EFFECTS OF WATER SOLUBLE ZINC ON GROWTH PERFORMANCE, IMMUNE RESPONSE, AND TISSUE ZINC TRANSPORTERS IN NURSERY PIGS

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Abstract: Regardless of adding pharmacological levels of dietary ZnO and $CuSO_4$, supplementing water soluble Zn amino acid complex (ZnAAC) via drinking water can offer excellent Zn bioavailability to support growth and immune function of nursery pigs. In the first experiment, effects of LPS injection on the immune response and tissue Zn transporter over 24-h following challenge were evaluated. Data revealed that rectal temperature and serum TNF-α increased ($P < 0.05$) within 3-h following LPS. While serum Zn and Cu concentrations decreased after injection until h 6 and h 12 ($P < 0.01$), respectively. Serum C-reactive protein (CRP) linearly increased over time ($P = 0.10$). Upregulations of hepatic IL-6, ZIP14, and MT-1 gene expressions were strongly correlated ($P \leq 0.0017$) and detected within 3-h ($P < 0.0001$) after LPS. Duodenal ZIP4 and MT-1 genes were upregulated ($P < 0.05$) at h 12 and 24, respectively. In the second experiment, pigs drinking titrated levels of water soluble ZnAAC (0, 20, 40, 80, and 160 mg Zn/L water) throughout a 42-d nursery period improved ADG ($P < 0.05$) and G:F ($P \leq 0.05$) when supplemented up to 80 mg Zn/L water. Estimation of optimal ZnAAC doses for ADG and G:F using non-linear broken-line models were 74 and 70 mg Zn/L, respectively. In the third experiment, the same ZnAAC treatments were supplemented to pigs with an addition of a single LPS challenge and data collection at h 0, 3, and 12 following LPS. Data reported that pigs drinking titrated levels of ZnAAC had lower serum CRP ($P = 0.04$) at baseline, and mitigated febrile response by decreasing rectal temperature ($P = 0.005$) and serum TNF-α ($P = 0.02$) at h 3 following LPS when supplemented up to 80 mg Zn/L water. LPS injection decreased serum Zn and Cu concentrations of all pigs ($P < 0.0001$). Hepatic IL-6, ZIP14, and MT-1 gene expressions were upregulated and correlated ($P \leq 0.0001$), while only duodenal IL-6 ($P \leq 0.0001$) was upregulated at h 3. Results of these experiments indicated that pigs drinking water soluble ZnAAC improved growth performance and mitigated febrile response under hepatic Zn homeostasis during inflammation.

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CHAPTER I

INTRODUCTION

Zinc (Zn) is an essential trace mineral that plays an important role in promoting growth performance and supporting the proper immune function of pigs. Zn is needed for structural components and the co-factor function of a large number of proteins including enzymes. At the cellular level, Zn activates signal transduction, transcription, and replication (Akira and Takeda, 2004; Murakami and Hirano, 2008). Thus, it is the most widely used metal in biology (Vallee and Falchuk, 1993). Consequences of Zn deficiency have been reported as stunting of growth and profound effects on host defense mechanisms (Kloubert et al., 2018; Wastney et al., 2018). Although swine feed ingredients, such as cereal grains and animal proteins contain Zn, the availability for intestinal absorption is likely inadequate. Therefore, supplementing additional Zn to meet the requirement is necessary to sustain life. The Swine National Research Council (NRC, 2012) recommends daily Zn intake ranging from 26.6 to 72.4 mg/d (100 mg Zn/kg of diet) for nursery pigs. However, those levels are to meet the requirement during normal circumstances. To keep up with the improved genetic lines and health challenge situations, Zn could be required in greater levels for its multifunction in the body.

In commercial settings, the use of pharmacological ZnO levels (2,000-3,000 mg/kg) are used to minimize the post-weaning lag of health and growth performance caused by various factors, including post-weaning diarrhea. However, the pharmacological ZnO doses are suggested to be used for short periods after weaning for a specific mode of action. Supplementing high Zn doses longer-term could lead to pathological effects (Zhou et al., 2017), and increase fecal Zn excretion as waste (Buff et al., 2005). Therefore, it is necessary to manage the Zn dose and feeding protocol to maximize response benefits.

It is logical to accept that the more relative bioavailability value (RBV) of Zn, the lower supplemental dose is needed to reach a certain amount of absorption, thus reducing Zn excretion. In the past years, studies have reported higher estimation in RBV from organic Zn sources (>100% RBV) (Buff et al., 2005; Hill et al., 2014). Therefore, using an organic Zn source with excellent RBV could prevent an over-formulation of low RBV Zn form, such as ZnO, to achieve equal growth and immune response of pigs (Carlson et al., 2004).

Given most of the studies have evaluated the effects of in-feed Zn, supplementing via drinking water could be a potential means to deliver Zn for nursery pigs. After 5-d post-weaning, pigs show a consistent parallel pattern between feed and water intake (McLeese et al., 1992) with a strong positive association, approximately a 1:3 ratio (Brooks et al., 1984). Escuredo et al. (2016) reported that supplementing organic acids via drinking water offered a higher level of acid intake than adding in-feed when administered at an equal amount. Furthermore, supplementing through the water system

is flexible for daily dose adjustment according to the needs of the herd, i.e., during stress or disease challenges.

Nursery pig management is one of the most challenging practices across all stages of swine production. Arising stress in this transitional period could affect the well-being, growth performance, and immune function of pigs to fight off invading pathogens. In global swine production, *Escherichia coli* infection causes significant impacts on morbidity and mortality of nursery pigs (Sun and Kim, 2017). Evaluation of inflammatory response to nutritional interventions can be induced by administrating endotoxin of *E. coli* in pigs. In the research setting, lipopolysaccharide (LPS) of *E.coli* has been widely used as a reproducible model to study innate immune response, which could be activated at concentrations below 1 nM of LPS (Ulevitch and Tobias, 1999). When body Zn was deficient, proinflammatory response increased significantly (Wong et al., 2015). On the other hand, Zn supplementation has been shown to mitigate the deleterious immune response. Therefore, the interplay of Zn homeostasis and inflammation could demonstrate mechanistic response and changes during LPS model induced systemic infection.

CHAPTER II

REVIEW OF LITERATURE

1. Nursery pig production

a. Factors affecting feed intake

In modern swine production, newborn piglets are usually weaned and removed from the sow herd at about 3-4 wk of age. The goal of early weaning is to achieve more efficient sow and growing herd production and maximize economic return. Though, this early weaning event may impact animal welfare, especially on the piglets. Many stressors, i.e., handling and transportation, different littermates and environment, and different forms and sources of food could disrupt the growth rate and health status of pigs.

A significant impact of transportation stress derived from tight space allowance, longer duration, and severe season of transportation is one of stress factors affecting feed intake after weaning. Transported weaned pigs spend more time to rest than non-transported ones, but longer transport duration increases motivation of pigs to eat and drink from longer water and feed deprivation than the short duration transport

(Lewis and Berry, 2006; Wamnes et al., 2008). Studies found that the smaller the group size of weaned pigs the more motivation to form linear hierarchy was established, whereas larger group size presented more of a complex hierarchy with the similar outcome of aggressive actions among members (Puppe et al., 2008; Fels et al., 2014). However, providing sufficient availability to feed and water could limit the duration of fighting (Fels et al., 2014).

Complexity of stress factors commonly leads to a transient drop in feed intake and impacts small intestinal structure and function reducing growth rate (Lallès et al., 2004). Digestive physiology contributing to growth performance may be limited during early weaning. It is also predicted that pigs would take approximately 2 wk post-weaning to be fully recovered from the first week of decreased metabolizable energy (ME) intake (Sève, 2000). According to Burrin and Stoll (2003), post-weaning changes in feed intake could be divided into two phases: acute phase and adaptive phase. The acute phase occurs for 5 d after weaning, while the adaptive phase occurs subsequently (Figure 2.1). In addition, digestive enzyme activities of weaned pigs increase with age and with an intake of solid diets (Lindemann et al., 1986).

It is even more difficult to satisfy post-weaning performance if piglets were weaned with low weaning weights. A study from Campbell (1990) provided a prediction equation on the relationship of weaning weight of pigs at 28 d of age (**W**), and the length of time taken to grow to 20 kg BW (**T**) as follows: **T** = 52.1 (± 1.69) – 3.39 (± 0.224) **W**. Data supported that small piglets had a disadvantage in survival rate than the heavier littermates (Milligan et al., 2002). On the opposite, the impact of low weaning weight on

lifetime growth performance could be resolved by feeding high density, high digestibility, or “complex” diets (Collins et al., 2017).

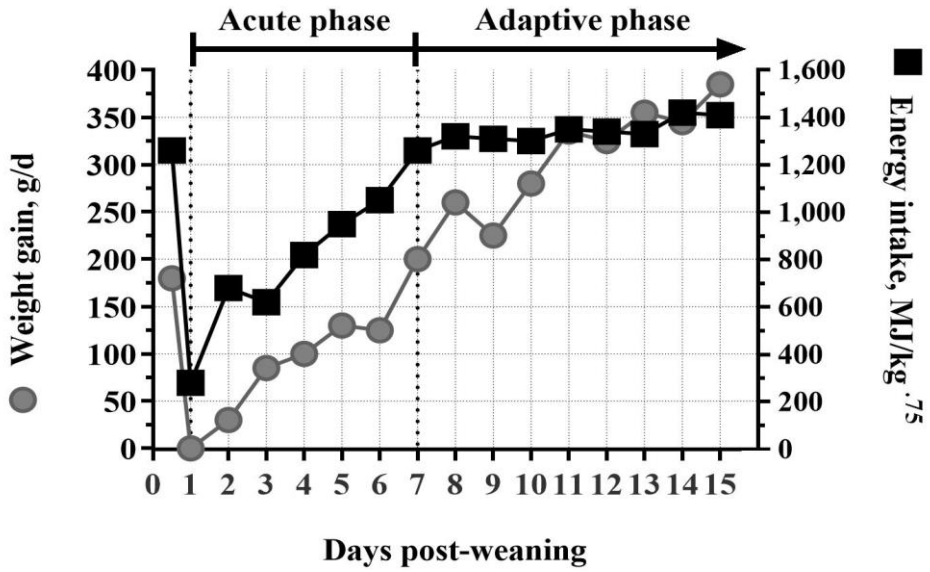

Figure 2.1. Development phases in feed intake of post-weaning pigs (adapted from Burrin and Stoll (2003) and De Lange et al. (2010)).

b. Post-weaning diarrhea

An abrupt change from different forms of diets and a decrease in dietary intake affects small intestinal architecture after weaning. The effect impairs absorptive capacity due to shortening of villi or villous atrophy by 25 to 30% within the first 24 h, and elongating crypt occurred over the first-week post-weaning (Hampson, 1986; Pluske et al., 1997). Besides, switching diets from liquid or semi-solid to solid form could rapidly change intestinal microbiota and lead to species imbalance, which modifies gut immunity. On the other hand, pigs are at the end of lactogenic immunity towards the weaning period, and they are not fully immunocompetent until about 4 wks of life (Poonsuk and Zimmerman, 2018). Therefore, post-weaning pigs are highly susceptible to infection. With the aforementioned factors, pathogenic bacteria such as *Escherichia coli*

could opportunistically proliferate along the intestinal tract, release toxin causing diarrhea, and worsen growth and health conditions.

An attempt to prevent post-weaning diarrhea (PWD) caused by *E. coli* infection was to use prophylactic antibiotic treatments. However, this practice has been ruled out of swine production due to concerns about antibiotic resistance found in some swine bacterial strains that cost substantial losses to producers and affected consumer's health. Relatively, the use of sub-therapeutic doses of antibiotics as antibiotic growth promoters (AGP) was banned from the global swine industry years ago. Feeding low-protein with ideal amino acids pattern for 7-10 d post-weaning could reduce PWD of pigs fed antibiotics-free diets (Heo et al., 2008). Manipulation of non-digestible carbohydrate composition could modulate PWD (Pluske et al., 2002). Supplementation of ZnO improved intestinal morphology by increasing villous height to crypt depth ratio subsequent decrease in intestinal permeability, reduce PDW occurrence, and increase feed intake of weaned pigs (Song et al., 2015).

Therefore, strategies to minimize post-weaning diarrhea and alleviate low feed intake have been focused on implementing sophisticated nutritional management. In conjunction with other nursery management strategies, the goal is for early-weaned pigs to have a smooth transition from the immediate post-weaning lag throughout the nursery period.

c. Importance of water consumption

Piglets gradually learn to locate and access food and water after being placed in a new environment. They have to adapt to the new source to satisfy their hunger and thirst

to maintain homeostasis and meet body requirements. After weaning, pigs consumed water more than eating feed according to the prediction equations of average daily water intake in liters (Y) and average daily feed intake in kg (X): $Y = 0.149 + 3.053X \pm 0.522$ (Brooks et al., 1984). The ratio of water to feed consumed is presented in Figure 2.2. However, there are factors affecting water intake as described in Table 2.1.

Since water is one of the six essential nutrients and constitutes about 70% of fat-free wet weight, it is offered *ad libitum* to pigs at any production stage. Also, pigs are diurnal drinkers as long as they are comfortable and healthy. Implementing drinking water feed additives could be the means to improve nutrient intakes, subsequently enhancing growth performance and health conditions. A study from Kaewtapee et al. (2010) reported an improved growth performance and intestinal benefits in supplementing liquid methionine via drinking water of nursery pigs. A high water-soluble phosphorus supplement improved phosphorus efficiency by increasing retention and reducing excretion (Liu et al., 2020). Adding water-soluble globulin (spray-dried animal serum, globulin, and plasma) through the water source of weanling pigs improved ADG and G:F in a 35-d nursery period (Steidinger et al., 2002).

Furthermore, daily observation for an individual pig in a larger herd production system could be limited by the number of caretakers and the amount of time spend per pig. On some occasions, the judgment of caretaker could be subjective to make further decisions. Monitoring daily water intake could offer verifiable information and time-effective means to observe the general herd health and well-being of pigs.

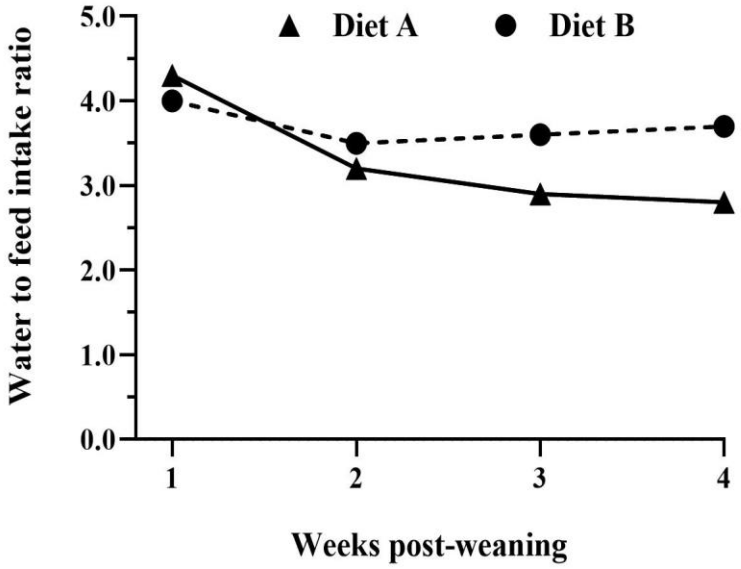

Figure 2.2. Water to feed intake ratio (g of water per 1 g of feed) of post-weaning pigs weaned at 21 d of age fed two commercial diets (adapted from Brooks et al. (1984)).

Table 2.1. Factors affecting water intake of post-weaning pigs (adapted from Brooks and Tsourgiannis (2003)).

Factors	**Outcomes**
Warm or cold environment and water	Warm water encourages intake in a cold environment, while cold water encourages intake at high ambient temperatures
Drinker design and positioning	Drinkers with a different type of water nipple affected water intake and wastage. Drinkers should be placed at the correct height, angle, and warm area of the pen.
Rate and velocity of water	The water delivery rate at 700 ml/minute better-improved growth performance than the 350 ml/minute rate because of increased water and feed intakes. Pigs would spend more time drinking at a lower water rate.
Taste of water	Pigs made more visits to drink the water supplemented with flavor or sweetener but resulted in less total water intake within the first-week post-weaning.
Disease or stress	Decrease water intake

2. Zinc properties and functions

Zinc, the 24th abundant metal element on earth (0.004%), is a robust transitional mineral of the periodic table with its atomic number 30 and atomic weight of 65.38 (Solomons, 2013). Zn does not participate in redox reactions because it is redox-inert, unlike other first-row transition metals that undergo the redox reaction and change their states, e.g., iron (Fe^{+}, Fe^{2+}, and Fe^{3+}), manganese (Mn^{+}, Mn^{2+}, and Mn^{3+}), and Cu (Cu^{+}, Cu^{2+}, and Cu^{3+}) (Williams, 1987). Instead, the Zn ion (Zn^{2+}) accepts a pair of electrons as a Lewis acid meaning that it forms complex ions or compounds in its +2 oxidation state, which makes it ideal as a metal cofactor (Butler, 1998).

Zn ions can form Zn salts with anionic molecules (e.g. O^{2-}, Cl^{-}, and SO_4^{2-}), complex with small molecules (e.g. acetate, and gluconate), or chelate with organic molecules (e.g. amino acids, polypeptide). Zn oxide is a white powder and likely insoluble in neutral pH water. Zn sulfate is a blue granule and highly water-soluble. Solomons (2013) reported that mineral chelates with amino acids are the most presentable form for human oral supplementation.

Zn is found in various organs and tissues. At cellular levels, it is found in the cytosol (50%), nucleus (30-40%), organelles, specialized vesicles, and the remainder is contacted with cell membranes (Vallee and Falchuk, 1993). With its abundant presence, Zn is in the right proximity for cooperation with cellular enzymes, such as metalloenzymes, and metalloproteins (Krężel and Maret, 2016). Therefore, the roles of Zn are categorized into three functions: catalytic, structural element, and regulatory (Table 2.2).

Table 2.2. Three functions of Zn (adapted from Kambe et al. (2015)).

Function of Zn	Details	Examples
Catalytic factor	Enzyme cofactor in six main enzyme classes	1. Oxidoreductase: alcohol dehydrogenase 2. Transferase: only 34% of this class are catalytic, not major 3. Hydrolase: carboxypeptidase, alkaline phosphatase 4. Lyase: carbonic anhydrase 5. Isomerase: phosphomannose isomerase 6. Ligase: only 39% of this class are catalytic, not major
Structural component	Zn fingers	Transcription factor Classical Zn finger motif Interprotein binding mediator
Signaling mediator	Extracellular signaling	Neuromodulating functions in central nervous system
	Intracellular signaling	Second messenger functions Zn flux Zn wave

a. Enzyme catalyst

Zn is indispensably required as a cofactor of more than 300 enzymes and 2,500 transcriptional factors (Vallee and Auld, 1990; Edelstein and Collins, 2005). In other words, Zn is involved with carbohydrate, protein, and energy metabolism (Coleman, 1992). Zn is required at the active site of the enzyme for catalytic function. Enzymes become inactive when the Zn atom is removed by either a chelating agent or others. A unique property for the catalytic Zn site, when compared with the structural Zn site, is the water molecule attach to the Zn-binding polyhedron (McCall et al., 2000). This component is crucial for ionization and polarization for the enzyme catalytic activity. The first Zn-dependent enzyme was identified as carbonic anhydrase in red blood cells (Keilin and Mann, 1939). Carbonic anhydrase is the Zn metalloenzyme (Zn-dependent enzyme)

and it functions in acid-base balance and gastric HCO_3^- secretion. Its activity declines with Zn deficiency while adding Zn can restore the activity (King, 2011). Other examples are enzyme hydrolases, e.g., carboxypeptidase A, alkaline phosphatase, and phospholipase C, which all require Zn atoms to perform their catalytic reaction (Andreini and Bertini, 2012). Overall Zn has been recognized for all six enzyme classes defined in the Enzyme Commission (EC) system.

b. Structural component

For the role of maintaining structure, Zn is coordinated by tetrahedral amino acid side chains. A structural role of Zn was discovered as Zn fingers, in which the molecules consist of 4 cysteine or histidine amino acids. Cysteine is frequently found as an amino acid ligand to the Zn molecule in this role followed by histidine (Andreini and Bertini, 2012). The tetrahedral Zn complexes are highly stable to enable the protein to achieve their functions (McCall et al., 2000). An example of a Zn role in maintaining enzyme structure is Cu-Zn superoxide dismutase 1 (SOD1), the anti-oxidant enzyme that breaks down superoxide radicals to defend against cell damage, where Zn is crucial to maintain the enzyme structure. Enzyme transferases and ligases such as galactose-1-phosphate uridylyltransferase mainly require Zn to stabilize their structure (Geeganage and Frey, 1999). Later Zn fingers were found to involve in cellular signaling or transcription.

c. Regulatory or signaling

Physiological roles of Zn have shown a significant cell communication for not only in the brain but in other tissues. Zn signals are described in three types: synaptic signal (neuromodulator), transmembrane signal (Zn translocation through plasma membrane), and intracellular Zn signal (Zn release within cells) (Frederickson and Bush, 2001). General pathways to create intracellular Zn ion movements are vesicular exocytosis via Zn release from reactive species modifying protein, and Zn release from vesicular stores (King, 2011). Intracellular Zn movement initiates cellular signals as Zn acts as a second messenger comparable to Ca (Yamasaki et al., 2007). The movement includes Zn transport through the plasma membrane, Zn move in and out of cell organelles, or binding proteins such as metallothionein (MT) and calprotectin. Intracellular Zn signaling can be classified according to the time onset of occurrence (Figure 2.3).

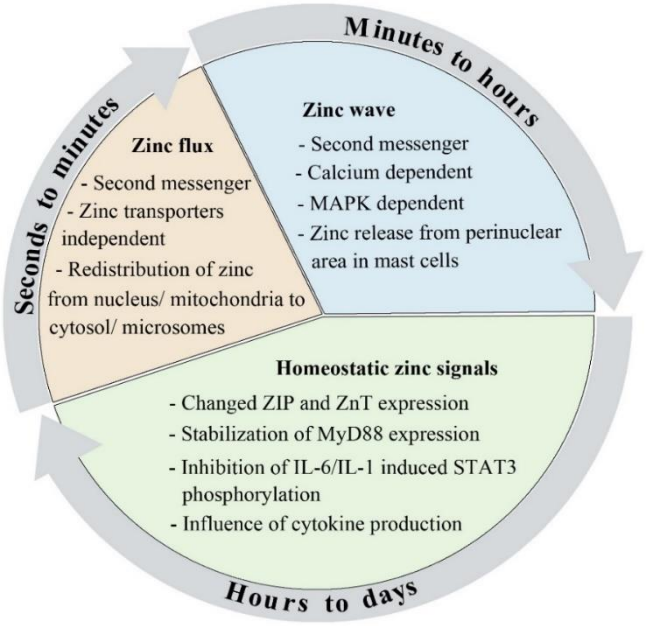

Figure 2.3. Classification of intracellular Zn signals by time scale of response (adapted from Maywald et al. (2017)).

3. Zinc absorption

Zn salts or complexes are required to dissolve with intraluminal liquid substances for absorption. The implication of Zn absorption is involved with solubility in the gastrointestinal lumen. Different Zn sources are not equal in solubility, i.e., Zn oxide and Zn carbonate are poorly soluble and absorbed by the enterocytes (Allen, 1998). When Zn passes along to the stomach, intragastric pH enhances Zn dissociation and stomach absorption, but it accounts for a small portion (Sturniolo et al., 1991). Martin et al. (2013) reported an upregulation of Zn transporter ZIP11 expression in the gastric mucosa of mice responding to dietary Zn intake. However, the expression was downregulated during dietary Zn restriction. Furthermore, digesta retention and gastric emptying time in pigs are as short as 1 and 3 h, respectively (Wilfart et al., 2007; Strathe et al., 2008).

Zn is absorbed along the small intestinal tract. Duodenum contributes to the largest amount of Zn absorbed from its high Zn concentration after a meal (King et al., 2015), while the highest rate of absorption is in the jejunum of the small intestine (King and Cousins, 2014). Digestive Zn can be either from food components or endogenous secretion and regulated by two mechanisms: passive and carrier-mediated transports, which the latter is satiable and the main route of transport. Passive transport or paracellular transport process occurs when large mineral concentration gradients create a driving force to push through the tight junction into the intestinal space (Nellans, 1991). Under normal physiological conditions, the body contains only 0.1% of serum Zn, 80% of which is loosely bound by albumin, and 20% bound by macroglobulin (Barnett et al., 2013) (Figure 2.4). Net Zn absorption is controlled by homeostasis depend on individual Zn status and dietary intake.

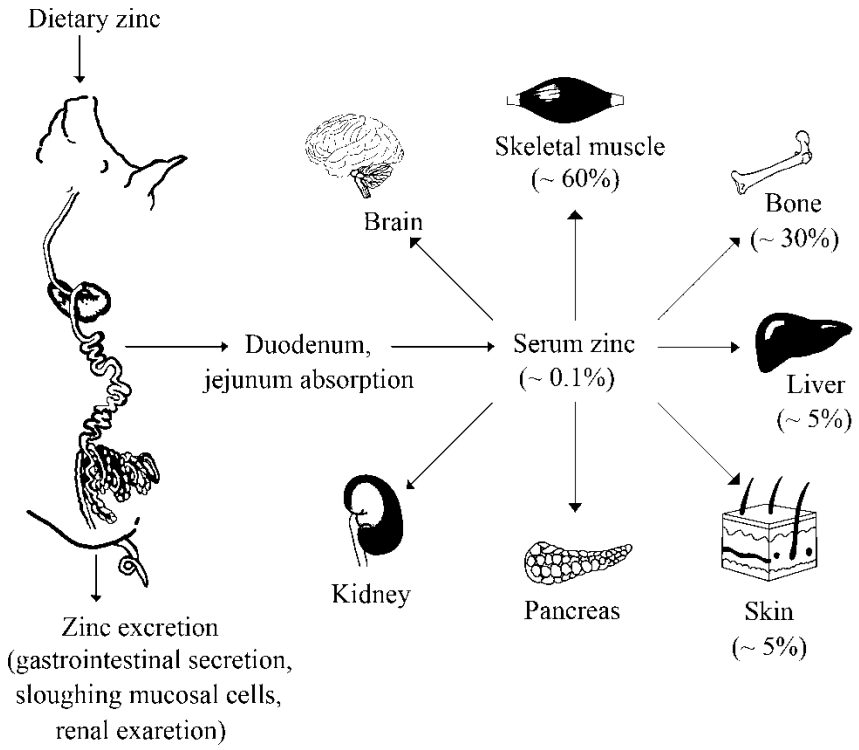

Figure 2.4. Body Zn distribution and excretion (adapted from Kambe et al. (2015)).

a. Factor affecting Zn absorption

Bioavailability of Zn can be affected by extrinsic and intrinsic factors. The extrinsic factors include dietary component of phytate, mineral levels, and protein quantity and quality, whereas the intrinsic factors are the nature of chemical Zn form, body Zn status, and physiological status. While the presence of Zn inhibitors is the most common factor influence Zn uptake, efforts to eliminate or improve nutritional strategies related to those causative factors may enhance Zn bioavailability and absorption.

Suboptimal Zn status can occur due to various factors including inadequate Zn intake and the presence of inhibitors of Zn absorption. Zn absorption depends on Zn intake, however, increasing Zn consumption reduces absorptive efficiency of Zn

(Lonnerdal, 2000). For example, when pigs are marginally Zn sufficient, they absorb greater Zn from the diets. In contrast, when pigs are overfeeding with dietary Zn, Zn absorption is reduced. This may be regulated by the presence of major Zn transporter ZIP4 that is responsible for Zn absorption in the small intestine. Expression of ZIP4 on the apical membranes of enterocytes is repressed when dietary Zn is sufficient (Dufner-Beattie et al., 2003).

A study from Sandström and Cederblad (1980) exhibited that meals based on animal protein intake enhanced the efficacy of Zn absorption. However, Zn absorption from soybean meal diet did not differ from an animal protein meal diet. Also, casein in milk has been reported to reduce Zn absorption due to casein phosphopeptides (CPP) (Lonnerdal, 2000). CPP contains phosphorylated threonine and serine residues that bind to Zn ion. However, demineralized bovine casein showed no inhibitory effect on Zn absorption (Hurrell et al., 1989).

Diets for pigs are mainly composed of plant products including grains. Approximately 50-85% of total phosphorus (P) in these ingredients are phytates. Phytic acid (*myo*inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate) is a secondary compound, that is a major P storage form in cereals, legumes, oilseeds, and nuts (Vats and Banerjee, 2004; Gupta et al., 2015). Phytic acid is negatively charged and capable of binding with cation minerals, i.e., Ca^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, and Mg^{2+}) at intestinal pH, thus it acts as an anti-nutritional factor to limit the absorption of minerals including Zn (Wilcock and Walk, 2016). The binding results in poor solubility and reduced Zn absorption. Pigs and other monogastric animals have low phytase (phytate degrading enzyme) activity along the gastrointestinal tract, it is excreted in manure to a large extent (Lu et al., 2020).

Therefore, supplementation of dietary phytase has shown beneficial effects on Zn and other minerals absorption. A study from Adeola et al. (1995) indicated that supplementation of phytase significantly increased plasma Zn concentration of nursery pigs. Other strategies implemented in human nutrition are the inclusion of organic acids, such as ascorbic acid, and ethylenediaminetetraacetic acid (EDTA) complexes to reduce the impact of phytate on Zn absorption (Gibson et al., 2010).

Antagonists of inorganic minerals within the digestive tract were suggested that the low gastric pH induce dissociation of mineral salts, resulting in complexes forming and impaired bioavailability and absorption (Henderson et al., 1995). Zinc absorption is influenced by the competition between Zn and similar chemical characteristic metal ions, such as Ca, Fe, Cu, and Mn for transporters or absorptive sites. For example, Zn must compete with Fe and Mn for binding sites of divalent metal transport 1 (DMT1) at the apical membrane of the enterocytes, and body Fe status can cause up- or downregulation of DMT1 expression (Goff, 2018). However, Zn absorption via DMT1 is considered a minor pathway. Zinc and Cu are antagonistic to one another. Most studies reported that high Zn repressed Cu absorption (Ogiso et al., 1974; Wapnir and Balkman, 1991), but when consuming high Cu:Zn ratio, Zn absorption was depleted (Van Campen, 1969).

Chemical structure of Zn form can affect Zn absorption through the gastrointestinal tract. Low-molecular-weight organic ligands such as amino acids and other organic acids may enhance the solubility of Zn complexes (Sandström and Lönnerdal, 1989). A study in Beagle dogs reported a significant increase in plasma Zn concentration when supplementing with Zn amino acid chelate over Zn oxide, and the peak concentration was later at 4.5 h compared with 2.25 h after dosing, respectively

(Lowe et al., 1994). Therefore, attributes of organic Zn sources might provide better bioavailability to enhance the absorptive efficiency of Zn, *i.e.*, Zn organic complex may be absorbed via peptide or amino acid transport systems (Nitrayova et al., 2012).

4. Zinc transporters

Because Zn is essential and much less dangerous than other metals, the body tries to conserve every bit of it for its use. Accordingly, Zn transport and binding proteins play important roles in the absorption and cellular localization of Zn (Table 2.3). Besides, Zn does not react to redox reactions to cross the cellular membrane, unlike Fe and Cu (Kambe et al., 2015). Thus, Zn mobilization is tightly controlled by Zn transporters and binding proteins. Two distinct classes of 24 Zn transport proteins interact in Zn translocation. The first class is the Zn transporter as a Zn exporter (ZnT), whose family contains 10 members from ZnT1 to ZnT10. These Zn exporters transport cytosolic Zn out of a cell or into cellular organelles. The second class is the Zrt-, Irt-like protein (ZIP) as a Zn importer, which contains 14 members from ZIP1 to ZIP14. These ZIP proteins work in the opposite of the ZnT proteins by transporting Zn from the extracellular space or cytosolic lumen into the cytosolic location (Lichten and Cousins, 2009). The opposite functions of ZnT and ZIP proteins that move Zn ions are termed as Zn mufflers. Genetic mutations of these transporters affect significantly the development, metabolism, and immune system of the host (Hojyo and Fukada, 2016). However, this dissertation will cover two focused ZIP transporters.

a. ZIP4

ZIP4 is a member of the solute carrier 39 A 4 (SLC39A4) Zn transporter family. In a human study, ZIP4 is essential to embryonic development and growth by transferring dietary Zn through the intestine (Dufner-Beattie et al., 2007). A mutation of the ZIP4 gene leads to acrodermatitis enteropathica (AE), a genetic disorder of Zn deficiency causing severe skin problems in humans (Küry et al., 2002).

ZIP4 mRNA is abundantly expressed in the small intestine, particularly in the apical membrane, and indispensable in enteric Zn absorption (Cousins, 2010). Zn homeostasis occurs in the gastrointestinal tract because it is an interface of Zn exchange between the host and intraluminal Zn (Wang and Zhou, 2010). According to Hashimoto et al. (2016), ZIP4 is responsive to Zn deficiency, thus accumulates rapidly to govern Zn absorption. During high Zn levels, ZIP4 is endocytosed, degraded in proteasome and lysosome (Mao et al., 2007), and removed from the apical membranes of the enterocytes within several hours (Dufner-Beattie et al., 2003). Therefore, dietary and body Zn levels regulated ZIP4 localization and trafficking affect dietary Zn absorption and cellular homeostasis (Kim et al., 2004).

b. ZIP14

ZIP14 is mainly expressed in the liver, small intestine, heart, and pancreas and also is important in non-transferrin-bound Fe uptake, Mn homeostasis, hepatocyte proliferation, and systemic growth (Zhao et al., 2010; Aydemir et al., 2012; Scheiber et al., 2019). Also, data showed that ZIP14 is expressed in the pituitary gland and chondrocytes involved in production of growth hormone and bone development (Mayo et

al., 1995; Karsenty et al., 2009). In the small intestine, ZIP14 is localized in the basolateral membrane and is required for Zn uptake and maintenance of tight junctions (Guthrie et al., 2015). During inflammation and infection, hepatic ZIP14 is upregulated by interleukin-6 (IL-6) to increase Zn uptake in the liver and leads to hypozincemia (decrease in plasma/serum Zn concentration) (Liuzzi et al., 2005). Deletion of ZIP14 in mice altered glucose homeostasis and insulin receptor functions by enhancing insulin receptor at the kinase domain led to increasing insulin function and glucose uptake to the liver (Aydemir et al., 2012).

c. Zinc binding protein

The major intracellular Zn-binding protein is metallothionein (MT). Metallothionein is a low molecular weight metal-binding protein that contains predominantly Zn, Cu, and Cd. The MT protein can bind 7 Zn atoms and 60-68 amino acids, and it is highly conserved 18-23 cysteine residues (Maret, 2000). There are mainly 4 isoforms of MTs: MT-1, MT-2, MT-3, and MT-4. The most widely expressed throughout the body is MT-1 and MT-2, while MT-3 is more specific in the brain (Masters et al., 1994), and MT-4 is detected in certain squamous epithelium and the maternal lining of the uterus (Quaife et al., 1994; Liang et al., 1996). Cellular Zn transients can be regulated by MT as it serves as Zn buffers. MT is a multifunctional stress protein that increases its presence during insult exposure, such as metal toxicity, oxidative stress, and inflammation involving in metal translocation (Andrews, 2000). Thus, functions of MT are involved with cell protection, metal (Zn) homeostasis, and regulation, and as inflammatory mediator. MT is highly expressed in the liver, intestine,

and kidney. For examples, research has shown enhanced apoptosis in MT-knockout cells (Kondo et al., 1997) and that nuclear MT protects DNA damage and regulates gene expression during cell proliferation and differentiation (Cherian and Apostolova, 2000), and cytoprotection of MT in LPS-induced inflammation (Inoue et al., 2006).

Table 2.3. Cellular localization and tissue expression of Zn transport and binding proteins (adapted from Myers et al. (2012) and Baltaci and Yuce (2018)).

