On d 35, MnSOD concentration was increased in diets that supplemented 12 mg/kg Mn compared to all other diets ($P < 0.05$).

4. Discussion

There was no effect of dietary Se on piglet growth performance. The NRC recommended concentration of dietary Se for swine (0.3 mg/kg; NRC 2012) has been shown to numerically

improve body weight and ADG in weaned pigs when compared to diets deficient (< 0.1 mg/kg) in dietary Se (Cao et al., 2014; Joksimović-Todorović et al., 2006). In this study, there was no negative effect of Se deficiency on piglet growth performance and it has been determined that feeding 0, 0.15 and 0.30 mg/kg Se had no effect on growth performance in a regional study (Mahan et al., 2014). In addition, since adequate vitamin E concentrations were present in these diets, the negative impacts of lower concentrations of Se may have been negated. Increased dietary Se has not been shown to have a significant effect on hepatic or muscular tissue concentrations of MnSOD activity (Schwarz et al., 2017), and there was no effect of Se on RBC MnSOD activity in this study.

Supplementation of dietary Mn has been of interest to researchers after the nursery period, but little research has been done with piglets immediately following weaning. The current NRC recommendation for Mn is 4 mg/kg added Mn for pigs 5-11 kg and 3 mg/kg added Mn for pigs 11-25 kg (NRC, 2012). Inclusion levels above 4 mg/kg have been shown to have positive effects on the growth performance of pigs. Grummer et al. (1950) reported that including 40 mg/kg Mn in grower swine diets increased ADG and improved feed efficiency in comparison with pigs fed no Mn. There have been effects on growth recorded in baby pigs or sows as a result of dietary Mn supplementation (Leibholz et al., 1962; Plumlee et al., 1956). In this study, it was determined that there was a linear response in ADG to increasing level of Mn in the diets, suggesting that innate levels of available Mn found in these nursery diets may not be adequate to support maximal growth, at least during the first week after weaning (Table 4-2). Piglets supplemented the highest level of dietary Mn (24 mg/kg) gained on average 41 more grams per day ($P < 0.05$; Table 2) than those piglets provided 0 mg/kg supplemental Mn, while piglets supplemented 12 mg/kg Mn gained an intermediate amount, not differing from the other

two levels ($P > 0.10$; Table 2). In addition, feed intake tended to increase linearly with increasing Mn concentrations in the diet, though not significantly ($P < 0.10$; Table 2). Feed efficiency tended to increase linearly with the level of increasing Mn during phase 1 and 2 of the nursery ($P < 0.10$). Improvements to ADG without piglets eating significantly more feed is an ideal situation when applied to a production system. Improved nursery ADG typically leads to a better grow-finish gain, which ultimately leads to reaching market weight more quickly. Including supplemental Mn, though a small dietary change, may have an important application to the industry in terms of improving ADG during the nursery phase of production.

MnSOD concentrations changed over the 35 days of the study ($P < 0.01$; Table 3). There is little research looking specifically at RBC MnSOD activity levels after weaning in swine. Gene expression and activity level of several antioxidant enzymes in the jejunum of piglets decreased following weaning (Cao et al., 2018; Zhu et al., 2012). Hepatic tissue levels of MnSOD averaged around 2.7 IU mg/protein while levels in the muscle averaged 1.03 IU mg/protein in grower pigs fed a control diet (Schwarz et al., 2017). Because red blood cells have no mitochondria, it was expected that levels of MnSOD would be minimal and would certainly have less enzymatic activity than those tissues with cells with more mitochondria (Liu et al., 2016; Miriyala et al., 2012; Schwarz et al., 2017). There tended to be an increase and peak in blood MnSOD activity on d 7 and subsequently a decrease on d 21 and 35 across all treatment groups (Table 4-3). This suggests there is a physiological need for antioxidant enzymes such as Mn superoxide-dismutase one week post-weaning. On d 35, MnSOD activity was decreased when compared to d 0 values (Table 4-3; $P < 0.05$). This trend of MnSOD activity suggests that at d 7, antioxidant activity was higher than any other day and more ROS needed to be eliminated. MnSOD activity decreased as pigs aged. The pigs supplemented with 0.3 mg/kg Se and 12

mg/kg Mn displayed the closest return to initial MnSOD activity levels on d 35 (Table 3). These pigs also had the highest peak in MnSOD activity on d 7 (Table 4-3).

5. Conclusion

The results of this study indicate that the level of supplemented Mn in swine diets has a linear effect on average daily gain and linear trends on feed intake and feed efficiency. Levels of MnSOD activity in RBC increased from the day of weaning until 7 d post-weaning and subsequently decreased through the remainder of the study. While there were no differences observed between the two levels of Se that were fed, piglets supplemented 0.3 mg/kg Se and 12 mg/kg Mn had the highest MnSOD activity on d 35 (0.58 IU mg/protein ($P < 0.05$)) when compared to the other dietary treatments. The NRC recommendation for 0.3 mg/kg Se is sufficient for normal piglet production based on the results of this study; there were no negative impacts on piglet performance at a lower concentration of supplemented Se, likely due to adequate vitamin E supplementation. These data indicate that Mn should be supplemented at 24 mg/kg to improve growth in the nursery. Nursery piglet performance can be improved by slight dietary modifications, which can have a positive impact on performance in the growing and finishing phases of production.

Conflict of interest statement

None of the authors have any conflicts of interest to declare.

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Table 4-1: Basal diet composition and analysis

Ingredients, %	Phase 1[a]	Phase 2[a]	Phase 3[a]
Corn	22.80	40.20	57.34
Soybean meal 47.5%	15.00	21.00	28.14
Whey	15.00	10.00	7.00
Oats	10.00	5.00	.
Hamlet protein	10.00	7.50	.
Lactose	10.00	3.00	.
Fish meal	5.00	3.00	3.00
Blood plasma	3.00	1.50	.
Fat	5.24	4.38	0.46
L-Lysine	0.32	0.40	0.30
DL Methionine	0.20	0.22	0.14
L-Threonine	0.12	0.16	0.12
Dicalcium phosphate	1.48	1.74	1.34
Limestone	0.32	0.38	0.64
Salt	0.26	0.26	0.26
UGA vitamin premix[1]	0.26	0.26	0.26
Trace mineral premix[2,3]	1.00	1.00	1.00
Analysis[4]			
ME[5,6], Mcal/kg	3511	3500	3319
Crude protein, %	21.1	21.2	21.3
Lysine[6], %	1.76	1.66	1.32
Crude fat, %	7.1	6.9	3.3
Ash, %	6.3	6.2	5.6
Crude fiber, %	1.6	7.4	7.7
Phosphorus (total), %	0.74	0.82	0.74
P (avail)[6], %	0.55	0.45	0.37
Calcium, %	0.97	1.1	0.93

[a]Nursery diets were formulated to meet NRC requirements (2012). Phase 1 was fed for 7 d, Phase 2 for 14 d, and Phase 3 for 14 d.

[1]Vitamin premix: supplied per kg of diet: vitamin A (4,134 IU); vitamin D (1,653 IU); vitamin E (66 IU); vitamin K (3.3 mg); riboflavin (8.27 mg); niacin (49.6 mg); vitamin B_{12} (0.033 mg); pantothenic acid (27.6 mg); ADM Alliance Nutrition, Quincy, IL 62305

[2]Trace minerals (with the exception of Se and Mn), were supplemented at 20 mg/kg Cu ($CuSO_4$); 100 mg/kg Fe ($FeSO_4$); 100 mg/kg Zn (ZnO); 0.1 mg/kg I (KIO_3). Ground corn was used as the carrier for trace mineral premixes. Analyzed values[4] (mg/kg diet): 20.7 mg Cu; 398.7 mg Fe; 141.8 mg Zn. Iodine levels were too low to detect in the complete diet.

[3]Selenium was supplemented at two levels: 0.1 or 0.3 mg/kg (Na_2SeO_3); manganese was supplemented at 0, 12, or 24 mg/kg ($MnSO_4$) for each level of Se, resulting in six different trace mineral premixes that were included at 1% of each basal pelleted diet. Analyzed[4] Mn values were 28.3, 39.9, and 51.7 mg/kg for 0, 12, and 24 mg/kg supplemented Mn dietary treatments. Selenium values were too low to detect in the complete diet.

