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The Effects of Zinc Supplementation from Inorganic and Organic Sources in the Diets of Weanling Pigs

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Suzanne L. Hoover

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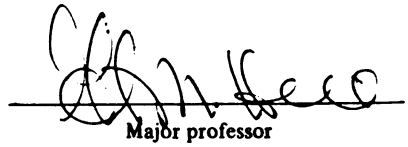

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THE EFFECTS OF ZINC SUPPLEMENTATION FROM INORGANIC AND ORGANIC SOURCES IN THE DIETS OF WEANLING PIGS

By

Suzanne Lynne Hoover

A THESIS

Submitted to
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ABSTRACT

THE EFFECTS OF ZINC SUPPLEMENTATION FROM INORGANIC AND ORGANIC SOURCES IN THE DIETS OF WEANLING PIGS

By

Suzanne L. Hoover

Three experiments were conducted with weanling pigs to determine the efficacy of zinc (Zn) supplementation from different forms during the nursery phase. Growth performance, plasma and tissue accumulation of Zn and Zn balance were evaluated. Supplementation of pharmacological concentrations of Zn in diets of nursery pigs did not result in improved growth performance. Pigs fed high Zn oxide (ZnO) had greater accumulation of Zn in plasma, liver, kidney, bone, urine and feces in all experiments ($P<.05$). In Exp. 2, pigs fed the control diet were in a positive Zn balance and pigs consuming diets containing Zn methionine complex, Zn amino acid complex and ZnO were in a negative Zn balance after 15 d of supplementation. Results of these studies indicate that nursery diets containing supplemental Zn at or above 500 ppm and fed for greater than two weeks results in excretion of Zn in excess of daily Zn consumption.

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LIST OF ABBREVIATIONS

ADFI	average daily feed intake
ADG	average daily gain
d	day
DM	dry matter
E. coli	*Escherichia coli*
et al.	et alia
G:F	gain to feed ratio
min	minute
MT	metallothionein
SEM	standard error of the mean
TGE	transmissible gastroenteritis
Tris	tris(hydroxymethyl)aminomethane
ZnAA	zinc amino acid complex
ZnMet	zinc methionine complex
ZnO	zinc oxide
$ZnSO_4$	zinc sulfate

INTRODUCTION:

Throughout the past decade the push toward increased production, as well as confinement, has led to heightened concerns about the nutritional status of livestock. Since the animals do not come in contact with soil or pasture, there is a greater potential of becoming deficient in one or more minerals (Pond, 1985). However, a mineral deficiency may be hard to discern. According to McDowell (1992), "Mineral disorders can vary from acute deficiency with clinical signs that are highly recognizable and pathological change, to mild conditions that are hard to diagnose because of the signs being decreases in performance."

Since, all living matter including feeds and body tissues is made up of minerals, meeting the animal's requirement is critical to maximize growth. The importance of minerals has been characterized by Underwood (1981), as stable components of tissues and organs, constituents of fluids and tissues in the body, and within enzyme and hormone systems, minerals act as catalyst. The skeleton consists of 80-85% of the total body mineral matter, with calcium comprising 46%, 29% phosphorus and together potassium, sulfur, sodium, chlorine, and magnesium contribute 25%, and .3% is made up of trace minerals.

Even though essential trace elements only contribute .3% of the total body mineral, deficiency or excess of any mineral can lead to harmful effects. Therefore, a greater concern on diet formulation with respect to minerals needs to be emphasized. One such mineral is zinc (Zn). Although characterized as a trace mineral, Zn has been used for thousands of years for practical purposes, such as an ointment for skin lesions, as well as for decorative and industrial purposes. Zn is an essential trace element that is required for numerous biological functions in all species of livestock throughout all stages of the life cycle. The essentialness of Zn for livestock was first reported in 1955 by Tucker and Salmon, as it was shown to prevent parakeratosis in swine, a characteristic of Zn deficiency disease.

Numerous other detrimental effects have been reported during Zn deficiency, therefore the inclusion of this trace element in the diets of livestock is a common practice and has been for decades. In recent years the inclusion of supplemental Zn above the requirement has been studied with results quite variable. Several reports have shown that the use of pharmacological or high concentrations of Zn as zinc oxide (> 2000 ppm) in the diets of nursery pigs improved growth (Hahn and Baker, 1993; Hill et al., 1996). On the contrary, Fryer (1992) reported no significant improvement in growth performance with the inclusion of high concentrations of Zn.

Initial reports from Europe linked this response to the suppression of *Escherichia coli* (*E. coli*) proliferation and believed that in herds where

postweaning scours were a problem, supplementation of Zn would be efficacious. Poulsen (1995) reported a decrease in the incidence of non-specific postweaning scours when pigs were fed pharmacological concentrations of ZnO for the first two weeks of the nursery phase. However, the effect that Zn has on pigs inoculated with *E. coli* has not been evaluated. At this physiological stage, diarrhea in the weanling pig is increased because of the stress of weaning and the loss of maternal immunity which increases the pig's susceptibility to infectious bacteria (Sarmiento et al., 1988). This infectious diarrhea results in substantial economic losses due to mortality, decreased growth rate, and costs for treatment. Due to the suggested growth promoting effects and suppression of diarrhea, an increasing number of commercial nursery diets now contain high concentrations of ZnO.

However, at these high concentrations, the amount of Zn excreted should be evaluated and the impact on the environment questioned since excess Zn in feces which in turn used as fertilizer could lead to soil accumulation and the possibility of plant toxicity. Ward et al. (1996) reported that the additional growth response in the nursery phase due to high ZnO supplementation could also be achieved with the supplementation of an organic form of Zn at a lower concentration. Numerous studies have evaluated the significance of organic Zn versus inorganic forms, and the results are inconclusive. Therefore, our objectives were (1) to determine the efficacy of dietary Zn additions from inorganic and organic forms of Zn on the growth performance of nursery pigs,

(2) assess the excretion and retention of different forms of Zn at the concentrations commonly used in the commercial industry, and (3) determine the effect of supplemental Zn from inorganic and organic forms on the health and growth of weanling pigs inoculated with *E. coli*.