Zn transport/binding proteins	**Cellular localization**	**Tissue expression**
ZnT proteins (SLC30)		
ZnT1 (SLC30A1)	Plasma membrane	Ubiquitously expressed
ZnT2 (SLC30A2)	Vesicles, lysosomes	Pancreas, kidney, testis, epithelial cells, small intestine
ZnT3 (SLC30A3)	Synaptic vesicles	Brain, testis
ZnT4 (SLC30A4)	Intracellular compartments	Mammary gland, brain, small intestine, placenta, blood, epithelial cells
ZnT5 (SLC30A5)	Secretory vesicles, Golgi apparatus	Ubiquitously expressed
ZnT6 (SLC30A6)	Secretory vesicles, Golgi apparatus	Small intestine, liver, brain, adipose tissue
ZnT7 (SLC30A7)	Golgi apparatus	Retina, small intestine, liver, blood, epithelial cells, spleen
ZnT8 (SLC30A8)	Secretory vesicles	Pancreatic B-cells
ZnT9 (SLC30A9)	Cytoplasm, nucleus	Ubiquitously expressed
ZnT10 (SLC30A10)	Unknown	Liver, brain
ZIP proteins (SLC39)		
ZIP1 (SLC39A1)	Plasma membrane	Ubiquitously expressed
ZIP2 (SLC39A2)	Plasma membrane	Blood, prostate, dermis
ZIP3 (SLC39A3)	Plasma membrane, intracellular compartment	Mammary gland, prostate
ZIP4 (SLC39A4)	Apical membrane	Small intestine, stomach, colon, kidney, brain
ZIP5 (SLC39A5)	Basolateral membrane	Pancreas, kidney, liver, spleen, colon, stomach, small intestine
ZIP6 (SLC39A6)	Plasma membrane	Ubiquitously expressed
ZIP7 (SLC39A7)	Golgi apparatus, endoplasmic reticulum	Ubiquitously expressed
ZIP8 (SLC39A8)	Vesicles	Ubiquitously expressed
ZIP9 (SLC39A9)	Trans-Golgi network	Ubiquitously expressed
ZIP10 (SLC39A10)	Plasma membrane	Ubiquitously expressed

Zn transport/binding proteins	Cellular localization	Tissue expression
ZIP11 (SLC39A11)	Unknown	Mammary gland, testis, gastric gland, ileum, cecum, colon
ZIP12 (SLC39A12)	Unknown	Retina, brain, testis, lung
ZIP13 (SLC39A13)	Golgi apparatus	Ubiquitously expressed
ZIP14 (SLC39A14)	Plasma membrane	Ubiquitously expressed
Metallothionein		
MT-1	Cytoplasm, nucleus, mitochondria	Ubiquitously expressed
MT-2	Cytoplasm, nucleus, mitochondria	Ubiquitously expressed
MT-3	Cytoplasm, nucleus,	Brain, testis
MT-4	Cytoplasm, nucleus,	Squamous epithelia

5. Zinc homeostasis and cellular distribution

Zn homeostasis is crucial for normal cell functions. Homeostatic control of Zn is a principle physiological response to maintain Zn in an optimal balance during exposures to various circumstances, e.g., disease challenge, ongoing loss, and increase Zn intake. Body Zn homeostasis during the acute phase response of inflammation changes Zn transiently. Serum Zn translocation to the liver causes hypozincemia or a decrease in serum Zn concentration. This event occurs simultaneously with increases in proinflammatory biomarkers, reactive oxygen species, and antimicrobial peptides that cause tissue damage in the lung, liver, and spleen (Knoell et al., 2009). Therefore, serum Zn concentration is sensitive to physiological changes and not a specific measure of Zn nutrition.

Intracellular Zn distribution is controlled tightly in every part of the cell compartment, e.g., organelles, and vesicles. Cytosolic Zn is floating freely or loosely bound in very low concentrations ranging from picomolar to a low nanomolar range (Outten and O'Halloran, 2001) (Figure 2.5). Most of the Zn ions in the cytosol are bound to metalloproteins and metallothionein. Although Zn is an essential metal, excessive Zn

can be toxic. Zn is excreted from the body via intestinal and pancreatic excretions (Krebs, 2000). The control mechanism is highly involved with the Zn sensing system by metal-response element-binding transcription factor-1 (MTF-1) (Laity and Andrews, 2007). MTF-1 is a 72.5 kDa protein composed of six Zn fingers and transcriptional domains (Heuchel et al., 1994). On the proximal promoters of MT genes, there is MTF-1 as the DNA motifs located to accompany metal-response element (MRE)-binding transcription (Stuart et al., 1984). Zn ions stimulate the DNA-binding activity of MTF-1 specifically by interacting with the Zn-finger domain. In other words, MTF-1 acts as a Zn sensor of free Zn pool and responds to changes in Zn concentration and cellular stresses including metal toxicity, heat shock, and oxidative stress. Evidence shows that these stressors induce DNA binding of MTF-1, thus upregulating MT gene transcription (Heuchel et al., 1994).

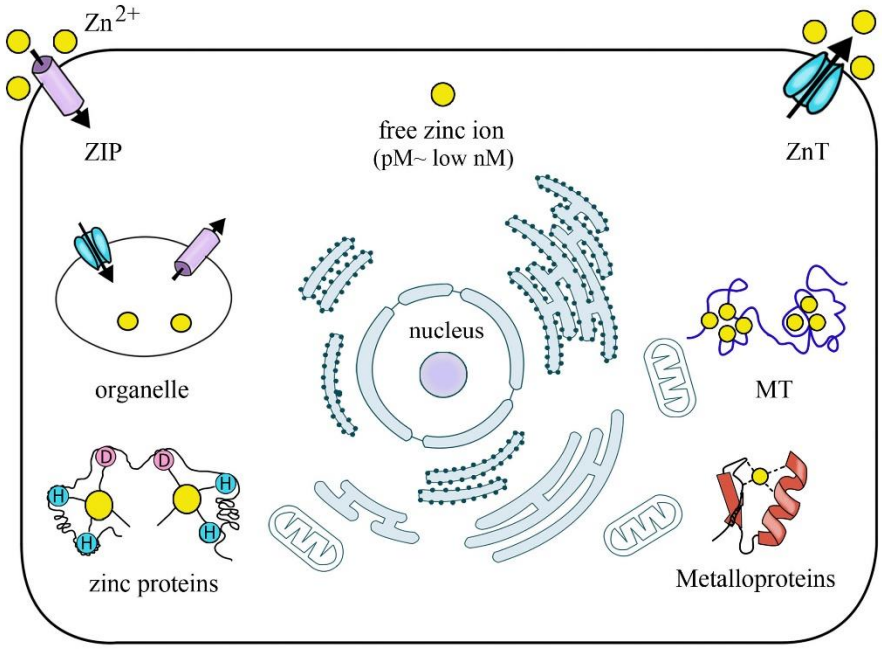

Figure 2.5. Cellular Zn distribution. ZIP proteins are the Zn importers (transfer Zn into cytoplasm) while ZnT proteins are the Zn exporters (transfer Zn out of cytoplasm). Metallothioneins are the Zn binding proteins. Free Zn ions are kept intracellularly as low as pM to low nM concentration (adapted from Kambe et al. (2015)).

6. Zinc use in swine nutrition

Back in the 1950s, Zn was supplemented to cure and prevent a skin disease termed parakeratosis in pigs (Stevenson and Earle, 1956). Parakeratosis is a hyperkeratinization of the skin including scabs and encrustations and induced by the onset by high dietary Ca (Luecke et al., 1957). This disease raised attention to more research on the effect, relationship, and requirement of Zn on health, growth, and physiological performance in pigs. Pond et al. (1964) reported that supplementing Zn no greater than 34 ppm in a corn-based diet containing 1.3% Ca could prevent parakeratosis and promote weight gain of pigs. In 1968, Zn-deficient pigs had been reported for impaired growth, development, and metabolism, e.g., decrease in growth rate, feed efficiency, feed intake, serum Zn, size, and strength of bone, thymus weight, and alkaline phosphatase enzyme (Miller et al., 1968). A study on dietary Zn levels for reproductive sows on litter performance was reported that the progeny of sows fed 33 ppm Zn had a lesser growth rate than pigs from sows fed 83 ppm (Hedges et al., 1976).

a. Zinc bioavailability

Currently, there are many feed grade Zn forms available for use in the swine industry. The responses of using different Zn forms are not consistent, which may be explained by relative bioavailability value (RBV) to some extent. From an animal nutrition perspective, Ammerman et al. (1995) defined bioavailability as “The degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal”. Whereas in the human nutrition perspective, bioavailability definition was provided by the subcommittee on Upper Reference Levels

of Nutrients as “Accessibility to normal metabolic and physiologic process. Bioavailability influences a nutrient’s beneficial effects at physiologic levels of intake and also may affect the nature and severity of toxicity due to excessive intakes” (Hambidge, 2010). Besides, RBV of Zn sources should be determined during normal physiological and nutritional states to avoid regulatory control by homeostatic mechanism in absorption and excretion (Paulicks et al., 2011). Bioavailability can be affected by various dietary and physiological factors (Figure 2.6). Therefore, the essence of feeding high RBV Zn is to use lower levels to improve intestinal absorption to benefit systemic use of Zn and lessen fecal Zn excretion. Although RBV may be used to indicate absorption and utilization of Zn source, over-formulated Zn doses could diverge RBV from homeostatic counter-regulation. Collectively, justification for RBV may be one of the prerequisites in selecting Zn source.

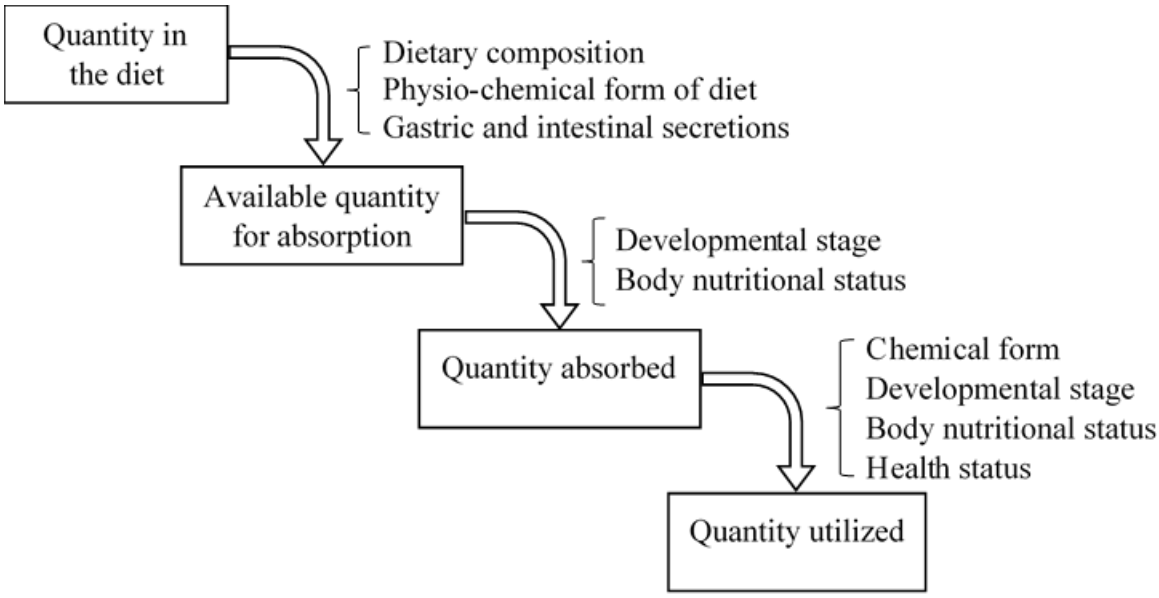

Figure 2.6. Stages of bioavailability and factors affected at each stage (adapted from Fairweather-Tait (1992)).

b. Zinc source

Two types of Zn sources available are inorganic and organic, which the two are described differently by their chemical and biological properties. Examples of feed grade inorganic Zn are ZnO, $ZnSO_4$, and $ZnCl_2$, while the organic ones are Zn that complexes with amino acids (specific and non-specific), proteins (polypeptides), or carbohydrates (polysaccharides). The difference of the Zn ligands affects stability, absorption, and utilization of Zn. For instance, ZnO as an inorganic source has RBV between 50-68% compared to other Zn sources, e.g., Zn sulfate and organic Zn (RBV ≥ 100%) (Wedekind et al., 1994; Hellman and Carlson, 2018). Cao et al. (2000) reported bioavailability of organic Zn forms relative to 100% of Zn sulfate were 113, 110, and 130% for Zn proteinate, Zn amino acid, and Zn methionine, respectively. A study from Lee et al. (2001) comparing supplementation of $ZnSO_4$, Zn amino acid chelate (ZAC) and Zn methionine complex (ZM) at 170 mg/kg diet with added 170 mg/kg diet of $CuSO_4$, Cu amino acid chelate, and Cu chloride, respectively in growing pigs revealed that serum Zn concentration of ZAC (1.55 mg/L) and ZM (1.74 mg/L) were significantly higher than the $ZnSO_4$ (1.44 mg/L) pigs at d 14 of supplementation. In addition, fecal Zn excretion of ZAC pigs was the lowest among others (1,240 vs 1,550 mg/kg). Another study reported pigs supplemented with 100 mg/kg of Zn chitosan chelate (Zn-CS) significantly increased serum Zn concentration, and mRNA expressions of duodenal Zn transporters ZnT1, ZIP4, and ZIP5 than the $ZnSO_4$ supplemented pigs (Lv et al., 2016). Thus, pigs fed with Zn-CS had improved average daily gain (ADG) (347.06 ± 61.33 vs 243.69 ± 47.05 g/d).

c. Growth performance

A recommendation by the Nutrient Requirements for Swine (NRC, 2012) suggests a 100 mg Zn/kg diet for 5 to 11 kg BW, and 80 mg Zn/kg diet for 11-25 kg BW. These amounts are required to support normal growth performance and prevent deficiency conditions during a steady state. In swine industry practice, supplemental Zn is usually included in greater levels than those recommended by NRC to compensate inefficiencies during the pre-absorptive state, to supply an increase physiological and special purpose requirements during challenging times, and to expect a dose-dependent performance and outcomes.

Zn has been used as a growth promoter because it is crucially required for metabolic and physiologic functions of the body. Feeding higher levels of Zn also exerts a positive outcome on growth rate. Industry levels of Zn fed to weaned pigs have been described as pharmacological doses (2,000-3,000 mg/kg diet), and the most commonly used form in North America is ZnO (Baker, 2000). The pharmacological doses are to give pharmacological effects to post-weaning pigs against the challenge state, i.e., growth lag from confronting to multiple stressors, and health challenges from post-weaning diarrhea. Feeding ZnO at 3,200 ppm to weaned pigs infected with *Escherichia coli* for 14 d revealed a decrease in post-weaning diarrhea without having toxic signs (Holm, 1990). A study by Li et al. (2001) indicated improved intestinal morphology, nutrient digestion, and absorption when weaned pigs were fed 3,000 mg/kg ZnO. A meta-analysis of pharmacological levels of ZnO supplementation on growth showed feasible outcomes when compared to the use of in-feed antibiotics to enhance growth performance (ADG, ADFI, and G:F) of nursery pigs (Sales, 2013). Although high levels of ZnO used are

effective to improve growth performance and treat diarrhea, there are studies recommending supplement only for short periods after weaning (Carlson and Hellman, 2003; Shelton et al., 2009). The use of high ZnO throughout the nursery phase can cause damage to intestinal lining epithelium (Zhou et al., 2017), and increase environmental problems from fecal Zn excretion (Meyer et al., 2002).

On the other hand, dietary Zn amino acid complex (ZnAAC) supplementation was reported to improve weight gain, feed efficiency, and increase serum antioxidant enzyme status of weaned pigs fed 20 to 120 ppm (Zhang et al., 2018). Significant lower doses with optimal outcomes have drawn attention as an alternative to ZnO. Weanling pigs fed Zn and Cu amino acid chelates exhibited improvements in feed efficiency, serum mineral concentration, and decrease fecal excretion over equal doses of inorganic sources (Lee et al., 2001). Therefore, strategies of Zn used in nursery pigs should be implemented to maximize the benefits of Zn source and doses to minimize adverse effects on pigs and the environment.

d. Immune attribution

Zn is vital for both the innate and adaptive immune systems. It was mentioned that Zn served as a second messenger of immune cells, and participated in intracellular signaling events (Prasad, 2014). Marginal Zn deficiency could impair immunity in various ways, such as chronic low-grade inflammation and increase oxidative stress (Sandström et al., 1994). Hallmarks of Zn deficiency are thymic atrophy and lymphopenia (decrease in $CD4^+$ T helper cell resulting in a decrease $CD4^+/CD8^+$ ratio) (Rink and Haase, 2007). One of the biological activities of the thymus gland is secreting

thymulin (thymic hormone). This hormone contains a Zn-bound form and is important for T-cells maturation and differentiation (Goldstein, 2013). Also, Zn is required for the extrathymic T-cell pathway (Mocchegiani et al., 1997). Zn supplementation increased lymphocyte proliferation and macrophage function of mice when supplemented for short periods (Lastra et al., 2001).

Zn is important in reducing free radical formation through the superoxide dismutase (SOD) enzyme. A study indicated piglets supplemented with Zn-chelate increased antioxidant capacity (Cu-ZnSOD and GSH-Px) and improved immune function (serum IgA, complement 3 and 4 levels) (Ma et al., 2014). Zn has been found to inhibit NF-κB signaling via A20 (Figure 2.7). A20 is a principal anti-inflammatory Zn-finger protein, that is important for tumor necrosis factor receptor (TNFR) and TLR signaling. The downstream action of A20 is to sequester NF-κB in the cytoplasm via I kappa B kinases (IKK) binding NF-κB, thus hindering the translocation of NF-κB to the nucleus for gene transcription (Maywald et al., 2017). In addition, Zn is required for the DNA binding activity of peroxisome proliferator-activated receptor-alpha (PPAR-α). PPAR-α plays a role in negatively interfering with NF-κB, thus preventing inflammatory gene expression, such as cytokines and adhesion molecules (Reiterer et al., 2004).

Weaned pigs fed additional Zn increased serum IgA concentration compared with the control group (Pei et al., 2019). Supplementing pharmacological ZnO modulated immune function by decreasing mRNA expression of proinflammatory cytokine interferon-g (IFN-g), but increasing anti-inflammatory cytokine transforming growth factor-b (TGF-b) in the jejunum of piglets (Zhu et al., 2017). Pigs fed Zn amino acid complex (ZnAAC) improved 56% of small intestinal integrity on transepithelial electrical

resistance (TER) during severe heat stress (Fernandez et al., 2014). However, supplementing Zn in the form of ZnO nanoparticles (~37 nm diameter) increased cell toxicity of macrophage by over generating intracellular reactive oxygen species (ROS), causing plasma membrane leakage, and impairing mitochondria function (Wang et al., 2014).

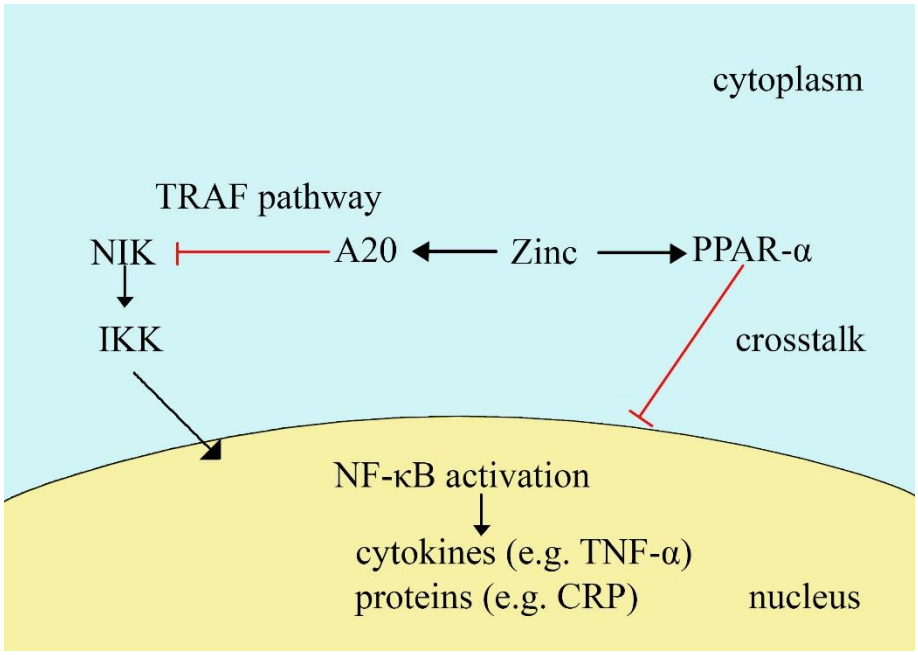

Figure 2.7. Anti-inflammatory mechanism of Zn (adapted from Maywald et al. (2017)).

e. Zinc and copper supplementation

Not only Zn has been used in swine diets, especially the nursery phase, but Cu is a widely used mineral. Zn and Cu play key roles in metabolic pathways and supporting growth. Supplementation of these two minerals in pharmacological concentrations (2000-3000 for Zn, and 100-250 for Cu) provides improvements in growth performance, independently (Hill et al., 2000). In contrast, no additive responses of high levels of Zn (3,000 ppm) and Cu (250 ppm) supplementation were found in growth performance of

weaned pigs (Smith et al., 1997). The research recommended that high levels of Zn should be fed after weaning until pigs reached 12 kg BW, then switched to feeding high Cu for the remainder of the nursery phase, would be a feasible and cost-effective approach (Jacela et al., 2010).

f. Zinc deficiency

From a notable human nutrition perspective by Golden (1996), there are two types of nutritional deficiencies: Type I and Type II. The explanation for Type I nutrient deficiency is that no primary effect on growth but decreases in body store which causes a specific metabolic problem, for example, Fe deficiency causes anemia, Ca causes bone and skin lesions, thiamin deficiency causes beriberi, and niacin deficiency causes pellagra. Thus, the diagnosis of Type I nutrient deficiency is straightforward. In contrast, Type II nutrient deficiency has major effects on growth failure, including stunting and wasting. The signs and symptoms may occur as non-specific as generalized dysfunction distressing most tissues and contribute to a protein-energy deficiency. Nutrients that fall into this category are Zn, magnesium, phosphorus, nitrogen, and essential amino acids. Therefore, the diagnosis of deficiency is not normally associated with specific signs. Instead, the body starts to limit excretion or breakdown of its tissues to supply the nutrients to the body.

Since Zn participates in many enzymatic activities for multiple systems, failure to absorb Zn adequately is exhibited by slow growth, skin lesions, diarrhea, alopecia, impaired wound healing, and compromised immune function (Henderson et al., 1995). It was reported that Zn deficiency was associated with decreased activity of enzyme

concentrations, i.e, hepatic leucine aminopeptidase, and ornithine transcarbamylase (Burch et al., 1975). A report from Sullivan et al. (1974) indicated that Zn-deficient pigs decreased pancreatic secretion, e.g., protease and amylase. Zn deficient with additional high Ca ration diets could induce the onset of a profound symptom called parakeratosis (Figure 2.8). This severe skin problem occurred simultaneously with decreased weight gain (Lewis Jr et al., 1956). However, the addition of Zn could eliminate or reverse the conditions (Lewis Jr et al., 1956; Hoefer et al., 1960).

Zn deficiency may be influenced by factors affecting Zn absorptive efficiency. The factors could be pathological conditions that affect the intestinal structure and Zn transporters, Zn solubility, Zn antagonists presenting in the intraluminal environment, such as dietary phytate found in plant-based ingredients, excessive Ca and Fe, and unabsorbed fat (Krebs, 2000). This agreed with Ognik et al. (2016) that diets containing high Ca and Fe can reduce the availability of Zn before being absorbed or a high Cu diet reduced Ca and Zn absorption. However, Zn deficiency is rarely found in commercial pigs nowadays when it is common to formulate swine diets to meet or exceed Zn recommendations.

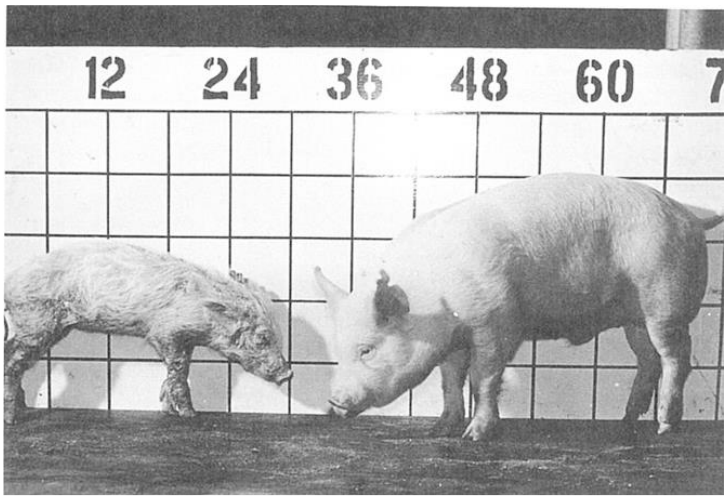

Figure 2.8. Zinc deficiency signs of parakeratosis and decreased growth in pig (left side). Obtained from Nielsen (2012) by courtesy of J.H. Conrad and W.M. Beeson, Purdue University.

g. Zinc toxicity

Although Zn is an essential metal, excess Zn can be toxic. Therefore, Zn homeostasis and detoxification are crucial for normal cell functions, i.e., Zn sensing system by metal-response element-binding transcription factor-1 (MTF-1) (Laity and Andrews, 2007). MTF-1 is a 72.5 kDa protein composed of six Zn fingers and transcriptional domains (Heuchel et al., 1994). On the proximal promoters of MT genes, there is MTF-1 as the DNA motifs located to accompany metal-response element (MRE)-binding transcription (Stuart et al., 1984). Zn stimulates the DNA-binding activity of MTF-1 specifically by interacting with the Zn-finger domain. In other words, MTF-1 acts as a Zn sensor of free Zn pool and responds to changes in Zn concentration and cellular stresses including metal toxicity, heat shock, and oxidative stress. Evidence showes that these stressors induce DNA binding of MTF-1, thus upregulates MT gene transcription (Heuchel et al., 1994).

Clinical signs of Zn toxicity in swine are detectable as anorexia, depression, gastroenteritis, and declined growth performance, i.e., reduced weight gain, reduced feed efficiency (Puls, 1988). Feeding ZnO at 4,000 mg/kg diet decreased ADG of pigs but did not induce toxic signs (Poulsen, 1995). While feeding ZnO at 6,000 mg/kg diet in weaned pig increased hepatic, pancreas, and serum Zn concentrations leading to pancreatic cells apoptosis, but decreased in serum Cu, and both serum and hepatic selenium (Burrough et al., 2019). Excessive feeding of 8,000 mg ZnO/kg of diet for 8 weeks led to chronic pancreatitis and wasting syndrome (Komatsu et al., 2020). Collectively, pharmacological levels of ZnO are recommended to feed for approximately 2 weeks during the nursery phase. For organic Zn, weanling pigs can tolerate up to 800 mg of Zn amino acids complex supplementation/kg diet (Zhang et al., 2018).

7. Lipopolysaccharide (LPS)

a. LPS component

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria, e.g., *Salmonella sp.* and *Escherichia coli*. LPS is a potent endotoxin and heat-stable complex molecule that plays a key role in the innate immune response of the host. It is called endotoxin because once released from the bacteria it can initiate a cascade of a host inflammatory response. The structure of LPS consists of three defined regions from the innermost to the outermost location: lipid A, core-oligosaccharide region, and O-antigen (Alexander and Rietschel, 2001) (Figure 2.9). Some bacterial LPS lack O-antigen and are called “rough (R)-type LPS” or “LOS”, while those comprised of all three regions are called “smooth (S)-type LPS” (Silipo and Molinaro, 2010). For

example, commercial LPS from *E. coli* O55:B5 is extracted from wild-type of S-type LPS, which is composed of three regions. This LPS is used in research studies to activate inflammation via a specific toll-like receptor 4 (TLR4). Therefore, the two different LPS forms could induce differences in oxidative burst level, host immune response, and kinetics of blood clearance.

Lipid A, a main virulent factor, is responsible for endotoxic activity of LPS molecule. Lipid A comprises of b (1-6) D-glucosamine disaccharide 1,4'-bisphosphate backbone and is embedded via hydrophobic bond in the phospholipid bilayer of the bacterial outer membrane (Kotani et al., 1985). However, structures of lipid A widely vary among different bacterial species, i.e., from a diverse phosphorylation pattern of the disaccharide backbone. The major contribution of structural differences is the immunogenic potential which defines LPS immunogenicity (Seydel et al., 2000). LPS which elicits strong inflammation of the host response is called "agonistic" lipopolysaccharide, i.e., *E.coli* has hexaacylated lipid A structure that stimulates the strongest proinflammatory response of the host cell (Zähringer et al., 1994). On contrary, LPS which triggers a weak inflammatory host response is named "weak agonistic" or "antagonistic" lipopolysaccharide, i.e., *Francisella tularensis* absences one or two phosphate groups of the lipid A structure and behaves in weak agonistic type (Phillips et al., 2004). Interestingly, the two types of lipid A may compete for host cell binding in a harsh environment (Steimle et al., 2016).

Linked to lipid A moiety is the core-oligosaccharide of LPS. Unlike lipid A that is a highly conserved region, the core oligosaccharide has some variations. The core often contains branched and phosphorylated heterooligosaccharide and can be divided into two

regions: the inner core and the outer core. At the inner core proximal to lipid A, the sugar chain contains residues of Kdo, which is the most conserved component, and L-glycerol-D-manno-heptose (Hep) (Wang and Quinn, 2010). The outer core is more diverse in structural components. Overall, core oligosaccharide gives serological specificity, unique antigenic properties, viability, and structural stability of the bacteria (Silipo and Molinaro, 2010).

O-antigen is located to the outermost part of LPS and extends into the environment. It consists of three to five sugars forming repeated oligosaccharide subunits. Those sugars are assembled into chains by glycosyltransferase enzymes, and they are much longer than core oligosaccharides. This region is found to give rise to large structural diversities of LPS. A wide variety of antigenic types is from strain to strain-specific (interstrain heterogeneity), or within strain-specific (intrastrain heterogeneity). Therefore, O-antigen modifications of bacterial LPS could impact host defense mechanisms, especially the adherence step (Lerouge and Vanderleyden, 2002).

Because LPS is located in the outer membrane, bacteria take benefit from LPS for their biological interactions with the surrounding environment. In particular, LPS has two major purposes. First, LPS maintains the stability of the outer membrane and provides a defensive barrier which enhances the survivability of bacteria in a stressful environment (Silipo et al., 2012). For that purpose, bacteria increase their rigidity and strength of the outer membrane by substituting the lipid A sugar moieties with phosphate groups to form a negative charge of the outer membrane to attract divalent cations from the neighboring milieu (Alexander and Rietschel, 2001). Second, LPS is a conserved structure recognized by the pathogen-associated molecular pattern (PAMP) of the innate immune system

(Akira and Takeda, 2004; Steimle et al., 2016). This LPS-host interaction activates immunological response for bacterial clearance.

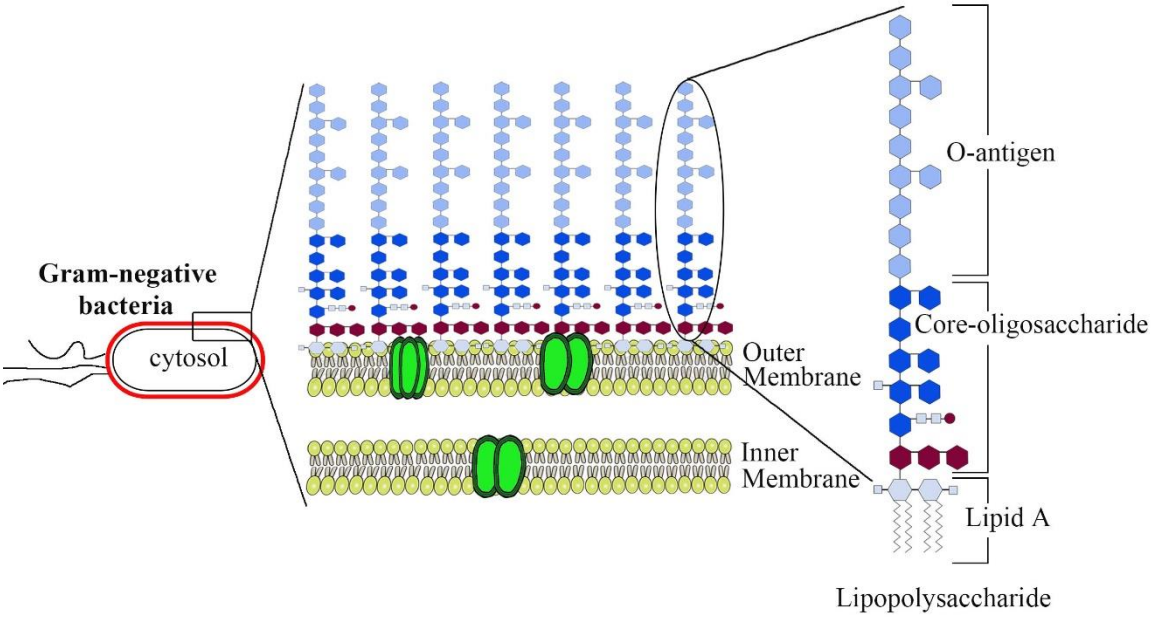

Figure 2.9. Lipopolysaccharide structural component of Gram-negative bacteria.

b. LPS recognition

To be recognized by the host innate immune system, LPS has to be released from the bacterial membrane either from bacterial cell death or cleaved off by the host LPS-binding protein (LBP) (Miyake, 2006). LBP is considered as an acute-phase protein produced by the liver. LBP is a soluble glycoprotein in plasma that facilitates binding (opsonizing) and transporting the lipid A moiety of bacterial LPS to surface markers on cellular membrane including the cluster of differentiation 14 (CD14) (Knapp et al., 2003). CD14 is a glycoprotein anchoring in the plasma membrane of myeloid lineage cells including monocytes and macrophages, and it serves as a pattern recognition receptor (PRR) and a co-receptor for toll-like receptors (TLRs). When CD14 is

upregulated upon LPS exposure, endocytosis of specific TLR4 is promoted. The extracellular domain of TLR4 is also bound to a secreted protein myeloid differentiation factor 2 (MD-2). MD-2 functions in supporting LPS signaling and stabilizing TLR4 (Shimazu et al., 1999). However, MD-2 could play a role in inhibiting LPS activation, which is useful to treat LPS toxicity (Viriyakosol et al., 2001). Stimulation of TLR4 then facilitates two inflammatory pathways: myeloid differentiation primary response protein 88 (MyD88)-dependent and MyD88 independent pathways (Akira and Takeda, 2004). Both pathways are crucial in signal transduction to induce immunological response including activation of nuclear factor kappa beta (NF-κB), a transcriptional factor, for cytokine release (Zanoni et al., 2011). Molecules within NF-κB include RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2), which can form homo- and heterodimers (Kambe et al., 2015). IκBs are the NF-κB inhibitors that hinder the translocation in the nucleus by sequestering the molecules and complexes in the cytoplasm (Jarosz et al., 2017). Thus, LPS once recognized by TLR4 mediates the inflammatory response of innate immune cells via PAMP and results in cytokine secretion through NF-κB transcription. Positive feedback of NF-κB can occur by the cytokines produced, which is specific to the cytokine receptor binding and signaling pathways. In contrast, NF-κB provides a negative feedback (feedback inhibition) to itself in concert with other anti-inflammatory factors in order for homeostatic regulation. (Figure 2.10).