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

[5]Metabolizable energy

[6]Calculated value

Table 4-2: The effect of varying dietary levels of supplementary manganese and selenium on the growth performance characteristics of nursery pigs (N = 214)

Dietary Treatments[1] Selenium, mg/kg	0.1			0.3				*P*-values[2]			
Manganese, mg/kg	0	12	24	0	12	24	SEM	Mn	Mn (Lin)	Se	Mn x Se
Body weight, kg											
d 0	5.19	5.22	5.18	5.14	5.22	5.29	0.1	0.79	0.51	0.84	0.74
d 7	5.86^{ab}	6.07^{ab}	6.13^{ab}	5.83^{b}	6.08^{ab}	6.22^{b}	0.1	0.05	0.02	0.85	0.89
d 21	10.81^{a}	11.39^{b}	11.65^{b}	10.82^{ab}	11.06^{ab}	11.70^{b}	0.3	0.04	0.01	0.74	0.80
d 35	19.01^{a}	20.38^{b}	20.46^{b}	19.25^{ab}	19.70^{ab}	20.66^{b}	0.5	0.03	0.01	0.85	0.62
Average daily gain (g/pig/day)											
d 0-7	96	115	129	97	124	133	15	0.06	0.02	0.68	0.97
d 7-21	352	381	392	348	356	397	19	0.08	0.03	0.61	0.75
d 21-35	585^{a}	643^{b}	628^{ab}	598^{ab}	618^{ab}	644^{b}	18	0.04	0.02	0.94	0.90
d 0-35	394^{a}	433^{ab}	434^{ab}	399^{a}	414^{ab}	443^{b}	14	0.03	0.01	0.90	0.61
Average daily feed intake (g/pig/day)											
d 0-7	141	150	173	143	168	168	12	0.07	0.02	0.62	0.61
d 7-21	486	514	518	489	490	494	20	0.64	0.37	0.35	0.75
d 21-35	941	975	983	946	920	951	50	0.71	0.44	0.26	0.61
d 0-35	559^{ab}	579^{ab}	598^{a}	554^{ab}	542^{b}	586^{ab}	19	0.15	0.08	0.27	0.69
Gain:Feed											
d 0-7	0.59	0.73	0.73	0.65	0.72	0.77	0.07	0.14	0.06	0.57	0.88
d 7-21	0.73	0.74	0.75	0.70	0.72	0.80	0.03	0.21	0.09	0.90	0.42
d 21-35	0.63	0.66	0.64	0.63	0.67	0.67	0.02	0.17	0.18	0.32	0.79
d 0-35	0.71	0.75	0.73	0.71	0.77	0.76	0.02	0.16	0.25	0.42	0.82

[a-b]LS Means within a row that do not share the same letter superscript differ significantly ($P < 0.05$).

[1]Dietary treatments were formulated in a 2 × 3 factorial design. Within each level of supplementary Se (0.1 and 0.3 mg/kg), there were three levels of supplementary Mn (0, 12, 24 mg/kg).

[2]*P*-values presented are for the main effects of manganese (Mn), selenium (Se), the linear orthogonal contrast of manganese levels Mn (Lin), and the interaction of Mn and Se.

Table 4-3: The effect of varying dietary levels of supplementary manganese and selenium on the activity of manganese-superoxide dismutase in the red blood cells of nursery piglets

	Dietary Treatments[1]										
Selenium, mg/kg	0.1			0.3				*P*-value[2-3]			
Manganese, mg/kg	0	12	24	0	12	24	SEM	Mn	Mn (Lin)	Se	Mn x Se
MnSOD Activity[4]											
d 0	0.75	0.65	0.71	0.79	0.76	0.77	0.05	0.53	0.59	0.13	0.79
d 7	0.84^{bc}	0.94^{c}	0.65^{a}	0.90^{c}	0.88^{c}	0.69^{ab}	0.06	0.01	0.01	0.82	0.57
d 21	0.77	0.85	0.84	0.85	0.81	0.80	0.05	0.89	0.74	0.98	0.44
d 35	0.40^{a}	0.45^{a}	0.46^{a}	0.43^{a}	0.58^{b}	0.45^{a}	0.03	0.03	0.28	0.11	0.19

[a-c] LS Means within a row that do not share the same letter superscript differ significantly ($P < 0.05$).

[1]Trace mineral dietary premixes were formulated in a 2 × 3 factorial design. Within each level of supplementary Se (0.1 and 0.3 mg/kg), there were three levels of supplementary Mn (0, 12, 24 mg/kg).

[2]*P*-values presented are for the main effects of manganese (Mn), selenium (Se), the linear orthogonal contrast of manganese levels Mn (Lin), and the interaction of Mn and Se.

[3]There was no time × Se × Mn effect ($P = 0.42$) or time × Se effect ($P = 0.58$), but there was a time effect ($P < 0.01$) and a time × Mn effect ($P < 0.01$).

[4]MnSOD specific activity is expressed in the internationally recognized unit: IU enzymatic activity/mg soluble protein.

CHAPTER 5

THE EFFECTS OF DIETARY MANGANESE AND SELENIUM ON THE GROWTH AND FECAL MICROBIOTA OF NURSERY PIGLETS

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Abstract

Weaning can be a stressful time for piglets and result in a compromised digestive system. The objective of this study was to determine the impact of altering dietary manganese and selenium concentrations, antioxidant cofactors, on the growth performance and fecal microbial populations of nursery pigs. Weaned piglets were blocked by weight (N = 120; 5.22 ± 0.7 kg; 21 ± 3 d) and sex (2 barrows and 2 gilts/pen). Pens (n=5/treatment) within a block were randomly assigned to dietary treatments in a 2 x 3 factorial design to examine the effects of Se (0.1 and 0.3 mg/kg added Se) and Mn (0, 12, and 24 mg/kg added Mn) and were fed in three phases (P1= d 1-7, P2= d 8-21, P3= d 22-35). Pigs and orts were weighed weekly. Fecal samples were collected on d 0 and 35 for 16S rRNA gene sequencing and VFA analysis. Data were analyzed as a factorial via the GLM procedure of SAS. There was a linear response ($P < 0.05$) in overall ADG across dietary Mn levels. Supplementing 24 mg/kg Mn numerically decreased ($P < 0.10$) the relative abundance of many bacteria possessing pathogenic qualities relative to the supplementation of 0 mg/kg Mn. Meanwhile increasing Mn concentration tended to foster the growth of bacteria beneficial to gut health and improved growth ($P < 0.10$). The data from this study provides preliminary evidence on the positive effects of manganese on the growth and gut health of nursery pigs.

Keywords: growth performance, manganese, microbiome, nursery swine, *Streptococcus*; *Roseburia*; *Turicibacter*

5.1: Introduction

Weaned piglets experience a number of physiological stressors in response to nutritional, social, and immunological changes (Cao et al., 2018). In addition, oxidative stress plays a large role in the physiological challenges that affect piglets at weaning (Cao et al., 2018; Lu et al., 2014) and is the result of reactive oxygen species (ROS) accumulating intracellularly and impairing normal cellular function in response to stress (Holley et al., 2011; Miriyala et al., 2012; Richards et al., 2010; Waris and Ahsan, 2006; Zhu et al., 2012). Accumulation of ROS can result in neurological disorders, abnormal cellular growth and changes, and several types of cancer (Brière et al., 2006; Lu et al., 2014; Valko et al., 2006; Waris and Ahsan, 2006). As a defense mechanism, antioxidant enzymes are produced by the animal to combat these harmful ROS (Mates et al., 1999) with mitochondrial manganese-superoxide dismutase (MnSOD), glutathione peroxidase, and catalase considered three of the most active (Valko et al., 2006). MnSOD utilizes manganese (Mn) as a cofactor for catalyzing the dismutation of the superoxide anion into hydrogen peroxide or molecular oxygen which are metabolized or eliminated by the body (Haikarainen et al., 2014; Holley et al., 2011). Glutathione peroxidase utilizes the trace element selenium (Se) to convert hydrogen peroxide into water (Dalto et al., 2018; Li et al., 2000). Therefore, altering supplemental dietary concentrations of manganese and selenium could have an impact on the antioxidant status of weaned piglets and as a result improve growth performance.

In swine, weaning is associated with changes in the gastrointestinal tract microbiota and immunology (Pluske, 2013). Immediately following weaning, piglets experience a period of sub-optimal growth and an increased incidence of intestinal disturbances with diarrhea (Pluske, 2013). These challenges may induce greater utilization of antibiotics such as Carbodox (Looft et

al., 2014; Lourenco et al., 2021), which may contribute to the problem of antimicrobial resistance. There has been limited research on the impact supplementing dietary manganese and selenium to nursery pigs has on their gut microbial populations or gut health. Manganese is supplemented to the diet because it plays major structural and anti-oxidative roles in swine and cattle (Hansen et al., 2006a, 2006b; Leibholz et al., 1962; Spears, 2019). We hypothesized that the microbial population of piglets might change due to dietary alterations of Mn and Se, and as a result, may impact animal performance and the overall gut health of those young animals. Thus, the objective of this study was to determine the impact of altering dietary concentrations of manganese and selenium in the diet of nursery piglets on their growth performance and fecal microbiota.