LITERATURE REVIEW

ZINC ABSORPTION:

Zn is absolutely essential for growth and development and to maintain cellular functions. The means by which regulation of body Zn occurs are still unclear and the regulatory roles that Zn absorption and excretion play within Zn homeostasis are debatable. The mechanism by which Zn absorption occurs has not been fully elicited and several theories exist. According to numerous studies with the laboratory rat, Zn can be absorbed throughout the total length of the small intestine. Various techniques in animal studies have yielded discrepant results as to which region of the small intestine has the maximum absorptive capacity. It has been postulated that the major site of absorption might shift with Zn status. Conclusive evidence has been provided that intestinal Zn absorption varies in response to an altered Zn supply status of the body (Flanagan et al., 1983; Johnson et al., 1988). It has been regularly displayed that inverted sacs from Zn deficient rats absorbed a greater amount of Zn than Zn supplemented rats. The intestine itself has been suggested to be the major site for regulation. Under normal conditions, absorption of Zn is far below the limit of saturation. Three different stages of absorption occur and each step (alone and in conjunction with the other steps) has been suggested to be the regulation point:

1) uptake from the lumen by cells lining the small intestine, 2) transport of Zn through cells, and 3) discharge of Zn out of basolateral membrane and into the vascular compartment (Kirchgessner and Weigand, 1983).

Gastrointestinal digestion produces both high and low molecular weight ligands and could affect Zn absorption by inhibiting or facilitating mucosal uptake. Therefore, the mucosa would appear to be the major site of regulation of the absorptive process. Several theories exist as to the mechanism by which Zn absorption is regulated. A majority of the theories suggest that metallothionein (MT) plays a key role in absorption. MT are cysteine rich proteins that bind heavy metals; their synthesis is also stimulated by these metals and other mechanisms such as stress (Andrews, 1990). MT binds several metals including copper (Cu) and Zn. Each molecule of MT binds 7 atoms of Zn(II) and 12 atoms of Cu(I). Metallothionein has a high affinity for Cu and can serve as a detoxification method. Hill et al. (1987) reported that oral Zn could be used as a therapy in Wilson's disease patients. This disease results in excessive accumulation of copper at toxic levels in the brain and liver. They concluded that oral Zn can be used as a therapy in decreasing the absorption of Cu. Zn stimulated MT and since MT preferentially binds Cu, excess Zn could be used as therapy to stimulate MT and bind Cu. Since the turnover of the MT complex is longer than that of the intestinal cells in which it is held, the bound Cu and Zn are sloughed off into the feces and excreted.

Nevertheless, with the potential of MT to bind Zn, MT has been suggested to play a role in absorption. Evans et al. (1975) reported that Zn absorption may be linked with a low molecular weight ligand as well as metal free albumin and receptor sites on the basolateral membrane. It was suggested that the Zn-metallothionein (MT) complex in the intestine had a longer life compared to turnover of villus cells and may reflect a regulatory mechanism for the disposal in feces of that Zn remaining in mucosa. The synthesis of MT in the intestine also increased with an excess amount of Zn in the diet. In 1980, Starcher et al., reported that when mice were fed diets that were adequate in Zn, that absorption was proportional to MT. On the contrary, Flanagan et al. (1983) concluded that a difference in the amount of intestinal MT from Zn deficient and adequate rats was found after five days of dietary treatment but not beyond five days. It was suggested that the deficiency state progressed, and therefore stress induced MT since differences were not seen after five days as would be expected since Zn deficiency progressively worsens with time.

Richards and Cousins (1975) reported that when rats were parentally loaded with Zn, serum concentrations initially increased and were followed by a decrease which was concomitant with a greater accumulation of Zn in the liver and also an increase in MT synthesis. From research completed by Richards and Cousins (1976), intestinal MT appeared to not be a major regulator of absorption and(or) binding component at all times. They suggested that when Zn status was elevated by dietary or parental administration that MT synthesis

was enhanced. Wang et al. (1993) suggested that MT plays a limited role in the regulation of Zn absorption. When weanling pigs were fed high and adequate Zn diets similar kinetic parameters were found and that the decrease in Zn absorption when pigs were fed high Zn diets may be due to increased mucosal passive diffusion of Zn rather than mediation of transport by a carrier. In 1979, Cousins proposed a mechanism of absorption shown in Figure 1. He concluded that,

"A portion of the dietary Zn which enters the lumen of the small intestine is transported across the mucosal brush border membrane. This transport process probably requires ATP. The Zn content of the intestinal cells which can be changed by Zn status may influence the amount of Zn that is transported via a carrier which is common to many divalent cations or uses a unique Zn transport system. Isotope experiments have shown that with the intestinal cells, newly acquired cytoplasmic Zn equilibriates with a Zn pool and is either shunted into high molecular weight proteins and MT or transferred to the plasma. The intracellular binding phase probably depends on rates of protein synthesis and degradation and availability of binding sites. In this regard MT may act as the expandable (inducible) intracellular Zn binding compartment."

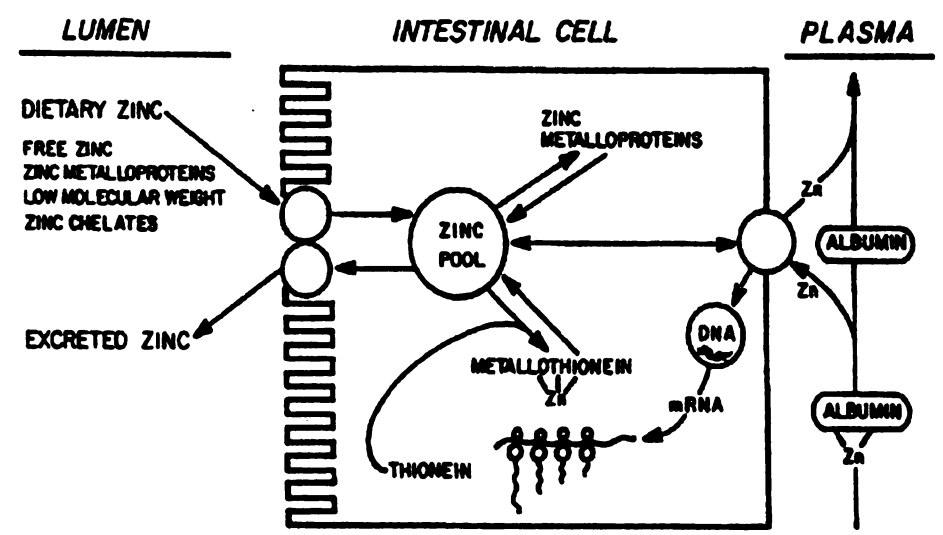

Figure 1. Regulatory pathway of dietary zinc processing by intestinal cells (Cousins, 1979).

These studies demonstrate that intestinal Zn absorption can respond rather quickly to fluctuations in Zn supply and intestinal MT may serve as a temporary storage protein for Zn. From MT, Zn may be passed through the vascular compartment or returned to the lumen of the intestine. Therefore, the intestinal wall may serve as the regulator of absorption and maintaining Zn homeostasis.