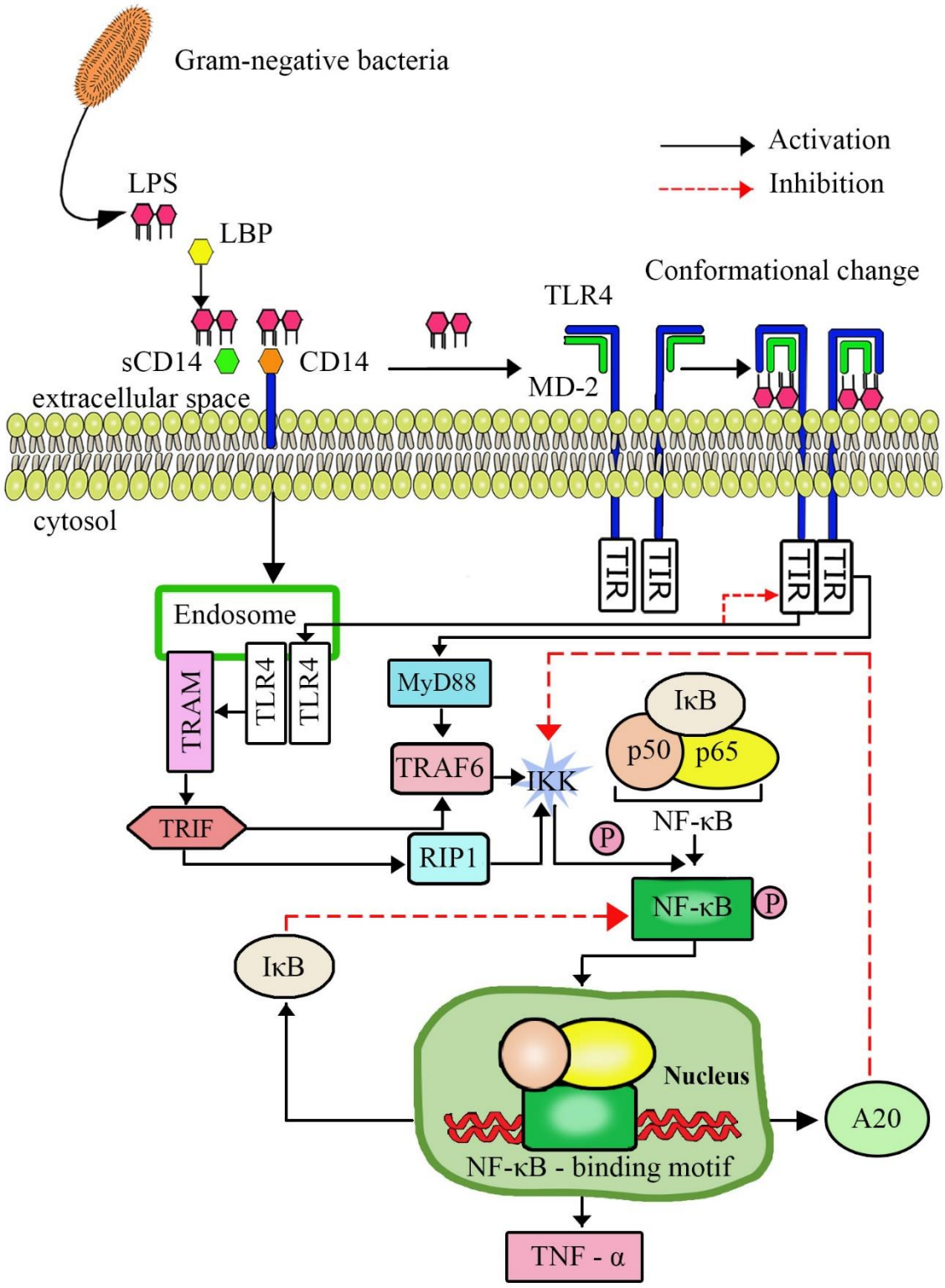

Figure 2.10. Lipopolysaccharide recognition and signaling (adapted from Akira and Takeda (2004) and Steimle et al. (2016)).

c. LPS use in swine research

In swine research, LPS is widely accepted as an experimental model studying an acute immune response. A small number of LPS can trigger an inflammatory response that mimics Gram-negative infection. The outcome yields reproducible results. The LPS model also provides a window to study the early inflammation of pigs and allows mechanistic and therapeutic insights to be tested. Nursery pigs challenged with a low dose of LPS (5 μg/kg BW) exhibited detectable salivary and plasma proinflammatory cytokines (Moya et al., 2006). However, the magnitude and amplitude of inflammatory response following LPS injection were influenced by the sex of pigs and varied among the proinflammatory cytokines (Williams et al., 2009).

Apart from dose injected, differences in LPS serotypes may affect inflammatory response and pattern. LPS from *E. coli* serotype O111:B4 was reported as the most frequently used in porcine research (Wyns et al., 2015). In contrast, a time-course study of LPS injected rats with LPS doses (2, 50, and 250 μg/kg BW) and serotypes (O55:B5, O127:B8, O111:B4) revealed that intraperitoneal injection of LPS from E. coli serotype O55:B5 induced the most consistent changes in rectal temperature pattern among different doses within 2-3 h post-challenge (Dogan et al., 2000). Also, different LPS preparations can offer to vary residual protein impurities that influence the transcriptional profile of inflammation from the TLR4 stimulation (Rutledge et al., 2012). Therefore, a justification for selecting LPS serotype and dose may depend on experience of researcher *in vivo* of pigs.

8. Proinflammatory cytokines

During an acute phase response (APR) of stress, trauma, infection, or inflammation, cytokines are synthesized by innate immune cells as immune regulators to counteract the challenge and return the body to homeostasis as soon as possible. Cytokines that promote pathophysiological response or worsen inflammation are called "proinflammatory cytokines", whereas the ones that suppress or promote healing of inflammation are called "anti-inflammatory cytokines" (Dinarello, 2000). Ideally, the body should balance the two arms of both cytokines to combat the insult and cost the least energy. In contrast, if there is a dysregulation of cytokine production, the outcomes may be detrimental to host cells. For example, proinflammatory cytokines can stimulate other immune modulators resulting in behavioral, neurological, and metabolic changes. The decision of either producing proinflammatory or anti-inflammatory cytokines is based on the genes coding for synthesis that is upregulated during inflammation. Factors that modulate gene regulations may play an important role in balancing the immune response. The most common proinflammatory cytokines stimulated by bacterial endotoxin are interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). In particular, proinflammatory cytokines share some overlapping functions but each one still has its characteristic properties (Akira et al., 1990).

Effects of proinflammatory cytokines on the liver, a key organ responsible during the acute phase response, were reported for three functions (Gruys et al., 2005): suppression of cytochrome P-450 enzyme impairing chemical and drug clearance, stimulation of heat shock proteins giving inhibitory feedback to inflammatory pathways, and synthesis of metallothionein increasing metal ion binding capacity. Importantly,

proinflammatory cytokines may cause growth reduction by inducing sickness behavior and increasing energy expenditure, i.e., induction of fever, and an increase in nitrogen catabolism. For this dissertation, TNF-α, and IL-6 will be discussed in more detail.

a. Tumor necrosis factor-alpha

Tumor necrosis factor (TNF) was named after its capability in cytotoxicity that caused necrosis or lysis in many tumor cell lines. TNF is a type II transmembrane protein arranged in three identical polypeptides as homodimers. Family of the tumor necrosis factor includes TNF-alpha (TNF-α), TNF beta (TNF-β; lymphotoxin), CD40 ligand (CD40L), Fas ligand (FasL), TNF-related apoptosis including ligand (TRAIL), and LIGHT (a receptor expressed by T-lymphocytes) (Chu, 2013). TNF-α is broadly produced by a variety of tissues including endothelial cells, lymphoid cells, mast cells, and neuronal cells, but mainly produced by macrophages (Kohn et al., 1992). Thus, TNF-α is a major pro-inflammatory cytokine released in response to LPS and other bacterial products.

There are two types of TNF-α derivatives: membrane-bound TNF (memTNF-α) and soluble TNF (sTNF-α). Both of them bind to two members of specific receptors: TNF-R1 (TNF receptor type 1; p55/60) and TNF-R2 (TNF receptor type 2; p75/80) (Wajant et al., 2003). MemTNF activates both receptors, while sTNF mainly activates TNF-R1 and is limited for TNF-R2. TNF-R1 plays a key role in TNF signaling that is found in most tissues, whereas TNF-R2 is important for the lymphoid system and found in cells of the immune system.

Although TNF-α has a similar pro-inflammatory effect via NF-κB like other cytokines including IL-1, there are also unique pathways of TNF-α in inducing cell apoptosis (program cell death) and necrosis. When TNF-α binds to TNF-R1, interaction with TNFR1-associated death domain protein (TRADD) occurs (Pobezinskaya and Liu, 2012). The following mechanism can either induce apoptosis via Fas-associated protein containing the death domain (FADD), receptor-interacting protein 1 (RIP1) kinases, and caspase proteins or proliferation for cytokines and chemokines production via TNF receptor-associated factor 2 (TRAF2) and TRAF5, leading to NF-κB activation (Chu, 2013). Interestingly, TNF-R2 does not have a death domain, but it can interact with TRAF2 and TRAF5 to either activate NF-κB for cytokine production or induce Dynamin-related peptide 1 (Drp1) subsequent tissue necrosis.

TNF-α is similar in a sequential manner to other cytokines including IL-6 and contributes to inflammatory cells infiltrating into the damaged site. Much research has investigated various outcomes of TNF-α production during infection and inflammation. For instance, TNF-α impairs gut barrier integrity by promoting intestinal mucosal disruption (Liu et al., 2008), TNF-α suppresses lipid deposition in adipose cells and alters adipose tissue metabolism (Ramsay et al., 2013), TNF-α decreases collagen deposition by decreasing cross-links of mature collagen fibers (Pischon et al., 2004), and TNF-α and glucocorticoids mediate muscle catabolism and peripheral amino acids transfer to the liver (Fischer and Hasselgren, 1991). Although the production of TNF-α during an immune response is essential to fight invading pathogens, it may cause detrimental effects on other systems. Hence, it is important to have a modest immune response to maintain the well-being of the whole body.

b. Interleukin-6

Interleukin-6 (IL-6) is a multifunctional cytokine involved in immune response, hematopoiesis, inflammation, oncogenesis, and nervous system (Akira et al., 1990; Kimura and Kishimoto, 2010). During the acute phase response, IL-6 upregulating by IL-1 is the principal cytokine that regulates hepatocytes to produce and release acute phase proteins (Choy and Rose-John, 2017). IL-6 is composed of four helical proteins of 184 amino acids (Bazan, 1990). There are two types of IL-6 receptors (IL-6R): membrane-bound IL-6R (mIL-6R) and soluble IL-6R (sIL-6R). An mIL-6R is only presented on some leukocytes, epithelial cells, and hepatic cells, thus cells without this receptor cannot respond to IL-6 (Rose-John, 2012). When IL-6R is produced in a soluble form it is called sIL-6R, which can be found in all cells including body fluids (urine and blood) (Novick et al., 1989). Thus, cells lacking mIL-6R can respond to IL-6 by binding to sIL-6R (Tisdale, 2009). The activation of IL-6 and mIL-6R is called classical signaling. On the other hand, activation of IL-6 and sIL-6R is called trans-signaling.

Complexes of IL-6 and its receptor (mIL-6R or sIL-6R) together with glycoprotein 130 (gp130), a signal-transducing and intrinsic protein, can initiate intracellular signaling (Hibi et al., 1990). The complexes of IL-6, IL-6R, and gp130 then requires members of the Janus Kinase (Jak) family, such as Jak1, Jak2, and tyrosine kinase 2 (Tyk2) (Stahl et al., 1994) to activate downstream pathways: the signal transducers and activators of transcription 1 and 3 (STAT1 and STAT3) and the mitogen-activated protein kinase (MAPK/ERK) cascade (Kushner, 1993; Ihle and Kerr, 1995). STAT1 was discovered to be 52% homologous with the interferon-stimulated gene factor (ISGF) 3 complex of subunit p91 (Akira et al., 1994). While fundamental STAT3

activation mediates hepatic APPs synthesis according to its former name as acute-phase response factor (APRF) (Wegenka et al., 1993). In addition, STAT3 and NF-κB interaction play a role in whether stimulation or inhibition of IL-6-mediated APP gene expression would occur, i.e., inhibitory activity in g-fibrinogen and α_2-macroglobulin during an acute phase response (Zhang and Fuller, 2000; Bode and Heinrich, 2001), and stimulatory activity in CRP and SAA (Betts et al., 1993; Agrawal et al., 2003).

According to Rose-John (2012), the activation of IL-6 via mIL-6R leads to more regenerative and anti-inflammatory functions, i.e., intestinal epithelial cell proliferation, epithelial cell apoptosis inhibition, and hepatic acute phase response initiation. Whereas the activation of IL-6 via sIL-6R contributes to proinflammatory effects, i.e., mononuclear cell recruitment, T-cell apoptosis inhibition, and T-reg differentiation inhibition. Also, the affinity of the two IL-6Rs is comparable (Rose-John and Heinrich, 1994).

In LPS-challenged mice, intestinal IL-6 increased significantly in marginal Zn mice (fed with 4 ppm Zn) compared with adequate Zn mice (fed with 40 ppm Zn), indicating that Zn status impacts intestinal cytokine response to LPS (Peterson et al., 2008). In pigs infected with respiratory disease, IL-6 gene expression was upregulated from white blood cells (~7.5-fold), liver (~12-fold), lymph nodes (~160-fold), and tonsil samples (~4-fold) (Skovgaard et al., 2007). Instillation of autologous feces via midline laparotomy to pigs induced inflammation by significantly increased serum IL-6 and TNF-α at 2 h after induction (Hoeger et al., 2015).

9. Acute phase proteins

The major site of acute phase proteins (APPs) production is mainly in the liver (intrahepatic), but extrahepatic locations, such as the gastrointestinal tract, respiratory tract, and lymphatic tissue are also reported among species (Vreugdenhil et al., 1999; Marques et al., 2017). Inflammatory mediators that regulate synthesis and release of APPs are categorized into four groups: IL-1 type cytokines (such as IL-1 and TNF-α), IL-6-type cytokines (such as IL-6 and IL-11), glucocorticoids, and growth factors (Ceciliani et al., 2002), but the main mediators are proinflammatory cytokines. During the acute phase response, IL-1 type cytokines circulate through the vascular system to the liver to activate more products of themselves and IL-6 of hepatic Kuffer cells (Jain et al., 2011). Interestingly, IL-6 can give negative feedback to inhibit the secretion of TNF-α and IL-1, while it auto-stimulates itself. Therefore, IL-6 is considered as a major stimulator of APPs production. Pathways of activating APPs synthesis of TNF-α, IL-1, and IL-6 are different resulting in a broad spectrum of APP products. For example, TNF-α binds to its receptor activates the NF-κB pathway for APP gene amplification, IL-6 activates transcription via NF-IL-6, whereas IL-1 activates APP transcription via both NF-κB and NF-IL-6 pathways (Joyce et al., 2001). However, all three cytokines are inhibited by glucocorticoids, hormones derived from the hypothalamic-pituitary-adrenal axis (Ceciliani et al., 2002).

Within a few hours following infection, liver metabolism is partly shifted to mediate APP synthesis according to proinflammatory cytokines stimuli. There are approximately 40 APPs discovered (Gabay and Kushner, 1999), but they can be divided into two classes based on circulating protein concentration: negative APPs and positive APPs. The negative APPs are the type that their serum concentrations drop, such as cortisol

binding globulin (CBG or transcortin), transthyretin (TTR or prealbumin), and retinol-binding protein (RBP), resulting in temporarily increased unbound proteins or hormones (Ingenbleek and Young, 1994). Whereas the positive APPs are the proteins that increase in serum concentrations during APR, such as C-reactive protein (CRP), haptoglobin (Hp), serum amyloid A (SAA), a major acute-phase protein, and fibrinogen. The magnitude of their decrease or increase concentration is species-specific and dependent on how strong and type of insult.

For the use of diagnostic tools, APPs could be markers to reflect the general health status of the animal as an individual or herd. There are several APP indexes generated to compare healthy vs unhealthy animals, i.e., acute phase index (API) by Toussaint et al. (1995), and nutritional and acute phase indicator (NAPI) by Gruys (2002). In addition, some APPs are useful to monitor disease progress or give prognosis because they can increase up to 1,000-fold (Uhlar and Whitehead, 1999). In particular, bacterial infections stimulate stronger APPs response than viral infections (Alsemgeest, 1994). Overall, APPs are better markers than cytokines because their levels remain detectable for 48 h or longer, while cytokines are rapidly cleared within a few hours (Gruys et al., 2005).

a. C-reactive protein

C-reactive protein (CRP) was discovered in the blood circulation of pneumonia patients during acute inflammation (Tillett and Francis Jr, 1930). The name was derived from its binding to the C-polysaccharide of pneumococci. CRP is primarily induced by IL-1 type cytokine and is considered as the first line APP with the presence in circulation within a few hours after infection (Ng et al., 2004; Petersen et al., 2004). In nature, there

are two distinct isoforms of CRP: pentameric CRP (pCRP) and monomeric CRP (mCRP) (Schwedler et al., 2006). pCRP exists primarily in plasma as a cyclic pentamer of 125-kDa contained five Ca-stabilized subunits (Volanakis, 2001). Thus, it makes CRP Ca-dependent and specific to bind phosphocholine in the bacterial cell membrane to initiate host defense functions (Thompson et al., 1999). pCRP can be dissociated to monomeric CRPs (mCRP) by bioactive lipids via the phospholipase A2 enzyme. mCRP is found to be a more potent activator of neutrophils (Khreiss et al., 2005), endothelial cells (Khreiss et al., 2004), and monocytes (Eisenhardt et al., 2009) resulting in worsening the inflammatory response.

Generally, CRP can activate the cascade of the classical complement pathway resulting in the deposition of C3b (trapping or opsonizing) on the surface of the microbe (Deban et al., 2011). This event enhances adherence of phagocytic cells to eliminate the trapped target cells. Thus, the function of CRP is commonly described as a host defense response towards the insults. However, a review article by Tilg et al. (1997) mentioned that CRP could downregulate proinflammatory cytokine production and decrease its production as well. As a result, the overproduction of CRP may provide an anti-inflammatory effect (Xia and Samols, 1997). In pigs, serum CRP was increased eight-fold with a peak at 48 h after 8 ml of subcutaneous turpentine injection and was considered as the best inflammatory biomarker among the acid-soluble glycoprotein, α_1-acid glycoprotein, ceruloplasmin, and haptoglobin (Eckersall et al., 1996). Yin et al. (2017) identified inflammatory biomarkers from pigs challenged with 15 μg/kg BW of intramuscular LPS injection that CRP significantly increases, but no difference in pig-major acute phase protein (Pig-MAP) and transthyretin (TTR) concentrations. In

summary, CRP is a prototypic first-line response APP that can be used as a non-specific marker of inflammation.

10. Conclusion

Weaned pigs are crucially required Zn to support growth and immune response during stressful change. Zn functions in enzyme catalyst and structure that is important for nutrient metabolism and muscle development. In addition, Zn plays as a signaling molecule and is involved profoundly during the acute phase response of inflammation. Mitigation of inflammatory response can enhance homeostasis restoration, thus, alleviate negative outcomes. The typical method of supplementation is applied through feed intake, however, supplementing via drinking water can be additional means for Zn delivery.

Therefore, a series of experiments were conducted to evaluate the effects of titrated levels of water soluble Zn amino acid complex (ZnACC) via drinking water on growth performance and immune response of nursery pigs challenged with lipopolysaccharide. Pigs were fed with pharmacological levels of Zn as ZnO and Cu as $CuSO_4$ for early and late phases of nursery pigs, respectively, as similar to a commercial setting. Zn dose estimation for an ideal response was generated using non-linear models. Expression of Zn transporter and intracellular metal-binding genes of liver and duodenum were assessed to observe change and relationship under inflammatory response. A time-course evaluation was included to evaluate inflammatory markers and Zn transporters kinetic and response pattern of pigs challenged with LPS.

CHAPTER III

TIME COURSE AND PEAK RESPONSE OF INFLAMMATION AND TISSUE ZINC TRANSPORTERS DURING LPS-INDUCED INFLAMMATION IN NURSERY PIGS FED PHARMACOLOGICAL LEVELS OF DIETARY ZINC AND COPPER

INTRODUCTION

Inflammation and the release of proinflammatory cytokines have been reported to cause profound effects on nutrient metabolism. They not only cause illness contributing to decreased appetite and intake (Johnson and Von Borell, 1994), but also alter the homeostasis of nutrients, including zinc. Commercial weaned pigs are fed either with pharmacological levels (2,000-3,000 mg Zn/kg diet) of Zn or adequate Zn levels to meet or exceed their requirements. In addition, research has contributed to our understanding of the role of Zn during inflammation that Zn is crucially needed for high metabolic and immunological tissues, such as the liver (Aydemir et al., 2012; Robinson et al., 2016). Limited knowledge of Zn homeostasis during inflammation has hindered advances in this matter of young pigs.

Numerous studies have evaluated the immune response of pigs challenged with lipopolysaccharide (LPS) in a time-course manner on changes and peak response of cytokines, and several other metabolites during the acute phase response (Webel et al., 1997; Choi et al., 2002; Terenina et al., 2017). Lipopolysaccharide is an endotoxin of Gram-negative bacteria that stimulates innate immune response and metabolic changes of the host. It provokes the production of the proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) from stimulated macrophages (Johnson, 1997). These cytokines mediate acute phase proteins, such as C-reactive protein (CRP) synthesis in hepatocytes. Pigs challenged with low dose LPS (5 μg/kg BW) exhibited sickness behavior and significantly increased proinflammatory cytokines and acute phase proteins (Moya et al., 2006).

In this study, we aimed to evaluate the inflammatory response and relationship of hepatic and duodenal IL-6, Zn transporters, and metallothionein expression following a single LPS injection to nursery pigs.

MATERIALS AND METHODS

This study was conducted at the Swine Research and Education Center at Oklahoma State University. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Experimental design, animal and treatment

This study evaluated the effects of LPS injection on the innate immune response and tissue Zn transporters of nursery pigs as illustrated in Figure 3.1. Thirty crossbred

weaned pigs (PIC USA; 19 d; 4.7 ± 0.8 kg BW) were allotted to 2 pens equally. They were fed and acclimated to the environmental-controlled room. All pigs were fed the same diets that included Zn from ZnO at 2,000 mg/kg from d 0-7, Zn from ZnO at 1,750 mg/kg from d 7-14, and switched to Cu from $CuSO_4$ at 200 mg/kg from d 14-23.

On d 23, pigs were randomly blocked by BW and sex into one of the five sampling times (n = 6 pigs/time; h 0, 3, 6, 12, and 24 after injection). Pigs were deprived of feed for 5 h before LPS challenge. Before injection, lyophilized powder of LPS purified by phenol extraction was diluted with 0.9% normal saline and vortexed to create 500 μg/mL LPS solution. At h 0, all pigs were injected with a single i.m. dose of LPS at 12 μg/kg BW (Escherichia coli serotype O55:B5; Sigma-Aldrich, St. Louis, MO) in the neck area. Barn temperature was set at 28.8 ºC.

At each time point, BW, rectal temperature (RT), and blood samples were collected before one group (n = 6 pigs) was euthanized for liver and duodenum collection. Sample size of BW, RT and blood samples at h 0 started at 30 pigs, and decreased by 6 pigs at the following time points.

Body weight, rectal temperature, and blood collection

Pigs were restrained in a cart scale for individual BW and rectal temperature (RT) recording. Blood sample was drawn from the jugular vein and placed into a trace element testing tube for 5 mL and into a non-additive tube (BD Vacutainer™, Franklin Lakes, NJ) for 10 mL. Blood samples were centrifuged at 2500 × *g*, 4 °C for 10 min to separate serum and aliquoted into 1.5-mL tubes, and stored at -20°C until further analysis.

Tissue sampling, RNA isolation, and cDNA synthesis

At each time point, six pigs were euthanized and liver tissue and duodenal mucosa scrapings were collected into separate tubes filled with RNAlater (Thermo Fisher Scientific, Waltham, MA), and stored at -20°C. Samples were homogenized in TriZol reagent (Invitrogen, Carlsbad, CA) before RNA isolation using Direct-Zol RNA Miniprep Plus (Zymo Research, Irvine, CA) with DNase I treatment to eliminate contaminated DNA. NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA) was used to determine RNA purity. The integrity of RNA samples was evaluated using agarose gel electrophoresis. Complementary DNA (cDNA) was created by reverse transcription of RNA using ProtoScript II Reverse Transcriptase kit (New England Biolabs, Ipswich, MA), and stored at -80°C until subsequent quantitative RT-PCR (RT-qPCR) analysis as previously described by Ojo et al. (2019).

Serum TNF-α and serum CRP concentrations analysis

Serum TNF-α concentrations were analyzed in duplicate using a commercial porcine ELISA kit (R&D Systems Inc., Minneapolis, MN). The intra- and inter-assay coefficient of variations (COVs) were less than 5%, respectively. Serum CRP concentrations were analyzed in duplicate using a commercial porcine ELISA kit (Lifediagnostics Co., Ltd., West Chester, PA). The intra- and inter-assay COVs of CRP samples were less than 3%.

Serum Zn and Cu concentrations analysis

Serum samples were diluted 1:50 in 0.1% high-purity nitric acid (GFS Chemicals, Inc., Powell, OH) before Zn and Cu concentrations analysis using Inductively Coupled

Plasma Mass Spectrometry (ICP-MS) (Elan 9000; Perkin Elmer Life and Analytical Sciences, Norwalk, CT). Gallium was used as an internal standard. The UTAK normal range control serum (UTAK Laboratories Inc, Valencia, CA) was analyzed to verify consistency after every 10^{th} sample as described by Joray et al. (2015).

Quantitative real-time polymerase chain reaction (RT-qPCR)

Hepatic and duodenal tissues were quantified for mRNA expression of interleukin-6 (IL-6), Zn transporters (ZIP4 for duodenum and ZIP14 for liver), and metallothionein-1 (MT-1) using SYBR Green Master Mix (Power Sybr, Applied Biosystems, Foster City, CA) for RT-qPCR on an ABI 7900HT sequence-detection instrument and 2.4 SDS software (Applied Biosystems, Foster City, CA) as described by Ojo et al. (2019). An internal reference gene was succinate dehydrogenase complex flavoprotein subunit A (SDHA). Intron-spanning primers were designed using Primer3 (Rozen and Skaletsky, 2000) and NCBI BLAST web interfaces (Johnson et al., 2008). Sequences of the primers and NCBI accession numbers are shown in Table 3.1.

Statistical analyses

This study was designed as a randomized complete block with a one-way treatment structure. Data of BW, RT, and serum markers were analyzed using a linear mixed model using PROC GLIMMIX of SAS (Version 9.4, SAS Institute Inc., Cary, NC). Pig was the experimental unit. Time was a fixed effect (0, 3, 6, 12, or 24 h after injection) and block (5 blocks) was a random effect. Orthogonal polynomial contrasts were used to evaluate linear or quadratic trends. The contrast coefficiency values were

generated using PROC IML. Means from all time points were separated using PDIFF option with the Tukey-Kramer adjustment for multiple comparisons.

For gene expression results, fold change of gene expression ($2^{-\Delta\Delta CT}$) was calculated based on h 0 as described by Livak and Schmittgen (2001) and was tested for the mean difference using PROC GLIMMIX. Time was a fixed effect and block was a random effect. Correlation of gene expression based on h 0 (ΔΔCT) was tested using PROC CORR, while linear regression equation was generated by using PROC REG.

All data were reported as least square means and standard error of the mean (lsmeans ± SEM). Tests were considered significant when $P \leq 0.05$ and a tendency of significance was reported when $0.05 < P \leq 0.10$.

RESULTS

Intramuscular injection of LPS increased rectal temperature (cubic, $P < 0.0001$), and serum TNF-α (cubic, $P < 0.0001$) over 12 h after injection (Figure 3.2). Rectal temperature peaked at h 3 from baseline (40.36 ºC vs 39.65 ºC) and carried to h 6 (40.19 ºC) after injection ($P < 0.05$). Rectal temperature returned to the basal as afebrile condition (38.91 ºC) at h 24. Serum TNF-α concentration peaked at h 3 after injection from baseline ($P < 0.05$; 5,406 pg/mL vs 150 pg/mL) and dropped significantly from h 6 (1487 pg/mL) to h 24 (551 pg/mL). Serum CRP concentration tended to increase over time from 12 μg/mL at h 0 to 20 μg/mL at h 24 (linear, $P = 0.10$).

In contrast, LPS injection significantly decreased serum Zn (cubic, $P = 0.007$) and Cu (cubic, $P = 0.0006$) concentrations (Figure 3.2). During sepsis, serum Zn concentration decreased significantly from baseline within 3 h ($P < 0.05$; 0.266 mg/L vs

0.807 mg/L) and continued to drop from h 0 to the lowest concentration at h 6 ($P < 0.05$; 0.221 mg/L; -72.5%) post injection. Serum Zn remained low until h 24. Serum Cu concentration decreased within 3-h after LPS injection ($P < 0.05$; 1.630 mg/L vs 2.220 mg/L), dropped to the lowest at h 12 ($P < 0.05$; 1.572 mg/L; -29.2%), and remained low until h 24 (1.869 mg/L) post injection. LPS injection did not significantly ($P > 0.05$) cause bodyweight loss in pigs.

Hepatic IL-6, ZIP14, and MT-1 expressions were increased after LPS injection (cubic, $P < 0.0001$) (Figure 3.3). Hepatic IL-6 peaked at h 3 from baseline ($P < 0.05$; 5-fold increased) and remained high until h 12 ($P < 0.05$) following LPS. Hepatic ZIP14 peaked at h 3 from baseline ($P < 0.05$; 3-fold increased) and remained high until h 6 ($P < 0.05$) after injection. The expression of hepatic ZIP14 returned to the basal at h 12 and lower than the basal at h 24 ($P < 0.05$). Hepatic MT-1 expression significantly increased at h 3 ($P < 0.05$), peaked at h 6 ($P < 0.05$; 5-fold increased), and remained high until h 12 ($P < 0.05$) after injection. Hepatic MT-1 expression returned to the basal at h 24. Correlation of relative expression of the hepatic genes revealed a positive relationship between IL-6, ZIP14, and MT-1 in pairwise comparisons ($P \leq 0.0017$) (Figure 3.4).

Duodenal IL-6 mRNA expression did not change after challenge ($P > 0.05$) (Figure 3.5). Duodenal ZIP4 and MT-1 increased after LPS injection (Quadratic, $P = 0.01$). Duodenal ZIP4 peaked at h 12 ($P < 0.05$; 2-fold increased), while duodenal MT-1 peaked at h 24 ($P < 0.05$; 4-fold increased). Correlation expression of the duodenal genes revealed no significant relationship between IL-6, ZIP4, and MT-1 in pairwise comparisons ($P > 0.05$) (Figure 3.6).

DISCUSSION

Our data indicated that LPS induced an acute febrile response by increasing rectal temperature within 3 h after injection and lasted until h 6. This is in agreement with Roberts et al. (2002) that i.m. LPS injection (serotype O55:B5) at 10 μg/kg BW induced short-term monophasic fever response of pigs within 6 h and returned to a normal level within 24 h. Pigs injected with an i.m. LPS (serotype O55:B5) at 15 μg/kg BW significantly increased tympanic temperature (ear temperature) at h 4 and returned to the basal temperature within 24 h post-challenge (Terenina et al., 2017). A short-term fever is associated with a mild form of systemic inflammation (Garami et al., 2018). On the other hand, pigs challenged with an i.m. LPS (serotype O55:B5) at 200 μg/kg BW had a higher body temperature for 24 h after injection (Van Heugten et al., 1994). Pattern of core body temperature depends on the dose of LPS and the ambient temperature, i.e., high LPS doses and low ambient temperature can induce hypothermic phase before a febrile increase of body temperature above the basal value (Töllner et al., 2000). A small dose of LPS can induce an initial phase of fever or *Phase 1* fever, which is the synthesis of prostaglandin E_2 (PGE_2) in the periphery and uniquely occurs with early sickness symptoms (Ivanov and Romanovsky, 2004). Since PGE_2 is a short-lived mediator and is rapidly acting, pigs challenged with LPS exhibited monophasic short-term fever as a febrile response.

TNF-α is a proinflammatory cytokine that can play roles as pyrogen or cryogen (Leon, 2004). In our study, serum TNF-α concentration peaked at h 3 (5,400 pg/mL) after injection, thus it acted as a pyrogenic cytokine according to the rectal temperature data. In

another study, plasma TNF-α concentration peaked at 2 h (11 pg/mL) after an i.p. LPS injection (serotype K-235) at 5 μg/kg BW from 2 h interval sampling for 24 h (Moya et al., 2006). A low dose of i.v. LPS injection (serotype O111:B4) at 1.5 μg/kg BW of pigs revealed the peak of serum TNF-α concentration at 1 h (4,000 pg/mL) from one-hour interval sampling for 4 hours post-challenge (Nordgreen et al., 2018). Although most LPS studies showed rapid onset of increased serum TNF-α concentration, a study on pigs challenged with an oral *Salmonella typhimurium*, a Gram-negative and endotoxin-producing bacteria, did not show an increase in plasma TNF-α concentration regardless of the peak increase of rectal temperature at 42 h after infection (Balaji et al., 2000).

C-reactive protein (CRP) is a major and positive acute phase protein (APP) synthesized by the hepatocytes in response to cytokines. CRP moderately responds to inflammation to promote pathogen clearance by activating the classical complement cascade and enhancing opsonization for phagocytosis (Mortensen and Zhong, 2000). During acute infection, inflammation, and stress of pigs, CRP can increase from 2 to 10-fold in blood circulation (Murata et al., 2004; Eckersall and Bell, 2010). In our study, serum CRP concentration increased from 12 μg/mL to 20 μg/mL (66.7% increased) over a 24-h period following LPS. This was similar to Nordgreen et al. (2018) who found serum CRP of endotoxemic pigs peaked at 12-h (34.1 μg/mL, 40.9% increased) and remained elevated until 48-h (27.1 μg/mL) after LPS injection. In contrast, pigs did not have a significant increase in serum CRP concentration after an i.m. LPS challenge at 25 or 50 μg/kg BW (Frank et al., 2005).

Serum Zn concentration dropped significantly after LPS injection. This phenomenon is called hypozincemia, a decrease in serum Zn concentration due to Zn translocation from blood to the liver. In our study, serum Zn concentration dropped drastically and remained low to h 24 following LPS challenge. Our data indicated strong positive relationships of upregulated hepatic IL-6, ZIP14, and MT-1 mRNA expression during inflammation. Also, we found that hepatic IL-6 highly correlated with hepatic ZIP14 at h 3 following LPS injection, while hepatic IL-6 vs hepatic MT-1 and hepatic ZIP14 vs hepatic MT-1 were highly correlated at h 6. Injection of LPS induced hepatic inflammation by increasing hepatic IL-6 gene expression. The presence of hepatic IL-6 regulated the Zn transporter ZIP14 in liver and contributed to hypozincemia during inflammation (Liuzzi et al., 2005). In a murine model, upregulation of ZIP14 expression leading to Zn influx is a key hepatic Zn mediator during the inflammatory state (Aydemir et al., 2012). Mice challenged with LPS exhibited hypozincemia and increased hepatic MT expression concomitantly with a 36% increase in hepatic Zn concentration (Rofe et al., 1996; Aydemir et al., 2012). In contrast, the MT-1 gene deleted mice had no changes in serum and liver Zn concentrations after LPS injection (Rofe et al., 1996; Philcox et al., 2000). Therefore, hepatic ZIP14 and MT-1 are significant mediators involved in hypozincemia and Zn influx to the liver following LPS-induced inflammation. Furthermore, hepatic Zn accumulation suggests that Zn plays a role as a signaling molecule to inhibit proinflammatory cytokines and intermediates at transcriptional levels (Haase and Rink, 2011).

Interestingly, our study showed that LPS did not upregulate duodenal IL-6 gene expression. In contrast, a study from Liu et al. (2008) reported nursery pigs injected with

i.p. LPS (E. coli serotype O55:B55) at 100 μg/kg BW significantly increased mRNA expression of duodenal IL-6 at 6 h after injection. We suggest two major differences contributing inconsistent results were the dose (15 vs 100 μg/kg BW) and route (intramuscular vs intraperitoneal) of LPS injection. Dose of LPS affects the amplitude of response in a dose-dependent manner, while the route of administration affects latency to fever onset and time to peak, i.e., intraperitoneal LPS injection had 10 to 20 mins shorter time to reach the fever onset compared to intramuscular injection (Cartmell et al., 2002). In addition, LPS escapes from the peritoneal cavity to systemic circulation via the portal vein and lymphatic system when administered intraperitoneally (Romanovsky et al., 2000). Increasing LPS dose may result in a greater increase in duodenal IL-6 expression.