5.2: Materials and methods

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

5.2.1: Animal Care and Experimental Design:

All care, handling, and sampling procedures of the piglets in this study were approved by the University of Georgia Institutional Animal Care and Use Committee prior to the start of this study (AUP#: A2018 08-012-A1). Piglets (N = 120; 5.22 ± 0.7 kg; 21 ± 3 d old) were blocked by weight and balanced by sex (2 barrows and 2 gilts per pen). Pens (n = 5 / dietary treatment) were in an environmentally controlled nursery room and piglets consumed feed and water *ad libitum* for 35 d post weaning (Large Animal Research Unit; University of Georgia, Athens, GA). Trace mineral premixes were formulated in a way that resulted in a 2 x 3 factorial arrangement: two levels of selenium (0.1 and 0.3 ppm) and three levels of manganese (0, 12, and 24 ppm). The

trace mineral premixes were included at 1% of the basal diet (Table 5-1), which was formulated to meet the current NRC requirements for all other trace minerals (NRC, 2012); and the resulting pelleted dietary treatments were randomly assigned within each weight block. Nursery diets were fed in three phases (Table 5-1): Phase I from d 0-7, Phase II from d 8-21, and Phase III from d 22-35. Piglet body weights and feed intake were recorded weekly.

5.2.2: Fecal Collection and Storage:

Fecal samples were taken from individual animals on d 0 and 35 of the study using a sterile cotton swab to stimulate fecal excretion. Samples were placed in 50 mL conical tubes and frozen at -20°C until DNA extraction and sequencing could be performed. Since pen was considered the experimental unit, samples were pooled by pen prior to DNA extraction occurred.

5.2.3: DNA Extraction and Sequencing:

Deoxyribonucleic acid (DNA) was extracted from fecal samples following the procedures described in detail by Welch et al. (2020) with some minor modifications. This procedure utilizes 0.25 g of sample placed in 2-mL Lysing Matrix E tubes (MP Biomedicals LLC, Irvine, CA, USA), which are homogenized using a QIAGEN vortex adapter (QIAGEN, Venlo, Netherlands) to mechanically disrupt the cells. Enzymatic inhibition was performed by using InhibitEX Buffer (QIAGEN, Venlo, the Netherlands), and DNA elution and purification were carried out using a spin column and a series of specialized buffers according to the manufacturer's instructions (QIAamp Fast DNA Stool Mini Kit; QIAGEN, Venlo, the Netherlands). Determination of DNA concentration and purity from the samples in the resulting eluate was performed spectrophotomically using the Synergy LX Multi-Mode Microplate Reader in conjunction with the Take3 Micro-Volume Plate (BioTek Instruments Inc; Winooski, VT, USA). Samples with a

minimum volume of 100 µL and 10 ng/µL of DNA were stored at 4-20°C. Samples that did not meet these requirements were rejected and subjected to a new DNA extraction cycle.

Following DNA extraction, samples were shipped to LC Sciences (Houston, TX, USA) for library preparation and 16S ribosomal ribonucleic acid (rRNA) gene sequencing. The library preparation included polymerase chain reaction (PCR) replications using the forwards: S-D-Bact-0341-b-S-17 (5'- CCTACGGGNGGCWGCAG-3') and reverse: S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3') primer pairs (Klindworth et al., 2013), followed by a PCR clean-up step using AMPure XP beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA). A second PCR step was carried out to attach Illumina's indices and sequencing adapters (Nextera XT Index Kit; Illumina Inc., San Diego, CA, USA), followed by another PCR clean-up step using AMPure XP beads. Following this final library clean-up, the library was quantified using qPCR, and the nucleotides were sequenced using an Illumina NovaSeq platform (paired-end reads, 2 x 250 bp; Illumina Inc., San Diego, CA, USA). A well characterized bacteriophage PhiX genome (Illumina Inc., San Diego, CA, USA) was used in the quality control of the sequencing runs.

Sequencing data were demultiplexed and converted into FASTQ files. Paired-end sequences were converted into QIIME 2 artifacts (Bolyen et al., 2019), and the non-biological nucleotides were removed, and sequences were denoised, dereplicated, and chimera-filtered using DADA2 (Callahan et al., 2016). Taxonomies were assigned by using a pre-trained Naïve Bayes classifier which was trained on the SILVA 138 SSU database (Quast et al., 2013), and reads were classified by taxon using the fitted classifier (Pedregosa et al., 2011). Samples were rarefied to 21,806 sequences per sample prior to computing alpha diversity using the "qiime" diversity plugin.

5.2.4: Volatile Fatty Acid Analysis:

Analysis of volatile fatty acids (VFA) was performed according to the procedure described by Lourenco et al. (2020). One gram of fecal material was diluted in 3 mL of distilled water, and placed into 15-mL conical tubes. Tubes were vortexed for 30 sec to produce a homogenous sample and 1.5 mL of the mixture was transferred to microcentrifuge tubes. The tubes were centrifuged at 10,000 x *g* for 10 min. One mL of the supernatant was transferred into a new microcentrifuge tube and mixed with 0.2 mL of metaphosphoric acid solution (25% w/v). Samples were vortexed for 30 sec and stored at -20°C overnight. The next morning, samples were thawed and subsequently centrifuged at 10,000 x *g* for 10 min. The supernatant was removed and transferred into polypropylene tubes combined with ethyl acetate in a 2:1 ratio of ethyl acetate to supernatant. The tubes were vortexed for 10 sec to thoroughly mix them and allowed to settle for 5 min for optimum separation. Then, 600 µL of the top layer was transferred into screw-thread vials. VFA analysis was performed using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector and capillary column (Zebron ZB-FFAP; 30 m x 0.32 mm x 0.25 µm; Phenomenex Inx., Torrance, CA, USA). Sample injection volume was set to 1.0 µL, and helium was used as a carrier gas. Column temperature started at 110°C and increased to 200°C over 6 minutes. Injector temperature was set to 250°C, while detector temperature was set to 350°C.

5.2.5: Statistical Analyses:

All growth performance analyses were performed using pen as the experimental unit and weight as the blocking factor. Initial body weight was utilized as a covariate for all growth performance parameters. Performance data were analyzed as a 2 x 3 factorial design via PROC GLM in SAS 9.4 (SAS Enterprise, Cary, NC). Indices of diversity and bacterial abundances at

the phylum, genus, and species level were run as a 2 x 3 factorial design using the same program. In addition, day was included into the model to detect differences attributed to sampling time and any interaction of day with manganese and selenium. Statistical significance was declared at $P < 0.05$ and tendencies were considered at $0.05 < P < 0.10$.

5.3: Results

5.3.1: Animal performance

Selenium and the interaction between Mn and Se did not have an effect on any growth performance parameters measured ($P > 0.14$; Table 5-2). However, Mn supplementation increased average daily gain ($P = 0.007$). There were no differences observed between treatments in body weight observed on d 0 or 35, average daily feed intake, or the ratio of gain-to-feed ($P > 0.10$).

5.3.2: Volatile fatty acids and alpha diversity

There was no significant interaction or main effect on any volatile fatty acid parameter except the molar proportion of acetate (Supplemental Table 5-1). There was a significant Mn x Se interaction and Mn x day interaction on the molar proportion of acetate ($P < 0.05$); however, the proportion of acetate did not differ on d 35. Mn and Se supplementation did not have an effect on any of the alpha diversity indices measured (Supplemental Table 5-1).

5.3.3: Microbial populations

Since there was an effect of Mn on average daily gain, the study focused on how the microbial population changed over time based on Mn level; however, information of the highlighted bacterial genera in regard to Se supplementation can be found in supplemental materials (Supplemental Figure 1). There was a significant increase in the relative abundance of *Streptococcus* in the non-Mn supplemented piglets on d 35 compared to the piglets supplemented

with Mn (P = 0.049; Figure 5-1) that was not present in the feces of Mn supplemented piglets. Mn supplementation tended to have an effect on the relative abundance of *Roseburia* (P = 0.079) on d 35 with the highest relative abundance being in the feces of the piglets received the highest Mn supplementation (24 mg/Kg). *Turicibatcer* decreased in the non-supplemented and 12 mg/Kg Mn piglets from d 0 to d 35; however, the piglets receiving the most Mn supplementation (24 mg/Kg) did not have a decrease in *Turicibacter* from d 0 to d 35 (P = 0.086).