ZINC EXCRETION:

Zinc is excreted primarily in one of three ways: surface of the skin, kidney and the gastrointestinal tract. A majority of Zn is excreted in feces. Pekas (1966) reported that pigs injected intravenously with labeled Zn excreted 75-90% in feces. Unabsorbed Zn is the primary component of fecal Zn; however, endogenous sources also contribute a small amount. As stated previously, Zn absorption varies depending on Zn status and intake. Excretion also follows a similar pattern in that as Zn status increases, a greater amount of Zn is excreted. Johnson et al. (1988) reported that in the rat, the current diet affects Zn absorption but, Zn of endogenous origin is not only affected by the current but also by the past Zn intake.

Although endogenous Zn contributes a minor amount, it may play a role in Zn homeostasis. Zinc accumulation in the lumen of the small intestine occurs from several sources. Within the enterocytes, a bidirectional flux of Zn may

occur and therefore deposit Zn back into the lumen. The pancreas and gall bladder also secrete Zn into the lumen. As stated previously, the low-molecular weight protein, MT, is synthesized via stimulation from excess Zn and has a slower turnover rate than the enterocytes within which the complex is held. Therefore, a large concentration of Zn is deposited into the lumen via sloughing of the epithelial cells. Weigand and Kirchgessner (1976) and Evans et al. (1979) suggested that Zn homeostasis may be regulated by the amount that is endogenously secreted back into the lumen and excreted in feces. Their conclusions were derived from the isotope dilution technique which determined the amount of Zn in the lumen that is of endogenous origin. It has also been suggested that Zn secretions in the lumen from endogenous origin, could impact absorption if intensive mixing of unabsorbed and endogenously secreted Zn occurs.

Excretion of Zn can also occur in urine. This will reflect dietary treatment and is thought to be from the plasma. Losses of Zn from body surfaces also occur and are primarily in sweat (Forbes, 1983). Milne et al. (1983) reported that in humans, the amount of Zn lost or excreted due to perspiration is directly correlated with dietary intake. However, Zn loss can also occur due to sloughing of skin and via hair, and in seminal fluid (Underwood, 1977).

ORGANIC FORMS OF ZINC:

In recent years much interest has developed in the area of organic trace minerals. Several early varieties of organic metals have been studied extensively such as EDTA chelates, proteinates, and metal polysaccharides. However, metals complexed with amino acids are the focus of the current discussion. The Association of American Feed Control Officials (AAFCO) has provided definitions to distinguish the differences in organic forms of trace minerals and are as follows:

> 57.151 Metal (Specific Amino Acid) Complex. The product resulting from complexing a soluble metal salt with a specific amino acid.
>
> 57.150 Metal Amino Acid Complex. The product resulting from the complexing of a soluble metal salt with an amino acid(s).
>
> 57.142 Metal Amino Acid Chelate. The product resulting from the reaction of a metal ion from a soluble metal salt with amino acids with a mole ratio of one mole of metal to one to three (preferable two) moles of amino acid to form coordinate covalent bonds. The total molecular weight of the hydrolyzed chelate must not exceed 800.
>
> 57.23 Metal Proteinate. The product resulting from the chelation of a soluble salt with amino acids and/or partially hydrolyzed protein.
>
> 57.29 Metal Polysaccharide Complex. The product resulting form complexing of a soluble salt with a polysaccharide solution declared as an ingredient as the specific metal complex.

Numerous studies have evaluated the significance of organic Zn forms compared to inorganic, but the results are inconclusive. These studies span across a wide range of species including poultry, dogs, cattle, sheep and swine.

Zinc is routinely added to the diets of animals, but the forms in which this mineral is typically added are zinc oxide and zinc sulfate, which are inorganic forms. In recent years there has been an increase in knowledge about the importance of Zn in the diet and the added benefits of high concentrations to weanling pigs. It has also led to heightened interest in complexed forms of Zn. Several studies conducted have suggested that bioavailability of Zn is increased from organic forms. Therefore the inclusion of these products has become more prevalent in commercial premixes for all species of livestock. Several theories have been suggested with regards to organic forms and the mechanism by which they are absorbed or metabolized. However, conclusive evidence has not been supplied to elicit the answers.

Bioavailability

The most widely used theory for the use of organic Zn forms is increased bioavailability of Zn from organic forms. There are major discrepancies in the literature with regards to the definition of bioavailability. Webster (1986) defines bioavailability as the rate at which a drug, trace element, etc. enters the bloodstream and is circulated to specific organs and tissues. However, O'Dell (1984) suggests that the term bioavailability of a trace element refers to that fraction of the element in a food that is absorbed and utilized such as the process of transport, cellular assimilation and conversion to an active form.

As stated previously, research with organic forms of Zn spans across many species. Pimental et al. (1991) completed two experiments with chicks and they concluded that the bioavailability of Zn from Zn methionine and Zn oxide were similar. These results were supported by a study done with isolated rat duodenal segments (Hemp and Cousins, 1989) where Zn methionine and Zn oxide were absorbed equally. However, several studies conducted using swine as the animal model, concluded that Zn oxide was the least available form of Zn and that the intermediates were Zn methionine and Zn lysine. In these studies, Zn sulfate proved to be the most available form of Zn when using parameters such as bone, plasma and numerous other tissues that accumulate Zn (Schell and Kornegay, 1996; Wedekind et al., 1994). These findings were also substantiated by experiments in other species. Nockels et al. (1993) concluded that feeding calves diets containing Zn sulfate or Zn methionine resulted in similar accumulation of Zn in plasma and tissues and therefore indicating that Zn was not more available from either source. No differences were reported by Spears (1989) when lambs and heifers were fed Zn oxide and Zn methionine in their diets when using plasma Zn and plasma alkaline phosphatase activity as availability parameters. However, a lower excretion of Zn in urine was reported for lambs fed Zn methionine. It was speculated that the difference in methionine content of inorganic versus organic Zn forms could be the cause. On the contrary, Rojas et al. (1995) concluded that lambs fed diets supplemented with Zn in the forms of Zn lysine, Zn methionine, Zn sulfate or Zn oxide had

increased accumulation of Zn in the liver, kidney and pancreas when fed Zn lysine. In addition, these lambs fed Zn lysine, had increased metallothionein concentrations in liver, kidney and pancreas compared to lambs fed the other dietary treatments. However, Carlson et al. (1997) reported that a difference was seen in the concentration of MT that was stimulated from organic and inorganic forms of Zn. Organic forms of Zn fed to weanling pigs were found to depress stimulation of MT in the intestine, compared to weanling pigs fed the control diet (Zn sulfate) or high Zn oxide supplemented diet.

Growth Performance

Growth performance of animals fed diets supplemented with inorganic versus organic Zn forms is as inconclusive as the information presented previously on bioavailability. In a study conducted with hens and their progeny, performance parameters were not different when fed diets with either Zn oxide of Zn methionine (Kidd et al., 1992).