Our data showed increased duodenal ZIP4 and MT-1 expressions at h 12 and 24 after LPS, respectively, displaying a delayed response of duodenal Zn homeostasis during acute inflammation. Under Zn-deficient conditions, the presence of ZIP4 on the apical membrane of the enterocytes is increased (Ohashi et al., 2019). On the opposite, the degradation of ZIP4 occurs during high Zn intake to protect against Zn cytotoxicity (Mao et al., 2007). Therefore, the effects of LPS reducing feed intake and long hours of fasting may temporarily increase the duodenal ZIP4 expression of pigs. A study in mice reported that intestinal metallothionein expression inversely correlated with serum Zn concentration (Davis et al., 1998). However, studies in pigs reported that mucosal MT-1 expression was directly associated with serum Zn concentration (Martínez et al., 2004; Carlson et al., 2007). Aside from Zn, metallothionein also functions as an intracellular Cu-binding protein. In this study, we observed that pigs recovered and started to eat and drink within 12-h following LPS injection. Therefore, upregulation of duodenal MT-1

expression at h 24 may be from increased feed intake (high dietary Cu concentration) and/or LPS-mediated intestinal MT-1 upregulation as described by Paradkar et al. (2004).

Our study found decreased serum Cu concentrations of approximately 29% at 3-h after LPS injection. This result is consistent with one of the experiments in Chapter V but opposed to some human studies (Malavolta et al., 2015; Giacconi et al., 2017). These studies concluded that an increased serum Cu concentration and a decreased serum Zn concentration resulting in a higher Cu/Zn ratio was an indicator of aging, nutritional and hormone imbalance, oxidative stress, and inflammation of the body. However, we suggest that decreased levels in serum Cu occurred for similar purposes of Zn homeostasis, but in a lesser magnitude. Further study may investigate the mechanistic explanation of decreased serum Cu during the LPS challenge of pigs.

CONCLUSION

Our data indicated the peak of inflammatory response in rectal temperature, serum TNF-α, and serum Zn and Cu concentrations were rapid within 3 h after LPS injection. Serum CRP was a more delayed response. Rapid upregulation of hepatic ZIP14 mediated by IL-6 leads to Zn accumulation in the liver, bound by MT-1, during the acute phase response. Therefore, liver is a central immunological and metabolic organ that regulates Zn homeostasis. Duodenal IL-6, ZIP4, and MT-1 genes are less responsive to a low dose i.m. LPS challenge. Furthermore, the data may provide the first evidence of tissue Zn transport and binding protein expressions of endotoxemic pigs in a time-course manner.

Table 3.1. Primer sequences used for RT-qPCR of hepatic and duodenal IL-6, Zn transporters, and MT-1 genes.

Genes[1]	Accession number	Primer sequences	Product size (bp)
SDHA	DQ_178128.1	5’-CTCAAGTTCGGGAAGGGCGG-3’ 5’-TCGTACCGCAGAGACCTTCCG-3’	104
IL-6	NM_214399.1	5’-CACCTCTCCGGACAAAACTGA-3’ 5’-TGCCAGTACCTCCTTGCTGTT-3’	118
ZIP4	XM_001925360.5	5’-CTGCTCCAGCAACAGCTGAGT-3’ 5’-GCAGAGGCAGATGAGCAGTGT-3’	123
ZIP14	XM_021072999.1	5’-TCTGTCTTCCAAGGCATCAGC-3’ 5’-CAGCAGGCAGAGAGGAAGTTG-3’	121
MT-1	NM_001001266	5’-TTGCTCTCTGCTTGGTCTCACCT-3’ 5’-GGGATGTAGCATGAAGTCAGTGCATGTG-3’	378

[1]SDHA, succinate dehydrogenase complex flavoprotein subunit A; IL-6, interleukin 6; ZIP4, solute carrier family 39 member 4 (SLC39A4); ZIP14, solute carrier family 39 member 14 (SLC39A14); and MT-1, metallothionein.

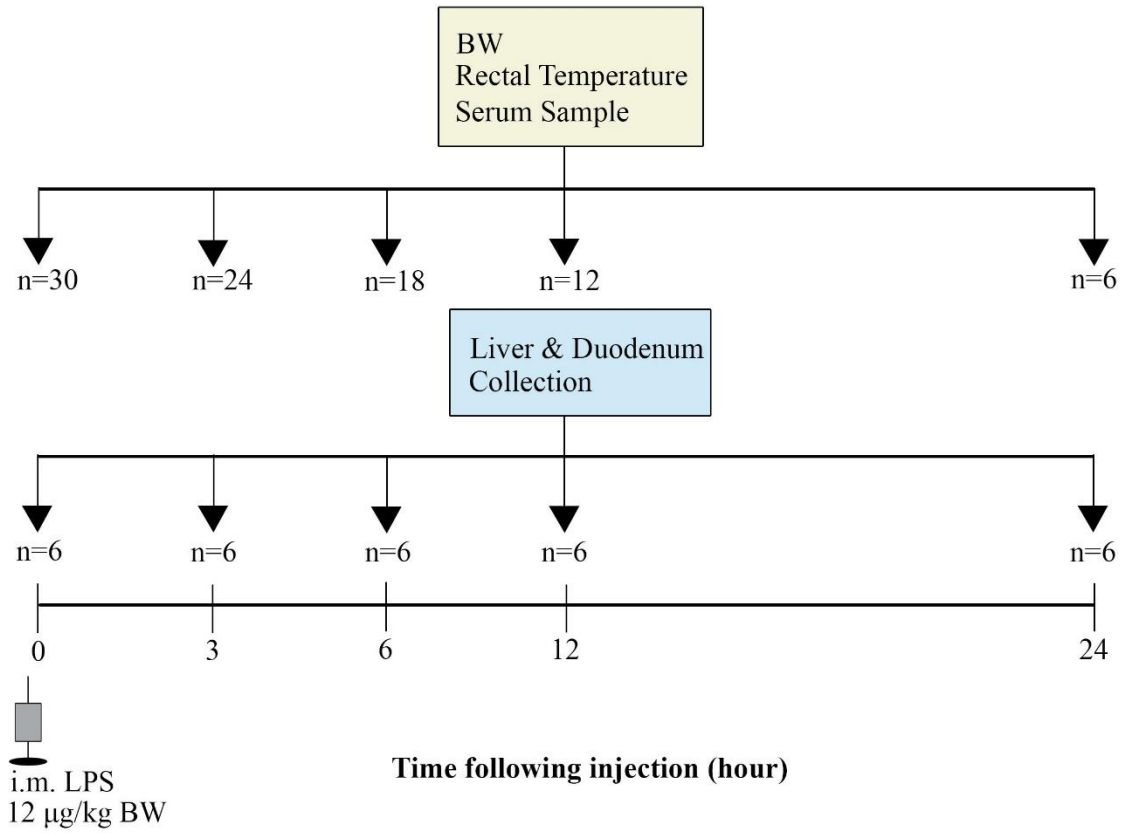

Figure 3.1. Timeline of measurements, sample collections and sample size over the five-time course following LPS (O55:B55 serotype) injection of nursery pigs. Serum samples were analyzed for TNF-α, CRP, Zn and Cu concentrations. Tissues collection was quantified for IL-6, ZIP4 (duodenum), ZIP14 (liver) and MT-1 mRNA expression.

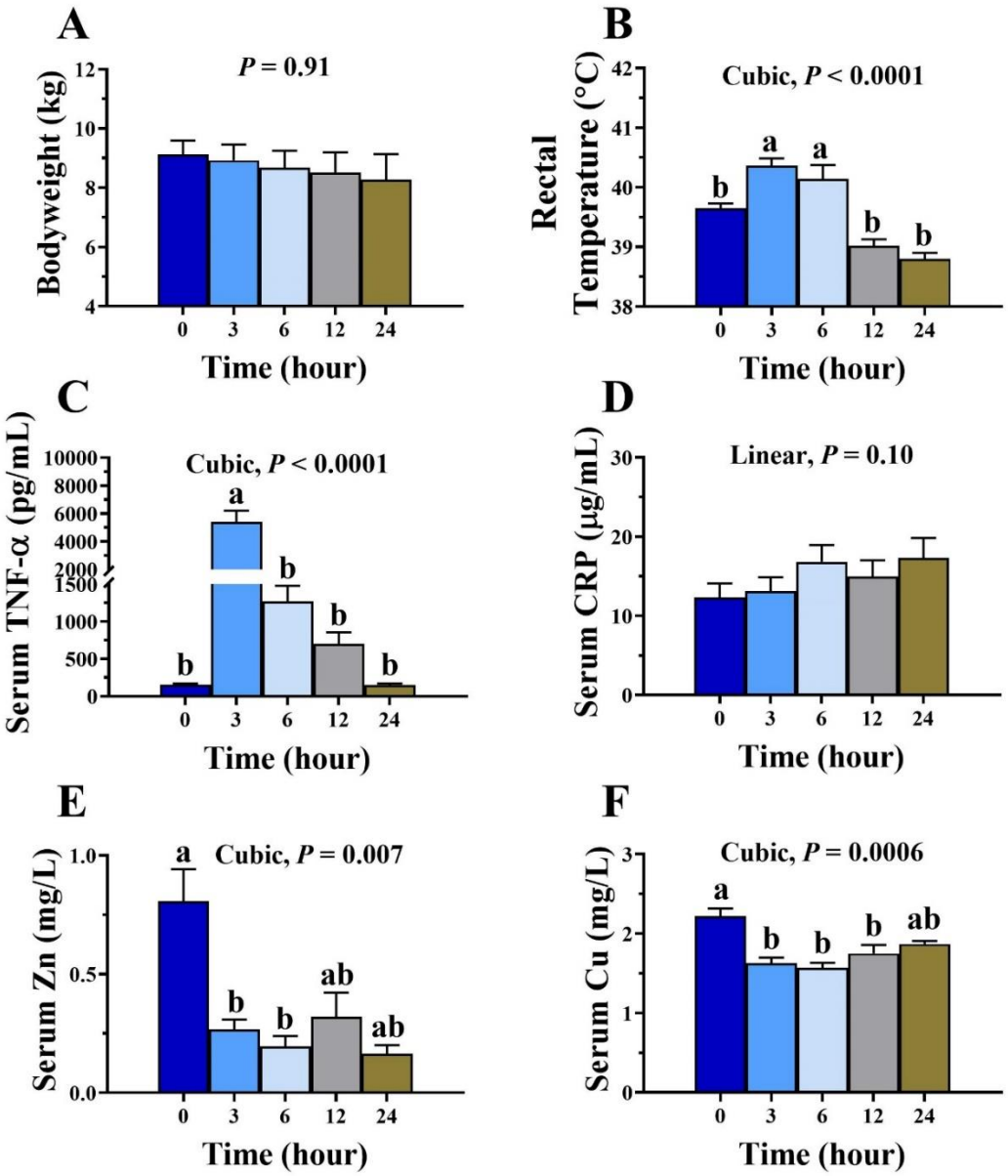

Figure 3.2. Five time-course of BW, rectal temperature, and serum biomarkers during LPS-induced inflammation of nursery pigs (n = 30, 24, 18, 12, and 6 for h 0, 3, 6, 12, and 24 following LPS, respectively. Data are least square means ± SEM. Superscripts denote differences between time points ($P < 0.05$).

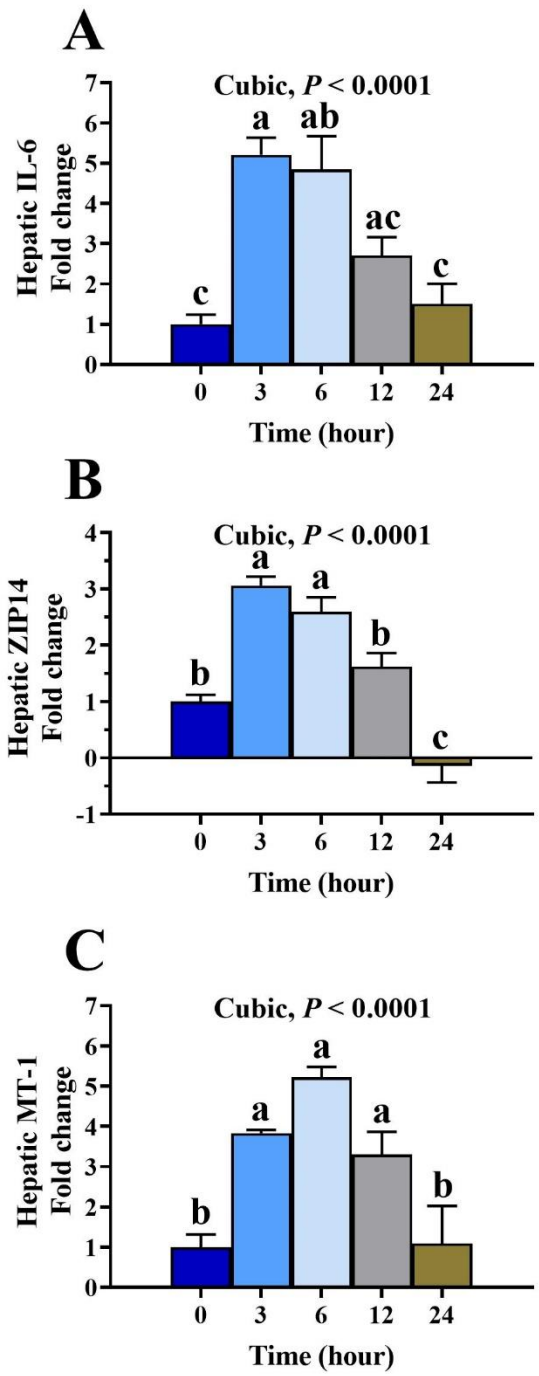

Figure 3.3. Five time-course of hepatic IL-6, ZIP14, and MT-1 mRNA expressions during LPS-induced inflammation of nursery pigs (n = 6/time). Data are least square means ± SEM of fold change based on h 0. Superscripts denote differences between time points ($P < 0.05$).

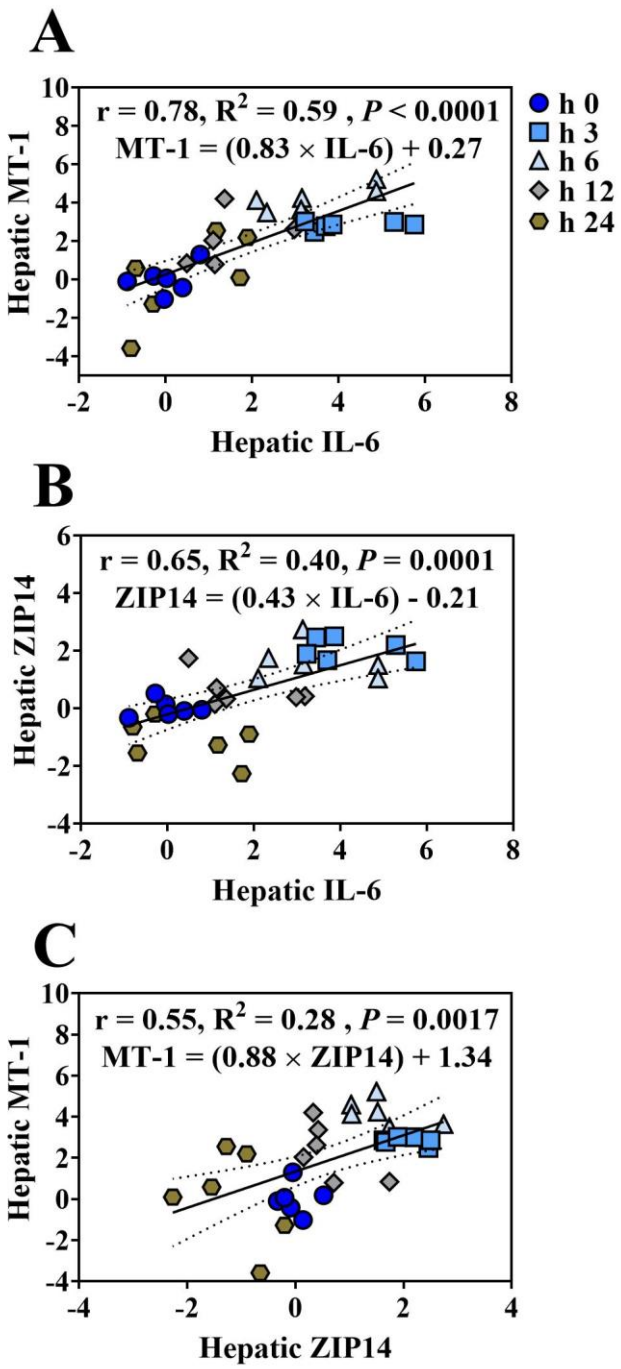

Figure 3.4. Pairwise correlation and linear regression equation of hepatic IL-6, ZIP14 and MT-1 mRNA expressions during LPS-induced inflammation of nursery pigs (n = 6/time). Data are means of relative expression (ΔΔCT) based on h 0 of the five-time course. Each plot overlays with the fitted solid line and a 95% confidence dotted line.

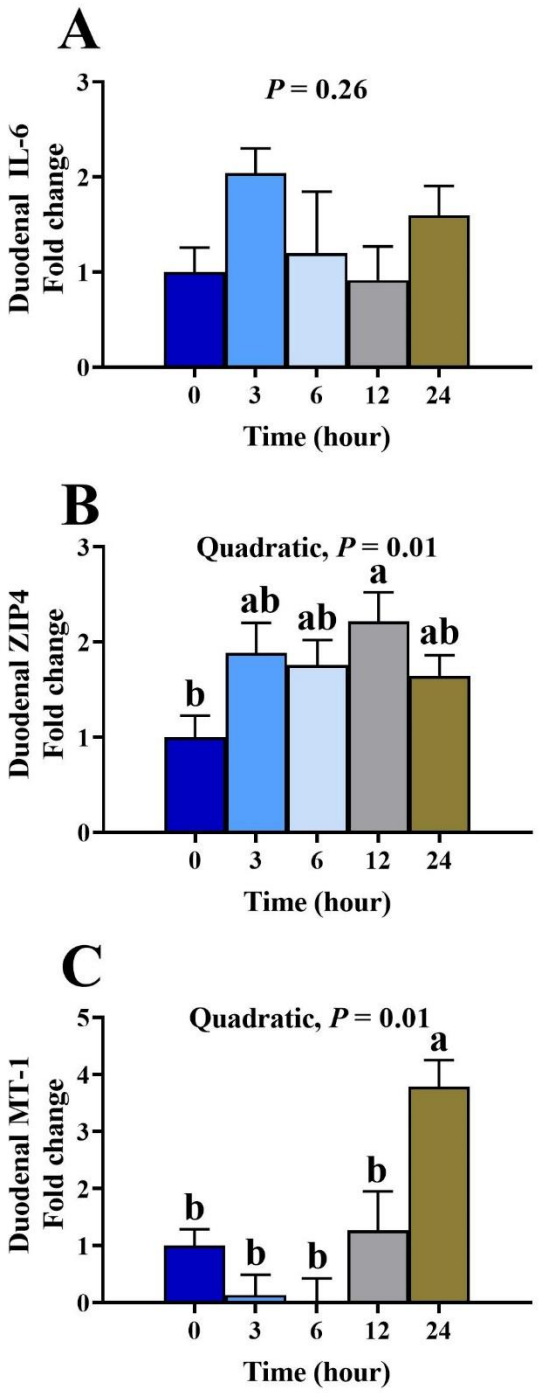

Figure 3.5. Five time-course of duodenal IL-6, ZIP4 and MT-1 mRNA expressions during LPS-induced inflammation of nursery pigs (n = 6/time). Data are least square means ± SEM of fold change based on h 0. Superscripts denote differences between time points ($P < 0.05$).

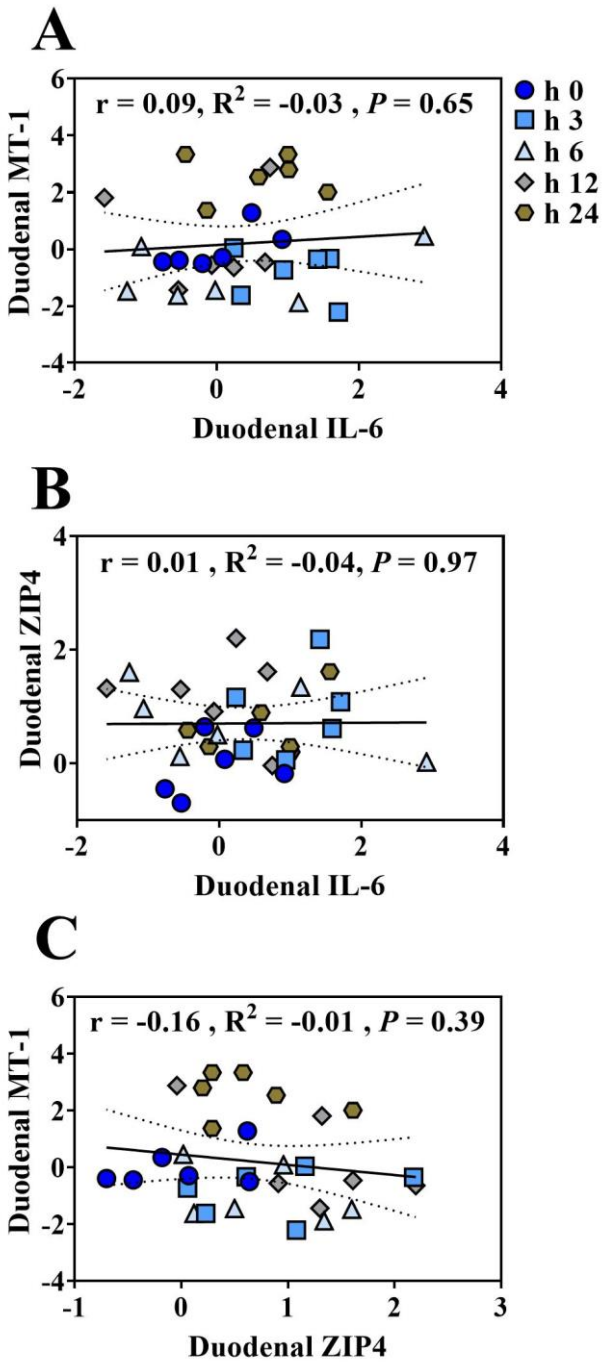

Figure 3.6. Pairwise correlation of duodenal IL-6, ZIP4 and MT-1 mRNA expressions during LPS-induced inflammation of nursery pigs (n = 6/time). Data are means of relative expression (ΔΔCT) based on h 0 of the five-time course. Each plot overlays with the fitted solid line and a 95% confidence dotted line.

CHAPTER IV

GROWTH RESPONSE AND DOSE ESTIMATION OF WEANED PIGS DRINKING TITRATED LEVELS OF WATER SOLUBLE ZINC AMINO ACID COMPLEX THROUGHOUT NURSERY PERIOD

INTRODUCTION

It is common practice to feed pharmacological levels (2,000-3,000 mg/kg diet) of dietary Zn during the early nursery period to enhance growth performance (Zhang et al., 2018) and decrease the incidence of post-weaning diarrhea (Pérez et al., 2011). However, Zn sources, especially ZnO used for the pharmacological effect, have low relative bioavailability (RBV), suggesting the primary mode of action is in the intestinal lumen and excreted via manure (Meyer et al., 2002; Jondreville et al., 2003). Estimated relative bioavailability of ZnO varies from 20% (Poulsen and Larsen, 1995), 50% (Hellman and Carlson, 2018), 68% (Wedekind et al., 1994), to 93% (Edwards III and Baker, 1999). Organic Zn sources offer greater bioavailability (> 100% RBV) over inorganic sources (Lee et al., 2001; Carlson and Hellman, 2003). Weaned pigs fed with pharmacological doses (2,000 – 3,000 mg Zn/kg diet) of organic Zn sources for 2 wks had significantly

higher Zn concentration in serum, liver, and kidney than pigs fed with equal levels of ZnO (Schell and Kornegay, 1996). Adding organic Zn at 40 mg/kg diet to corn-soybean meal diet in pigs increased Zn absorption (46.58 vs 35.91 mg/d), retention (42.95 vs 31.52 mg/d), and apparent total tract digestibility (ATTD) (53.78 vs 45.11%) compared to adding the same dose of inorganic Zn form (Liu et al., 2014). Therefore, supplementing organic sources may contribute to more available Zn for systemic purposes.

A conventional method of supplementing nutritional additives, including Zn, is administered via dietary intake. However, adding water soluble nutrients to drinking water has successfully improved growth performance of weanling pigs (Kaewtapee et al., 2010; Escuredo et al., 2016). Therefore, a possible means to increase systemic Zn in the presence of pharmacological levels of dietary inorganic Zn sources would be to offer water soluble organic Zn via drinking water to increase growth performance in nursery pigs.

In this study, weanling pigs were supplemented with water soluble Zn amino acid complex (ZnAAC) (TruCare®, Zinpro Corporation, Eden Prairie, MN) via drinking water in the presence of pharmacological levels of dietary Zn or Cu during a 42-d nursery period. We hypothesized improvement in growth performance in a dose-dependent pattern. A series of two experiments administering titrated levels of water soluble ZnAAC on growth performance and optimal Zn dose estimation using nonlinear models were reported.

MATERIALS AND METHODS

A series of two experiments were conducted at the Oklahoma State University Swine Research and Education Center. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Experimental design, animals and treatment

The objective of this study was to evaluate the growth performance of nursery pigs drinking titrated levels of water soluble ZnAAC (TruCare®, Zinpro Corporation, Eden Prairie, MN). In Exp 1, water ZnAAC treatments were 0, 20, 40, and 80 mg Zn/L of water (**Trt 0**, **Trt 20**, **Trt 40**, and **Trt 80**). In Exp 2, the treatments were 0, 40, 80, and 160 mg Zn/L of water (**Trt 0**, **Trt 40**, **Trt 80**, and **Trt 160**). In each experiment, 280 crossbred pigs (19 ± 2 d of age; 5.6 ± 0.3 kg BW) were randomly assigned to one of four water ZnAAC treatments (10 pigs/pen; 7 pens/treatment).

Housing, feeding, watering, and vaccinating methods

Pigs were housed in an environmentally controlled room and offered feed and water ad libitum. Each pen was equipped with one 4-hole dry feeder and one water meter above the waterer cup. Pigs were fed corn-soybean meal-based diets formulated to meet or exceed swine NRC (2012) recommendations. There were four dietary phases fed to all pigs with added Zn from ZnO or Cu from $CuSO_4$: Phase 1 (2,500 mg added Zn/kg) from d 0-7, Phase 2 (1,750 mg added Zn/kg) from d 7-14, Phase 3 (200 mg added Cu/kg) from d 14-23, and Phase 4 (200 mg added Cu/kg) from d 23-41 (Table 4.1). On day 4 and 25, pigs were vaccinated with i.m. Circumvent® PCV-M G2 (Merck Animal Health, USA).

All pens were supplied drinking water via the same water system. The stock solutions of each water Zn treatment, including **Trt 0** were prepared for 48 h use. Water regulators (Dosatron D128R, Clearwater, FL) were used to blend Zn stock solutions with incoming water (1:100) and delivered through a waterline to each pen. Daily water disappearance was recorded as average daily water intake (ADWI) and calculated for water Zn intake. Water and dietary Zn concentrations were analyzed at a commercial laboratory (ServiTech, Dodge City, KS) and reported in Table 4.2.

Growth performance data

Pigs and feeders were weighed weekly for calculations of average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). Additionally, average daily water intake (ADWI) was calculated from water disappearance obtained from the water meter to report water to feed ratio (W:F). Average dietary Zn intake, water Zn intake, and total Zn intake were calculated based on analyzed dietary and water Zn concentrations. Data were reported for d 0-14 (high dietary Zn phases), d 14-41 (high dietary Cu phases), and d 0-41 period.

Statistical analyses

Data from this one-way treatment structure with a randomized complete block design were analyzed using PROC GLIMMIX of SAS 9.4 software (SAS Institute, Cary, NC) for linear mixed model methods. In each experiment, water ZnAAC treatment was a fixed effect and block was a random effect. In combined data of the two experiments, fixed effects were treatment and experiment, and a random effect was block. Orthogonal

polynomial contrasts were conducted to determine linear and quadratic effects for increasing levels of water ZnAAC treatment. Because of unequal spacing among treatments, PROC IML was used to calculate the coefficients for the orthogonal polynomial contrasts. Pen was considered the experimental unit.

Data from both experiments were combined and analyzed for Zn dose estimation. Data for ADG and G:F ratio from two experiments were modeled for the optimal dose-response. PROC NLIN was used to generate initial parameters of non-linear quadratic broken line models (Fadel, 2004) before estimating the breaking points of the models, including experiment as a random component using PROC NLMIXED (Robbins et al., 2006).

Data were presented as least squares means ± standard error of the means (lsmeans ± SEM). All tests were conducted at the nominal 0.05 level of significance, while the level between 0.05 to 0.10 was considered as a trend toward significance. Graphs were illustrated using GraphPad Prism (GraphPad Software, San Diego, CA).

RESULTS

Experiment 1

We observed positive effects of increasing water ZnAAC treatment in increasing BW at d 14 (linear, $P = 0.05$), ADFI (linear, $P = 0.07$) during d 0-14, ADG (linear, $P = 0.04$) during d 0-14, and G:F during d 0-14 (linear, $P = 0.10$) and d 0-41 (linear, $P = 0.02$) (Table 4.3). In addition, there was no reduction in water intake ($P > 0.05$) among all

ZnAAC treatments. Water intake tended to increase (linear, $P = 0.09$) during d 0-14 with increasing water ZnAAC treatment.

Pigs drinking titrated levels of water soluble ZnAAC during feeding high dietary levels of ZnO tended to increase (linear, $P = 0.08$) dietary Zn intake during d 0-14 and d 0-41 (Figure 4.1A). Water Zn intake increased (quadratic, $P < 0.05$) among the four treatments throughout the experiment (Figure 4.1B). Overall, total Zn intake increased (linear, $P < 0.001$) with increasing water ZnAAC treatment throughout the study (Figure 4.1C).

A regression equation of average water intake (abbreviated as ADWI; L/pig) by day in experiment exhibited a strong positive association ($r = 0.95$; $R^2 = 0.91$; $P < 0.0001$; ADWI = (0.08407 × Day) + 0.29755) (Figure 4.3A).

Experiment 2

Pigs drinking titrated levels of water soluble ZnAAC in Exp 2 had improved ADG (quadratic, $P = 0.04$) and G:F (quadratic, $P = 0.01$) during d 14-41, which were the phases pigs were fed high dietary Cu (200 mg/kg) from $CuSO_4$ (Table 4.4). These positive outcomes influenced overall improvements during d 0-41 in increasing ADG (quadratic, $P = 0.07$), G:F (quadratic, $P = 0.05$), and final BW (quadratic, $P = 0.07$) at d 41.

There were no differences ($P > 0.05$) in dietary Zn intake observed during d 0-14, 14-41, and 0-41 among the four water ZnAAC treatments (Figure 4.2A). Calculated water Zn intake increased significantly (linear, $P < 0.0001$) with increasing ZnAAC

treatment (Figure 4.2B). Total Zn intake increased (linear, $P < 0.001$) throughout the study (Figure 4.2C).

In Exp 2, a regression equation indicated that ADWI increased linearly by day in experiment ($r = 0.92$; $R^2 = 0.84$; $P < 0.0001$; ADWI = (0.11922 × Day) + 0.14279) (Figure 4.3B).

Combined results of Exp 1 and Exp 2

Data of **Trt 0**, **Trt 40**, and **Trt 80** of Exp 1 and 2 were combined and analyzed for water ZnAAC effects on growth performance. As illustrated in Table 4.5, supplementing ZnAAC via drinking water tended to increase ADFI (quadratic, $P = 0.08$) during the early weaning period (d 0-14). Average daily gain (ADG) increased (linear, $P = 0.03$) during d 0-41. Feed efficiency (G:F) improved (linear, $P < 0.05$) during d 14-41 and d 0-41. A linear regression exhibited ADWI increased linearly by day in the combined experiment ($r = 0.95$; $R^2 = 0.91$; $P < 0.0001$; ADWI = (0.10164 × Day) + 0.11850) (Figure 4.3C).

Zn dose estimation

The optimal Zn doses were estimated using non-linear regression for the ideal ADG and G:F responses for the entire period from the five treatments of both experiments: **Trt 0**, **Trt 20**, **Trt 40**, **Trt 80**, and **Trt 160**. The model estimated that supplementing water soluble ZnAAC at 73.7 and 69.9 mg Zn/L of water were the best to increase ADG and G:F, respectively, in nursery pigs (Figure 4.4).

DISCUSSION

The main difference among treatments leading to our results was the levels of water soluble ZnAAC provided through drinking water. Regardless of the high levels of dietary Zn and Cu used, pigs improved ADG and G:F ratio in a linear fashion from drinking titrated levels of water soluble ZnAAC when supplemented up to 80 mg Zn/L (Exp 1). In addition, pigs improved ADG and G:F ratio in a quadratic fashion when treated up to 160 mg Zn/L in Exp 2. The best response was detected in pigs supplemented with 80 mg Zn/L compared with those non-supplemented with ZnAAC during high dietary $CuSO_4$ phases (33 g of ADG and 37 g of G:F greater). The plateau of Zn response at 160 mg Zn/L may be from the reduced absorption of a higher dose via saturation of transport mechanisms and body Zn status (Lonnerdal, 2000).

Our combined results indicated that the five water soluble ZnAAC doses enhanced growth response in a non-linear fashion and fitted a quadratic curve as previously described (Robbins et al., 2006). The model included a random component for parameter L, which accounted for the blocking of pigs. Our model estimated ZnAAC doses at 70 to 74 mg Zn/L for the optimized growth response. A study from Hill et al. (2014) reported that supplementing 75 mg/kg of organic Zn maximized growth and health response of early-weaned pigs when compared to 0, 25, 50, and 100 mg Zn/kg of an organic, inorganic, and equal combination of both. Supplementing nursery pigs with 20-80 mg Zn/kg from ZnAAC increased ADG compared to a non-supplemental group, while pigs fed 800 mg Zn/kg exhibited comparable performance to 80 mg/kg without showing significant health effects (Zhang et al., 2018). In addition, nursery pigs fed

dietary Zn amino acid chelate at 100 mg/kg or ZnO at 3,000 mg/kg achieved similar growth performance and serum Cu/Zn superoxide dismutase and alkaline phosphatase activities with less fecal Zn excretion in the Zn-chelated group (Wang et al., 2010). Therefore, lower doses of organic Zn and pharmacological doses of ZnO may provide comparable systemic Zn utilization in pigs. From an absorption standpoint, Lee et al. (2001) reported nursery pigs fed with 170 mg/kg diet of Zn amino acid chelate had higher serum Zn concentration than pigs fed with 170 mg/kg diet of $ZnSO_4$ (1.55 mg/L vs 1.44 mg/L of serum Zn). Additionally, feeding nursery pigs 3,000 mg/kg of ZnO resulted in a significantly lower plasma Zn concentration than feeding the same level of Zn methionine (1.29 mg/L vs 4.82 mg/L) (Hahn and Baker, 1993), suggesting ZnO had lower digestibility, intestinal absorption, and availability for systemic utilization.

Supplementing water soluble ZnAAC via drinking water may enhance Zn absorptive efficiency due to its high solubility. Organic Zn, including ZnAAC, is a complex of Zn atoms bound to low-molecular-weight ligands or chelators, such as amino acids. The organic ligands have been used in efforts to increase the solubility of Zn complex and enhance Zn bioavailability (Lonnerdal, 2000). On the other hand, inorganic Zn can be bound with other dietary molecules forming insoluble complexes and excreted out, thus, reducing bioavailability and absorption across the small intestine. From a solubility and absorptive standpoint, supplementing water soluble ZnAAC might leverage interfering effects with other dietary substances that prevent Zn absorption. A human study reported greater Zn absorption from drinking water Zn solution than a single meal consumption (peak concentration at 80-100 μmol vs 18-20 μmol) (Sandström and Sandberg, 1992; Lonnerdal, 1997).