In the present study, supplementary dietary Mn tended to affect the relative abundance of *Acidaminococcus fermentans* (P = 0.070; Figure 5-2) over time. The piglets receiving the most Mn supplementation (24 mg/Kg) did not have an increased relative abundance of *A. fermentans* from d 0 to 35 (P > 0.05); whereas, the non-supplemented and lower supplementation (12 mg/Kg) piglets had a fecal increase from d 0 to 35 (P < 0.05). Mn supplementation tended to have an effect on the relative abundance of *Lactobacillus ruminis* (P = 0.077) from d 0 to 35. On d 35, the relative abundance of *L. ruminis* was significantly increased (P < 0.05) in piglets fed 0 mg/kg Mn compared to those fed 12 mg/kg or 24 mg/kg Mn. The relative abundance of *Massiliomicrobiota timonensis* was impacted by Mn supplementation over time (P = 0.095). On d 35, relative abundance of *M. timonensis* decreased in 24 mg/kg piglets compared to d 0 (P < 0.05); whereas, there was no change in relative abundance in either 0 mg/kg and 12 mg/kg Mn piglets from d 0 to 35 (P > 0.05). The relative abundance of *Roseburia hominis* was affected by Mn supplementation over time (P = 0.077). Piglets receiving the highest Mn supplementation (24 mg/Kg) had an increase in the relative abundance of *R. hominis* from d 0 to 35 in the feces (P < 0.05) whereas the other dietary groups did not.

5.4: Discussion

Supplementation of dietary manganese after the nursery stage of production has drawn interest to researchers, but little Mn research has been performed with piglets immediately following weaning. The current NRC recommendation for Mn is 4 mg/kg added Mn for piglets that weigh 5-11 kg and 3 mg/kg added Mn for pigs weighing 11-25 kg (NRC, 2012). Inclusion levels above the published requirements have been shown to have positive effects on the growth performance of pigs. Grummer et al. (1950) reported that including 40 mg/kg Mn in grower swine diets increased ADG and improved feed efficiency in comparison with pigs fed no Mn. There have been effects on growth reported in sows and baby pigs as a result of dietary Mn supplementation (Leibholz et al., 1962; Plumlee et al., 1956). The current study supports these previous findings that including supplemental Mn may have critical application to the swine industry in terms of improving ADG during the nursery phase of production.

Although there were no direct effects of Mn on microbial diversity as a whole, Mn supplementation did impact specific bacterial relative abundances that could ultimately have an impact on the overall health of the piglets. *Streptococcus* is generally considered to be a pathogenic genus in the majority of food animal species and humans. There are various streptococci that are of ecological importance to the microbial flora of humans and animals; but there are also some that cause disease and can be harmful in humans and animals (Patterson, 1996). Torres-Pitarch et al. (2020) found that ileal *Streptococcus* was negatively correlated to growth. The addition of copper and zinc supplemented to the diet decreased colonic digesta levels of Streptococcus along with other pathogenic genera like Enterobacter and Escherichia (Villagómez-Estrada et al., 2020). It is known that copper and zinc have growth promoting (Cromwell et al., 1998; Dove, 1993) and antimicrobial effects (Højberg et al., 2005; Namkung et

al., 2006). Less is known about manganese and its potential effects on the gut microbiome; however, this study suggests that Mn has a similar impact by increasing ADG while decreasing fecal relative abundance of Streptococcus. This study may serve as preliminary evidence that supplemental dietary manganese may have some antimicrobial effects on the genus Streptococcus, especially following a five-week nursery period.

The genus *Roseburia* produces short-chain fatty acids from the metabolism of complex polysaccharides, especially butyrate, which can positively affect gut motility, maintain host immunity, and decrease inflammatory properties (Hillman et al., 2020; Tamanai-Shacoori et al., 2017). *Roseburia* is a common genus found in the gut of piglets during the post-weaning phase of production and is generally considered a beneficial genera for the host (Chen et al., 2017; Choudhury et al., 2021). Though little research has directly studied the impact of dietary manganese on the presence of *Roseburia* in the gut of weaned piglets, Torres-Pitarch et al. (2020) found that cecal *Roseburia faecis* was positively correlated with ADG in older pigs. In the current study, the increase in both the genus *Roseburia* and *Roseburia hominis* in the feces of piglets with the highest Mn supplementation serves as preliminary evidence that supplementary dietary manganese may foster the growth of the beneficial genus *Roseburia* in the gut of piglets 35 d post-weaning resulting in an increase in ADG.

Recent research has indicated that the genus *Turicibacter* can be beneficial to the health of the gastrointestinal tract; however, there is limited research as to how Mn affects this bacterium. It has been reported that *Turicibacter* has been associated with intestinal butyrate in rats fed high-fat diets (Zhong et al., 2015). In swine production, *Turicibacter* was increased in pigs fed increased starch diets which investigators concluded lead to an increase in metabolic pathways that were beneficial to the host's gastrointestinal health (Sun et al., 2006).

Additionally, research has identified *Turicibacter* as an important bacterium for increasing body weight in pigs (Wang et al., 2019). The present study's results that the relative abundance of *Turicibacter* is maintained from d 0 to d 35 in the group with the highest Mn supplementation suggests the addition of Mn in the diet can have positive effects of the growth of this genus, ultimately increasing body weight while maintaining gastrointestinal health in weaned piglets.

Acidaminococcus fermentans was isolated by Fuller (1966) from a porcine alimentary tract. *A. fermentans* thrives primarily via glutamate fermentation in the intestinal tract of homeothermic animals like pigs (Chang et al., 2010; Rogosa, 1969). Although previous research has not investigated the effect of trace minerals on the gastrointestinal tract, fiber inclusion rates impact the gut microbial populations. Pigs fed a low fiber diet had increased abundances of *A. fermentans* (Li et al., 2016). They concluded that dietary fiber modulates the host microbial population toward more beneficial butyrate-producing bacteria instead of more pathogenic bacteria including *A. fermentans*, *Clostridium perfringens,* and *C. rectum*. The present study's findings suggest Mn inclusion may have similar impacts on *A. fermentans* as fiber inclusion since the piglets receiving the highest level of Mn supplementation had the lowest levels of *A. fermentans* on d 35. This further supports the hypothesis that Mn positively impacts host gastrointestinal health by decreasing pathogenic bacteria.

Lactobacillus ruminis is a commensal species of bacteria found in the gastrointestinal tract of several species of animals including monogastric animals like pigs (Al Jassim, 2003). In pigs, *L. ruminis* is considered to be the dominant lactic acid bacteria in the large intestine (Al Jassim, 2003; O'Donnell et al., 2015). Various species of *Lactobacillus* are considered to be beneficial to the pig and have been used as feed additives either as pure or mixed cultures (Fuller et al., 1999). However multiple studies have found *L. ruminis* to be associated with increased

levels of either interlukin-6 (IL-6) and IL-8 leading to an increase in inflammation (Neville et al., 2012; Yamashiro et al., 2017). This study may provide preliminary evidence that Mn supplementation may reduce fecal relative abundance of the *L. ruminis* in piglets 35 d post-weaning, potentially reducing gut inflammation.

Massiliomicrobiota timonensis is a bacteria that is gram-negative, rod-shaped and found in chains (Ndongo et al., 2016). Little is known about *M. timonensis*. It was first isolated from an 87-year-old patient hospitalized in 2015 for cognitive impairment experiencing weight loss complications (Ndongo et al., 2016). To the best of our knowledge, this is the first time *M. timonensis* has been isolated in the feces of pigs. Combining previous findings that the patient was experiencing weight loss at the time of isolation with the fact that in the current study the piglets that had a higher relative abundance of *M. timonensis* had a lower ADG, this bacterium might be associated with less nutrient absorption and this could be due to the host having a less "healthy" gut. Although more research is needed to determine the role *M. timonensis* plays in the gastrointestinal microbial consortium, the current data suggests that *M. timonensis* may possess pathogenic tendencies by being more abundant in less efficient piglets that also had increased relative abundances of other pathogenic bacteria.

5.5.5: Conclusions

The results from this study highlight the importance of Mn supplementation on increasing growth of nursery piglets following weaning. Although dietary concentrations of manganese did not alter the fecal microbiota as a whole, it did have an effect on specific microorganisms. Although the present study had a relatively small sample size, it highlights the importance for future research into how Mn impacts the gut microbial populations. Specifically, our data suggest Mn may have some antimicrobial effects by reducing key bacteria that exhibit

pathogenic behavior and create gut inflammation, consequently decreasing growth rates. Additionally, dietary Mn supplementation may also foster the growth of beneficial microorganisms that increase body weight gain while maintaining gastrointestinal health. The results of this study provide preliminary evidence of the importance of increasing Mn in the diet of nursery piglets to a higher level studied (12 or 24 mg/Kg) to increase ADG and gut health. Additionally, the reduction of harmful pathogenic microorganisms is of great importance to the food animal industry and Mn may be a solution to the reduction of these pathogens.