Several studies have shown that the addition of high concentrations of Zn oxide can improve growth performance of weanling pigs (Hill et al., 1996; Hahn and Baker, 1993). Although, the improvement in performance has been variable, the supplementation of high Zn in nursery diets has become a common practice. Many of the studies with organic forms of Zn have suggested improved bioavailability, therefore supplementation of these products has been tested to

determine if the enhanced performance from inorganic Zn could also be achieved with supplementation of an organic form of Zn at a lower concentration. Ward et al. (1996) reported that supplementing nursery pig diets with Zn in the form of Zn methionine at 250 ppm enhanced growth performance equal to that of 2000 ppm Zn oxide. These results indicate that if the bioavailability of Zn from organic products is greater than Zn oxide than a lower concentration can be fed and the improvement in performance can still be achieved. On the contrary, Schell and Kornegay (1996) reported no improvement in growth performance from dietary additions of organic or inorganic forms of Zn in the diets of weanling pigs at three different concentrations (1000, 2000, or 3000 mg/kg of diet). The addition of pharmacological concentrations of Zn oxide in the diets of nursery pigs improved feed intake and weight gain; however, the inclusion of Zn methionine or Zn lysine did not enhance performance above those fed the control diet, when fed at the same concentrations as Zn oxide (Hahn and Baker, 1993). The growth performance of heifers fed Zn methionine was not enhanced over that of heifers fed dietary treatments supplemented with inorganic Zn forms (Spears, 1989).

In addition to performance, the quality of the products from livestock supplemented with organic products has been evaluated. Kienholz et al. (1992) reported that the inclusion of Zn methionine at one g/kg of diet to laying hens during a low calcium stress, maintained egg size and produced more eggs ($P < .05$) during the stress period compared to hens fed no supplemental Zn

methionine or Zn methionine addition of two g/kg of diet. Shell quality was also evaluated and the results suggest that the addition of Zn methionine in the diet of laying hens receiving sodium chloride in the water at two g/L helped overcome the negative effects of the sodium chloride and improved shell quality (Moreng et al., 1992). Carcass characteristic for steers were improved with the addition of an organic forms of Zn in the diet. More specifically, Greene et al. (1988) reported that steers fed Zn methionine diets had a higher quality grade and greater amount of marbling. Although average daily gain and feed efficiency were not affected by treatment, the increase in the previously mentioned carcass parameters could prove to be beneficial economically.

Immune Response

In addition to the previous suggested benefits of organic Zn forms, it has also been reported that these products may enhance immune response. Kidd et al. (1992) reported that when chicks were fed Zn methionine in the diet, antibody titers were significantly increased in chicks that had been challenged with salmonella pullonem antigen. The addition of Zn methionine in the diets of turkey poults helped clear a strain of *E. coli* that had been administered to the poults (Kidd et al., 1994). Similar improvements in immune response have also been reported in cattle. Antibody titers against *bovine herpesvirus-1* increased in steers fed supplemental Zn methionine compared to control steers however, in *parainfluenza* the antibody titers were not increased (Spears et al., 1991).

Steers challenged with *infectious bovine rhino tracheitis virus* and consuming diets with Zn methionine had lower mean rectal temperatures and mean daily dry matter intakes returned to pretrial levels sooner than control fed steers (Chirase et al., 1991).

LINK WITH *ESCHERICHIA COLI*:

Mortality and reduced efficiency due to disease are costly to the swine industry in achieving maximum production efficiency in the competitive U.S. and world market. The area where great opportunity to reduce costs for treatment is in post-weaning scours. In the North-Central region of the United State, Crooks et al. (1993) reported that the increased profit potential due to a 10% reduction in scours would be $39 to $434 depending on herd size. Hurley et al. (1995) reported that the incidence of scours in pigs is significantly greater in the Midwest than the Southeast region. These losses cost Midwestern producers $2.05 per pig compared to $0.62 in the Southeast. Relative to the weanling pig, 16.11% of the pigs up to 40 pounds, show signs of scouring.

The growth response associated with pharmacological concentration of Zn in the diets of nursery pigs maybe linked to the suppression of *E. coli*. Poulsen (1995) reported a decrease in the incidence of non-specific scours when pigs were fed pharmacological concentrations of zinc oxide for the first two weeks for the nursery phase. It was reported by Kidd et al. (1994) that turkey poults administered E. coli and fed a Zn methionine supplemented diet, cleared

the strain at a faster rate than control or inorganic supplemented diets.

As noted by Holland (1990), bacteria cause diarrhea by (1) producing enterotoxins that influence the crypt cells to hyper secrete, (2) eliciting an inflammatory response from the invasion of the intestinal mucosa and (3) destroying the absorptive capacity of the villi resulting in malabsorption. *Escherichia coli* is a gram-negative bacteria and is an anaerobic, non-spore forming member of the *Enterobacteriaceae* family. It causes a profuse and watery diarrhea with dehydration of up to 10 to 12% loss of body weight in a six hour period.

The importance of *enterotoxigenic E. coli* as the cause of diarrhea among young farm animals is well recognized. *Escherichia coli* is a common inhabitant of the intestinal tract of humans and animals, but some strains cause infections and diarrhea in young farm animals. This bacteria may act alone or in conjunction with other bacteria such as rotavirus (Holland, 1990).

The family of *Enterobacteriaceae* is made up of a series of interrelated bacterial types. Each member of the family has an endotoxin which is a lipopolysaccharide complex in the outer membrane and contains an O-specific serologic group. Additionally, some members, especially *E. coli*, produce enterotoxins. *Escherichia coli* produce two enterotoxins a heat-labile and a heat-stable. The family is further divided into subgroups on the basis of serology of the O antigens of the bacteria. The O antigen subgroups are divided into serotype, which are characterized by the possession of particular O antigen

fractions, K antigens or H antigens. The K antigen denotes polysaccharide capsular antigens. The K antigen is further divided into three different types according to physical characteristics: L, A, and B (Sojka, 1965). According to Holland (1990) and Wilson (1986) the most common cause of diarrhea in pigs is from the *enterotoxigenic E. coli* K88 strain, which has been reported to cause diarrhea in pigs pre and postweaning. Sarmiento et al. (1988) reported a reproducible model of postweaning scours caused by *enterotoxigenic E. coli* utilizing antigen K88.