In this study, water disappearance (referring to water intake) of pigs increased linearly by day in the experiment. More emphasis should be made on drinking water as pigs exhibited a strong association in eating and drinking behaviors (Dybkjær et al., 2006). Furthermore, daily water intake could reflect the well-being of pigs in the pen. As an example, on d 4 and d 25, we observed a marked decrease in water intake from vaccination. In addition, all pigs received an LPS injection on d 23, which consequently decreased water intake on d 24. Throughout the nursery period, our results exhibited ranges of 3.7 to 4.1 in water to feed intake ratio among all water ZnAAC treatments. These numbers were consistent with the study of adding liquid methionine to drinking water for nursery pigs, which ranged from 3.1 to 4 in water to feed intake ratio (Kaewtapee et al., 2010). Therefore, drinking water could be a potential oral route of delivering nutrients to the pig. Monitoring water intake can be used to address general herd health status.

CONCLUSION

Our results suggested that supplementing water soluble ZnAAC via drinking water linearly improved weight gain and feed efficiency when supplemented at 70-74 mg Zn/L in the presence of pharmacological levels of dietary ZnO during early phases or $CuSO_4$ during late phases of nursery pigs. We did not observe an additive response on growth with adding ZnAAC up to 160 mg Zn/L. Our study provided insights on the average daily water intake as a reasonable parameter to predict the daily status of herd health. Lastly, our data indicated no reduction in water and feed intake when pigs were supplemented ZnAAC via drinking water for the entire 42-d of the nursery period.

Table 4.1. Ingredients and nutritional composition of nursery diets fed to pigs.

Dietary phase	1 (d 0-7)	2 (d 14-23)	3 (d 23-35)	4 (d 35-41)
Ingredients, %				
Corn, yellow dent	32.21	38.30	54.00	59.15
Soybean meal, 47.5% CP	15.00	20.00	26.32	34.30
Dried whey	25.00	25.00	10.00	0.00
Lactose	7.00	0.00	0.00	0.00
Spray-dried animal plasma	6.00	2.50	0.00	0.00
Spray-dried blood cells	0.00	1.25	1.25	0.00
Fish meal, menhaden	6.00	4.00	2.00	0.00
Soy protein concentrate	2.21	2.12	0.00	0.00
Soybean oil	4.00	4.00	3.00	3.00
Dicalcium phosphate, 18.5% P	0.67	0.93	1.40	1.58
Limestone	0.45	0.44	0.72	0.74
Salt	0.50	0.50	0.50	0.50
L-Lysine HCl	0.17	0.21	0.27	0.25
DL-Methionine	0.18	0.21	0.17	0.11
L-Threonine	0.07	0.09	0.12	0.09
Vitamin premix[1]	0.05	0.05	0.05	0.05
Mineral premix[2]	0.06	0.06	0.06	0.06
Selenium premix[3]	0.05	0.05	0.05	0.05
Choline chloride	0.03	0.03	0.03	0.03
Zn oxide, 72% Zn	0.347	0.243	0.00	0.00
Cu sulfate, 25.2% Cu	0.00	0.00	0.08	0.08
Total	100.00	100.00	100.00	100.00
Chemical composition				
ME, kcal/kg	1,600	1,593	1,569	1,571
CP, %	23.00	23.05	21.03	21.57
Crude fat, %	6.68	6.83	6.19	6.34
Calcium, %	0.89	0.85	0.85	0.75
Phosphorus, % (available)	0.59	0.55	0.45	0.37
Sodium, %	0.63	0.54	0.33	0.22
Cu, mg/kg diet	19.00	20.00	217.00	218.00
Zn, mg/kg diet	2,500.00	1,750.00	100.00	100.00

[1] Contributed per kilogram of diet: vitamin A, 4,995 IU; vitamin D3, 749 IU; vitamin E, 325 IU; biotin, 100 μg; folic acid, 0.55 mg; niacin, 22.5 mg; pantothenic acid, 20.5 mg; riboflavin, 4.50 mg; thiamine, 1.00 mg; vitamin B6, 0.62 mg; and vitamin B12, 20.4 μg.
[2] Mineral premix provided per kilogram of diet: Zn, 99 mg; iron, 90.65 mg; manganese, 31.8 mg; Cu, 9.96 mg; and iodine, 0.36 mg.
[3] Selenium premix provided per kilogram of diet: selenium as selenium yeast, 0.3 mg.

Table 4.2. Analyses of water and dietary Zn concentrations supplemented to nursery pigs of Exp 1 and Exp 2.

Item	Water Zn concentration (mg/L)[1]					Dietary Zn concentration (mg/kg)[2]			
	0	20	40	80	160	Phase 1	Phase 2	Phase 3	Phase 4
Experiment 1	0.34	20.0	33.5	84.0	-	2,625	1,877	129	130
Experiment 2	0.18	-	42.9	79.7	158.0	2,310	1,710	124	112

[1]Pooled samples from pens of each treatment (n = 7/Trt).

[2]Phase 1, 2, 3, and 4 diets were fed to pigs from d 0-7, d 7-14, d 14-23, and d 23-41, respectively.

Table 4.3. Growth performance of nursery pigs supplemented with titrated levels of water soluble ZnAAC via drinking water during d 0-14, d 14-41, and d 0-41 of Exp 1.[1]

Item	Treatment[2]				SEM	*P*-value	
	0	20	40	80		Linear	Quadratic
Body Weight, kg							
d 0	5.68	5.69	5.69	5.70	0.13	0.76	0.97
d 14	8.18	8.10	8.21	8.53	0.21	0.05	0.31
d 41	22.49	22.56	23.49	23.11	0.59	0.37	0.49
ADFI, kg							
d 0-14	0.202	0.202	0.206	0.226	0.01	0.07	0.44
d 14-41	0.713	0.705	0.737	0.715	0.03	0.83	0.64
d 0-41	0.512	0.504	0.511	0.508	0.01	0.96	0.89
ADG, kg							
d 0-14	0.170	0.172	0.180	0.203	0.01	0.04	0.61
d 14-41	0.511	0.521	0.528	0.516	0.02	0.89	0.50
d 0-41	0.393	0.401	0.424	0.421	0.01	0.13	0.39
G:F Ratio							
d 0-14	0.838	0.850	0.872	0.900	0.03	0.10	0.99
d 14-41	0.717	0.738	0.719	0.721	0.01	0.83	0.46
d 0-41	0.767	0.796	0.831	0.830	0.02	0.02	0.18
ADWI, L							
d 0-14	0.837	0.778	0.834	0.976	0.06	0.09	0.27
d 14-41	2.573	2.570	2.663	2.799	0.20	0.41	0.88
d 0-41	1.884	1.851	1.924	2.066	0.14	0.33	0.70
Water to Feed Intake Ratio							
d 0-14	4.12	3.84	4.04	4.40	0.24	0.30	0.34
d 14-41	3.63	3.64	3.61	3.92	0.28	0.45	0.65
d 0-41	3.71	3.67	3.68	4.00	0.24	0.39	0.58

[1]Data represent least square means ± SEM adjusted using the Tukey-Kramer method (n = 7/Trt/time). Pigs were on high dietary ZnO (1,750-2,500 mg Zn/kg diet) on d 0-14, then switched to high dietary $CuSO_4$ (200 mg Cu/kg diet) on d 14-41.
[2]Treatment was applied via drinking water (mg Zn/L water).

Table 4.4. Growth performance of nursery pigs supplemented with titrated levels of water soluble ZnAAC via drinking water during d 0-14, d 14-41, and d 0-41 of Exp 2.[1]

Item	Treatment[2]				SEM	*P*-value	
	0	40	80	160		Linear	Quadratic
Body Weight, kg							
d 0	5.48	5.49	5.49	5.49	0.10	0.87	0.87
d 14	8.48	8.26	8.37	8.28	0.20	0.27	0.52
d 41	23.73	23.97	24.55	23.70	0.45	0.96	0.07
ADFI, kg							
d 0-14	0.218	0.202	0.211	0.203	0.01	0.22	0.57
d 14-41	0.795	0.796	0.800	0.777	0.02	0.49	0.58
d 0-41	0.597	0.594	0.596	0.581	0.02	0.42	0.75
ADG, kg							
d 0-14	0.214	0.198	0.206	0.200	0.01	0.28	0.49
d 14-41	0.545	0.561	0.578	0.546	0.01	0.99	0.04
d 0-41	0.434	0.440	0.454	0.434	0.01	0.97	0.07
G:F Ratio							
d 0-14	0.984	0.981	0.971	0.983	0.02	0.96	0.70
d 14-41	0.686	0.706	0.723	0.702	0.01	0.21	0.01
d 0-41	0.727	0.740	0.763	0.749	0.01	0.08	0.05
ADWI, L							
d 0-14	0.870	0.821	0.813	0.791	0.05	0.31	0.68
d 14-41	3.107	3.380	3.456	3.193	0.25	0.93	0.32
d 0-41	2.342	2.514	2.540	2.365	0.17	0.96	0.36
Water to Feed Intake Ratio							
d 0-14	3.99	4.07	3.84	3.87	0.16	0.46	0.91
d 14-41	3.93	4.27	4.37	4.10	0.33	0.82	0.36
d 0-41	3.93	4.24	4.30	4.07	0.31	0.85	0.40

[1]Data represent least square means ± SEM adjusted using the Tukey-Kramer method (n = 7/Trt/time). Pigs were on high dietary ZnO (1,750-2,500 mg Zn/kg diet) on d 0-14, then switched to high dietary $CuSO_4$ (200 mg Cu/kg diet) on d 14-41.
[2]Treatment was applied via drinking water (mg Zn/L water).

Table 4.5. Combined data on growth performance of nursery pigs supplemented with titrated levels of water soluble ZnAAC via drinking water during d 0-14, d 14-41, and d 0-41 of Exp 1 and Exp 2.[1]

Item	Treatment[2]			SEM	*P*-value		
	0	40	80		Trt × Exp	Linear	Quadratic
Body Weight, kg							
d 0	5.58	5.59	5.59	0.11	1.00	0.69	0.94
d 14	8.33	8.24	8.45	0.16	0.41	0.29	0.13
d 41	23.11	23.73	23.83	0.68	0.60	0.13	0.52
ADFI, kg							
d 0-14	0.210	0.204	0.219	0.01	0.14	0.22	0.08
d 14-41	0.754	0.766	0.757	0.04	0.81	0.87	0.57
d 0-41	0.555	0.556	0.548	0.05	0.74	0.57	0.70
ADG, kg							
d 0-14	0.192	0.189	0.204	0.01	0.11	0.20	0.28
d 14-41	0.528	0.545	0.547	0.02	0.53	0.20	0.57
d 0-41	0.413	0.432	0.438	0.02	0.45	0.03	0.45
G:F Ratio							
d 0-14	0.911	0.926	0.936	0.06	0.47	0.34	0.90
d 14-41	0.702	0.713	0.722	0.01	0.19	0.03	0.93
d 0-41	0.747	0.781	0.803	0.03	0.18	0.0001	0.56
ADWI, L							
d 0-14	0.854	0.827	0.894	0.04	0.15	0.44	0.31
d 14-41	2.840	3.022	3.127	0.35	0.93	0.23	0.85
d 0-41	2.153	2.248	2.325	0.24	0.89	0.28	0.94
Water to Feed Intake Ratio							
d 0-14	4.06	4.05	4.12	0.16	0.36	0.75	0.83
d 14-41	3.78	3.94	4.14	0.30	0.86	0.26	0.94
d 0-41	3.90	4.05	4.25	0.21	0.86	0.23	0.92

[1]Data represent least square means ± SEM adjusted using the Tukey-Kramer method (n = 14/Trt/time). Pigs were on high dietary ZnO (1,750-2,500 mg Zn/kg diet) on d 0-14, then switched to high dietary $CuSO_4$ (200 mg Cu/kg diet) on d 14-41.
[2]Treatment was applied via drinking water (mg Zn/L water).

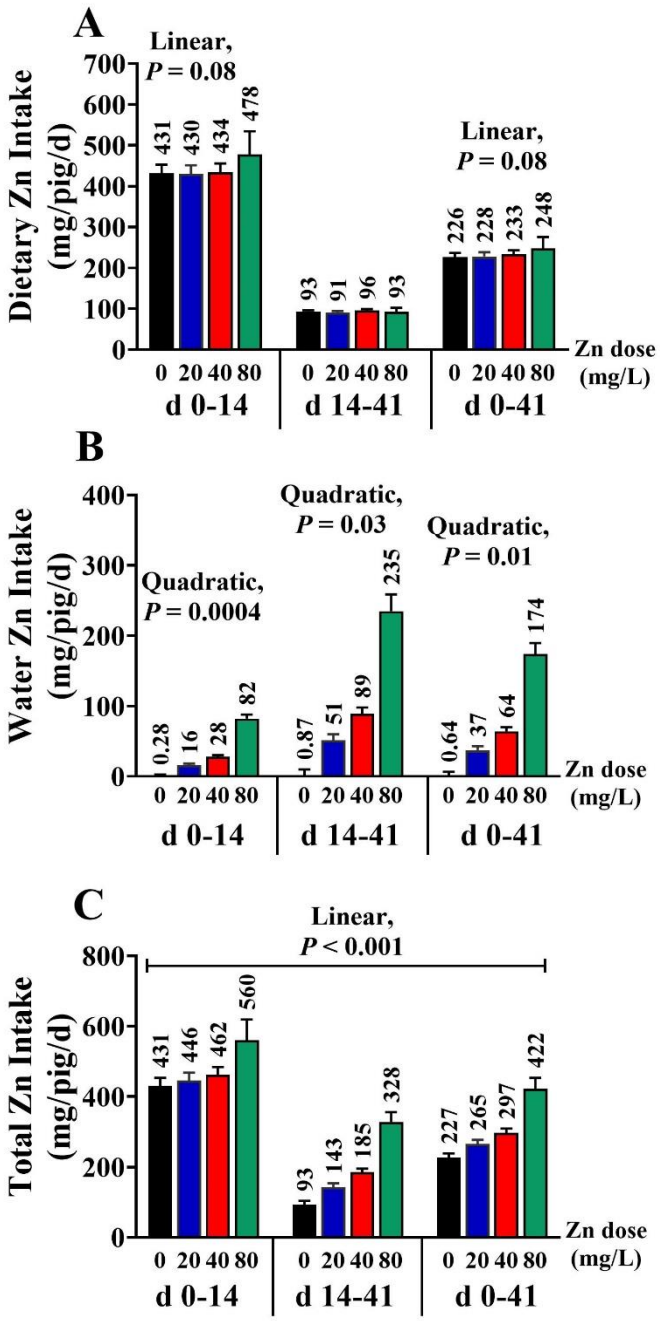

Figure 4.1. Zn intake of nursery pigs from d 0-14, d 14-41, and d 0-41 of Exp 1. (A) Dietary Zn intake tends to increase (linear, $P = 0.08$) with increasing water ZnAAC treatment from d 0-14 (added 1,750 to 2,500 mg/kg of ZnO) and d 0-41. (B) Water Zn intake significantly increases (quadratic, $P < 0.05$) with increasing water ZnAAC treatment. (C) Total Zn intake significantly increases (linear, $P < 0.001$) with increasing water ZnAAC treatment. Calculation of dietary and water Zn intakes are based on the total intake of analyzed Zn concentrations as received by pig days.

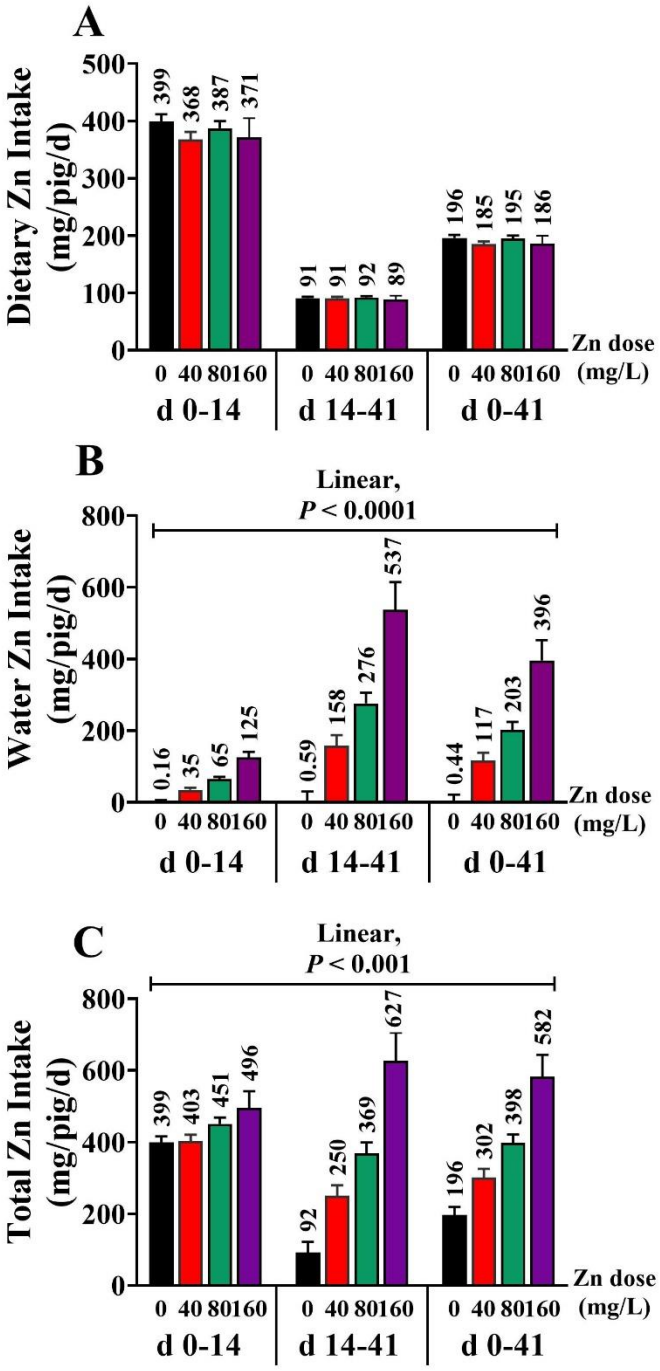

Figure 4.2. Zn intake of nursery pigs from d 0-14, d 14-41, and d 0-41 of Exp 2. (A) No differences ($P > 0.05$) in dietary Zn intake among the four water ZnAAC treaments. (B) Water Zn intake significantly increases (linear, $P < 0.0001$) with increasing water ZnAAC treatment. (C) Total Zn intake significantly increases (linear, $P < 0.001$) with increasing water ZnAAC treatment. Calculation of dietary and water Zn intakes are based on the total intake of analyzed Zn concentrations as received by pig days.

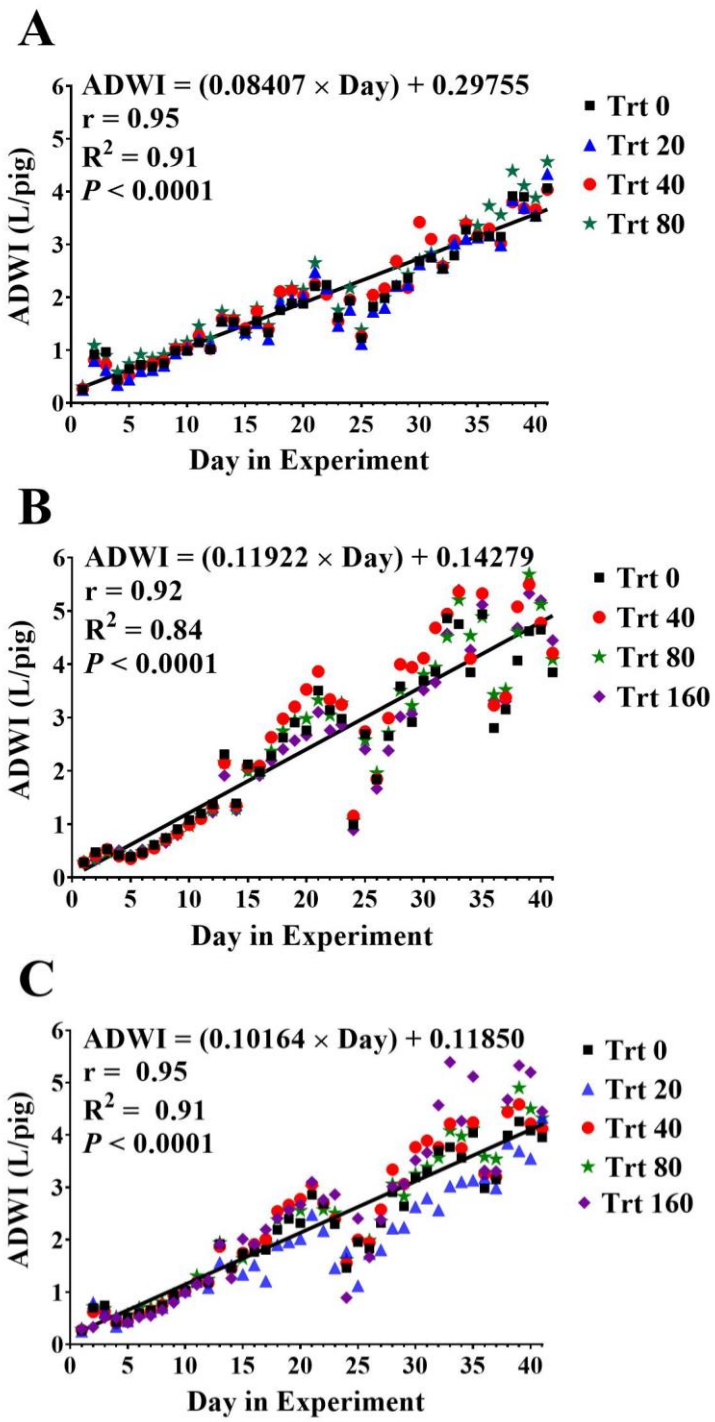

Figure 4.3. Scatterplots and linear regression equations of ADWI by day in experiment of Exp 1, Exp 2, and combined data of both experiments. (A) A positive relation of Exp 1 ($r = 0.95$; $R^2 = 0.91$; $P < 0.0001$). (B) A positive relation of Exp 2 ($r = 0.92$; $R^2 = 0.84$; $P < 0.0001$). (C) A positive relation of the combined data from both experiments ($r = 0.95$; $R^2 = 0.91$; $P < 0.0001$).

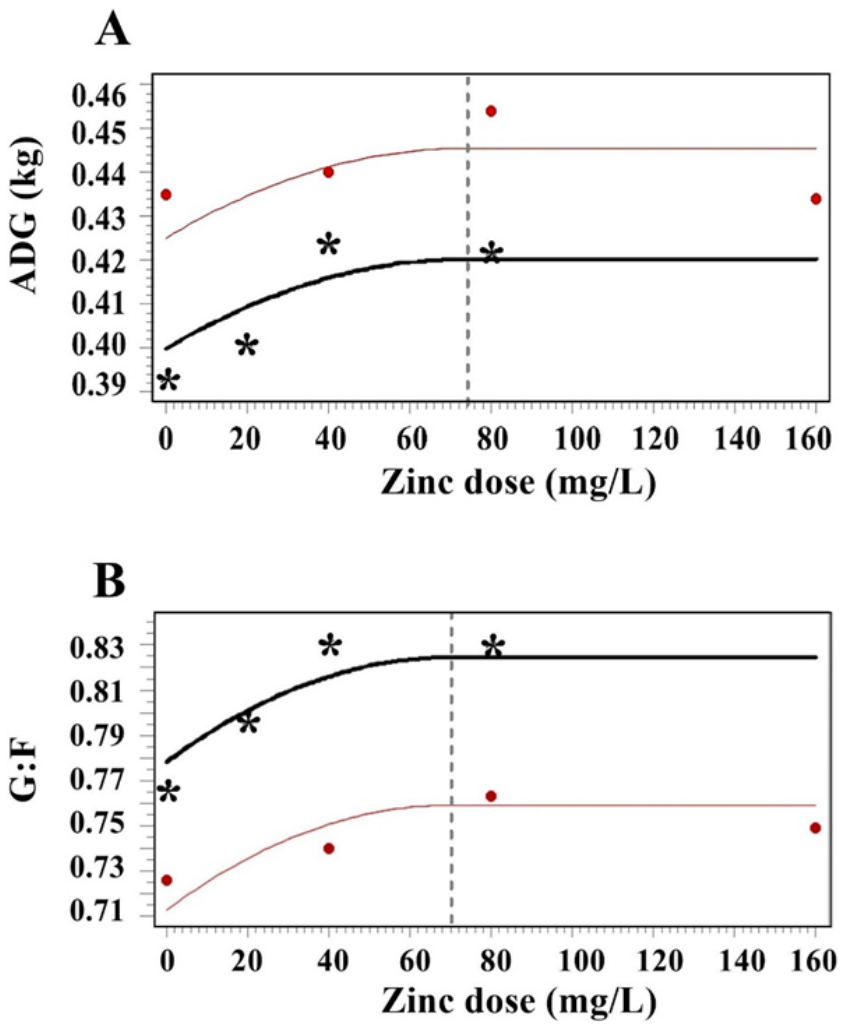

Figure 4.4. Estimation of water soluble ZnAAC dose generated by using quadratic broken-line models on growth performance of nursery pigs (Liu et al., 2016). (A) Estimated ideal dose for ADG is 73.7 mg Zn/L of water (model parameters L = 0.42395, U = -0.00001, R = 67; R^2 = 0.8286; SE = 14.5). (B) Estimated ideal dose for G:F ratio is 69.9 mg Zn/L of water (model parameters L = 0.83251, U = -0.00002, R = 61; R^2 = 0.9273; SE = 11.1). L = values at breaking point, U = slope ratio of the line at X < R, and R = value of X at the breaking points from Exp 1 and Exp 2, respectively. Asterisk (*****) and dot (•) represent treatment means from Exp 1 and Exp 2, respectively. Thick line and thin line are the prediction lines of data.

CHAPTER V

EFFECTS OF SUPPLEMENTING TITRATED LEVELS OF WATER SOLUBLE ZINC AMINO ACID COMPLEX ON IMMUNE RESPONSE AND TISSUE ZINC TRANSPORTERS IN LPS-INDUCED INFLAMMATION OF NURSERY PIGS

INTRODUCTION

Zinc plays an important role in immune function, including the innate and adaptive immune systems (Kloubert et al., 2018). The control of Zn homeostasis occurs in the small intestine, liver, and pancreas through the regulation of Zn transporters (Krebs, 2000). During inflammation, proinflammatory cytokines mediating Zn transporters induce Zn dyshomeostasis (Liuzzi et al., 2005). On the opposite, Zn supplementation reduces the severity of the host immune response during polymicrobial sepsis (Wessels and Cousins, 2015). Studies reported anti-inflammatory effects of Zn supplementation in mitigating proinflammatory cytokines and acute-phase protein secretion at various tissues (Bao et al., 2010; Hu et al., 2013; Paulk et al., 2015). Besides, Zn has been proposed as one of the alternatives to in-feed antibiotics in pigs (Roselli et al., 2005).

Although pharmacological concentrations of dietary Zn supplementation (2,000-3,000 ppm) are commonly used to prevent and treat post-weaning diarrhea, it is suggested to use for short periods for gut health purposes and to avoid negative environmental impacts (Shannon and Hill, 2019). To date, supplementing Zn with greater relative bioavailability value (RBV) in lower levels, such as Zn amino acid complex, has been reported to mitigate inflammation during infection or stress conditions of pigs (Roselli et al., 2005; Leite et al., 2018). However, Zn supplementation is applied mainly in-feed. Few studies have evaluated supplementing Zn via drinking water, an indispensable nutrient, that is offered *ad libitum* for pigs.

In this study, we evaluated the effects of supplementing titrated levels of Zn amino acid complex via drinking water on immune response, and tissue Zn transporter and metallothionein during the acute phase response of lipopolysaccharide-challenged pigs. Also, we emphasized that the altered Zn homeostasis during inflammation was involved with cytokine-mediated hepatic Zn influx via Zn transporter and caused hypozincemia.

MATERIALS AND METHODS

Two experiments were conducted at the Swine Research and Education Center at Oklahoma State University. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Experimental design, animals, and treatment

The two studies aimed to evaluate the effects of supplementing Zn amino acid complex via drinking water on the innate immune response and tissue Zn transporters of nursery pigs fed pharmacological Zn and Cu doses and challenged with LPS. A total of five hundred and sixty crossbred weaned pigs (PIC USA; 19 ± 2 d; 5.6 ± 0.3 kg BW) were utilized. The experimental treatments were the titrated concentrations of Zn amino acid complex (ZnAAC) powder (TruCare®, Zinpro Corporation, Eden Prairie, MN) supplemented via drinking water.

In each experiment (Exp), two hundred and eighty weaned pigs were randomly allotted (10 pigs/pen) and assigned to one of four water Zn treatments (Trt; 7 pens/Trt) upon arrival. Water Zn treatments for Exp 1 were **Trt 0**, **Trt 20**, **Trt 40**, and **Trt 80** (mg Zn/L of water), while for Exp 2 were **Trt 0**, **Trt 40**, **Trt 80**, and **Trt 160**. Each pen had one water meter installed to the waterline above the pen. The stock solution of water Zn treatments was prepared according to the product instruction. **Trt 0** was prepared without the addition of Zn and supplied similarly via water regulators set at the rate of 1:100.

On d 23, pigs were fasted for 5 h before injected with a single i.m. LPS (*Escherichia coli* serotype O55:B5; Sigma-Aldrich, St. Louis, MO) dose at 12 μg/kg BW. Three-time courses of measurement were h 0 as a baseline, h 3, and h 12 following LPS challenge.

Housing and feeding methods

Pigs were housed in an environmentally-controlled nursery facility with slatted plastic flooring and a mechanical ventilation system. The environmental temperature was

maintained at 30˚C for the first week of the experiment and was reduced by 1˚C in the following weeks. Each pen was equipped with one 4-hole dry feeder, and one nipple waterer with a cup.

The dietary phase feeding program was employed in both experiments, with diets formulated to meet or exceed swine NRC 2012 (Table 5.1). Pigs were fed the same corn-soybean meal-based diets with added Zn from ZnO or Cu from $CuSO_4$: Phase 1 (2,500 mg Zn/kg) from d 0-7, Phase 2 (1,750 mg Zn/kg) from d 7-14, and Phase 3 (200 mg Cu/kg) from d 14-23.

Body weight, rectal temperature, and blood collection

Two pigs/pen (14 pigs/Trt) of each experiment were obtained BW, rectal temperature (RT), and collected blood samples at h 0 before LPS injection, and repeated the sample collection post-challenge as described in Figure 5.1. Blood samples were drawn from the jugular vein when pigs laid in dorsal recumbency position using a single-use blood collection needle (Monoject™, Dublin, OH). Five and ten mL of blood were collected into a trace element testing tube, and a non-additive tube for serum (BD Vacutainer™, Franklin Lakes, NJ), respectively. Blood samples were centrifuged for serum at 2500 × *g*, 4 °C for 10 min, aliquoted into 1.5-mL tubes, and stored at -20°C until further analysis.

Saliva collection

In Exp 2, saliva was collected using three-strand cotton ropes (2.5-cm diameter) on the challenge day at h 0 and h 12. One rope was tied to the gate and left the three end

knots hanging in front of the feeder at the level of the pig's shoulder without possible contact from pigs in other pens. Pigs were allowed to chew ropes for 20 min before saliva collection. Ropes were cut above the wet parts into a zip-lock plastic bag for each pen. Collected wet ropes were squeezed and collected saliva fluid into 15 ml tubes. Saliva samples were processed by centrifugation at 2500 × *g*, 4°C for 15 min to separate dirt and feed particles. The clear liquid portion was transferred into 1.5-mL tubes and stored at -20°C until salivary C-reactive protein (CRP) analysis.

Tissue sampling, RNA isolation, and cDNA synthesis

In Exp 1, one pig/pen (7 pig/Trt) was euthanized subsequently hepatic tissue sample and duodenal mucosa scrapings were collected in separate tubes filled with RNAlater (Thermo Fisher Scientific, Waltham, MA). The tissue samples were stored at -20°C until homogenization. Samples were homogenized in TriZol reagent (Invitrogen, Carlsbad, CA) before RNA isolation by using Direct-Zol RNA Miniprep Plus (Zymo Research, Irvine, CA) with DNase I treatment to eliminate contaminated DNA. RNA quantity was measured by the A260/A280 ratio using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA). RNA quality was evaluated for 18s/28s ribosomal RNA integrity by agarose gel electrophoresis. Total RNA was reverse-transcribed similarly to methods described by Ojo et al. (2019) to create complementary DNA (cDNA) using ProtoScript II Reverse Transcriptase kit (New England Biolabs, Ipswich, MA), and stored at -80°C until subsequent quantitative RT-PCR (RT-qPCR) analysis.

Serum TNF-α, serum CRP, and salivary CRP concentrations

Serum TNF-α concentration was analyzed in duplicate using a commercial porcine ELISA kit (R&D Systems Inc., Minneapolis, MN). From both experiments, the intra- and inter-assay coefficient of variations were equal or less than 6.9% and 9.2%, respectively for both experiments. Serum samples were not analyzed for TNF-α concentration at h 12 in Exp 1. Serum and salivary CRP concentrations were analyzed in duplicate using a commercial porcine ELISA kit (Lifediagnostics Co., Ltd, West Chester, PA). From both experiments, the intra- and inter-assay coefficient of variations of serum and saliva samples were equal or less than 10.8% and 15.5%, respectively.

Serum Zn and Cu concentrations

Before analysis, serum samples were diluted 1:50 in 0.1% high-purity nitric acid (GFS Chemicals, Inc., Powell, OH). Serum Zn and Cu concentrations were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Elan 9000; Perkin Elmer Life and Analytical Sciences, Norwalk, CT) with gallium as an internal standard. After every 10^{th} sample, UTAK normal range control serum (UTAK Laboratories Inc., Valencia, CA) was analyzed to verify consistency as described by Joray et al. (2015).

Quantitative real-time polymerase chain reaction (RT-qPCR)

The relative abundance of genes encoding for interleukin-6 (IL-6), Zn transporters (ZIP4 for duodenum and ZIP14 for liver), and metallothionein-1 (MT-1) were evaluated by RT-qPCR as previously described (Ojo et al., 2019). Succinate dehydrogenase complex flavoprotein subunit A (SDHA) was an internal reference gene used for data

normalization of the target genes. Intron-spanning primers were designed using Primer3 (Rozen and Skaletsky, 2000) and NCBI BLAST web interfaces (Johnson et al., 2008). Primers sequences and NCBI accession numbers are shown in Table 5.2.

Before analysis, cDNA was diluted 1:4 with DNase/RNase-free water. Samples were run in duplicate and analyzed by RT-qPCR using SYBR Green Master Mix (Power Sybr, Applied Biosystems, Foster City, CA) on an ABI 7900HT sequence-detection instrument and 2.4 SDS software (Applied Biosystems, Foster City, CA).

Statistical analyses

In both experiments, BW, RT, serum, and salivary chemicals data were analyzed using PROC GLIMMIX of SAS (Version 9.4, SAS Institute, Cary, NC, USA). The linear mixed model method for randomized complete block design was used to evaluate Zn and LPS effects at the three-time course and their changes. Water ZnAAC treatment and time were fixed effects, while block was a random effect. Pig was the experimental unit. Orthogonal polynomial contrasts were included to assess the trend of titrated water Zn levels effect. The contrast efficiencies were generated using PROC IML.

For gene expression results, threshold cycles (CTs) of target genes generated by the qPCR system were normalized to the CT value of the reference gene ($\Delta CT = CT_{target} - CT_{reference}$) and calculated for fold change expression ($2^{-\Delta\Delta CT}$) as described by Livak and Schmittgen (2001). Data were analyzed using PROC GLIMMIX with randomized complete block design and presented for different fold change expression during h 0 and h 3. Gene correlations were tested using PROC CORR, while regression equations were generated by using PROC REG.