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Table 5-1: Basal diet composition and analysis

Ingredients, %	Phase 1[a]	Phase 2[a]	Phase 3[a]
Corn	22.80	40.20	57.34
Soybean meal 47.5%	15.00	21.00	28.14
Whey	15.00	10.00	7.00
Oats	10.00	5.00	.
Hamlet protein	10.00	7.50	.
Lactose	10.00	3.00	.
Fish meal	5.00	3.00	3.00
Blood plasma	3.00	1.50	.
Fat	5.24	4.38	0.46
L-Lysine	0.32	0.40	0.30
DL Methionine	0.20	0.22	0.14
L-Threonine	0.12	0.16	0.12
Dicalcium phosphate	1.48	1.74	1.34
Limestone	0.32	0.38	0.64
Salt	0.26	0.26	0.26
UGA vitamin premix[1]	0.26	0.26	0.26
Trace mineral premix[2,3]	1.00	1.00	1.00
Analysis[4]			
ME[5,6], Mcal/kg	3511	3500	3319
Crude protein, %	21.1	21.2	21.3
Lysine[6], %	1.76	1.66	1.32
Crude fat, %	7.1	6.9	3.3
Ash, %	6.3	6.2	5.6
Crude fiber, %	1.6	7.4	7.7
Phosphorus (total), %	0.74	0.82	0.74
P (avail)[6], %	0.55	0.45	0.37
Calcium, %	0.97	1.1	0.93

[a]Nursery diets were formulated to meet NRC requirements (2012). Phase 1 was fed for 7 d, Phase 2 for 14 d, and Phase 3 for 14 d.

[1]Vitamin premix: supplied per kg of diet: vitamin A (4,134 IU); vitamin D (1,653 IU); vitamin E (66 IU); vitamin K (3.3 mg); riboflavin (8.27 mg); niacin (49.6 mg); vitamin B_{12} (0.033 mg); pantothenic acid (27.6 mg); ADM Alliance Nutrition, Quincy, IL 62305

[2]Trace minerals (with the exception of Se and Mn), were supplemented at 20 mg/kg Cu ($CuSO_4$); 100 mg/kg Fe ($FeSO_4$); 100 mg/kg Zn (ZnO); 0.1 mg/kg I (KIO_3). Ground corn was used as the carrier for trace mineral premixes. Analyzed values[4] (mg/kg diet): 20.7 mg Cu; 398.7 mg Fe; 141.8 mg Zn. Iodine levels were too low to detect in the complete diet.

[3]Selenium was supplemented at two levels: 0.1 or 0.3 mg/kg (Na_2SeO_3); manganese was supplemented at 0, 12, or 24 mg/kg ($MnSO_4$) for each level of selenium, resulting in six different trace mineral premixes that were included at 1% of each basal pelleted diet. Analyzed Mn values were 28.3, 39.9, and 51.7 mg/kg for 0, 12, and 24 mg/kg supplemented Mn dietary treatments. Selenium values were too low to detect in the complete diet.

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

[5]Metabolizable energy

[6]Calculated value

Table 5-2: The effect of varying dietary levels of supplementary manganese and selenium on the growth performance characteristics of nursery pigs (*N* = 120)

Dietary Treatments[1] Selenium, mg/kg	0.1			0.3				*P*-values[2]		
Manganese, mg/kg	0	12	24	0	12	24	SEM	Mn	Se	Mn x Se
Body weight, kg										
d 0	5.12	5.33	5.19	5.19	5.26	5.24	0.7	0.216	0.455	0.914
d 35	18.81	20.23	19.60	19.02	20.77	6.64	0.7			
Growth Performance d 0-35										
ADG (g/pig/day)	393[a]	429[ab]	414[ab]	397[a]	445[b]	442[b]	13	0.007	0.140	0.661
ADFI (g/pig/day)	541	584	583	547	559	581	22	0.221	0.702	0.767
Gain:Feed	0.73	0.74	0.71	0.71	0.80	0.76	0.03	0.144	0.141	0.191

[a-b]LS Means within a row that do not share the same letter superscript differ significantly ($P < 0.05$).

[1]Dietary treatments were formulated in a 2 × 3 factorial design. Within each level of supplementary Se (0.1 and 0.3 mg/kg), there were three levels of supplementary Mn (0, 12, 24 mg/kg).

[2]*P*-values presented are for the main effects of manganese (Mn), selenium (Se), and the interaction of Mn and Se (Mn x Se).

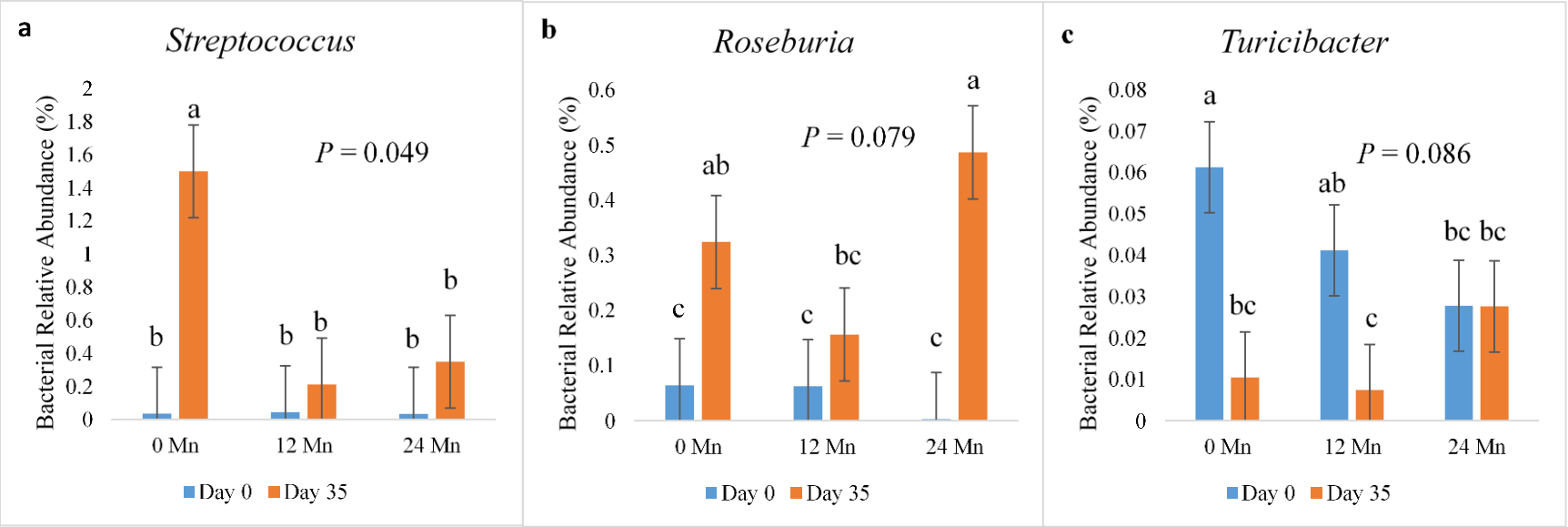


Figure 5-1. The effect of varying dietary manganese and selenium on bacterial relative abundance for the genera *Streptococcus* (**a**), *Roseburia* (**b**), and *Turicibacter* (**c**) in nursery pigs ($n = 30$ pens). *P*-values reported for Mn x Day effect. [abcd] indicates significant differences ($P < 0.05$).