The pathogenesis of the *E. coli* is initiated by the adherence of the bacteria to the epithelia of the small intestine. The colonization is mediated by appendages called pili or fimbriae that adhere to the intestinal mucosa. More specifically, on the tip of the fimbriae is a protein, called adhesin, which is the major factor in adherence to the intestine. Some animals may be genetically resistant to some strains of *E. coli*. In order for the bacteria to colonize the intestine of animal species, the villous epithelium must have receptors for the fimbriae (Nagy et al., 1992). If the receptor is not present on the epithelial cell, the animal will be resistant to that strain of *E. coli*. The presence of the receptor is genetically mediated. Age-related resistance to other strains of *E. coli* such as 987P+ strains have been reported by Dean-Nystrom and Samuel (1994). Neonatal pigs less than six days of age were susceptible to colonization of the small intestine by the 987 strain but, weaned pigs were resistant. Katouli et al. (1995) reported phenotypical characteristics of intestinal *E. coli* of pigs during

suckling, postweaning and finishing periods. They concluded that the colonization and persistence of *E. coli* in pigs is dependent on both the strain and host specificity.

Weaning has been suggested to reduce resistance to infection because of associated circumstances such as stress. These factors include the loss of maternal contact, introduction into strange pens and pen-mates, and the loss of maternal immunity from milk. Hampson (1986) reported that weaned pigs, in contrast to unweaned pigs, experienced alterations in intestinal architecture after weaning. Changes included dramatic increases in crypt depth and an increase in the complexity of villus morphology with a large reduction in villus height. He concluded that due to the changes in morphology primarily with the absorptive area of the small intestine, susceptibility of piglets to infection or bacterial invasion may be increased. Opportunistic bacteria are given a chance to invade since the pigs immune system may be compromised due to these factors. Consequently, infectious diarrhea may occur and result in substantial economic losses due to mortality, decreased growth rate and cost for treatment. However, Sarmiento et al. (1988) reported that weaning itself is not required to induce diarrhea but may increase the severity of the scouring.

THE EFFECTS OF ZINC SUPPLEMENTATION FROM INORGANIC AND ORGANIC SOURCES IN THE DIETS OF WEANLING PIGS

ABSTRACT

Three experiments were conducted with weanling pigs to determine the efficacy of zinc (Zn) supplementation from different forms of Zn during the nursery phase. In Exp. 1, 172 PIC pigs were weaned (average 19 d and 6.47 kg) and allotted to dietary treatments for a total of 33 d: basal (control), basal + 250 ppm zinc methionine complex (ZnMet), basal + 500 ppm zinc oxide (ZnO), basal + 250 ppm zinc amino acid complex (ZnAA), basal + 500 ppm ZnAA, basal + 250 ppm ZnAA + 500 ppm ZnO, and basal + 3000 ppm ZnO. In Exp. 2, 12 PIC barrows (average 19 d and 6.68 kg) were allotted to a dietary treatment: basal (control), basal + 250 ppm ZnMet, basal + 250 ppm ZnAA, and basal + 3000 ppm ZnO. Pigs were housed in individual stainless steel metabolism cages for a 20 d trial. Feces, orts and urine were collected for 5 d following a 15 d adjustment period. In Exp. 3, 72 crossbred pigs were weaned (average 16 d and 4.86 kg) and allotted to a 28 d trial with a 3 x 2 factorial arrangement of treatments. Each dietary treatment was represented by three replicates of *Escherichia coli* (*E. coli*) inoculated and three sham inoculated pigs. Dietary

treatments were: basal (control), basal + 250 ppm ZnAA, and basal + 3000 ppm ZnO. Blood samples were obtained on d 0 and 33 of Exp. 1 and d 0 and 20 of Exp. 2. On d 33 of Exp. 1, two pigs per replicate (n=70), on d 20 of Exp. 2 and d 28 of Exp. 3, all pigs were killed and tissues collected. Supplementation of pharmacological concentrations of Zn in the diets of nursery pigs did not result in improved growth performance in Exp. 1 or 3. Pigs fed ZnO, had greater accumulation of Zn in plasma, liver, kidney, bone, urine and feces in all experiments (P<.05). In Exp. 2, pigs fed the control diet were in a positive Zn balance, and pigs consuming diets containing ZnMet, ZnAA and ZnO were in a negative Zn balance. Results of these studies indicate that nursery diets containing supplemental Zn at or above 500 ppm and fed for greater than two weeks results in excretion of Zn in excess of daily Zn consumption.

Key words: Pigs, Zinc, Tissues, Plasma

INTRODUCTION

The use of pharmacological concentrations of Zn as ZnO in the diets of nursery pigs has been reported to have growth promoting properties (Hahn and Baker, 1993; Hill et al., 1996). Initial reports from Europe linked this response to the suppression of *E. coli* proliferation. Pigs at weaning have an increased

susceptibility to diarrhea due to opportunistic infection during stress (Sarmiento et al., 1988). This infectious diarrhea results in substantial economic losses due to mortality, decreased growth rate, and costs for treatment. Poulsen (1995) reported a decrease in the incidence of non-specific post-weaning scours when pigs were fed pharmacological concentrations of ZnO for the first two weeks of the nursery phase.

Due to the suggested growth promoting effects and suppression of diarrhea, nursery diets now commonly contain high concentrations of ZnO. However, at these high concentrations, the amount of Zn excreted should be evaluated and the impact on the environment questioned. Ward et al. (1996) reported that the additional growth response in the nursery phase due to high ZnO supplementation could also be achieved with the supplementation of an organic form of Zn at a lower concentration. Numerous studies have evaluated the significance of organic Zn versus inorganic forms and the results are inconclusive. Therefore, our objectives were (1) to determine the efficacy of dietary Zn additions from inorganic and organic forms of Zn on the growth performance of nursery pigs, (2) assess the excretion and retention of different forms of Zn at the concentrations commonly used in nursery diets today, and (3) determine the effect of supplemental Zn from inorganic and organic forms on the health and growth of weanling pigs inoculated with *E. coli*.

MATERIALS AND METHODS

All experiments were approved by the Michigan State University Animal Care and Use Committee prior to initiation of the study. Exp. 1 and 2 were completed at Consolidated Nutrition Research Center, Decatur, IN., and Exp. 3 was completed at Michigan State University.

Experiment 1. One hundred sixty-seven pigs of Pig Improvement Company (PIC) genetics (Line 326 X C22) were weaned at 19 d of age and 6.47 kg, and used in a randomized complete block experiment. Pigs were allotted to one of seven dietary treatments: basal (control), basal + 250 ppm Zn as ZnMet, basal + 500 ppm Zn as ZnO, basal + 250 ppm Zn as ZnAA, basal + 500 ppm Zn as ZnAA, basal + 250 ppm Zn as ZnAA + 500 ppm Zn as ZnO, and basal + 3000 ppm Zn as ZnO. Phase 1 diets were fed from d 0-14, with the basal diet containing 250 ppm calculated Zn as zinc sulfate ($ZnSO_4$), and 100 ppm calculated copper (Cu) as copper lysine and 25 ppm Cu as copper sulfate ($CuSO_4$). Phase 2 diets were fed from d 14-33, and the basal diet contained 160 ppm calculated Zn as $ZnSO_4$ and 50 ppm calculated Cu as copper lysine and 16 ppm Cu as $CuSO_4$. All diets (Table 1 and Table 2) met or exceeded NRC nutrient recommendations (NRC, 1988) for weanling pigs. Four or five pigs were allotted by sex and litter to a dietary treatment with five weight replicates. Pigs were weighed on d 0, 14, and 33.