Least square means were calculated and the difference among treatments was tested using the Tukey-Kramer method for all datasets. Tests were considered significant when $P \leq 0.05$ and a trend toward significance when $0.05 < P \leq 0.10$.

RESULTS

Experiment 1

Pigs injected with a single i.m. LPS dose at 12 μg/kg BW, regardless of water ZnAAC treatment, exhibited significant signs of sickness behavior, i.e., vomiting, hyperventilating, losing appetite, and lethargy. LPS led to decreases in BW, serum Zn, and copper concentrations, while increases in rectal temperature, serum TNF-α, and CRP concentrations were seen (Figure 5.2).

As shown in Table 5.3, rectal temperature tended to increase (quadratic, $P = 0.06$) with increasing water ZnAAC treatment at h 12 following LPS. The rectal temperature changes from h 3 to 12 dropped (quadratic, $P = 0.03$) among the four water ZnAAC treatments. Serum CRP concentration decreased at the baseline (h 0) (quadratic, $P = 0.04$) and tended (quadratic, $P = 0.06$) to maintain this pattern at h 3 following LPS. The serum CRP concentration changes tended to drop (quadratic, $P = 0.09$) with increasing water ZnAAC treatment during h 3 to 12. Serum Zn concentration over 12-h following LPS tended to decrease (quadratic, $P = 0.06$). Serum copper concentration during h 3 to 12 and overall from h 0 to 12 depicted a significant decrease in a quadratic pattern ($P < 0.001$), which **Trt 0** remained the highest in serum copper concentration at h 12 among the four water ZnAAC treatments.

LPS upregulated ($P < 0.0001$) all three hepatic genes (IL-6, ZIP14, and MT-1) and duodenal IL-6 gene expression within 3 h post-challenge for approximately 20 to 30-fold and 2 to 3-fold, respectively (Figure 5.3). Hepatic ZIP14 expression was upregulated ($P < 0.0001$) following LPS challenge, while duodenal ZIP4 did not change ($P > 0.05$). Upregulation of hepatic MT-1 gene expression was increased ($P < 0.0001$) after LPS injection (approximately 2 to 5-fold), while there was no difference detected ($P > 0.05$) for duodenal MT-1 gene expression. For gene expression correlation (Figure 5.4), the three hepatic gene expressions were positively correlated ($P \leq 0.0001$) in pairwise comparisons, while only ZIP4 and MT-1 genes of the duodenum were found positively correlated ($P = 0.02$).

Experiment 2

As shown in Table 5.4, pigs supplemented with increasing water ZnAAC treatment significantly reduced BW loss (linear, $P = 0.05$) over 12-hour following the LPS challenge. Rectal temperature significantly decreased at the baseline (h 0) ($P = 0.02$) and at h 3 ($P = 0.005$) after challenge in a linear fashion. Baseline (h 0) serum TNF-α concentration decreased (linear, $P = 0.04$) in pigs drinking higher doses of water ZnAAC amino acid complex. Following the LPS challenge, serum TNF-α concentration and serum TNF-α concentration changes decreased (quadratic, $P \leq 0.05$) with increasing water ZnAAC treatment. Serum Zn concentration increased at h 3 (linear, $P = 0.08$) and 12 (linear, $P = 0.001$) post-LPS challenge, whereas serum Cu concentration tended to increase (linear, $P = 0.07$) with increasing water ZnAAC dose.

Similar to Exp1, LPS had significant effects ($P < 0.0001$) on BW loss, increased rectal temperature, increased serum TNF-α concentration and decreased serum Zn and copper concentrations of pigs (Figure 5.2). No significant difference ($P > 0.05$) was found for serum CRP concentration within a 12 h period following the LPS challenge.

Combined results of Exp 1 and Exp 2

Data of **Trt 0**, **Trt 40**, and **Trt 80** of Exp 1 and 2 were combined and analyzed for water ZnAAC effects on the immune response. As illustrated in Table 5.5, supplementing ZnAAC via drinking water increased rectal temperature at h 12 post-LPS injection (quadratic, $P = 0.02$) due to rectal temperature decreased during h 3-12 is significant (quadratic, $P = 0.003$). There was a significant Trt × Exp effect ($P = 0.01$) in mitigating rectal temperature increase during h 0-3 with increasing water ZnAAC dose. Effect of Trt × Exp in decreasing serum TNF-α concentration was detected ($P = 0.09$) at h 3 following challenge, while pigs significantly mitigated the increase of serum TNF-α during h 0-3 (linear, $P = 0.04$). Pigs drinking water ZnAAC tended to increase serum Zn concentration at baseline and h 3 post-LPS (linear, $P = 0.07$), while significantly increased serum Zn at h 12 (linear, $P = 0.03$). There was a significant Trt × Exp effect in decreasing serum Cu concentration with increasing ZnAAC dose at h 12 ($P = 0.003$).

DISCUSSION

Our study showed that LPS challenged pigs exhibited several signs of sickness behavior, including a decrease in BW. Significantly increases in serum TNF-α concentration and rectal temperature of all water ZnAAC treatments within 3 h following LPS challenge were observed. Pigs drinking increasing water soluble ZnAAC had a lower rectal temperature and reduced the increase in serum TNF-α in a quadratic manner following the LPS challenge in Exp 2. However, we did not find Zn effects on the febrile response of attenuating the rise of rectal temperature as reported by Roberts et al. (2002). The increasing temperature was induced by proinflammatory cytokines, e.g., TNF-α, IL-1β, and IL-6 that were released from activated macrophages during the acute phase response. These cytokines are pleiotropic and they have similar biological functions, i.e. thermogenesis, anorexia, and lethargy (Johnson, 1997). Kim and Jeong (2015) reported that Zn treated-macrophage and exposed to LPS could downregulate TNF-α expression via the Zn finger protein A20. The A20 protein is a negative regulator of the nuclear transcription factor NF-κB that stimulates proinflammatory cytokines. Pigs supplemented with ZnAAC decreased TNF-α production during the heat stress recovery period (Mayorga et al., 2018). In contrast, Krones et al. (2004) found a significant increase in TNF-α and IL-6 productions following the LPS challenge of pigs infused with Zn over the control pigs. Due to a severe decrease in Zn binding capacity correlated with albumin levels, administration of Zn during sepsis may increase free Zn concentrations that could be more toxic caused by direct interaction of Zn with monocytes (Wellinghausen et al., 1997; Hoeger et al., 2015).

Although the effects of Zn supplementation on inflammatory responses are debatable, this study only reported a Zn role for anti-inflammatory effects. In Exp 1, pigs drinking increasing water soluble ZnAAC had lower serum CRP concentrations following the LPS challenge. However, a spike increased from **Trt 40** during h 3 to 12, and the trend of increasing serum CRP was not consistent between the two experiments. Likewise, Moya et al. (2006) reported that plasma CRP concentration significantly increased at 12 h following low-dose LPS challenge (5 μg/kg BW) of nursery pigs. However, Sassu et al. (2016) found increased serum CRP concentration at 68 h after *Actinobacillus pleuropneumoniae* infection. Production of CRP is mediated by hepatic proinflammatory cytokines IL-6, IL-1, and IL-17, however, extrahepatic production could contribute to systemic concentrations (Eklund, 2009). Although CRP is reported as the first line and fast response acute phase protein in pigs, its production is affected by multiple factors, e.g., age, sex, health status, and stress (Sorensen et al., 2006; Hennig-Pauka et al., 2019). To reduce blood collection stress, we also measured salivary CRP as a non-invasive sampling approach. We reported the two-time points of salivary CRP measurements (h 0 as baseline and h 12 post-challenge) because pigs rejected to chew on ropes during the peak febrile onset at h 3. There was no difference in salivary CRP concentration among the four water ZnAAC treatments, but LPS increased salivary CRP concentration. Our findings agreed with Escribano et al. (2014) who reported a significant elevation in salivary CRP concentration of endotoxemic pigs. They also addressed that the amplitude decreased over time from repeated LPS injections.

Basal serum Zn concentration in this study numerically increased with increasing water Zn doses, i.e., 1.059, 1.273, 1.355, and 1.349 for **Trt 0**, **Trt 40**, **Trt 80**, and **Trt**

160, respectively. These results corresponded with Roberts et al. (2002) who reported that pigs fed 10, 50, and 150 mg/kg of dietary $ZnSO_4$ resulted in 0.97, 1.04, and 1.35 mg/L in serum Zn concentrations, respectively. Regardless of water ZnAAC treatment, all pigs significantly decreased in serum Zn concentration (hypozincemia) within 3-h post-challenge. We also observed the magnitude between the two experiments was different, *i.e.*, decreases in serum Zn concentration at an average of 15% and 38% of Exp 1 and 2, respectively. Hypozincemia is a Zn redistribution from blood circulation to hepatocyte induced by IL-6 upregulated hepatic ZIP14 to increase Zn influx (Liuzzi et al., 2005). Of significance was serum Cu concentration decreased following the LPS challenge, which was opposed to other findings that inflammation stimulated ceruloplasmin (Cp; copper-containing protein) production from hepatocytes resulting in increased serum copper concentration (Cousins, 1985; Humann-Ziehank et al., 2014; Malavolta et al., 2015). In addition, our data exhibited an opposite pattern of serum copper concentration with increasing water ZnAAC treatment at h 12 of both experiments. However, the basal serum copper concentrations (h 0) of both experiments were not suppressed by increasing water ZnAAC dose.

Our study reported strong positive correlations in LPS upregulating hepatic IL-6, ZIP14, and MT-1 expression, which may support hepatic Zn sequestration. Increasing IL-6 stimulated the release of MT-1 and contributed to Zn sequestering in the liver (Schroeder and Cousins, 1990), as mentioned by a 20-fold increase in hepatic Zn concentration of wild-type mice compared to the MT-1 knockout mice by Philcox et al. (1995). Liver is an important organ for homeostatic control of inflammation. Not only is it responsible for inflammatory protein production, but it acts as a buffer between the gut

environment and systemic circulation via the portal vein (Robinson et al., 2016). On the other hand, we only found a positive relation between duodenal ZIP4 and MT-1. No changes in duodenal ZIP4 and MT-1 at 3 h following LPS might not be the accurate timing to see changes in duodenal Zn homeostasis as reported in Chapter III. Upregulation of duodenal ZIP4 expression occurred when dietary Zn was restricted or reflected in an attempt to restore Zn homeostasis (Cousins et al., 2006). However, LPS upregulated duodenal IL-6 expression was similar to previously mentioned by Liu et al. (2008). Our evidence indicated that LPS independently stimulated duodenal IL-6 from duodenal ZIP4 or duodenal MT-1 expression.

CONCLUSION

Our study confirms that the liver plays an important role in Zn homeostasis, while the duodenum is less responsive during the acute phase response. Upregulation of hepatic IL-6 positively mediates ZIP14 and MT-1 expressions, which contributes to hypozincemia. Therefore, serum Zn concentration is not an ideal marker to indicate normal Zn status but can be an early indication of non-specific inflammation.

Taken together, supplementing titrated levels of water soluble Zn amino acid complex to nursery pigs under an acute immune challenge leads to the mitigation of systemic febrile response. The immune response is not further mitigated when supplementing the highest water ZnAAC dose, indicating no dose-dependent effects. We suggest that Zn might not function in additive response when Zn intake is adequate and satisfied with the body's need.

Table 5.1. Ingredients and nutritional composition of nursery diets fed to pigs.

Dietary phase	1 (d 0-7)	2 (d 14-23)	3 (d 23-35)
Ingredients, %			
Corn, yellow	32.21	38.30	54.00
Soybean meal, 47.5% CP	15.00	20.00	26.32
Whey	25.00	25.00	10.00
Lactose	7.00	0.00	0.00
Spray-dried animal plasma	6.00	2.50	0.00
Spray-dried blood cells	0.00	1.25	1.25
Fish meal	6.00	4.00	2.00
Soy protein concentrate	2.21	2.12	0.00
Soybean oil	4.00	4.00	3.00
Dicalcium phosphate, 18.5% P	0.67	0.93	1.40
Limestone	0.45	0.44	0.72
Salt	0.50	0.50	0.50
L-Lysine HCl	0.17	0.21	0.27
DL-Methionine	0.18	0.21	0.17
L-Threonine	0.07	0.09	0.12
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.06	0.06	0.06
Selenium premix [3]	0.05	0.05	0.05
Choline chloride	0.03	0.03	0.03
Zn oxide, 72% Zn	0.347	0.243	0.00
Cu sulfate, 25.2% Cu	0.00	0.00	0.08
Total	100.00	100.00	100.00
Chemical composition			
ME, kcal/kg	1,600	1,593	1,569
CP, %	23.00	23.05	21.03
CF, %	6.68	6.83	6.19
Calcium, %	0.89	0.85	0.85
Phosphorus, % (available)	0.59	0.55	0.45
Sodium, %	0.63	0.54	0.33
Cu, mg/kg diet	19.00	20.00	217.00
Zn, mg/kg diet	2,500.00	1,750.00	100.00

[1] Contributed per kilogram of diet: vitamin A, 4,995 IU; vitamin D3, 749 IU; vitamin E, 325 IU; biotin, 100 μg; folic acid, 0.55 mg; niacin, 22.5 mg; pantothenic acid, 20.5 mg; riboflavin, 4.50 mg; thiamine, 1.00 mg; vitamin B6, 0.62 mg; and vitamin B12, 20.4 μg.
[2] Mineral premix provided per kilogram of diet: Zn, 99 mg; iron, 90.65 mg; manganese, 31.8 mg; Cu, 9.96 mg; and iodine, 0.36 mg.
[3] Selenium premix provided per kilogram of diet: selenium as selenium yeast, 0.3 mg.

Table 5.2. Primer sequences used for RT-qPCR of hepatic and duodenal IL-6, Zn transporters, and MT-1 genes of Exp 1.

Genes[1]	Accession number	Primer sequences	Product size (bp)
SDHA	DQ_178128.1	5’-CTCAAGTTCGGGAAGGGCGG-3’ 5’-TCGTACCGCAGAGACCTTCCG-3’	104
IL-6	NM_214399.1	5’-CACCTCTCCGGACAAAACTGA-3’ 5’-TGCCAGTACCTCCTTGCTGTT-3’	118
ZIP4	XM_001925360.5	5’-CTGCTCCAGCAACAGCTGAGT-3’ 5’-GCAGAGGCAGATGAGCAGTGT-3’	123
ZIP14	XM_021072999.1	5’-TCTGTCTTCCAAGGCATCAGC-3’ 5’-CAGCAGGCAGAGAGGAAGTTG-3’	121
MT-1	NM_001001266	5’-TTGCTCTCTGCTTGGTCTCACCT-3’ 5’-GGGATGTAGCATGAAGTCAGTGCATGTG-3’	378

[1]SDHA, succinate dehydrogenase complex flavoprotein subunit A; IL-6, interleukin 6; ZIP4, solute carrier family 39 member 4 (SLC39A4); ZIP14, solute carrier family 39 member 14 (SLC39A14); and MT-1, metallothionein.

Table 5.3. Effects of titrated levels of water soluble ZnAAC on immune response indices following LPS-challenged of pigs at d 23 of Exp 1.[1]

	Water ZnAAC treatment, mg/L					*P*-value[2]		
Item	0	20	40	80	SEM	L	Q	Trt 0 vs others
Body Weight, kg								
h 0	11.10	9.93	10.87	10.68	0.42	0.92	0.42	0.24
h 3	10.72	9.52	10.46	10.31	0.41	0.94	0.35	0.21
h 12	10.52	9.34	10.25	10.13	0.34	0.96	0.26	0.15
Δ h 0-3	-0.37	-0.41	-0.41	-0.37	0.05	0.84	0.45	0.67
Δ h 3-12	-0.20	-0.18	-0.21	-0.17	0.10	0.88	0.93	0.89
Δ h 0-12	-0.57	-0.59	-0.62	-0.54	0.09	0.79	0.57	0.89
Rectal Temperature, °C								
h 0	38.99	39.19	39.20	39.09	0.16	0.75	0.25	0.27
h 3	40.74	40.54	40.59	40.63	0.17	0.76	0.44	0.39
h 12	39.24	39.57	39.76	39.44	0.18	0.54	0.06	0.11
Δ h 0-3	1.76	1.40	1.40	1.57	0.19	0.63	0.12	0.12
Δ h 3-12	-1.51	-0.97	-0.84	-1.21	0.20	0.48	0.03	0.04
Δ h 0-12	0.24	0.41	0.56	0.34	0.25	0.79	0.33	0.42
Serum TNF-α concentration, pg/mL								
h 0	161	161	149	169	14	0.69	0.42	0.96
h 3	1731	1699	1630	1605	152	0.52	0.87	0.61
Δ h 0-3	1700	1538	1482	1436	143	0.23	0.55	0.20
Serum CRP concentration, μg/mL								
h 0	82.71	61.14	49.43	61.14	9.05	0.13	0.04	0.02
h 3	105.16	79.86	63.14	83.43	12.93	0.31	0.06	0.07
h 12	109.52	85.14	96.29	85.43	8.61	0.16	0.46	0.07
Δ h 0-3	24.43	18.60	13.91	22.00	7.95	0.84	0.23	0.38
Δ h 3-12	1.16	5.37	33.00	2.26	11.39	0.84	0.09	0.44
Δ h 0-12	24.18	23.94	46.91	24.27	10.03	0.85	0.19	0.58
Serum Zn Concentration, mg/L								
h 0	0.978	1.038	1.101	1.023	0.06	0.63	0.20	0.28
h 3	0.854	0.883	0.923	0.869	0.04	0.82	0.28	0.44
h 12	0.932	0.857	0.958	0.986	0.06	0.28	0.61	0.98
Δ h 0-3	-0.124	-0.155	-0.178	-0.155	0.05	0.67	0.50	0.45
Δ h 3-12	0.078	-0.026	0.035	0.117	0.06	0.36	0.18	0.59
Δ h 0-12	-0.046	-0.182	-0.143	-0.038	0.05	0.56	0.06	0.26
Serum Cu Concentration, mg/L								
h 0	2.940	2.952	2.863	2.860	0.10	0.48	0.90	0.67
h 3	2.517	2.411	2.250	2.325	0.11	0.21	0.25	0.14
h 12	2.888[a]	2.243[b]	2.131[b]	2.403[b]	0.10	0.007	<0.0001	<0.0001
Δ h 0-3	-0.423	-0.541	-0.612	-0.535	0.09	0.43	0.21	0.17
Δ h 3-12	0.371[a]	-0.169[b]	-0.120[b]	0.077[ab]	0.09	0.13	0.0002	0.0003
Δ h 0-12	-0.052[a]	-0.710[b]	-0.732[b]	-0.457[b]	0.10	0.02	<0.0001	<0.0001

[1] Data were analyzed using the linear mixed models with block as a random effect (n = 14 pigs/Trt/Time).

[2] *P*-value is reported for L as a linear response and Q as a quadratic response.

[a-b] Means within the same row without a common letter differ significantly ($P \leq 0.05$).

Table 5.4. Effects of titrated levels of water soluble ZnAAC on immune response indices following LPS-challenged of pigs at d 23 of Exp 2.[1]

Item	Water ZnAAC treatment, mg/L					*P*-value[2]		
	0	40	80	160	SEM	L	Q	Trt 0 vs others
Body Weight, kg								
h 0	13.09	12.99	12.92	12.89	0.40	0.67	0.85	0.66
h 3	12.72	12.76	12.63	12.62	0.40	0.76	0.99	0.88
h 12	12.52	12.49	12.47	12.50	0.39	0.97	0.92	0.93
Δ h 0-3	-0.37	-0.23	-0.29	-0.27	0.07	0.52	0.47	0.21
Δ h 3-12	-0.20	-0.19	-0.15	-0.12	0.06	0.29	0.96	0.47
Δ h 0-12	-0.57	-0.49	-0.45	-0.39	0.06	0.05	0.57	0.07
Rectal Temperature, °C								
h 0	39.78	39.68	39.64	39.49	0.09	0.02	0.92	0.07
h 3	41.06[a]	40.92[ab]	40.93[ab]	40.65[b]	0.13	0.005	0.72	0.06
h 12	40.53	40.69	40.31	40.50	0.10	0.43	0.46	0.82
Δ h 0-3	1.27	1.24	1.29	1.16	0.11	0.39	0.63	0.66
Δ h 3-12	-0.53	-0.23	-0.62	-0.15	0.16	0.14	0.42	0.26
Δ h 0-12	0.74	1.01	0.67	1.01	0.13	0.28	0.56	0.30
Serum TNF-α concentration, pg/mL								
h 0	94	83	73	69	8.21	0.04	0.46	0.06
h 3	4145	1787	1877	2166	659	0.09	0.05	0.005
h 12	368[a]	179[b]	160[b]	191[ab]	50	0.04	0.02	0.001
Δ h 0-3	4052	1704	1807	2098	657	0.10	0.05	0.005
Δ h 3-12	3778	1608	1742	1975	613	0.10	0.06	0.006
Δ h 0-12	274[a]	95[b]	86[b]	122[ab]	49	0.07	0.02	0.002
Serum CRP concentration, μg/mL								
h 0	127.37	128.47	129.94	131.32	17.79	0.86	0.98	0.89
h 3	152.15	133.75	132.53	140.10	16.23	0.71	0.40	0.36
h 12	143.83	130.16	138.32	138.45	12.03	0.93	0.64	0.55
Δ h 0-3	24.78	5.28	2.59	8.78	10.59	0.40	0.19	0.11
Δ h 3-12	-8.32	-3.59	5.79	-1.65	10.38	0.56	0.36	0.38
Δ h 0-12	16.36	1.69	8.38	7.13	12.27	0.67	0.52	0.31
Salivary CRP concentration, ng/mL								
h 0	3.59	2.29	2.79	2.93	1.01	0.80	0.48	0.38
h 12	14.14	8.84	9.46	11.64	3.39	0.78	0.28	0.28
Δ h 0-12	10.56	6.57	6.67	8.76	3.58	0.85	0.40	0.43
Serum Zn Concentration, mg/L								
h 0	1.059	1.273	1.355	1.349	0.13	0.16	0.29	0.08
h 3	0.596	0.687	0.924	0.919	0.15	0.08	0.43	0.13
h 12	0.747[b]	0.812[ab]	0.987[ab]	1.088[a]	0.07	0.001	0.60	0.03
Δ h 0-3	-0.463	-0.586	-0.431	-0.431	0.11	0.61	0.78	0.88
Δ h 3-12	0.150	0.125	0.063	0.170	0.18	0.94	0.67	0.87
Δ h 0-12	-0.313	-0.461	-0.367	-0.261	0.16	0.64	0.50	0.77

Item	Water ZnAAC treatment, mg/L					*P*-value[2]		
	0	40	80	160	SEM	L	Q	Trt 0 vs others
Serum Cu Concentration, mg/L								
h 0	2.840	2.970	3.052	3.019	0.22	0.58	0.63	0.48
h 3	1.953	1.787	2.333	2.218	0.26	0.30	0.75	0.61
h 12	2.371	2.443	2.567	2.773	0.17	0.07	0.94	0.23
Δ h 0-3	-0.887	-1.184	-0.719	-0.801	0.27	0.57	0.93	0.96
Δ h 3-12	0.418	0.656	0.235	0.555	0.33	0.93	0.75	0.85
Δ h 0-12	-0.468	-0.528	-0.484	-0.246	0.29	0.53	0.69	0.88

[1] Data were analyzed using the linear mixed models with block as a random effect (n = 14 pigs/Trt/Time).

[2] *P*-value is reported for L as a linear response and Q as a quadratic response.

[a-b] Means within the same row without a common letter differ significantly ($P \leq 0.05$).

Table 5.5. Effects of titrated levels of water soluble ZnAAC on immune response indices following LPS-challenged of pigs at d 23 of combined results of Exp 1 and Exp 2.[1]

Item	Water ZnAAC treatment, mg/L			SEM	*P*-value[2]		
	0	40	80		Trt × Exp	L	Q
Body Weight, kg							
h 0	12.09	11.93	11.80	0.33	0.50	0.71	0.79
h 3	11.72	11.61	11.47	0.34	0.47	0.62	0.70
h 12	11.52	11.37	11.30	0.33	0.42	0.77	0.72
Δ h 0-3	-0.37	-0.32	-0.33	0.07	1.00	0.43	0.59
Δ h 3-12	-0.20	-0.24	-0.17	0.07	0.74	0.25	0.93
Δ h 0-12	-0.57	-0.56	-0.49	0.05	0.61	0.45	0.59
Rectal Temperature, °C							
h 0	39.39	39.44	39.36	0.12	0.25	0.44	0.41
h 3	40.90	40.76	40.78	0.12	0.13	0.99	0.34
h 12	39.88	40.23	39.87	0.10	0.95	0.79	0.02
Δ h 0-3	1.51	1.31	1.43	0.10	0.01	0.41	0.07
Δ h 3-12	-1.02	-0.53	-0.91	0.11	0.38	0.50	0.003
Δ h 0-12	0.49	0.79	0.51	0.12	0.85	0.90	0.07
Serum TNF-α concentration, pg/mL							
h 0	127	116	122	8.65	0.37	0.65	0.44
h 3	2934	1709	1799	384.26	0.09	0.05	0.17
Δ h 0-3	2874	1593	1680	0.12	0.12	0.04	0.15
Serum CRP concentration, μg/mL							
h 0	105	89	96	9.98	0.40	0.47	0.32
h 3	130	99	108	10.57	0.67	0.17	0.12
h 12	130	113	112	7.97	0.59	0.18	0.51
Δ h 0-3	23	10	12	6.54	0.43	0.27	0.32
Δ h 3-12	-4	15	4	8.19	0.17	0.50	0.13
Δ h 0-12	20	24	16	8.72	0.25	0.74	0.55
Serum Zn Concentration, mg/L							
h 0	1.018	1.186	1.189	0.07	0.39	0.07	0.30
h 3	0.725	0.805	0.896	0.07	0.18	0.07	0.94
h 12	0.839	0.885	0.986	0.05	0.31	0.03	0.61
Δ h 0-3	-0.294	-0.382	-0.293	0.06	0.72	0.96	0.21
Δ h 3-12	0.114	0.080	0.090	0.08	0.77	0.83	0.81
Δ h 0-12	-0.179	-0.302	-0.202	0.07	0.93	0.80	0.17

Item	Water ZnAAC treatment, mg/L			SEM	*P*-value[2]		
	0	40	80		Trt × Exp	L	Q
Serum Cu Concentration, mg/L							
h 0	2.889	2.916	2.955	0.11	0.49	0.60	0.96
h 3	2.235	2.018	2.329	0.11	0.10	0.50	0.03
h 12	2.629	2.287	2.485	0.12	0.003	0.23	0.01
Δ h 0-3	-0.655	-0.898	-0.627	0.15	0.58	0.88	0.13
Δ h 3-12	0.395	0.268	0.156	0.17	0.12	0.21	0.97
Δ h 0-12	-0.26	-0.63	-0.471	0.15	0.21	0.24	0.09

[1] Data were analyzed using the linear mixed models with block as a random effect (n = 28 pigs/Trt/Time).
[2] *P*-value is reported for L as a linear response and Q as a quadratic response.

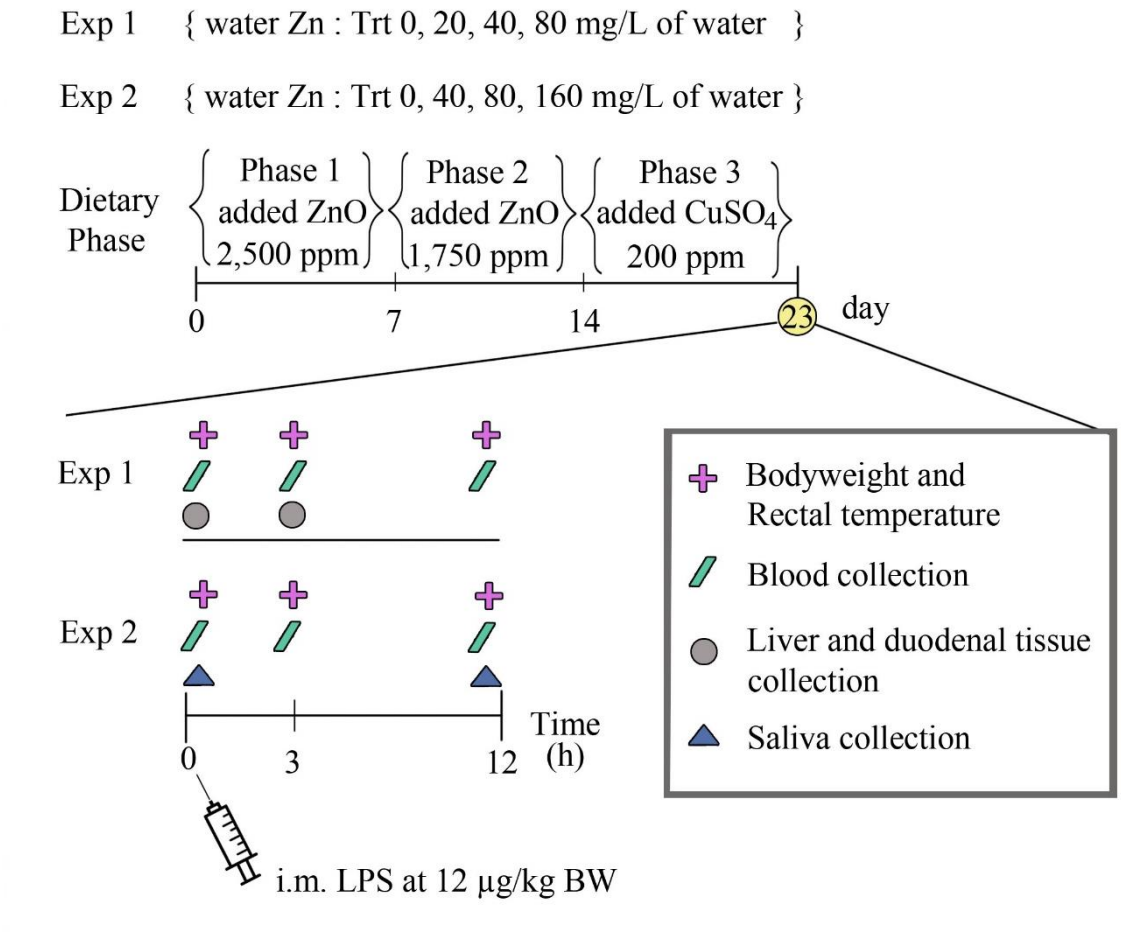

Figure 5.1. Timeline of phase feeding, LPS injection, and sample collection of Exp 1 and Exp 2.

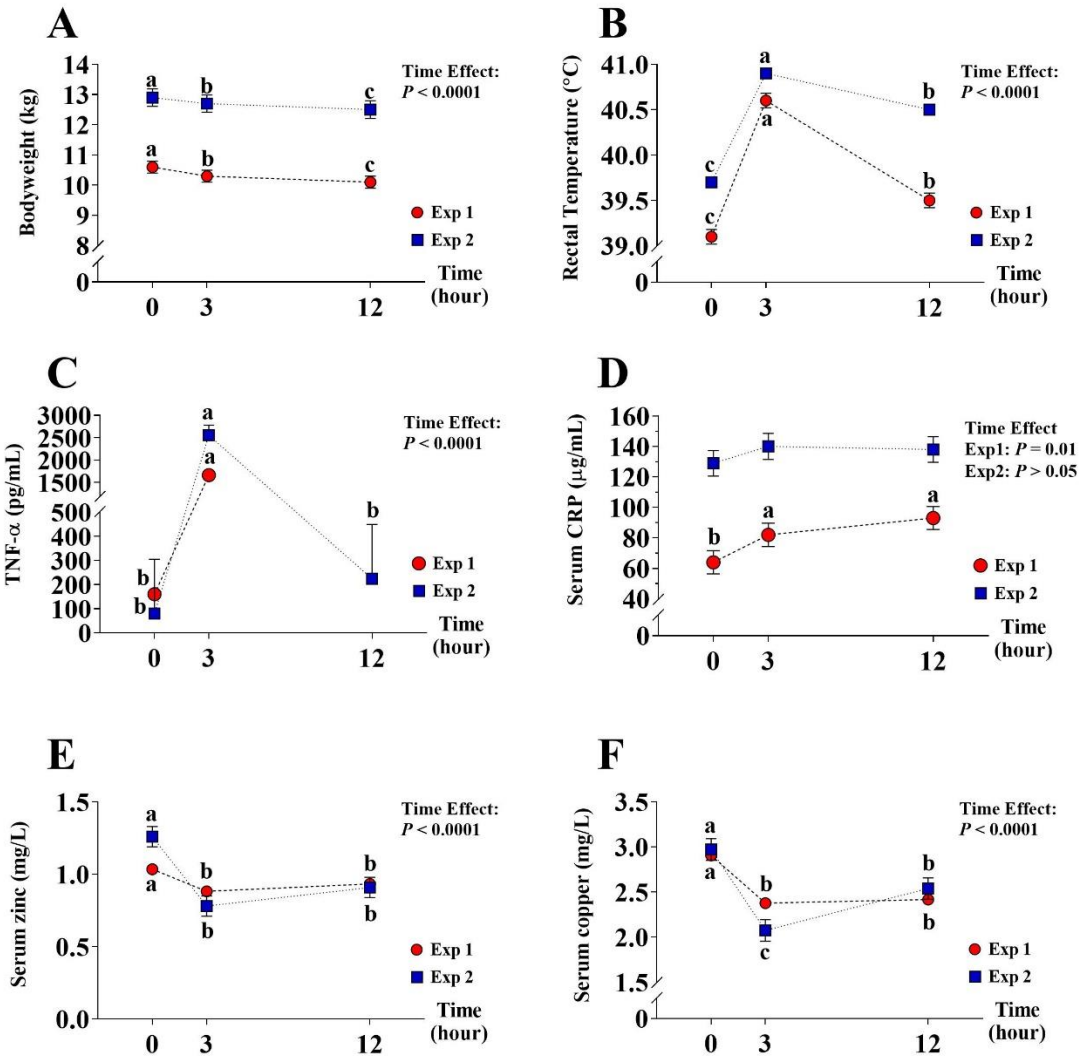

Figure 5.2. Effects of LPS injection on BW, rectal temperature, and serum biomarkers of nursery pigs on d 23 of Exp 1 and Exp 2. Different letters indicate significantly differences among the three time-course of each experiment ($P \leq 0.05$).