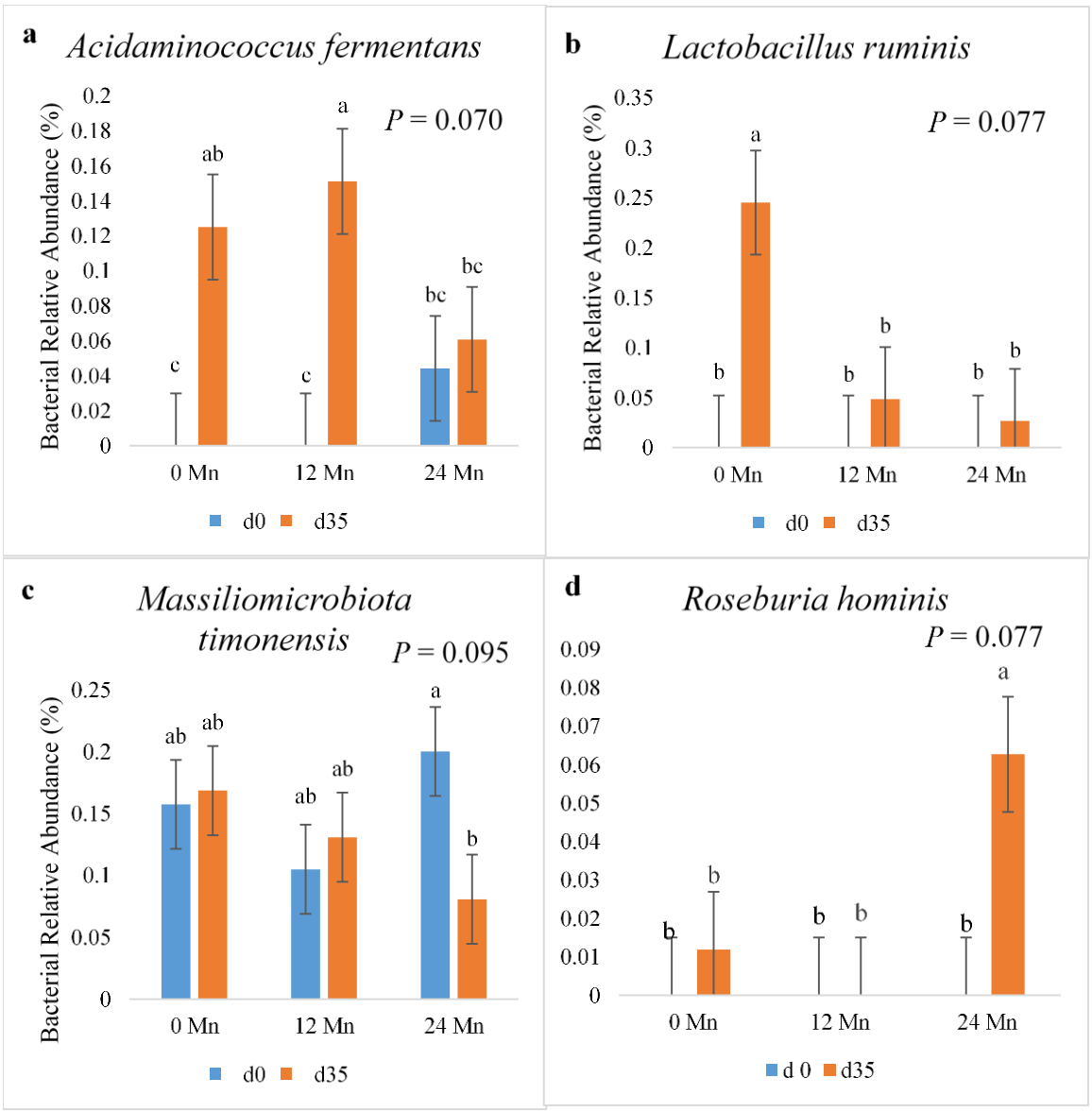


Figure 5-2. The effect of varying dietary manganese and selenium on bacterial relative abundance for the genera *Acidaminococcus fermentans* (**a**), *Lactobacillus ruminis* (**b**), *Massiliomicrobiota timonensis* (**c**), and *Roseburia hominis* (**d**) in nursery pigs ($n = 30$ pens). *P* values reported for Mn x Day effect. ^{abc} indicates significant differences ($P < 0.05$).

Supplemental Table 5-1: The effect of varying dietary manganese and selenium on volatile fatty acid profile and microbial diversity indices

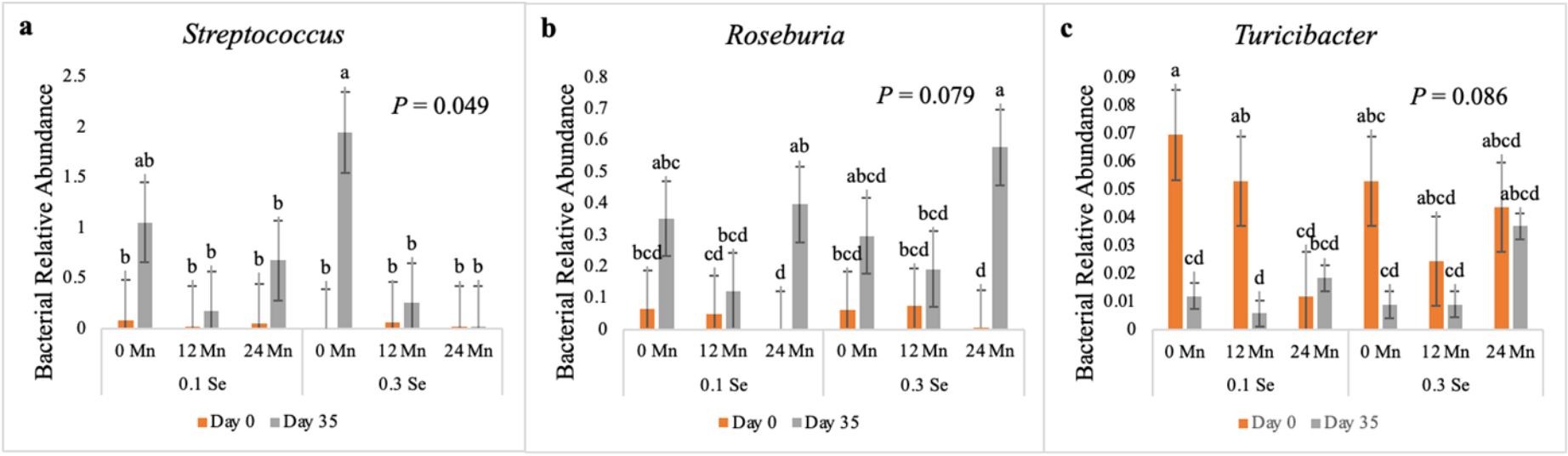
Dietary Treatments[1] Selenium, mg/kg	0.1			0.3				*P*-values[3]			
Manganese, mg/kg	0	12	24	0	12	24	SEM	Mn	Se	Mn x Se	Mn x Day
VFA Concentration											
Acetate (MP[2])											
d 0	76.06[a]	68.48[ab]	61.16[c]	70.64[ab]	74.00[ab]	72.61[ab]	2.4	0.214	0.064	0.004	0.045
d 35	55.14[cd]	55.71[cd]	54.19[d]	54.17[d]	55.65[cd]	57.79[cd]	2.4				
Propionate (MP[2])											
d 0	14.91	18.51	16.79	18.29	17.00	16.6	1.4	0.703	0.639	0.147	0.803
d 35	28.54	29.00	29.67	29.95	29.47	28.16	1.4				
Butyrate (MP[2])											
d 0	4.92	8.22	9.54	6.79	5.37	6.45	1.49	0.758	0.187	0.326	0.310
d 35	11.55	10.78	10.98	10.90	10.40	9.87	1.49				
Valerate (MP[2])											
d 0	4.11	4.79	12.52	4.28	3.64	4.34	1.74	0.094	0.071	0.326	0.093
d 35	4.78	4.51	5.16	4.97	4.48	4.19	1.74				
A:P											
d 0	5.40	3.75	3.87	4.19	4.40	4.87	0.46	0.499	0.721	0.083	0.437
d 35	1.92	1.89	1.81	1.78	1.86	2.04	0.46				
Total VFAs											
d 0	38.98	49.70	48.59	39.62	39.00	37.18	12	0.298	0.144	0.750	0.090
d 35	153.15	128.58	137.23	147.53	115.45	122.43	12				
Alpha diversity											
OTUs											
d 0	638	618	595	683	596	638	56	0.903	0.705	0.359	0.209
d 35	665	644	628	529	691	725					
Shannon											
d 0	7.84	7.51	7.79	7.87	7.73	7.85	0.21	0.739	0.561	0.333	0.544
d 35	7.79	7.72	7.49	7.42	7.70	8.00					
Evenness											
d 0	0.84	0.81	0.86	0.84	0.85	0.84	0.02	0.570	0.607	0.712	0.598
d 35	0.84	0.83	0.81	0.83	0.82	0.84					

[a-c]LSMeans within a row that do not share a superscript letter are significantly different from one another ($P < 0.05$).

[1]Dietary treatments were formulated in a 2 x 3 factorial design. Within each level of supplementary selenium (0.10 and 0.30 mg/kg) there were three levels of supplementary manganese (0, 12, and 24 mg/kg).

[2]Molar proportion

[3]P-values are for the main effects of manganese (Mn), selenium (Se), their interaction (Mn x Se), and the interaction of Mn with day (Mn x day).



Supplemental Figure 1. The effect of varying dietary manganese and selenium on bacterial relative abundance for the genera *Streptococcus* (**a**), *Roseburia* (**b**), and *Turicibacter* (**c**) in nursery pigs (n = 30 pens). *P* values reported for MnXDay effect. [abcd] indicates significant differences ($P < 0.05$).