Experiment 2. Twelve PIC barrows (Line 326 x C22) were weaned (19 d of age and 6.68 kg) and allotted by litter to one of four dietary treatments as follows: basal (control), basal + 250 ppm Zn as ZnMet, basal + 250 Zn as ZnAA, and basal + 3000 ppm Zn as ZnO. All diets (Table 1) met or exceeded NRC nutrient recommendations for weanling pigs (NRC, 1988). Basal diet was the same as the phase 1 basal diet in Exp. 1. Pigs were assigned to individual stainless steel pens equipped with stainless steel feeders and waters in a temperature controlled room. For the first 14 d, pigs were given ad libitum access to feed and distilled water. On d 15, pigs were fed 90% of daily ad libitum intake divided into three feedings per day. This regime was followed for the next 5 d and weigh backs were recorded as necessary. Feces, orts and urine were collected for five, 24 h periods. Pigs were weighed daily for the first 15 d and every other day thereafter.

Experiment 3. Seventy-two crossbred pigs (Duroc x (Yorkshire x Landrace)), having received no creep feed prior to weaning, were weaned at an average age of 16 d and average weight of 4.86 kg and allotted by sex and litter to a 28 d trial with a 3 x 2 factorial arrangement of treatments. Six separate temperature controlled rooms were used with three pens per room and four pigs per pen with ad libitum access to feed and water. Each dietary treatment was represented by three replicates of *E. coli* inoculated and sham inoculated pigs. The dietary treatments across the two health status were as follows: basal (control), basal + 250 ppm Zn as ZnAA, and basal + 3000 ppm Zn as ZnO.

Phase 1 (d 0-14) and phase 2 (d 14-28) basal diets were the same as those in Exp. 1. All diets (Table 1 and Table 2) were pelleted and met or exceeded NRC nutrient recommendations (NRC, 1988). Individual pigs were weighed on d 0, 7, 14, 21, and 28 post-weaning and feed disappearance was recorded.

At weaning, three rooms of pigs received an oral dose of 5 mL of enterotoxigenic *Escherichia coli* (serotype 0149:k88 + non motile) inoculum (1.3 x 10^{-10} CFU/mL), in peptone saline. A sham of peptone saline was orally administered to pigs in the remaining three rooms. On d 5, pigs that had previously received *E. coli* were given another 5 mL of *E. coli* inoculum (3.2 x 10^{-10} CFU/mL). On d 7, one pig per replicate group (n=18) was killed. The remaining pigs (n=54) continued on test for 21 d. Pigs were observed daily for evidence of diarrhea. Scour scoring was done by an individual who was blind to the study protocol. Rooms inoculated with sham were scored first and then rooms inoculated with *E. coli* to avoid cross-contamination. The index used for scoring was as follows: 1=no loose stools in pen, 2=one-half of the stools in pen are loose, 3=all stools in pen are loose, 4=one-half of stools in pen are watery and one-half loose, and 5=all stools are very watery.

Plasma. Blood samples for all pigs were taken in Exp. 1 on d 0 and 32 and in Exp. 2 on d 0 and 20, from the anterior vena cava into heparinized vacutainer tubes and placed on ice. Plasma was separated by centrifugation at 3000 x g for 15 min at 5°C and stored at -80°C until analyzed for Cu, Fe, and Zn. Mineral concentrations in plasma were determined by flame atomic absorption

spectrophotometry (Smith-Hieftje 4000, Thermo Jarrell Ash Corporation, Franklin, MA) after a 1:7 dilution with deionized distilled water.

Tissues. In Exp. 1, d 33, two pigs per replicate and on d 20 of Exp. 2, all 12 pigs, were killed by electric-immobilization and exsanguination. Fifty-four pigs in Exp. 3, were killed by injecting penibarbitol (1 mL/4.5 kg of BW) into the jugular vein. From pigs in Exp. 3, the liver and several segments of the intestine were removed. Further description of methods used for obtaining intestinal segments is listed in Appendix A. Liver, left kidney and right front foot were removed from all pigs in Exp. 1 and 2 and a portion of the longissimus muscle from pigs in Exp. 2. Samples were immediately weighed and then placed on ice. Dry matter of liver and kidney were determined separately by drying in vacuum oven (Heinicke, Portland, OR). Frozen metacarpal bones were cleaned of all adhering tissue and thawed before being weighed and dry ashed in muffle oven (Barnstead/Thermolyne, Dubuque, IA). Bone ash was weighed and digested with 30 mL of 10 M nitric acid. Longissimus muscle samples were freeze dried (Vitris 25 SRC, The Vitris Company, Gardiner, NY) for dry matter determination and then ground in a stainless steel blender. Tissue samples were prepared for mineral analysis by nitric-perchloric acid wet digestion (AOAC, 1990) modified using 3 mL of 10 M perchloric acid and 20 mL of 10 M nitric acid. Mineral concentrations were determined by flame atomic absorption spectrophotometry.

Urine, Feces, and Feed. In Exp. 2, urine was collected twice every 24 h period, pooled, mixed and two aliquots were frozen at -80°C for later mineral

analysis. Feces were collected twice every 24 h period, weighed and also stored at -80°C. Fecal samples were freeze dried for determination of dry matter content then ground in stainless steel blender. Feces and feed samples were prepared for mineral analysis according to nitric-perchloric acid wet digestion, using 3 mL of 10 M perchloric acid and 20 mL of 10 M nitric acid. Mineral concentration of feed, feces and urine were determined by flame atomic absorption spectrophotometry.

Statistical Analysis. Data for the three experiments were analyzed using GLM procedures and least squares of means by treatment (SAS, 1995). Some of the data were log transformed to increase normal distribution after detection of heterogeneity of variance problem using a modified Levene's test. The mean differences between the treatments were detected by comparison of least square means. Differences were considered significant at $P < .05$. Several preplanned comparisons were used when analyzing data from Exp. 1 and they are as follows: ZnMet vs. ZnAA, 250 ZnAA, 500 ZnO, 250 ZnAA + 500 ZnO effect and a linear and quadratic effect for the ZnAA dietary treatment.