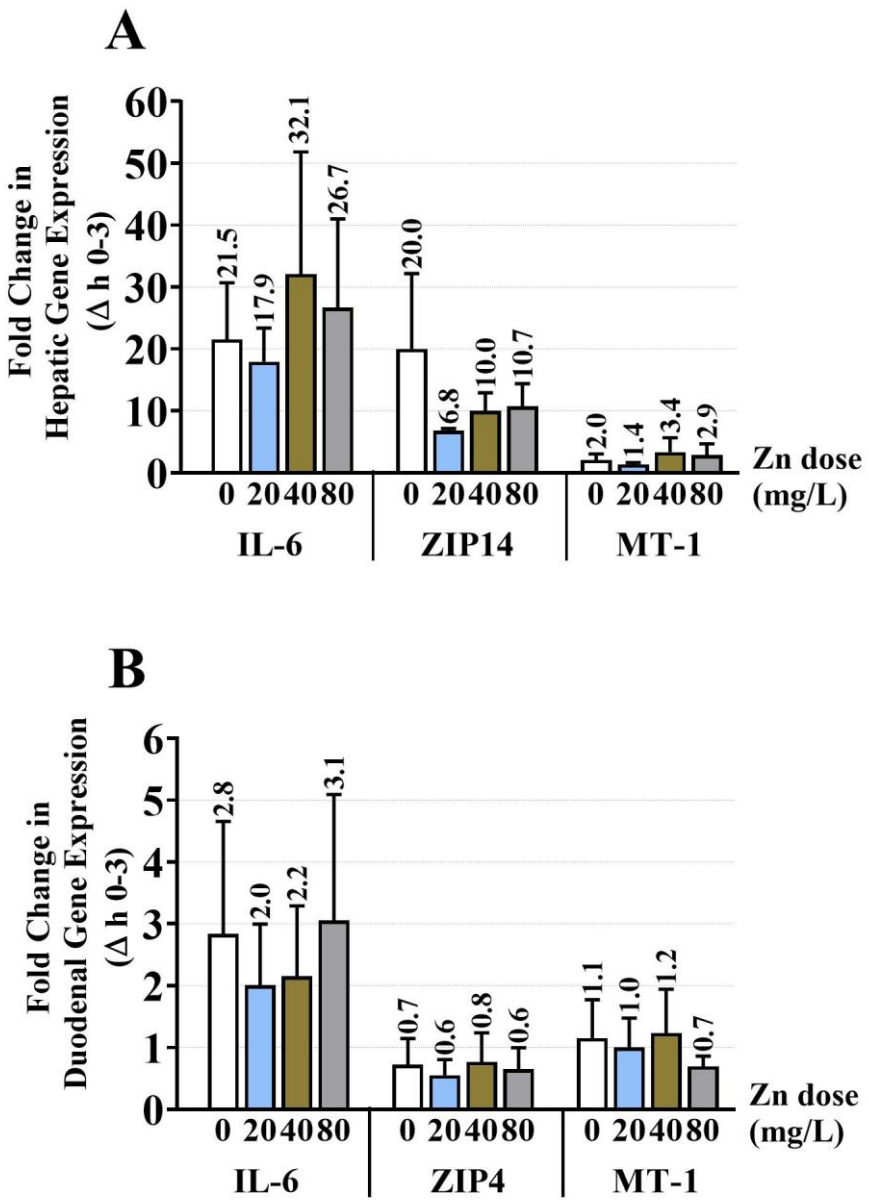

Figure 5.3. Mean fold change of hepatic and duodenal IL-6, Zn transporters, and MT-1 mRNA expressions during h 0-3 of LPS-challenged pigs from Exp 1 (n = 6-7/ treatment). (A) Upregulation of hepatic IL-6, ZIP14, and MT-1 genes following LPS. (B) Upregulation of duodenal IL-6 gene following LPS.

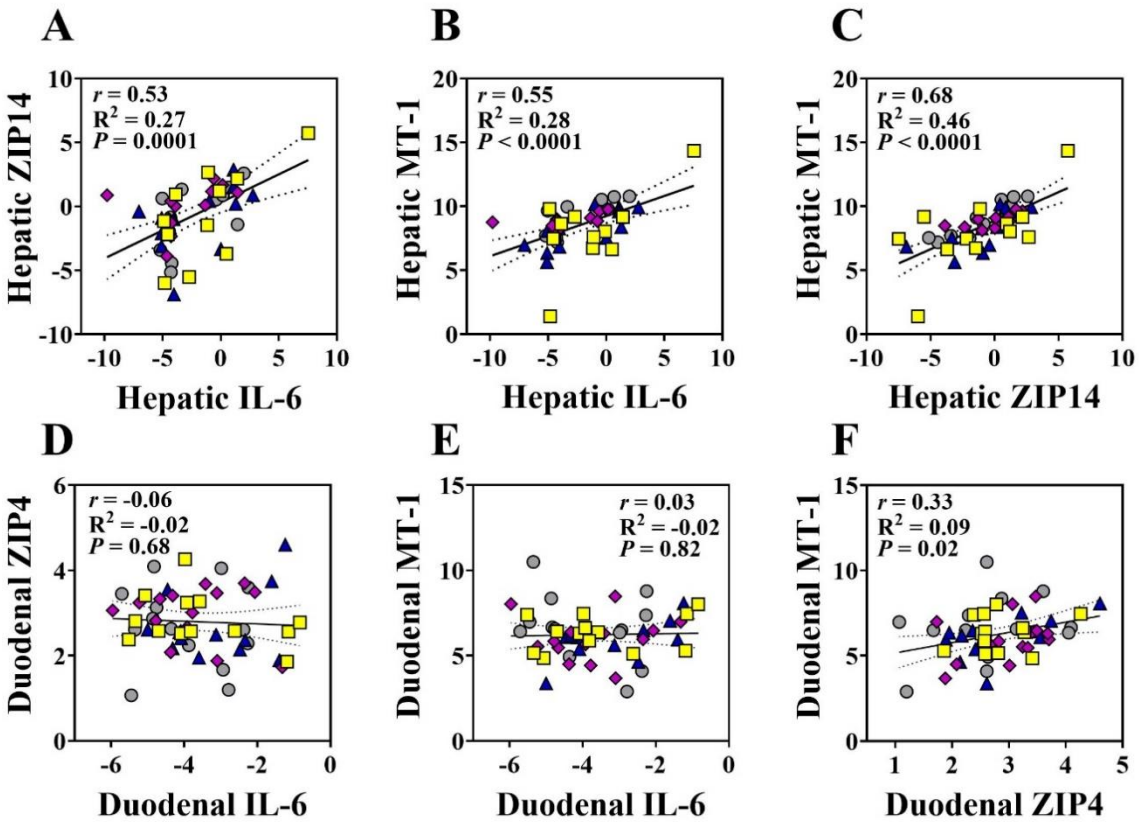

Figure 5.4. Pearson correlations of hepatic and duodenal IL-6, Zn transporters, and MT-1 mRNA expressions of LPS-challenged pigs from Exp 1.
(A) Positive relation of hepatic ZIP14 by IL-6; ZIP14 = (0.43846 × IL-6) + 0.31935.
(B) Positive relation of hepatic MT-1 by IL-6; MT-1 = (0.31449 × IL-6) + 9.24486.
(C) Positive relation of hepatic MT-1 by ZIP14; MT-1 = (0.44606 × ZIP14) + 8.88355.
(D) Duodenal ZIP4 by IL-6.
(E) Duodenal MT-1 by IL-6.
(F) Positive relation of duodenal MT-1 by ZIP4; MT-1 = (0.60174 × ZIP4) + 4.56622.
Each plot overlays with the fitted solid line and a 95% confidence dotted line. Data are displayed as the CT value normalized to SDHA mRNA expression (ΔCT, n = 12-14/ treatment). Each treatment is formatted as **Trt 0** (□), **Trt 20** (◊), **Trt 40** (Δ), and **Trt 80** (○).

CHAPTER VI

SUMMARY

A low dose of lipopolysaccharide (LPS) injection at 12 μg/kg BW in nursery pigs induced rapid inflammatory response including increased rectal temperature and serum proinflammatory cytokine (TNF-α) but led to decreased serum Zn and Cu concentrations. Pigs reached the peak of inflammatory response within 3-h and recovered to normal after 12-h following injection, except for a slight increase in serum C-reactive protein as an acute phase protein synthesized by the liver. Thus, measuring peaks of the inflammatory response can be done at h 3 following LPS injection. In addition, decreased serum Zn concentration or hypozincemia condition of pigs resulted from increased liver inflammation indicated by upregulation of hepatic IL-6 increased Zn transporter ZIP14 in the liver. Then, intracellular free Zn can upregulate metallothionein expression. Hepatic IL-6, ZIP14, and MT-1 genes were suggested to work in concert from the evidence of strong positive correlation data. Whereas duodenal IL-6, ZIP4, and MT-1 mRNA expressions were less responsive after an intramuscular LPS injection indicated by delayed increases in duodenal ZIP4 and MT-1 gene expressions.

Pigs drinking titrated levels of water soluble Zn amino acid complex (ZnAAC) mitigated the increase of inflammatory response following a low dose LPS-injection at h 3 by decreasing rectal temperature, serum TNF-α, serum CRP, and increasing serum Zn and Cu concentrations. Also, pigs drinking ZnAAC had lower basal serum TNF-α and CRP concentrations. Hepatic and duodenal IL-6, Zn transporter (duodenal ZIP4 and hepatic ZIP14), and MT-1 gene expression changes were not different among the four water ZnAAC treatments following LPS injection. However, LPS injection upregulated hepatic IL-6, ZIP14, and MT-1 gene expressions, while only the duodenal IL-6 gene was upregulated within 3-h.

Supplementing ZnAAC via drinking water to nursery pigs improved average daily feed intake (ADFI), average daily gain (ADG), gain to feed ratio (G:F), and body weight (BW) at d 14 in a linear manner when supplemented up to 80 mg Zn/L of water. Whereas supplementing up 160 mg Zn/L, pigs improved ADG, G:F, and final BW in a quadratic manner, which 80 mg Zn/L was the best dose. The quadratic broken-line model estimating ideal water ZnAAC doses for ADG and G:F were 74 and 70 mg Zn/L, respectively.

REFERENCES

Adeola, O.,Lawrence, B.,Sutton, A. and Cline, T. 1995. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. Journal of Animal Science 73:3384-3391.

Agrawal, A.,Cha-Molstad, H.,Samols, D. and Kushner, I. 2003. Overexpressed nuclear factor-κB can participate in endogenous C-reactive protein induction, and enhances the effects of C/EBPβ and signal transducer and activator of transcription-3. Immunology 108:539-547.

Akira, S.,Hirano, T.,Taga, T. and Kishimoto, T. 1990. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). The FASEB journal 4:2860-2867.

Akira, S.,Nishio, Y.,Inoue, M.,Wang, X.-J.,We, S.,Matsusaka, T.,Yoshida, K.,Sudo, T.,Naruto, M. and Kishimoto, T. 1994. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 77:63-71.

Akira, S. and Takeda, K. 2004. Toll-like receptor signalling. Nature reviews immunology 4:499-511.

Alexander, C. and Rietschel, E. T. 2001. Bacterial lipopolysaccharides and innate immunity (2001) J. Endotoxin Res 7:167-120.

Allen, L. H. 1998. Zinc and micronutrient supplements for children. The American journal of clinical nutrition 68:495S-498S.

Alsemgeest, S. P. M. 1994. Blood concentrations of acute-phase proteins in cattle as markers for disease. Ph. D Thesis. Department of Pathology, Faculty of Veterinary Medicine, Utrecht University, Netherlands:39-53.

Ammerman, C. B.,Baker, D. P. and Lewis, A. J. 1995. Bioavailability of nutrients for animals: Amino acids, minerals, vitamins, Elsevier.

Andreini, C. and Bertini, I. 2012. A bioinformatics view of zinc enzymes. Journal of inorganic biochemistry 111:150-156.

Andrews, G. K. 2000. Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochemical pharmacology 59:95-104.

Aydemir, T. B.,Chang, S.-M.,Guthrie, G. J.,Maki, A. B.,Ryu, M.-S.,Karabiyik, A. and Cousins, R. J. 2012. Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia). PloS one 7:e48679.

Aydemir, T. B.,Sitren, H. S. and Cousins, R. J. 2012. The zinc transporter Zip14 influences c-Met phosphorylation and hepatocyte proliferation during liver regeneration in mice. Gastroenterology 142:1536-1546. e1535.

Baker, D. H. 2000. Bioavailability of minerals and vitamins Swine Nutrition, CRC Press: 377-400.

Balaji, R.,Wright, K.,Hill, C.,Dritz, S.,Knoppel, E. and Minton, J. 2000. Acute phase responses of pigs challenged orally with Salmonella typhimurium. Journal of Animal Science 78:1885-1891.

Baltaci, A. K. and Yuce, K. 2018. Zinc transporter proteins. Neurochemical Research 43:517-530.

Bao, B.,Prasad, A. S.,Beck, F. W.,Fitzgerald, J. T.,Snell, D.,Bao, G. W.,Singh, T. and Cardozo, L. J. J. T. A. j. o. c. n. 2010. Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. 91:1634-1641.

Barnett, J. P.,Blindauer, C. A.,Kassaar, O.,Khazaipoul, S.,Martin, E. M.,Sadler, P. J. and Stewart, A. J. 2013. Allosteric modulation of zinc speciation by fatty acids. Biochimica et Biophysica Acta (BBA)-General Subjects 1830:5456-5464.

Bazan, J. F. 1990. Haemopoietic receptors and helical cytokines. Immunology today 11:350-354.

Betts, J.,Cheshire, J.,Akira, S.,Kishimoto, T. and Woo, P. 1993. The role of NF-kappa B and NF-IL6 transactivating factors in the synergistic activation of human serum amyloid A gene expression by interleukin-1 and interleukin-6. Journal of Biological Chemistry 268:25624-25631.

Bode, J. G. and Heinrich, P. C. 2001. Interleukin-6 signaling during the acute-phase response of the liver. The liver: biology and pathobiology 4:565-580.

Brooks, P.,Russell, S. and Carpenter, J. 1984. Water intake of weaned piglets from three to seven weeks old. Vet. Rec 115:513-515.

Brooks, P. and Tsourgiannis, C. 2003. Factors affecting the voluntary feed intake of the weaned pig. Weaning the Pig, Concepts and Consequences. Wageningen, The Netherlands: Wageningen Academic Publishers:81-116.

Buff, C.,Bollinger, D.,Ellersieck, M.,Brommelsiek, W. and Veum, T. J. J. o. a. s. 2005. Comparison of growth performance and zinc absorption, retention, and excretion in weanling pigs fed diets supplemented with zinc-polysaccharide or zinc oxide. 83:2380-2386.

Burch, R. E.,Williams, R. V.,Hahn, H. K.,Jetton, M. M. and Sullivan, J. F. 1975. Serum and tissue enzyme activity and trace-element content in response to zinc deficiency in the pig. Clinical Chemistry 21:568-577.

Burrin, D. and Stoll, B. 2003. 12 Intestinal nutrient requirements in weanling pigs. Weaning the pig: Concepts and consequences:301.

Burrough, E. R.,De Mille, C. and Gabler, N. K. 2019. Zinc overload in weaned pigs: tissue accumulation, pathology, and growth impacts. Journal of Veterinary Diagnostic Investigation 31:537-545.

Butler, A. 1998. Acquisition and utilization of transition metal ions by marine organisms. Science 281:207-209.

Campbell, R. 1990. The nutrition and management of pigs to 20 kg liveweight. Pig Rations: Assessment and Formulation, Proceedings of the Refresher Course for Veterinarians 132:123-126.

Cao, J.,Henry, P.,Guo, R.,Holwerda, R.,Toth, J.,Littell, R.,Miles, R. and Ammerman, C. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. Journal of animal science 78:2039-2054.

Carlson, D.,Beattie, J. and Poulsen, H. D. 2007. Assessment of zinc and copper status in weaned piglets in relation to dietary zinc and copper supply. Journal of animal physiology and animal nutrition 91:19-28.

Carlson, M.,Boren, C.,Wu, C.,Huntington, C.,Bollinger, D. and Veum, T. 2004. Evaluation of various inclusion rates of organic zinc either as polysaccharide or proteinate complex on the growth performance, plasma, and excretion of nursery pigs. Journal of Animal Science 82:1359-1366.

Carlson, M. S. and Hellman, H. 2003. Feeding Organic and Inorganic Sources of Trace Minerals for Swine Production (2003). Extension publications (MU).

Cartmell, T.,Mitchell, D.,Lamond, F. and Laburn, H. 2002. Route of administration differentially affects fevers induced by Gram-negative and Gram-positive pyrogens in rabbits. Experimental physiology 87:391-399.

Ceciliani, F.,Giordano, A. and Spagnolo, V. 2002. The systemic reaction during inflammation: the acute-phase proteins. Protein and peptide letters 9:211-223.

Cherian, M. and Apostolova, M. D. 2000. Nuclear localization of metallothionein during cell proliferation and differentiation. Cellular and molecular biology (Noisy-le-Grand, France) 46:347.

Choi, I. S.,Shin, N. R.,Shin, S. J.,Lee, D. Y.,Cho, Y. W. and Yoo, H. S. 2002. Time course study of cytokine mRNA expression in LPS-stimulated porcine alveolar macrophages. Journal of Veterinary Science 3:97-102.

Choy, E. and Rose-John, S. 2017. Interleukin-6 as a multifunctional regulator: inflammation, immune response, and fibrosis. Journal of Scleroderma and Related Disorders 2:S1-S5.

Chu, W.-M. 2013. Tumor necrosis factor. Cancer letters 328:222-225.

Coleman, J. E. 1992. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. Annual review of biochemistry 61:897-946.

Collins, C. L.,Pluske, J. R.,Morrison, R. S.,McDonald, T. N.,Smits, R. J.,Henman, D. J.,Stensland, I. and Dunshea, F. R. 2017. Post-weaning and whole-of-life performance of pigs is determined by live weight at weaning and the complexity of the diet fed after weaning. Animal Nutrition 3:372-379.

Cousins, R. 1985. Absorbtion, transport, and hepatic metabolism of Cu and Zn: special reference to MTs and ceruloplasmin. Physiol. Rev 65.

Cousins, R. J. 2010. Gastrointestinal factors influencing zinc absorption and homeostasis. International Journal for Vitamin and Nutrition Research 80:243.

Cousins, R. J.,Liuzzi, J. P. and Lichten, L. A. 2006. Mammalian zinc transport, trafficking, and signals. Journal of Biological Chemistry 281:24085-24089.

Davis, S. R.,McMahon, R. J. and Cousins, R. J. 1998. Metallothionein knockout and transgenic mice exhibit altered intestinal processing of zinc with uniform zinc-dependent zinc transporter-1 expression. The Journal of nutrition 128:825-831.

De Lange, C.,Pluske, J.,Gong, J. and Nyachoti, C. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science 134:124-134.

Deban, L.,Jaillon, S.,Garlanda, C.,Bottazzi, B. and Mantovani, A. 2011. Pentraxins in innate immunity: lessons from PTX3. Cell and tissue research 343:237-249.

Dinarello, C. A. 2000. Proinflammatory cytokines. Chest 118:503-508.

Dogan, M. D.,Ataoglu, H. and Akarsu, E. S. 2000. Effects of different serotypes of Escherichia coli lipopolysaccharides on body temperature in rats. Life sciences 67:2319-2329.

Dufner-Beattie, J.,Wang, F.,Kuo, Y.-M.,Gitschier, J.,Eide, D. and Andrews, G. K. 2003. The acrodermatitis enteropathica gene ZIP4 encodes a tissue-specific, zinc-regulated zinc transporter in mice. Journal of Biological Chemistry 278:33474-33481.

Dufner-Beattie, J.,Weaver, B. P.,Geiser, J.,Bilgen, M.,Larson, M.,Xu, W. and Andrews, G. K. 2007. The mouse acrodermatitis enteropathica gene Slc39a4 (Zip4) is essential for early development and heterozygosity causes hypersensitivity to zinc deficiency. Human molecular genetics 16:1391-1399.

Eckersall, P. and Bell, R. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The veterinary journal 185:23-27.

Eckersall, P.,Saini, P. and McComb, C. 1996. The acute phase response of acid soluble glycoprotein, α1-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. Veterinary Immunology and Immunopathology 51:377-385.

Edelstein, L. C. and Collins, T. 2005. The SCAN domain family of zinc finger transcription factors. Gene 359:1-17.

Edwards III, H. M. and Baker, D. H. 1999. Bioavailability of zinc in several sources of zinc oxide, zinc sulfate, and zinc metal. J. Anim. Sci. 77:2730-2735. doi:10.2527/1999.77102730x.

Eisenhardt, S. U.,Habersberger, J.,Murphy, A.,Chen, Y.-C.,Woollard, K. J.,Bassler, N.,Qian, H.,von zur Muhlen, C.,Hagemeyer, C. E. and Ahrens, I. 2009. Dissociation of pentameric to monomeric C-reactive protein on activated platelets localizes inflammation to atherosclerotic plaques. Circulation research 105:128-137.

Eklund, C. M. 2009. Proinflammatory cytokines in CRP baseline regulation. Advances in clinical chemistry 48:111-136.

Escribano, D.,Campos, P. H.,Gutiérrez, A. M.,Le Floc'h, N.,Cerón, J. J. and Merlot, E. 2014. Effect of repeated administration of lipopolysaccharide on inflammatory and stress markers in saliva of growing pigs. The Veterinary Journal 200:393-397.

Escuredo, J. A. M.,van der Horst, Y.,Carr, J. and Maes, D. 2016. Implementing drinking water feed additive strategies in post-weaning piglets, antibiotic reduction and performance impacts: case study. Porcine health management 2:25.

Fairweather-Tait, S. J. 1992. Bioavailability of trace elements. Food Chemistry 43:213-217.

Fels, M.,Hartung, J. and Hoy, S. 2014. Social hierarchy formation in piglets mixed in different group compositions after weaning. Applied Animal Behaviour Science 152:17-22.

Fernandez, M. S.,Pearce, S.,Gabler, N.,Patience, J.,Wilson, M.,Socha, M.,Torrison, J.,Rhoads, R. and Baumgard, L. 2014. Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. Animal: an international journal of animal bioscience 8:43.

Fischer, J. E. and Hasselgren, P.-O. 1991. Cytokines and glucocorticoids in the regulation of the “hepato-skeletal muscle axis” in sepsis. The American journal of surgery 161:266-271.

Frank, J.,Mellencamp, M.,Carroll, J.,Boyd, R. and Allee, G. 2005. Acute feed intake and acute-phase protein responses following a lipopolysaccharide challenge in pigs from two dam lines. Veterinary immunology and immunopathology 107:179-187.

Frederickson, C. J. and Bush, A. I. 2001. Synaptically released zinc: physiological functions and pathological effects. Biometals 14:353-366.

Gabay, C. and Kushner, I. 1999. Acute-phase proteins and other systemic responses to inflammation. New England journal of medicine 340:448-454.

Garami, A.,Steiner, A. A. and Romanovsky, A. A. 2018. Fever and hypothermia in systemic inflammation Handbook of clinical neurology, Elsevier. 157: 565-597.

Geeganage, S. and Frey, P. A. 1999. Significance of metal ions in galactose-1-phosphate uridylyltransferase: an essential structural zinc and a nonessential structural iron. Biochemistry 38:13398-13406.

Giacconi, R.,Costarelli, L.,Piacenza, F.,Basso, A.,Rink, L.,Mariani, E.,Fulop, T.,Dedoussis, G.,Herbein, G. and Provinciali, M. 2017. Main biomarkers associated with age-related plasma zinc decrease and copper/zinc ratio in healthy elderly from ZincAge study. European journal of nutrition 56:2457-2466.

Gibson, R. S.,Bailey, K. B.,Gibbs, M. and Ferguson, E. L. 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. Food and nutrition bulletin 31:S134-S146.

Goff, J. P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science 101:2763-2813.

Golden, M. H. 1996. Specific deficiencies versus growth failure: type I and type II nutrients. Journal of Nutritional & Environmental Medicine 6:301-308.

Goldstein, A. 2013. Thymic Hormones and Lymphokines: Basic Chemistry and Clinical Applications, Springer Science & Business Media.

Gruys, E. 2002. Acute phase proteins in bovine medicine Proceedings American Veterinary Medical Association Congress: 317-321.

Gruys, E.,Toussaint, M.,Niewold, T. and Koopmans, S. 2005. Acute phase reaction and acute phase proteins. Journal of Zhejiang University. Science. B 6:1045.

Gupta, R. K.,Gangoliya, S. S. and Singh, N. K. 2015. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. Journal of food science and technology 52:676-684.

Guthrie, G. J.,Aydemir, T. B.,Troche, C.,Martin, A. B.,Chang, S.-M. and Cousins, R. J. 2015. Influence of ZIP14 (slc39A14) on intestinal zinc processing and barrier function. American Journal of Physiology-Gastrointestinal and Liver Physiology 308:G171-G178.

Haase, H. and Rink, L. 2011. Zinc signaling. Zinc in human health 76:94.

Hahn, J. D. and Baker, D. H. 1993. Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. Journal of animal science 71:3020-3024.

Hambidge, K. M. 2010. Micronutrient bioavailability: Dietary Reference Intakes and a future perspective. The American journal of clinical nutrition 91:1430S-1432S.

Hampson, D. 1986. Alterations in piglet small intestinal structure at weaning. Research in veterinary science 40:32-40.

Hashimoto, A.,Nakagawa, M.,Tsujimura, N.,Miyazaki, S.,Kizu, K.,Goto, T.,Komatsu, Y.,Matsunaga, A.,Shirakawa, H. and Narita, H. 2016. Properties of Zip4 accumulation during zinc deficiency and its usefulness to evaluate zinc status: a study of the effects of zinc deficiency during lactation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 310:R459-R468.

Hedges, J. D.,Kornegay, E. and Thomas, H. 1976. Comparison of dietary zinc levels for reproducing sows and the effect of dietary zinc and calcium on the subsequent performance of their progeny. Journal of animal science 43:453-463.

Hellman, H. and Carlson, M. 2018. Feeding organic and inorganic sources of trace minerals for swine production.

Henderson, L. M.,Brewer, G. J.,Dressman, J. B.,Swidan, S. Z.,DuRoss, D. J.,Adair, C. H.,Barnett, J. L. and Berardi, R. R. 1995. Effect of intragastric pH on the absorption of oral zinc acetate and zinc oxide in young healthy volunteers. Journal of Parenteral and Enteral Nutrition 19:393-397.

Hennig-Pauka, I.,Menzel, A.,Boehme, T. R.,Schierbaum, H.,Ganter, M. and Schulz, J. J. F. i. v. s. 2019. Haptoglobin and C-reactive protein–non-specific markers for nursery conditions in swine. 6:92.

Heo, J.-M.,Kim, J.-C.,Hansen, C. F.,Mullan, B. P.,Hampson, D. J. and Pluske, J. R. 2008. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. Archives of Animal Nutrition 62:343-358.

Heuchel, R.,Radtke, F.,Georgiev, O.,Stark, G.,Aguet, M. and Schaffner, W. 1994. The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. The EMBO journal 13:2870-2875.

Hibi, M.,Murakami, M.,Saito, M.,Hirano, T.,Taga, T. and Kishimoto, T. 1990. Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell 63:1149-1157.

Hill, G.,Cromwell, G.,Crenshaw, T.,Dove, C.,Ewan, R.,Knabe, D.,Lewis, A.,Libal, G.,Mahan, D. and Shurson, G. C. 2000. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). Journal of animal science 78:1010-1016.

Hill, G.,Mahan, D. and Jolliff, J. J. J. o. a. s. 2014. Comparison of organic and inorganic zinc sources to maximize growth and meet the zinc needs of the nursery pig. 92:1582-1594.

Hoefer, J.,Miller, E.,Ullrey, D.,Ritche, H. and Luecke, R. 1960. Interrelationships between calcium, zinc, iron and copper in swine feeding. Journal of Animal Science 19:249-259.

Hoeger, J.,Simon, T.-P.,Doemming, S.,Thiele, C.,Marx, G.,Schuerholz, T. and Haase, H. 2015. Alterations in zinc binding capacity, free zinc levels and total serum zinc in a porcine model of sepsis. Biometals 28:693-700.

Hojyo, S. and Fukada, T. 2016. Zinc transporters and signaling in physiology and pathogenesis. Archives of biochemistry and biophysics 611:43-50.

Holm, A. 1990. E. coli associated diarrhoea in weaner pigs: zinc oxide added to the feed as a preventive measure Proceedings, International Pig Veterinary Society, 11th Congress, July 1-5, 1990, Lausanne, Switzerland., Swiss Association of Swine Medicine.

Hu, C.,Song, J.,Li, Y.,Luan, Z. and Zhu, K. 2013. Diosmectite–zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. British journal of nutrition 110:681-688.

Humann-Ziehank, E.,Menzel, A.,Roehrig, P.,Schwert, B.,Ganter, M. and Hennig-Pauka, I. 2014. Acute and subacute response of iron, zinc, copper and selenium in pigs experimentally infected with Actinobacillus pleuropneumoniae. Metallomics 6:1869-1879.

Hurrell, R. F.,Lynch, S. R.,Trinidad, T. P.,Dassenko, S. A. and Cook, J. D. 1989. Iron absorption in humans as influenced by bovine milk proteins. The American journal of clinical nutrition 49:546-552.

Ihle, J. N. and Kerr, I. M. 1995. Jaks and Stats in signaling by the cytokine receptor superfamily. Trends in Genetics 11:69-74.

Ingenbleek, Y. and Young, V. 1994. Transthyretin (prealbumin) in health and disease: nutritional implications. Annual review of nutrition 14:495-533.

Inoue, K. i.,Takano, H.,Shimada, A.,Wada, E.,Yanagisawa, R.,Sakurai, M.,Satoh, M. and Yoshikawa, T. 2006. Role of metallothionein in coagulatory disturbance and systemic inflammation induced by lipopolysaccharide in mice. The FASEB journal 20:533-535.

Ivanov, A. I. and Romanovsky, A. A. 2004. Prostaglandin E2 as a mediator of fever: synthesis and catabolism. Front Biosci 9:1977-1983.

Jacela, J. Y.,DeRouchey, J. M.,Tokach, M. D.,Goodband, R. D.,Nelssen, J. L.,Renter, D. G. and Dritz, S. S. 2010. Feed additives for swine: fact sheets–high dietary levels of copper and zinc for young pigs, and phytase. Kansas Agricultural Experiment Station Research Reports:87-91.

Jain, S.,Gautam, V. and Naseem, S. 2011. Acute-phase proteins: As diagnostic tool. Journal of Pharmacy and Bioallied Sciences 3:118.

Jarosz, M.,Olbert, M.,Wyszogrodzka, G.,Młyniec, K. and Librowski, T. 2017. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology 25:11-24.

Johnson, M.,Zaretskaya, I.,Raytselis, Y.,Merezhuk, Y.,McGinnis, S. and Madden, T. L. 2008. NCBI BLAST: a better web interface. Nucleic acids research 36:W5-W9.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: an integrated view. Journal of animal science 75:1244-1255.

Johnson, R. W. and Von Borell, E. 1994. Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin. Journal of Animal Science 72:309-314.

Jondreville, C.,Revy, P. and Dourmad, J. J. L. P. S. 2003. Dietary means to better control the environmental impact of copper and zinc by pigs from weaning to slaughter. 84:147-156.

Joray, M. L.,Yu, T.-W.,Ho, E.,Clarke, S. L.,Stanga, Z.,Gebreegziabher, T.,Hambidge, K. M. and Stoecker, B. J. 2015. Zinc supplementation reduced DNA breaks in Ethiopian women. Nutrition research 35:49-55.

Joyce, D.,Albanese, C.,Steer, J.,Fu, M.,Bouzahzah, B. and Pestell, R. G. 2001. NF-κB and cell-cycle regulation: the cyclin connection. Cytokine & growth factor reviews 12:73-90.

Kaewtapee, C.,Krutthai, N.,Poosuwan, K.,Poeikhampha, T.,Koonawootrittriron, S. and Bunchasak, C. 2010. Effects of adding liquid dl-methionine hydroxy analogue-free acid to drinking water on growth performance and small intestinal morphology of nursery pigs. Journal of animal physiology and animal nutrition 94:395-404.

Kambe, T.,Tsuji, T.,Hashimoto, A. and Itsumura, N. 2015. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiological reviews 95:749-784.

Karsenty, G.,Kronenberg, H. M. and Settembre, C. 2009. Genetic control of bone formation. Annual Review of Cell and Developmental 25:629-648.

Keilin, D. and Mann, T. 1939. Carbonic anhydrase. Nature 144:442-443.

Khreiss, T.,József, L.,Potempa, L. A. and Filep, J. G. 2004. Conformational rearrangement in C-reactive protein is required for proinflammatory actions on human endothelial cells. Circulation 109:2016-2022.

Khreiss, T.,József, L.,Potempa, L. A. and Filep, J. G. 2005. Loss of pentameric symmetry in C-reactive protein induces interleukin-8 secretion through peroxynitrite signaling in human neutrophils. Circulation research 97:690-697.

Kim, B.-E.,Wang, F.,Dufner-Beattie, J.,Andrews, G. K.,Eide, D. J. and Petris, M. J. 2004. Zn2+-stimulated endocytosis of the mZIP4 zinc transporter regulates its location at the plasma membrane. Journal of Biological Chemistry 279:4523-4530.

Kim, M.-H. and Jeong, H.-J. 2015. Zinc oxide nanoparticles suppress LPS-induced NF-κB activation by inducing A20, a negative regulator of NF-κB, in RAW 264.7 macrophages. Journal of nanoscience and nanotechnology 15:6509-6515.

Kimura, A. and Kishimoto, T. 2010. IL-6: regulator of Treg/Th17 balance. European journal of immunology 40:1830-1835.

King, J. and Cousins, R. 2014. Chapter 11: Zinc. Modern Nutrition in Health and Disease, 11th ed, Philadelphia Wolters Kluwer Health, Lippincott Williams & Wilkins, Philadelphia.

King, J. C. 2011. Zinc: an essential but elusive nutrient. The American journal of clinical nutrition 94:679S-684S.

King, J. C.,Brown, K. H.,Gibson, R. S.,Krebs, N. F.,Lowe, N. M.,Siekmann, J. H. and Raiten, D. J. 2015. Biomarkers of Nutrition for Development (BOND)—zinc review. The Journal of nutrition 146:858S-885S.

Kloubert, V.,Blaabjerg, K.,Dalgaard, T. S.,Poulsen, H. D.,Rink, L. and Wessels, I. 2018. Influence of zinc supplementation on immune parameters in weaned pigs. Journal of Trace Elements in Medicine and Biology 49:231-240.

Knapp, S.,de Vos, A. F.,Florquin, S.,Golenbock, D. T. and van der Poll, T. 2003. Lipopolysaccharide binding protein is an essential component of the innate immune response to Escherichia coli peritonitis in mice. Infection and immunity 71:6747-6753.

Knoell, D. L.,Julian, M. W.,Bao, S.,Besecker, B.,Macre, J. E.,Leikauf, G. D.,DiSilvestro, R. A. and Crouser, E. D. 2009. Zinc deficiency increases organ damage and mortality in a murine model of polymicrobial sepsis. Critical care medicine 37:1380.

Kohn, F.,Phillips, G. and Klingemann, H. 1992. Regulation of tumor necrosis factor-alpha production and gene expression in monocytes. Bone marrow transplantation 9:369.

Komatsu, T.,Sugie, K.,Inukai, N.,Eguchi, O.,Oyamada, T.,Sawada, H.,Yamanaka, N. and Shibahara, T. 2020. Chronic pancreatitis in farmed pigs fed excessive zinc oxide. Journal of Veterinary Diagnostic Investigation:1040638720944368.

Kondo, Y.,Rusnak, J. M.,Hoyt, D. G.,Settineri, C. E.,Pitt, B. R. and Lazo, J. S. 1997. Enhanced apoptosis in metallothionein null cells. Molecular pharmacology 52:195-201.

Kotani, S.,Takada, H.,Tsujimoto, M.,Ogawa, T.,Takahashi, I.,Ikeda, T.,Otsuka, K.,Shimauchi, H.,Kasai, N. and Mashimo, J. 1985. Synthetic lipid A with endotoxic and related biological activities comparable to those of a natural lipid A from an Escherichia coli re-mutant. Infection and immunity 49:225-237.

Krebs, N. F. 2000. Overview of zinc absorption and excretion in the human gastrointestinal tract. The Journal of nutrition 130:1374S-1377S.

Krężel, A. and Maret, W. 2016. The biological inorganic chemistry of zinc ions. Archives of biochemistry and biophysics 611:3-19.

Krones, C. J.,Klosterhalfen, B.,Fackeldey, V.,Junge, K.,Rosch, R.,Schwab, R.,Stumpf, M.,Klinge, U. and Schumpelick, V. 2004. Deleterious effect of zinc in a pig model of acute endotoxemia. Journal of Investigative Surgery 17:249-256.

Küry, S.,Dréno, B.,Bézieau, S.,Giraudet, S.,Kharfi, M.,Kamoun, R. and Moisan, J.-P. 2002. Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. Nature genetics 31:239-240.

Kushner, I. 1993. Regulation of the acute phase response by cytokines. Perspectives in biology and medicine 36:611-622.

Laity, J. H. and Andrews, G. K. 2007. Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1). Archives of biochemistry and biophysics 463:201-210.

Lallès, J.-P.,Boudry, G.,Favier, C.,Le Floc'h, N.,Luron, I.,Montagne, L.,Oswald, I. P.,Pié, S.,Piel, C. and Sève, B. 2004. Gut function and dysfunction in young pigs: physiology. Animal Research 53:301-316.