CHAPTER 6

THE EFFECT OF DIETARY IODINE LEVEL ON THE GROWTH PERFORMANCE AND THYROID HORMONE LEVELS OF NURSERY PIGS

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Abstract

The nursery period is a critical growth period for the piglet and iodine (I) is a trace element that is critical for growth. This study examined the impact of evaluating dietary iodine supplementation on nursery pig growth performance and thyroid hormone concentrations. Weaned piglets were blocked by weight (N = 100; 5.37 ± 0.75 kg; 21 ± 3 d old) and sex (2 barrows and 2 gilts/pen). Pens (n = 5 pens/treatment) within a block were randomly assigned to dietary treatments: CON (0 mg/kg I), NRC (0.14 mg/kg), NRC10 (1.4 mg/kg), NRC100 (14 mg/kg), and NRC1000 (140 mg/kg). Diets were fed in three phases (P1 = d 0-10, P2 = d 11-21, P3 = d 22-35). Piglets and orts were weighed weekly and blood was collected on d 21 and 35 for analysis of circulating thyroid hormone concentration. Data were analyzed as a randomized complete block design via the GLM procedure of SAS. Overall average daily gain (d 0-35), was impacted by dietary treatment ($P < 0.05$). NRC10 (422 g/day) and NRC100 (412 g/day) pens gained more weight, on average, than CON pens (369 g/day; $P < 0.05$). NRC (396 g/day) and NRC1000 (407 g/day) did not grow differently from the other treatments ($P > 0.10$). On d 21, thyroid hormone concentrations did not differ in response to dietary treatments ($P > 0.10$), though thyroid hormone ratio tended to increase with dietary iodine concentration ($P < 0.10$). On d 35, thyroid hormone concentration increased with dietary iodine concentration ($P < 0.05$). Based on overall ADG and circulating thyroid hormone concentrations on d 35, a conclusion can be made that iodine supplementation levels above NRC recommendations have growth promoting effects on nursery pigs.

Keywords: iodine, nursery swine, trace minerals

6.1: Introduction

Iodine (I) is an essential trace mineral for humans and animals. It is required for the synthesis of the thyroid hormones (THs) triiodothyronine (T3) and thyroxine (T4) that are regulators of metabolic processes and metabolic rate (Flachowsky, 2007; Franke et al., 2008; Stepien and Huttner, 2019). Thyroid hormones regulate growth of an organism, brain development, and reproduction (Franke et al., 2008). Thyroxine has low affinity for nuclear TH receptors and is primarily a biologically inactive prohormone (Stepien and Huttner, 2019; Wassen et al., 2004). In humans, approximately 20% of T3 is secreted by the thyroid gland; most of the circulating T3 is derived from the de-iodination of T4 in peripheral tissues (Chanoine et al., 1993; Van Doorn et al., 1983). A severe deficiency of iodine can lead to hypothyroidism and related symptoms such as goiter, diminished growth, and physical and mental deformities (Andrews et al., 1948; Delange, 1994; Flachowsky, 2007; Kaufmann and Rambeck, 1998; Kotwal et al., 2007). Because iodine is necessary for thyroid hormone function in the animal, T3 and T4 are great indicators of iodine status.

The dietary requirement for iodine is not well established in pigs. In addition, the requirement can be increased in the presence of goitrogens, which are found in certain feedstuffs like peanuts, rapeseed, and soybeans (Kaufmann and Rambeck, 1998; McCarrison, 1933; Schöne, 1999). A concentration of 0.14 mg/kg iodine in a corn-soybean meal diet is sufficient to prevent thyroid hypertrophy in grower pigs (Cromwell et al., 1975), while 800 mg/kg iodine decreased growth, hemoglobin, and liver iron concentrations (Newton and Clawson, 1974). The range between an iodine concentration low enough to prevent a hypertrophic thyroid gland (0.14 mg/kg) and inducing a depression of growth (800 mg/kg) is quite wide, therefore, optimizing the dietary concentration with growth promotion would be ideal from an industry perspective. The

objective of this study was to determine the effect of dietary iodine on the growth performance and circulating thyroid hormone levels in nursery pigs.

6.2: Materials and methods

Care, handling, and sampling procedures were approved by the University of Georgia Institutional Animal Care and Use Committee prior to the start of this study (AUP#: A2019 08-038-R1).

6.2.1: Animal care and experimental design:

Weaned piglets (N = 100; 5.37 ± 0.75 kg; 21 ± 3 d old) were blocked by weight into pens of 4 with 2 gilts and 2 barrows per pen. Pigs from this herd were considered of good health status, were vaccinated as per typical commercial vaccine protocols, and were not allowed access to creep feed during lactation. Pens (n = 5 / treatment) were placed in an environmentally controlled nursery room and piglets consumed feed and water *ad libitum* (Large Animal Research Unit (LARU); University of Georgia, Athens, GA). Trace mineral premixes were formulated with no supplemented iodine (CON), 0.14 mg/kg (NRC), 1.4 mg/kg (NRC10), 14 mg/kg (NRC100), and 140 mg/kg (NRC1000). The premixes were included at 1% of the basal diet which was formulated to meet NRC requirements for all other minerals (NRC, 2012). The resulting diets were randomly assigned within each weight block as this was a randomized complete block design. Nursery diets were fed in three phases (Table 6-1): phase 1 from d 0-10, phase 2 from d 10-21, phase 3 from d 21-35. Piglet body weights and feed intake were recorded weekly. Animals were bled via orbital sinus (Dove and Alworth, 2015) on d 21 and 35 for circulating thyroid hormone concentrations. Piglets were not food-deprived prior to bleeding. Blood was collected in heparinized blood collection tubes (BD Vacutainer®, Franklin Lakes, NJ) and transported on ice to the laboratory. Blood plasma was aliquoted and frozen at -20°C

following centrifugation (2000 x *g*, 10 min, 4°C) and samples were sent to the USDA Meat Animal Research Center (Clay Center, NE) for analysis of circulating T3 and T4 concentrations.

6.2.2: Statistical analysis

All analyses were performed using pen as the experimental unit and weight as a blocking factor. Initial body weight (d 0) was utilized as a covariate for all growth performance parameters. Growth performance and thyroid concentration data were analyzed as a randomized complete block design via PROC GLM in SAS 9.4 (SAS Enterprise, Cary, NC). Comparisons between the least square means of the factor level comparisons were computed using the PDIFF option of the LSMEANS statement. Statistical significance was declared at $P < 0.05$ and trends were considered at $0.05 < P < 0.10$.

6.3: Results

6.3.1: Growth Performance

Dietary treatment tended to effect piglet body weights on d 10 and 21 of the nursery period (Table 6-2; $P < 0.10$). Body weights numerically increased from CON to NRC10 piglets, then decreased in NRC100 and NRC1000 piglets. Piglet body weights were affected by dietary treatment on d 35 ($P < 0.05$). NRC10 and NRC100 piglets weighed significantly more than CON piglets ($P < 0.05$), while these piglets' body weights did not differ from NRC or NRC1000 groups ($P > 0.10$). Average daily gain during phase 1 of the nursery (d 0 -10) tended to differ across dietary treatments ($P < 0.10$). Average daily gain tended to increase from CON to NRC10 groups, while NRC100 and NRC1000 groups gained numerically less weight per day. Dietary treatment did not impact growth during phase 2 (d 10-21) or phase 3 (d 21-35) of the nursery ($P > 0.10$). Overall ADG (d 0-35) was affected by dietary treatment ($P < 0.05$) and followed the same pattern as body weights on d 35. ADG increased across CON (369 g/day), NRC (396

g/day), and NRC10 (422 g/day), then decreased for NRC100 (412 g/day) and NRC1000 (407 g/day). NRC10 and NRC100 pens gained significantly more per day than CON pens ($P < 0.05$), while they did not differ significantly from NRC or NRC1000 pens ($P > 0.10$). Average daily feed intake was impacted by dietary treatment during phase 1 ($P < 0.05$), while it was not affected for the remainder of the study ($P > 0.10$). During phase 1, ADFI followed a similar trend to overall ADG with slight differences. NRC100 pens ate more feed per day (239 g/day; $P < 0.05$) compared to CON (195 g/day) and NRC1000 (202 g/day), while NRC and NRC10 pens did not eat differently than NRC100 pens ($P > 0.10$). Overall ADFI was not affected by dietary treatment ($P > 0.10$). Feed efficiency was not impacted by dietary treatment during any phase of the nursery study ($P > 0.10$). Overall feed efficiency statistically tended to be affected by dietary treatment ($P < 0.10$), but there was no true pattern observed across the treatments.

6.3.2: Circulating thyroid hormone concentrations

Thyroid hormone concentrations (T3 and T4) on d 21 were not impacted by dietary concentration of iodine (Table 6-3; $P > 0.10$). There was a tendency for T4:T3 to numerically increase in response to increasing dietary concentration of iodine on d 21 ($P < 0.10$).

On d 35, there was a difference in T4 concentration among dietary treatments ($P < 0.05$). NRC10 pens (58.4 nmol/L) had significantly increased T4 concentrations compared to CON (50.9 nmol/L) and NRC (50.4 nmol/L) pens ($P < 0.05$). NRC100 pens did not differ in T4 concentration compared to the other treatment groups (53.9 nmol/L; $P > 0.10$). NRC1000 pens did not differ in T4 concentration (50.4 nmol/L; $P > 0.10$) compared to CON, NRC10, and NRC100 pens, but did differ from NRC pens ($P < 0.05$). On d 35, circulating T3 hormone concentration was also affected by dietary iodine concentration ($P < 0.05$). CON (1.39 nmol/L) and NRC10 (1.37 nmol/L) pens had significantly increased ($P < 0.05$) circulating T3

concentration compared to NRC (1.16 nmol/L) and NRC100 (1.14 nmol/L). The T3 concentration in NRC1000 pens did not differ among the other dietary treatments ($P > 0.10$). On d 35, T4:T3 ratio was affected by dietary treatment ($P < 0.05$). CON (45.2), NRC100 (49.6), and NRC1000 (48.3) pens had a significantly increased ($P < 0.05$) T4:T3 ratio compared to CON pens. NRC10 pens did not differ significantly among the other dietary treatments ($P > 0.10$).