RESULTS

Growth Performance. The effects of source and concentration of supplemental Zn on average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (GF) are summarized in Table 3. From d 0 to 14 in Exp. 1,

ADG of pigs fed the diet with 3000 ppm supplemental Zn as ZnO was lower ($P < .05$) than that of pigs fed the diets supplemented with Zn at 500 ppm ZnO, 250 ppm Zn as ZnAA, or 250 ppm Zn as ZnAA + 500 ppm Zn as ZnO. Similar results were achieved for ADFI with those pigs fed 3000 ppm Zn as ZnO, consuming less feed per day during phase 1. Overall, pigs fed diets containing an additional 500 ppm Zn as ZnO tended to gain faster and consume more feed than those fed the other diets from d 0 to 14. Due to the restricted feed intake of pigs in Exp. 2 for the 5 d collection period, the weight gains and feed consumption of these pigs are not discussed however, growth performance data are presented in Table 3. All growth performance parameters measured in Exp. 3 (Table 4) were not statistically different between dietary treatments or across the two health statuses ($P > .05$).

Plasma. The effects of source and concentration of supplemental Zn on plasma minerals of pigs in Exp. 1 and 2 are listed in Table 5. In Exp. 1, plasma Zn concentration was greater ($P < .05$) from pigs fed the 3000 ppm ZnO. Plasma Zn concentration of pigs fed 250 ZnAA + 500 ZnO diet was not different than the plasma Zn concentration of pigs fed 250 ZnMet, 500 ppm ZnO, 250 ZnAA, or 500 ZnAA. The lowest plasma Cu concentrations were seen when pigs were fed the control diet or 500 ppm ZnO but not 3000 ppm ZnO which would be expected.

In Exp. 2 there were no differences in plasma Zn between pigs fed the control diet or diets containing ZnMet or ZnAA even though, the concentration of

Zn in the organic supplemented diets was calculated to contain 250 ppm more Zn than in the control diet. However, pigs fed the ZnO diet had greater concentrations of Zn in plasma on d 20 (P=0.0001). Plasma Cu and Fe concentrations were not different between dietary treatments (P > .05).

Organ weights . In Exp. 1 (data not shown), the kidney weight as a percentage of body weight was increased (P < .05) in pigs fed 250 ZnAA compared to those fed control diet, 250 ZnMet, or 250 ZnAA + 500 ZnO diet. There was also a ZnMet versus ZnAA , 250 ZnAA + 500 ZnO effect, and ZnAA quadratic effect (P<.05), for kidney weight as a percentage of body weight.

Tissues. The effects of source and concentration of supplemental Zn on the Zn, Cu and Fe concentrations of tissues are presented in Table 6, 7, and 8. The percent dry matters of all tissues analyzed in Exp. 1, 2, and 3 were not statistically different between dietary treatments. Hepatic and renal Zn concentrations were greater from pigs fed the 3000 ZnO diet than pigs fed all other dietary treatments (P = .0001). Liver Cu was not different between dietary treatments (P > .05) however, renal Cu concentration was increased when pigs were fed 3000 ppm supplemental ZnO. Hepatic and renal Fe were elevated from pigs fed the 250 ZnAA diet. Pigs fed 250 ZnAA had greater renal Fe concentrations compared to control, 250 ZnMet, 500 ZnO, 250 ZnAA + 500 ZnO pigs at P<.05.

Zn concentrations in the metacarpal bone (Table 6) followed the same pattern as plasma, liver and kidney Zn, in that the pigs fed the high Zn oxide

(3000 ppm) retained more Zn in their metacarpal bone than pigs fed all other dietary treatments (P<.0001). Bone Cu was not different due to dietary treatment however, bone Fe was decreased in the bones of pigs consuming the control diet compared to pigs fed 3000 ZnO (P<.05).

In Exp. 2, liver Zn (Table 7) was greater in those pigs fed ZnO than those consuming the control diets or diets containing either of the organic Zn products (P=0.0001). Zn concentrations in the liver were not significantly different between pigs fed diets containing control, ZnMet or ZnAA. The concentrations of hepatic Cu and Fe were not different between dietary treatments. Renal Zn and Cu were greater in those pigs fed ZnO however no differences in kidney Fe were seen. No significant differences in Cu, Fe or Zn concentrations in muscle were seen between dietary treatments. Pigs fed ZnMet appear to be depositing a greater amount of Zn in muscle tissue compared to pigs fed the other dietary treatments however, they are only numerically and not different. There was a trend for greater Cu concentration in muscle when pigs were fed ZnMet although not significant. The Fe and Cu concentrations in bone were not different between dietary treatments. Pigs fed the diet containing ZnO, retained a greater amount of Zn in bone than pigs fed control, ZnMet, or ZnAA diets, (P=0.0001).

In Exp. 3, (Table 8) the hepatic Zn, Cu and Fe concentrations were not different between pigs fed the control or ZnAA supplemented diets with or without E. coli or sham inoculation. However, liver Zn was elevated (P < .05)in ZnO fed pigs (E. coli and sham inoculated), compared to control and ZnAA fed

pigs. E. coli inoculated pigs fed ZnO had decreased Zn concentration in liver compared to sham inoculated pigs within the same dietary treatment.

Scour Scoring. In Exp. 3 (data not shown), scores were assigned to each pen to determine the effect of Zn supplementation on scouring. The most prominent differences in scour scores were seen from d 4 to d 7. On d 4, pigs that were inoculated with E. coli and were consuming the ZnAA and ZnO supplemented diets had looser stools than pigs fed the control , ZnAA and ZnO supplemented diets and inoculated with sham ($P < .05$). Similar results were seen on d 5 however, pigs fed the control diet and inoculated with E. coli had looser stools than control sham inoculated pigs ($P < .05$). On d 6, again E. coli inoculated pigs had looser stools across all dietary treatments than pigs given sham inoculation. Pigs receiving the ZnAA supplemented diet and inoculated with the sham had looser stools than control sham inoculated pigs ($P < .05$). On d 7, stools followed the same pattern with E. coli inoculated pigs with looser stools however, the pigs with the firmest stools were those consuming the ZnO diet and inoculated with sham. After d 7, stools were at a similar consistency until d 19 when pigs consuming the ZnAA supplemented diet and inoculated with sham had the firmest stools. Pigs consuming the ZnO supplemented diet and inoculated with sham exhibited the firmest feces on d 21, 22, 25 and 26.