Lastra, M. D.,Pastelin, R.,Camacho, A.,Monroy, B. and Aguilar, A. 2001. Zinc intervention on macrophages and lymphocytes response. Journal of trace elements in medicine and biology 15:5-10.

Lee, S.,Choi, S.,Chae, B.,Lee, J. and Acda, S. 2001. Evaluation of metal-amino acid chelates and complexes at various levels of copper and zinc in weanling pigs and broiler chicks. Asian-Australasian Journal of Animal Sciences 14:1734-1740.

Lee, S.,Choi, S.,Chae, B.,Lee, J. and Acda, S. J. A.-A. J. o. A. S. 2001. Evaluation of metal-amino acid chelates and complexes at various levels of copper and zinc in weanling pigs and broiler chicks. 14:1734-1740.

Leite, F. L.,Vasquez, E.,Vannucci, F. A.,Gebhart, C. J.,Rendahl, A.,Torrison, J.,Mueller, A.,Winkelman, N. L.,Rambo, Z. J. and Isaacson, R. E. 2018. The effects of zinc amino acid complex supplementation on the porcine host response to Lawsonia intracellularis infection. Veterinary research 49:1-9.

Leon, L. R. 2004. Hypothermia in systemic inflammation: role of cytokines. Frontiers in bioscience: a journal and virtual library 9:1877-1888.

Lerouge, I. and Vanderleyden, J. 2002. O-antigen structural variation: mechanisms and possible roles in animal/plant–microbe interactions. FEMS microbiology reviews 26:17-47.

Lewis Jr, P.,Hoekstra, W.,Grummer, R. and Phillips, P. 1956. The effect of certain nutritional factors including calcium, phosphorus and zinc on parakeratosis in swine. Journal of Animal Science 15:741-751.

Lewis, N. J. and Berry, R. J. 2006. Effects of season on the behaviour of early-weaned piglets during and immediately following transport. Applied Animal Behaviour Science 100:182-192.

Li, B.,Van Kessel, A.,Caine, W.,Huang, S. and Kirkwood, R. 2001. Small intestinal morphology and bacterial populations in ileal digesta and feces of newly weaned pigs receiving a high dietary level of zinc oxide. Canadian Journal of Animal Science 81:511-516.

Liang, L.,Fu, K.,Lee, D. K.,Sobieski, R. J.,Dalton, T. and Andrews, G. K. 1996. Activation of the complete mouse metallothionein gene locus in the maternal deciduum. Molecular Reproduction and Development: Incorporating Gamete Research 43:25-37.

Lichten, L. A. and Cousins, R. J. 2009. Mammalian zinc transporters: nutritional and physiologic regulation. Annual review of nutrition 29:153-176.

Lindemann, M.,Cornelius, S.,El Kandelgy, S.,Moser, R. and Pettigrew, J. 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. Journal of animal science 62:1298-1307.

Liu, L.,Guo, Y.,Tu, Y.,Zhang, N.,Bai, Z.,Chadwick, D.,Dou, Z. and Ma, L. 2020. A higher water-soluble phosphorus supplement in pig diet improves the whole system phosphorus use efficiency. Journal of Cleaner Production 272:122586.

Liu, S.,Liao, X.,Lu, L.,Li, S.,Wang, L.,Zhang, L.,Jiang, Y. and Luo, X. J. P. s. 2016. Dietary non-phytate phosphorus requirement of broilers fed a conventional corn-soybean meal diet from 1 to 21 d of age. 96:151-159.

Liu, Y.,Huang, J.,Hou, Y.,Zhu, H.,Zhao, S.,Ding, B.,Yin, Y.,Yi, G.,Shi, J. and Fan, W. 2008. Dietary arginine supplementation alleviates intestinal mucosal disruption induced by Escherichia coli lipopolysaccharide in weaned pigs. British Journal of Nutrition 100:552-560.

Liu, Y.,Ma, Y.,Zhao, J.,Vazquez-Añón, M. and Stein, H.-H. 2014. Digestibility and retention of zinc, copper, manganese, iron, calcium, and phosphorus in pigs fed diets containing inorganic or organic minerals. Journal of animal science 92:3407-3415.

Liuzzi, J. P.,Lichten, L. A.,Rivera, S.,Blanchard, R. K.,Aydemir, T. B.,Knutson, M. D.,Ganz, T. and Cousins, R. J. 2005. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proceedings of the National Academy of Sciences 102:6843-6848.

Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods 25:402-408.

Lonnerdal, B. 2000. Dietary factors influencing zinc absorption. The Journal of nutrition 130:1378S-1383S.

Lowe, J. A.,Wiseman, J. and Cole, D. 1994. Absorption and retention of zinc when administered as an amino-acid chelate in the dog. The Journal of nutrition 124:2572S-2574S.

Lu, H.,Shin, S.,Kuehn, I.,Bedford, M.,Rodehutscord, M.,Adeola, O. and Ajuwon, K. M. 2020. Effect of phytase on nutrient digestibility and expression of intestinal tight junction and nutrient transporter genes in pigs. Journal of Animal Science 98:skaa206.

Luecke, R.,Hoefer, J.,Brammell, W. and Schmidt, D. 1957. Calcium and zinc in parakeratosis of swine. Journal of Animal Science 16:3-11.

Lv, M.,Fu, X.,Hu, L.,Yue, X. and Han, X. 2016. The expression of zinc transporters changed in the intestine of weaned pigs exposed to zinc chitosan chelate. Biological trace element research 174:328-334.

Ma, Y.,Huang, Q.,Lv, M.,Wu, Z.,Xie, Z.,Han, X. and Wang, Y. 2014. Chitosan-Zn chelate increases antioxidant enzyme activity and improves immune function in weaned piglets. Biological trace element research 158:45-50.

Malavolta, M.,Piacenza, F.,Basso, A.,Giacconi, R.,Costarelli, L. and Mocchegiani, E. 2015. Serum copper to zinc ratio: Relationship with aging and health status. Mechanisms of ageing and development 151:93-100.

Mao, X.,Kim, B.-E.,Wang, F.,Eide, D. J. and Petris, M. J. 2007. A histidine-rich cluster mediates the ubiquitination and degradation of the human zinc transporter, hZIP4, and protects against zinc cytotoxicity. Journal of Biological Chemistry 282:6992-7000.

Maret, W. 2000. The function of zinc metallothionein: a link between cellular zinc and redox state. The Journal of nutrition 130:1455S-1458S.

Marques, A. T.,Nordio, L.,Lecchi, C.,Grilli, G.,Giudice, C. and Ceciliani, F. 2017. Widespread extrahepatic expression of acute-phase proteins in healthy chicken (Gallus gallus) tissues. Veterinary immunology and immunopathology 190:10-17.

Martin, A. B.,Aydemir, T. B.,Guthrie, G. J.,Samuelson, D. A.,Chang, S.-M. and Cousins, R. J. 2013. Gastric and colonic zinc transporter ZIP11 (Slc39a11) in mice responds to dietary zinc and exhibits nuclear localization. The Journal of nutrition 143:1882-1888.

Martínez, M. M.,Hill, G. M.,Link, J. E.,Raney, N. E.,Tempelman, R. J. and Ernst, C. W. 2004. Pharmacological zinc and phytase supplementation enhance metallothionein mRNA abundance and protein concentration in newly weaned pigs. The Journal of nutrition 134:538-544.

Masters, B. A.,Quaife, C. J.,Erickson, J. C.,Kelly, E. J.,Froelick, G. J.,Zambrowicz, B. P.,Brinster, R. L. and Palmiter, R. D. 1994. Metallothionein III is expressed in neurons that sequester zinc in synaptic vesicles. Journal of Neuroscience 14:5844-5857.

Mayo, K. E.,Godfrey, P. A.,Suhr, S. T.,Kulik, D. J. and Rahal, J. O. 1995. Growth hormone-releasing hormone: synthesis and signaling Proceedings of the 1993 Laurentian Hormone Conference, Elsevier: 35-73.

Mayorga, E. J.,Kvidera, S. K.,Horst, E. A.,Al-Qaisi, M.,Dickson, M. J.,Seibert, J. T.,Lei, S.,Keating, A. F.,Ross, J. W.,Rhoads, R. P.,Rambo, Z. J.,Wilson, M. E. and Baumgard, L. H. 2018. Effects of zinc amino acid complex on biomarkers of gut integrity and metabolism during and following heat stress or feed restriction in pigs1. Journal of Animal Science 96:4173-4185. doi:10.1093/jas/sky293.

Maywald, M.,Wessels, I. and Rink, L. 2017. Zinc signals and immunity. International journal of molecular sciences 18:2222.

McCall, K. A.,Huang, C.-c. and Fierke, C. A. 2000. Function and mechanism of zinc metalloenzymes. The Journal of nutrition 130:1437S-1446S.

McLeese, J.,Tremblay, M.,Patience, J. and Christison, G. 1992. Water intake patterns in the weanling pig: effect of water quality, antibiotics and probiotics. Animal Science 54:135-142.

Meyer, T.,Lindemann, M.,Cromwell, G.,Monegue, H. and Inocencio, N. 2002. Effects of pharmacological levels of zinc as zinc oxide on fecal zinc and mineral excretion in weanling pigs. The Professional Animal Scientist 18:162-168.

Miller, E.,Luecke, R.,Ullrey, D.,Baltzer, B. V.,Bradley, B. and Hoefer, J. 1968. Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. The Journal of nutrition 95:278-286.

Milligan, B. N.,Fraser, D. and Kramer, D. L. 2002. Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weight gain, and variation in weaning weights. Livestock Production Science 76:181-191.

Miyake, K. 2006. Invited review: Roles for accessory molecules in microbial recognition by Toll-like receptors. Journal of endotoxin research 12:195-204.

Mocchegiani, E.,Verbanac, D.,Santarelli, L.,Tibaldi, A.,Muzzioli, M.,Radosevic-Stasic, B. and Milin, C. 1997. Zinc and metallothioneins on cellular immune effectiveness during liver regeneration in young and old mice. Life sciences 61:1125-1145.

Mortensen, R. F. and Zhong, W. 2000. Regulation of phagocytic leukocyte activities by C-reactive protein. Journal of leukocyte biology 67:495-500.

Moya, S. L.,Boyle, L.,Lynch, P. and Arkins, S. J. A. S. 2006. Pro-inflammatory cytokine and acute phase protein responses to low-dose lipopolysaccharide (LPS) challenge in pigs. 82:527-534.

Murakami, M. and Hirano, T. 2008. Intracellular zinc homeostasis and zinc signaling. Cancer science 99:1515-1522.

Murata, H.,Shimada, N. and Yoshioka, M. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. The Veterinary Journal 168:28-40.

Myers, S. A.,Nield, A. and Myers, M. 2012. Zinc transporters, mechanisms of action and therapeutic utility: implications for type 2 diabetes mellitus. Journal of nutrition and metabolism 2012.

Nellans, H. 1991. Mechanisms of peptide and protein absorption:(1) Paracellular intestinal transport: Modulation of absorption. Adv Drug Delivery Rev 7:339-364.

Ng, P. M.,Jin, Z.,Tan, S. S.,Ho, B. and Ding, J. L. 2004. C-reactive protein: a predominant LPS-binding acute phase protein responsive to Pseudomonas infection. Journal of endotoxin research 10:163-174.

Nielsen, F. H. 2012. History of zinc in agriculture. Advances in Nutrition 3:783-789.

Nitrayova, S.,Windisch, W.,Von Heimendahl, E.,Müller, A. and Bartelt, J. 2012. Bioavailability of zinc from different sources in pigs. Journal of animal science 90:185-187.

Nordgreen, J.,Munsterhjelm, C.,Aae, F.,Popova, A.,Boysen, P.,Ranheim, B.,Heinonen, M.,Raszplewicz, J.,Piepponen, P. and Lervik, A. 2018. The effect of lipopolysaccharide (LPS) on inflammatory markers in blood and brain and on behavior in individually-housed pigs. Physiology & behavior 195:98-111.

Novick, D.,Engelmann, H.,Wallach, D. and Rubinstein, M. 1989. Soluble cytokine receptors are present in normal human urine. The Journal of experimental medicine 170:1409-1414.

NRC. 2012. Nutrient requirements of swine. 11th ed. Washington (DC): The National Academies Press.

Ogiso, T.,Moriyama, K.,Sasaki, S.,Ishimura, Y. and Minato, A. 1974. Inhibitory effect of high dietary zinc on copper absorption in rats. Chemical and Pharmaceutical Bulletin 22:55-60.

Ognik, K.,Stępniowska, A.,Cholewińska, E. and Kozłowski, K. 2016. The effect of administration of copper nanoparticles to chickens in drinking water on estimated intestinal absorption of iron, zinc, and calcium. Poultry science 95:2045-2051.

Ohashi, W.,Hara, T.,Takagishi, T.,Hase, K. and Fukada, T. 2019. Maintenance of Intestinal epithelial homeostasis by zinc transporters. Digestive diseases and sciences:1-12.

Ojo, B. A.,O'Hara, C.,Wu, L.,El-Rassi, G. D.,Ritchey, J. W.,Chowanadisai, W.,Lin, D.,Smith, B. J. and Lucas, E. A. 2019. Wheat Germ Supplementation Increases Lactobacillaceae and Promotes an Anti-inflammatory Gut Milieu in C57BL/6 Mice Fed a High-Fat, High-Sucrose Diet. The Journal of nutrition.

Outten, C. E. and O'Halloran, T. V. 2001. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. Science 292:2488-2492.

Paradkar, P. N.,Blum, P. S.,Berhow, M. A.,Baumann, H. and Kuo, S.-M. 2004. Dietary isoflavones suppress endotoxin-induced inflammatory reaction in liver and intestine. Cancer letters 215:21-28.

Paulicks, B. R.,Ingenkamp, H. and Eder, K. 2011. Bioavailability of two organic forms of zinc in comparison to zinc sulphate for weaning pigs fed a diet composed mainly of wheat, barley and soybean meal. Archives of animal nutrition 65:320-328.

Paulk, C.,Burnett, D.,Tokach, M.,Nelssen, J.,Dritz, S. S.,DeRouchey, J.,Goodband, R.,Hill, G.,Haydon, K. and Gonzalez, J. 2015. Effect of added zinc in diets with ractopamine hydrochloride on growth performance, carcass characteristics, and ileal mucosal inflammation mRNA expression of finishing pigs. Journal of animal science 93:185-196.

Pei, X.,Xiao, Z.,Liu, L.,Wang, G.,Tao, W.,Wang, M.,Zou, J. and Leng, D. 2019. Effects of dietary zinc oxide nanoparticles supplementation on growth performance, zinc status, intestinal morphology, microflora population, and immune response in weaned pigs. Journal of the Science of Food and Agriculture 99:1366-1374.

Pérez, V.,Waguespack, A.,Bidner, T.,Southern, L.,Fakler, T.,Ward, T.,Steidinger, M. and Pettigrew, J. 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiota of pigs after weaning. Journal of animal science 89:414-425.

Petersen, H. H.,Nielsen, J. P. and Heegaard, P. M. H. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary research 35:163-187.

Peterson, D. G.,Scrimgeour, A. G.,McClung, J. P. and Koutsos, E. A. 2008. Moderate zinc restriction affects intestinal health and immune function in lipopolysaccharide-challenged mice. The Journal of nutritional biochemistry 19:193-199.

Philcox, J. C.,Coyle, P.,Michalska, A.,Choo, K. and Rofe, A. 1995. Endotoxin-induced inflammation does not cause hepatic zinc accumulation in mice lacking metallothionein gene expression. Biochemical journal 308:543-546.

Philcox, J. C.,Sturkenboom, M.,Coyle, P. and Rofe, A. M. 2000. Metallothionein in mice reduces intestinal zinc loss during acute endotoxin inflammation, but not during starvation or dietary zinc restriction. The Journal of nutrition 130:1901-1909.

Phillips, N. J.,Schilling, B.,McLendon, M. K.,Apicella, M. A. and Gibson, B. W. 2004. Novel modification of lipid A of Francisella tularensis. Infection and immunity 72:5340-5348.

Pischon, N.,Darbois, L. M.,Palamakumbura, A. H.,Kessler, E. and Trackman, P. C. 2004. Regulation of collagen deposition and lysyl oxidase by tumor necrosis factor-α in osteoblasts. Journal of Biological Chemistry 279:30060-30065.

Pluske, J. R.,Hampson, D. J. and Williams, I. H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock production science 51:215-236.

Pluske, J. R.,Pethick, D. W.,Hopwood, D. E. and Hampson, D. J. 2002. Nutritional influences on some major enteric bacterial diseases of pig. Nutrition research reviews 15:333-371.

Pobezinskaya, Y. L. and Liu, Z. 2012. The role of TRADD in death receptor signaling. Cell Cycle 11:871-876.

Pond, W.,Jones, J. and Kroening, G. 1964. Effect of level of dietary zinc and source and level of corn on performance and incidence of parakeratosis in weanling pigs. Journal of Animal Science 23:16-20.

Poonsuk, K. and Zimmerman, J. J. A. h. r. r. 2018. Historical and contemporary aspects of maternal immunity in swine. 19:31-45.

Poulsen, H. D. 1995. Zinc oxide for weanling piglets. Acta Agriculturae Scandinavica A-Animal Sciences 45:159-167.

Poulsen, H. D. and Larsen, T. 1995. Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. Livestock Production Science 43:235-242.

Prasad, A. S. 2014. Impact of the discovery of human zinc deficiency on health. Journal of trace elements in medicine and biology 28:357-363.

Puls, R. 1988. Mineral levels in animal health. Diagnostic data, Sherpa International.

Puppe, B.,Langbein, J.,Bauer, J. and Hoy, S. 2008. A comparative view on social hierarchy formation at different stages of pig production using sociometric measures. Livestock science 113:155-162.

Quaife, C. J.,Findley, S. D.,Erickson, J. C.,Froelick, G. J.,Kelly, E. J.,Zambrowicz, B. P. and Palmiter, R. D. 1994. Induction of a new metallothionein isoform (MT-IV) occurs during differentiation of stratified squamous epithelia. Biochemistry 33:7250-7259.

Ramsay, T.,Stoll, M.,Conde-Aguilera, J. A. and Caperna, T. 2013. Peripheral tumor necrosis factor α regulation of adipose tissue metabolism and adipokine gene expression in neonatal pigs. Veterinary research communications 37:1-10.

Reiterer, G.,Toborek, M. and Hennig, B. 2004. Peroxisome proliferator activated receptors α and γ require zinc for their anti-inflammatory properties in porcine vascular endothelial cells. The Journal of nutrition 134:1711-1715.

Rink, L. and Haase, H. 2007. Zinc homeostasis and immunity. Trends in immunology 28:1-4.

Roberts, E.,Heugten, E. v.,Lloyd, K.,Almond, G. and Spears, J. 2002. Dietary zinc effects on growth performance and immune response of endotoxemic growing pigs. Asian-australasian journal of animal sciences 15:1496-1501.

Robinson, M. W.,Harmon, C. and O'Farrelly, C. 2016. Liver immunology and its role in inflammation and homeostasis. Cellular & molecular immunology 13:267-276.

Rofe, A. M.,Philcox, J. C. and Coyle, P. 1996. Trace metal, acute phase and metabolic response to endotoxin in metallothionein-null mice. Biochemical Journal 314:793-797.

Romanovsky, A. A.,Ivanov, A. I.,Lenczowski, M. J.,Kulchitsky, V. A.,Van Dam, A.-M.,Poole, S.,Homer, L. D. and Tilders, F. J. 2000. Lipopolysaccharide transport from the peritoneal cavity to the blood: is it controlled by the vagus nerve? Autonomic Neuroscience 85:133-140.

Rose-John, S. 2012. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. International journal of biological sciences 8:1237.

Rose-John, S. and Heinrich, P. C. 1994. Soluble receptors for cytokines and growth factors: generation and biological function. Biochemical Journal 300:281-290.

Roselli, M.,Finamore, A.,Britti, M. S.,Bosi, P.,Oswald, I. and Mengheri, E. 2005. Alternatives to in-feed antibiotics in pigs: Evaluation of probiotics, zinc or organic acids as protective agents for the intestinal mucosa. A comparison of in vitro and in vivo results. Animal Research 54:203-218.

Rozen, S. and Skaletsky, H. 2000. Primer3 on the WWW for general users and for biologist programmers Bioinformatics methods and protocols, Springer: 365-386.

Rutledge, H. R.,Jiang, W.,Yang, J.,Warg, L. A.,Schwartz, D. A.,Pisetsky, D. S. and Yang, I. V. 2012. Gene expression profiles of RAW264. 7 macrophages stimulated with preparations of LPS differing in isolation and purity. Innate immunity 18:80-88.

Sales, J. 2013. Effects of pharmacological concentrations of dietary zinc oxide on growth of post-weaning pigs: a meta-analysis. Biological trace element research 152:343-349.

Sandström, B. and Cederblad, A. 1980. Zinc absorption from composite meals II. Influence of the main protein source. The American journal of clinical nutrition 33:1778-1783.

Sandström, B.,Cederblad, Å.,Lindblad, B. S. and Lönnerdal, B. 1994. Acrodermatitis enteropathica, zinc metabolism, copper status, and immune function. Archives of pediatrics & adolescent medicine 148:980-985.

Sandström, B. and Lönnerdal, B. 1989. Promoters and antagonists of zinc absorption Zinc in human biology, Springer: 57-78.

Sassu, E. L.,Frömbling, J.,Duvigneau, J. C.,Miller, I.,Müllebner, A.,Gutiérrez, A. M.,Grunert, T.,Patzl, M.,Saalmüller, A. and von Altrock, A. 2016. Host-pathogen interplay at primary infection sites in pigs challenged with Actinobacillus pleuropneumoniae. BMC veterinary research 13:64.

Scheiber, I. F.,Wu, Y.,Morgan, S. E. and Zhao, N. 2019. The intestinal metal transporter ZIP14 maintains systemic manganese homeostasis. Journal of Biological Chemistry 294:9147-9160.

Schell, T. and Kornegay, E. 1996. Zinc concentration in tissues and performance of weanling pigs fed pharmacological levels of zinc from ZnO, Zn-methionine, Zn-lysine, or ZnSO4. Journal of animal science 74:1584-1593.

Schroeder, J. J. and Cousins, R. J. 1990. Interleukin 6 regulates metallothionein gene expression and zinc metabolism in hepatocyte monolayer cultures. Proceedings of the National Academy of Sciences 87:3137-3141.

Schwedler, S. B.,Filep, J. G.,Galle, J.,Wanner, C. and Potempa, L. A. 2006. C-reactive protein: a family of proteins to regulate cardiovascular function. American journal of kidney diseases 47:212-222.

Sève, B. 2000. Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. Domestic animal endocrinology 19:63-74.

Seydel, U.,Oikawa, M.,Fukase, K.,Kusumoto, S. and Brandenburg, K. 2000. Intrinsic conformation of lipid A is responsible for agonistic and antagonistic activity. European Journal of Biochemistry 267:3032-3039.

Shannon, M. C. and Hill, G. M. 2019. Trace mineral supplementation for the intestinal health of young monogastric animals. Frontiers in Veterinary Science 6:73.

Shelton, N.,Jacob, M.,Tokach, M. D.,DeRouchey, J. M.,Amachawadi, R.,Shi, X.,Nagaraja, T. G.,Nelssen, J. L.,Goodband, R. D. and Dritz, S. S. 2009. Effects of copper sulfate, zinc oxide, and neoterramycin on weanling pig growth and antibiotic resistance rate for fecal Escherichia coli.

Shimazu, R.,Akashi, S.,Ogata, H.,Nagai, Y.,Fukudome, K.,Miyake, K. and Kimoto, M. 1999. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. The Journal of experimental medicine 189:1777-1782.

Silipo, A.,Leone, M. R.,Lanzetta, R.,Parrilli, M.,Lackner, G.,Busch, B.,Hertweck, C. and Molinaro, A. 2012. Structural characterization of two lipopolysaccharide O-antigens produced by the endofungal bacterium Burkholderia sp. HKI-402 (B4). Carbohydrate research 347:95-98.

Silipo, A. and Molinaro, A. 2010. The diversity of the core oligosaccharide in lipopolysaccharides Endotoxins: Structure, Function and Recognition, Springer: 69-99.

Skovgaard, K.,Mortensen, S.,Poulsen, K. T.,Angen, Ø. and Heegaard, P. M. 2007. Validation of putative reference genes for qRT-PCR normalization in tissues and blood from pigs infected with Actinobacillus pleuropneumoniae. Veterinary Immunology and Immunopathology 118:140-146.

Smith, J.,Tokach, M.,Goodband, R.,Nelssen, J. and Richert, B. 1997. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. Journal of Animal Science 75:1861-1866.

Solomons, N. W. 2013. Update on zinc biology. Annals of Nutrition and Metabolism 62:8-17.

Song, Z. H.,Xiao, K.,Ke, Y. L.,Jiao, L. F. and Hu, C. H. 2015. Zinc oxide influences mitogen-activated protein kinase and TGF-β1 signaling pathways, and enhances intestinal barrier integrity in weaned pigs. Innate Immunity 21:341-348.

Sorensen, N.,Tegtmeier, C.,Andresen, L. O.,Pineiro, M.,Toussaint, M.,Campbell, F. M.,Lampreave, F. and Heegaard, P. M. 2006. The porcine acute phase protein response to acute clinical and subclinical experimental infection with Streptococcus suis. Veterinary immunology and immunopathology 113:157-168.

Stahl, N.,Boulton, T. G.,Farruggella, T.,Ip, N. Y.,Davis, S.,Witthuhn, B. A.,Quelle, F. W.,Silvennoinen, O.,Barbieri, G. and Pellegrini, S. 1994. Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. Science 263:92-95.

Steidinger, M.,Goodband, R.,Tokach, M.,Nelssen, J.,Dritz, S.,Borg, B. and Campbell, J. 2002. Effects of providing a water-soluble globulin in drinking water and diet complexity on growth performance of weanling pigs. Journal of animal science 80:3065-3072.

Steimle, A.,Autenrieth, I. B. and Frick, J.-S. 2016. Structure and function: Lipid A modifications in commensals and pathogens. International Journal of Medical Microbiology 306:290-301.

Stevenson, J. and Earle, I. 1956. Studies on parakeratosis in swine. Journal of Animal Science 15:1036-1045.

Strathe, A. B.,Danfær, A. and Chwalibog, A. 2008. A dynamic model of digestion and absorption in pigs. Animal feed science and technology 143:328-371.

Stuart, G. W.,Searle, P. F.,Chen, H. Y.,Brinster, R. L. and Palmiter, R. D. 1984. A 12-base-pair DNA motif that is repeated several times in metallothionein gene promoters confers metal regulation to a heterologous gene. Proceedings of the National Academy of Sciences 81:7318-7322.

Sturniolo, G.,Montino, M.,Rossetto, L.,Martin, A.,D'inca, R.,D'odorico, A. and Naccarato, R. 1991. Inhibition of gastric acid secretion reduces zinc absorption in man. Journal of the American College of Nutrition 10:372-375.

Sullivan, J. F.,Burch, R. E.,Quigley, J. and Magee, D. F. 1974. Zinc deficiency and decreased pancreatic secretory response. American Journal of Physiology-Legacy Content 227:105-108.

Sun, Y. and Kim, S. W. 2017. Intestinal challenge with enterotoxigenic Escherichia coli in pigs, and nutritional intervention to prevent postweaning diarrhea. Animal Nutrition 3:322-330.

Terenina, E.,Sautron, V.,Ydier, C.,Bazovkina, D.,Sevin-Pujol, A.,Gress, L.,Lippi, Y.,Naylies, C.,Billon, Y. and Liaubet, L. 2017. Time course study of the response to LPS targeting the pig immune gene networks. BMC genomics 18:988.

Thompson, D.,Pepys, M. B. and Wood, S. P. 1999. The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure 7:169-177.

Tilg, H.,Dinarello, C. A. and Mier, J. W. 1997. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunology today 18:428-432.

Tillett, W. S. and Francis Jr, T. 1930. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. The Journal of experimental medicine 52:561.

Tisdale, M. J. 2009. Mechanisms of cancer cachexia. Physiological reviews 89:381-410.

Töllner, B.,Roth, J.,Störr, B.,Martin, D.,Voigt, K. and Zeisberger, E. 2000. The role of tumor necrosis factor (TNF) in the febrile and metabolic responses of rats to intraperitoneal injection of a high dose of lipopolysaccharide. Pflügers Archiv 440:925-932.

Toussaint, M.,Van Ederen, A. and Gruys, E. 1995. Implication of clinical pathology in assessment of animal health and in animal production and meat inspection. Comparative Haematology International 5:149-157.

Uhlar, C. M. and Whitehead, A. S. 1999. Serum amyloid A, the major vertebrate acute-phase reactant. European journal of biochemistry 265:501-523.

Ulevitch, R. J. and Tobias, P. S. 1999. Recognition of gram-negative bacteria and endotoxin by the innate immune system. Current opinion in immunology 11:19-22.

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

Vallee, B. L. and Falchuk, K. H. 1993. The biochemical basis of zinc physiology. Physiological reviews 73:79-118.

Van Campen, D. R. 1969. Copper interference with the intestinal absorption of zinc-65 by rats. The Journal of Nutrition 97:104-108.

Van Heugten, E.,Spears, J. and Coffey, M. 1994. The effect of dietary protein on performance and immune response in weanling pigs subjected to an inflammatory challenge. Journal of Animal Science 72:2661-2669.

Vats, P. and Banerjee, U. C. 2004. Production studies and catalytic properties of phytases (myo-inositolhexakisphosphate phosphohydrolases): an overview. Enzyme and Microbial Technology 35:3-14.

Viriyakosol, S.,Tobias, P. S.,Kitchens, R. L. and Kirkland, T. N. 2001. MD-2 binds to bacterial lipopolysaccharide. Journal of Biological Chemistry 276:38044-38051.

Volanakis, J. E. 2001. Human C-reactive protein: expression, structure, and function. Molecular immunology 38:189-197.

Vreugdenhil, A. C.,Dentener, M. A.,Snoek, A. P.,Greve, J.-W. M. and Buurman, W. A. 1999. Lipopolysaccharide binding protein and serum amyloid A secretion by human intestinal epithelial cells during the acute phase response. The Journal of Immunology 163:2792-2798.

Wajant, H.,Pfizenmaier, K. and Scheurich, P. 2003. Tumor necrosis factor signaling. Cell Death & Differentiation 10:45-65.

Wamnes, S.,Lewis, N. and Berry, R. 2008. The behaviour of early-weaned piglets following transport: Effect of season and weaning weight. Canadian Journal of Animal Science 88:357-367.

Wang, B.,Zhang, Y.,Mao, Z.,Yu, D. and Gao, C. 2014. Toxicity of ZnO nanoparticles to macrophages due to cell uptake and intracellular release of zinc ions. Journal of nanoscience and nanotechnology 14:5688-5696.

Wang, X. and Quinn, P. J. 2010. Lipopolysaccharide: biosynthetic pathway and structure modification. Progress in lipid research 49:97-107.

Wang, X. and Zhou, B. 2010. Dietary zinc absorption: a play of Zips and ZnTs in the gut. IUBMB life 62:176-182.

Wapnir, R. A. and Balkman, C. 1991. Inhibition of copper absorption by zinc. Biological trace element research 29:193-202.

Wastney, M. E.,McDonald, C. M. and King, J. C. 2018. A dynamic model for predicting growth in zinc-deficient stunted infants given supplemental zinc. The American Journal of Clinical Nutrition 107:808-816.

Webel, D.,Finck, B.,Baker, D. and Johnson, R. W. 1997. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. Journal of animal science 75:1514-1520.

Wedekind, K.,Lewis, A.,Giesemann, M. and Miller, P. S. J. J. o. A. S. 1994. Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. 72:2681-2689.

Wegenka, U. M.,Buschmann, J.,Lütticken, C.,Heinrich, P. C. and Horn, F. 1993. Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. Molecular and cellular biology 13:276-288.

Wellinghausen, N.,Kirchner, H. and Rink, L. 1997. The immunobiology of zinc. Immunology today 18:519.

Wessels, I. and Cousins, R. J. 2015. Zinc dyshomeostasis during polymicrobial sepsis in mice involves zinc transporter Zip14 and can be overcome by zinc supplementation. American Journal of Physiology-Gastrointestinal and Liver Physiology 309:G768-G778.

Wilcock, P. and Walk, C. 2016. Low phytate nutrition–what is the pig and poultry industry doing to counter dietary phytate as an antinutrient and how is it being applied, Wageningen Academic Publishers The Netherlands.

Wilfart, A.,Montagne, L.,Simmins, H.,Noblet, J. and van Milgen, J. 2007. Digesta transit in different segments of the gastrointestinal tract of pigs as affected by insoluble fibre supplied by wheat bran. British Journal of Nutrition 98:54-62.

Williams, P.,Collier, C.,Carroll, J.,Welsh Jr, T. and Laurenz, J. 2009. Temporal pattern and effect of sex on lipopolysaccharide-induced stress hormone and cytokine response in pigs. Domestic Animal Endocrinology 37:139-147.

Williams, R. 1987. The biochemistry of zinc. Polyhedron 6:61-69.

Wong, C. P.,Rinaldi, N. A. and Ho, E. 2015. Zinc deficiency enhanced inflammatory response by increasing immune cell activation and inducing IL6 promoter demethylation. Molecular nutrition & food research 59:991-999.

Wyns, H.,Plessers, E.,De Backer, P.,Meyer, E.,Croubels, S. J. V. i. and immunopathology. 2015. In vivo porcine lipopolysaccharide inflammation models to study immunomodulation of drugs. 166:58-69.

Xia, D. and Samols, D. 1997. Transgenic mice expressing rabbit C-reactive protein are resistant to endotoxemia. Proceedings of the National Academy of Sciences 94:2575-2580.

Yamasaki, S.,Sakata-Sogawa, K.,Hasegawa, A.,Suzuki, T.,Kabu, K.,Sato, E.,Kurosaki, T.,Yamashita, S.,Tokunaga, M. and Nishida, K. 2007. Zinc is a novel intracellular second messenger. The Journal of cell biology 177:637-645.

Yin, C.,Liu, W.,Liu, Z.,Huang, Y.,Ci, L.,Zhao, R. and Yang, X. 2017. Identification of potential serum biomarkers in pigs at early stage after Lipopolysaccharide injection. Research in veterinary science 111:140-146.

Zähringer, U.,Lindner, B. and Rietschel, E. T. 1994. Molecular structure of lipid A, the endotoxic center of bacterial lipopolysaccharides. Advances in carbohydrate chemistry and biochemistry 50:211.

Zanoni, I.,Ostuni, R.,Marek, L. R.,Barresi, S.,Barbalat, R.,Barton, G. M.,Granucci, F. and Kagan, J. C. 2011. CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. Cell 147:868-880.

Zhang, Y.,Ward, T. L.,Ji, F.,Peng, C.,Zhu, L.,Gong, L. and Dong, B. 2018. Effects of zinc sources and levels of zinc amino acid complex on growth performance, hematological and biochemical parameters in weanling pigs. Asian-Australasian journal of animal sciences 31:1267.

Zhang, Z. and Fuller, G. M. 2000. Interleukin 1β inhibits interleukin 6–mediated rat γ fibrinogen gene expression. Blood, The Journal of the American Society of Hematology 96:3466-3472.

Zhao, N.,Gao, J.,Enns, C. A. and Knutson, M. D. 2010. ZRT/IRT-like protein 14 (ZIP14) promotes the cellular assimilation of iron from transferrin. Journal of Biological Chemistry 285:32141-32150.

Zhou, X.,Li, Y.,Li, Z.,Cao, Y.,Wang, F. and Li, C. 2017. Effect of dietary zinc on morphological characteristics and apoptosis related gene expression in the small intestine of Bama miniature pigs. Acta Histochemica 119:235-243.

Zhu, C.,Lv, H.,Chen, Z.,Wang, L.,Wu, X.,Chen, Z.,Zhang, W.,Liang, R. and Jiang, Z. 2017. Dietary zinc oxide modulates antioxidant capacity, small intestine development, and jejunal gene expression in weaned piglets. Biological trace element research 175:331-338.

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