6.4: Discussion and conclusion

Based on the present data, it was evident that dietary supplementation of iodine did have significant impact on animal growth and circulating thyroid hormone concentrations. It is well known that iodine is a critical cofactor for the synthesis of the thyroid hormones T3 and T4 (Li et al., 2013, 2012). These hormones play a pivotal role in an animal's energy metabolism, growth, and brain development (Stepien and Huttner, 2019). As mentioned previously, iodine-deficient diets (no supplemented iodine) have a negative effect on growth (Flachowsky, 2007), and that was reflected in this data with CON pens gaining significantly less per day, on average, than diets supplemented with 10 and 100 times the recommended iodine concentration. The decreased circulating T4 concentration and T4:T3 ratio in CON pens on d 35 supported suppressed ADG (d 0-35) in CON pens. Although it does not appear in the present study that 1000 times the NRC recommendation (140 mg/kg) was detrimental to the animal, such as the side effects observed when 800 mg/kg was supplemented (Newton and Clawson, 1974), there was still a slight decrease in overall ADG observed. Thyroid hormone concentrations do not necessarily reflect the growth data in the NRC1000 pens though. NRC1000 pens did not experience significant differences in thyroid hormone concentrations compared NRC10 pens.

Preliminary recommendations of dietary iodine could be made from this study alone. If one were optimizing piglet growth, the recommendation would be somewhere between 10 and

100 times the NRC recommendation for iodine. If one were recommending based on optimizing thyroid hormone concentrations, the recommendation would be to supplement at least 10 times the NRC recommendation. Ultimately, this decision would come down to the producer and what is most beneficial for their operation. It would be of great benefit to repeat and research this topic more to approach a narrower range of what concentration of dietary iodine is optimal for the animal from a growth perspective and physiological perspective. In addition, experimental repetition would be beneficial to parse out whether our data was unique to this particular trial or whether the trends are observed across additional trials and give a stronger argument to the dietary iodine recommendation for optimal growth and iodine status of nursery pigs.

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Table 6-1: Basal diet composition and analysis

	Phase 1[a]	Phase 2[a]	Phase 3[a]
Ingredient, %			
Corn	24.14	43.48	62.83
Soybean meal	18.64	27.01	32.34
Oats	10.00	5.00	.
HP 300	10.00	5.00	.
Whey	15.00	5.00	.
Lactose	10.00	5.00	.
Fish meal	5.00	3.00	.
Fat	3.35	2.90	1.33
Lysine	0.40	0.30	0.35
Methionine	0.16	0.11	0.12
Threonine	0.11	0.06	0.05
Limestone	0.74	0.85	0.84
Dicalcium phosphate	0.64	0.91	1.55
Salt	0.20	0.25	0.35
Vitamin premix[1]	0.25	0.25	0.25
Antibiotic	1.00	0.50	.
Nutrient Content[2]			
ME[3], kcal/kg	3400	3400	3330
Crude Protein, %	23.19	23.06	21.36
Lysine, SID %	1.50	1.35	1.20
TSAA, SID %	0.82	0.74	0.68
Tryptophan, SID %	0.25	0.24	0.23
Threonine, SID %	0.88	0.79	0.73
Calcium, %	0.85	0.80	0.80
Phosphorus, STTD	0.45	0.40	0.40

[a]Nursery diets were formulated to meet NRC requirements (2012). Phase 1 was fed for 10 d, Phase 2 for 11 d, and Phase 3 for 14 d.

[1]Vitamin premix: supplied per kg of diet: vitamin A (4,134 IU); vitamin D (1,653 IU); vitamin E (66 IU); vitamin K (3.3 mg); riboflavin (8.27 mg); niacin (49.6 mg); vitamin B_{12} (0.033 mg); pantothenic acid (27.6 mg); ADM Alliance Nutrition, Quincy IL 62305

[2]Mineral premix: supplied per kg of diet: copper (20 mg/kg Cu from $CuSO_4$); zinc (100 mg/kg Zn from ZnO); iron (100 mg/kg Fe from $FeSO_4$); manganese (24 mg/kg from $MnSO_4$); selenium (0.3 mg/kg Se from Na_2SeO_3).

[3]Iodine was supplied at five concentrations from KI: 0 mg/kg (CON), 0.14 mg/kg (NRC), 1.4 mg/kg (NRC10), 14 mg/kg (NRC100), and 140 mg/kg (NRC1000). Concentrations were too low to detect in the complete diet.

[3]Calculated value

[4]Metabolizable energy

Table 6-2: The effect of dietary iodine on the growth performance of nursery pigs (N = 100)

Treatment:	CON	NRC	NRC10	NRC100	NRC1000		
Iodine, mg/kg	0	0.14	1.4	14	140	SEM	P-value[1]
Body Weight, kg							
d 0	5.37	5.40	5.37	5.33	5.39	0.04	0.94
d 10	6.49	6.69	6.86	6.81	6.63	0.11	0.09
d 21	10.24	10.88	11.37	11.13	10.84	0.28	0.09
d 35	18.04[b]	18.90[ab]	19.87[a]	19.68[a]	19.31[ab]	0.53	0.05
ADG, g/d							
d 0-10	117	137	154	149	131	12	0.09
d 10-21	344	387	413	394	388	21	0.13
d 21-35	581	591	620	615	620	28	0.13
d 0-35	369[b]	396[ab]	422[a]	412[a]	407[ab]	15	0.03
ADFI, g/d							
d 0-10	195[c]	226[ab]	226[ab]	239[a]	202[bc]	11	0.03
d 10-21	542	618	630	671	616	25	0.36
d 21-35	872	946	978	953	911	32	0.83
d 0-35	575	637	654	660	616	18	0.35
G:F							
d 0-10	0.60	0.61	0.68	0.62	0.64	0.04	0.32
d 10-21	0.64	0.63	0.66	0.59	0.63	0.02	0.17
d 21-35	0.67	0.63	0.63	0.65	0.68	0.02	0.21
d 0-35	0.64	0.62	0.64	0.63	0.66	0.02	0.09

[a-c]LSMeans within a row that do not share a letter superscript differ significantly ($P < 0.05$).

[1]The P-value presented is for the dietary treatment alone which differs by iodine concentration.

Table 6-3: The effect of dietary iodine on thyroid hormone concentrations on d 21 and d 35 in the nursery

Treatment:	CON	NRC	NRC10	NRC100	NRC1000		
Iodine, mg/kg	0	0.14	1.4	14	140	SEM	*P*-value[1]
d 21							
T4, nmol/L	58.5	60.4	64.7	66.4	61.0	2.9	0.30
T3, nmol/L	1.65	1.63	1.65	1.57	1.44	0.08	0.30
T4:T3	36.3	38.4	40.1	43.7	43.8	2.2	0.08
d 35							
T4, nmol/L	50.9^{bc}	50.4^{c}	58.4^{a}	53.9^{abc}	57.1^{ab}	2.3	0.05
T3, nmol/L	1.39^{a}	1.16^{b}	1.37^{a}	1.14^{b}	1.23^{ab}	0.07	0.03
T4:T3	38.4^{b}	45.2^{a}	43.7^{ab}	49.6^{a}	48.3^{a}	2.2	0.009

$^{a-c}$LSMeans within that do not share a letter superscript differ significantly ($P < 0.05$).

[1]The *P*-value presented is for differences detected between dietary treatments alone, which differ by iodine concentration.

CHAPTER 7

CONCLUSION

In summary, though trace minerals make up such a small part of a pig's diet relative to other essential nutrients, they can have a large impact on the growth and physiology of pigs. In this series of experiments, it was determined that maternal Mn supplementation had a positive impact on piglet birthweights, growth, and weaning weights. In addition, analyzed immune marker parameters in the sow were impacted by dietary Mn. Manganese supplementation in nursery pigs was shown to: improve average daily gain, affect plasma MnSOD activity, have potential antimicrobial effects on harmful pathogens, and foster the growth of healthy gut microbes. Increasing supplemental iodine above NRC recommendations was shown to have impacts on nursery piglet growth and thyroid hormone concentrations. Additional research is needed to narrow down the recommendations of these particular minerals. This research can serve as a stepping stone for future researchers in the swine nutrition industry.

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