Urine and Feces Mineral Concentrations and Balance. The effects of supplemental Zn source and concentration on excretory products and balance are given in Table 9, and Figures 2, 3, and 4. As Zn increased in the diet

regardless of form, urinary and fecal Zn increased. Pigs that were fed the dietary treatment containing 3,000 ppm supplemental ZnO had increased Zn concentration in urine and feces compared to pigs fed the control diet, ZnMet or ZnAA supplemented diets ($P < .05$). Fecal Cu was not different between dietary treatments. The greatest concentration of Fe that was excreted in the feces was from pigs fed diets containing supplemental Zn in the form of ZnMet and ZnO. Pigs fed the supplemental Zn as ZnAA were excreting greater amounts of Zn and Cu in urine than pigs on control diet. Urinary Cu from pigs fed control and ZnMet was less than pigs fed ZnAA or ZnO ($P < .05$). Contrast to urinary Zn and Cu excretion from pigs fed ZnMet or ZnAA products in the diet, pigs fed ZnMet excreted a greater amount of Fe in feces compared to pigs fed ZnAA, however this was not significant (157 mg vs. 133 mg of Zn in feces from pigs fed ZnMet or ZnAA, respectively). Urinary excretion of Fe was greatest for ZnAA pigs but only different from control and ZnO fed pigs ($P<.05$).

Although, data suggest trends for differences in Zn balance (Figure 2) between dietary treatments, differences were not significant. The only pigs in positive balance were those fed the control diet. However, Zn balance was negative for all other dietary treatment meaning more Zn was excreted than taken in each day after receiving the respective diet for 15 d prior to initiation of balance. Pigs fed the control and ZnAA diets were in a positive balance for Cu (Figure 3) unlike ZnMet and ZnO fed pigs. However, Fe balance (Figure 4) was positive for pigs fed all dietary treatments.

DISCUSSION

In this study, feeding supplemental Zn in the diets of weanling pigs resulted in variable performance and are similar to findings reported by Fryer et al. (1992) and Schell and Kornegay (1996). The increased growth performance due to the supplementation of high concentrations of ZnO reported by Hahn and Baker (1993) and Hill et al. (1996) were not seen in this study. The results of Exp. 1 show a decrease in performance especially during the first two weeks of the nursery phase when pigs were fed diets containing 3000 ppm Zn as ZnO compared to other dietary treatments. Numerous studies have been completed to test the hypothesis that high concentrations of Zn in the diet of nursery pigs improves growth performance also indicating inconsistent performance.

It has been speculated that health status of the pigs plays a key role. Poulsen (1995) reported that the addition of high concentrations of ZnO in the diet of nursery pigs for two weeks reduced the incidence of diarrhea. It has been postulated that whether the pigs are suffering from post-weaning scours influences the effect of high zinc in the diet. However, in Exp. 3 pigs inoculated with *E. coli* and(or) sham did not grow differently relative to Zn concentration or form in the diet. Growth performance parameters measured were not different between dietary treatments or between pigs inoculated with sham or *E. coli*. Health status may not be the only influence in whether positive or negative

results are seen due to Zn supplementation. Factors such as age and sanitation of the facility in which the pigs are housed may have an impact. Pigs in Exp. 3 were housed in a pristine facility built specifically for research with infectious bacteria. This facility is new and the sanitation is impeccable compared to on-farm trials. The ability of additional bacteria to proliferate was minimal if not void since rooms and pens were washed and sanitized daily. Due to the strict sanitation of the facility and the inclusion of an antibiotic in the diet, results of this study may differ from on-farm trials.

Ward et al. (1996) reported that the additional growth response in the nursery phase due to high ZnO supplementation could also be achieved with the addition of zinc methionine at a lower concentration. In Exp. 1, pigs receiving diets containing 250 ppm ZnAA or 250 ppm ZnAA + 500 ppm ZnO had improved growth performance above that achieved by pigs supplemented with 3000 ppm ZnO during phase 1 ($P < .05$).

The concentration of Zn in plasma, liver, kidney, bone , urine and feces were greater ($P < .05$) from pigs fed the high ZnO diets than from pigs fed all other dietary treatments in Exp. 1, 2, and 3. It has been postulated that the importance of the chelated or complexed minerals is the increased bioavailability. While tissue storage is not the same as bioavailability, there were minimal differences in tissue Zn concentrations between dietary treatments containing no supplemental Zn or supplemental Zn in the form of ZnMet or ZnAA in Exp. 2. Results of Exp. 2, show that after the 15-d adjustment period, pigs

receiving the control diet were in a positive Zn balance, therefore indicating that they were retaining more Zn than what they were taking in on a daily basis. However, pigs receiving diets containing supplemental Zn from ZnMet, ZnAA or ZnO were excreting more Zn per day than these pigs were consuming during the third week of dietary intervention. When comparing plasma, liver, kidney and bone Zn concentrations over the three studies, it is evident that metabolism of Zn must change after several weeks of consuming a diet containing supplemental Zn above the requirement for the weanling pig. The concentration of Zn in the basal diet of pigs in Exp. 1 during phase two (d 14-33) was only calculated to be 90 ppm less than what pigs in the Exp. 2 were receiving for 20 d. However, plasma Zn concentration in pigs from Exp. 1, were almost half as much as plasma Zn on d 20 of Exp. 2. Hepatic Zn concentrations also followed this same trend. For example, from data in Table 6, 7, and 8, the hepatic Zn concentration of pigs in Exp. 2 on d 20 that were fed 3000 ZnO was 1166.22 μg/g of wet tissue but, the liver Zn concentration of pigs fed 3000 ppm ZnO in Exp. 1 on d 33 was 580.79 μg/g and in Exp. 3 the sham inoculated pigs fed ZnO had hepatic Zn concentration on d 28 of 611.35 μg/g. These differences were also seen in kidney and bone and for dietary treatments that were similar across all three studies including 250 ZnMet, 250 ZnAA and 3000 ZnO. These results indicate transitory changes in Zn concentrations in body compartments relative to age and Zn source and concentration. It has been reported that Zn absorption is affected by Zn status and that as dietary Zn increases absorption

decreases in pigs and rats (Wang et al., 1993; Jackson et al., 1981) Therefore, by the third week, the accumulation of Zn in the plasma and tissues may have reached a threshold and the ability to absorb Zn is decreased. A greater amount of dietary Zn is not absorbed but is excreted via feces. Although, not evaluated in this study, it is likely that at this time, the amount of endogenously secreted Zn may also increase. Pekas (1966) reported that fecal Zn of pigs on an adequate Zn intake included unabsorbed as well as endogenously secreted Zn. Our work demonstrates that supplementation of Zn in the diets of weanling pigs at or above 500 ppm from inorganic and organic sources of Zn during the third week of the nursery phase results in excess Zn excreted in urine and feces. The pigs consuming ZnAA, ZnMet of ZnO supplemented diets during the third week of Exp. 2 were in a negative Zn balance and therefore excreted more Zn than what they were consuming on a daily basis (Figure 2). Supplementation during this time period appears to not play a role in enhancing performance.

Carlson et al. (1996) reported that feeding pharmacological concentrations of Zn in the diets of nursery pigs for the first two weeks improved growth performance as much as four weeks of supplementation. Our results would indicate that after two weeks, excess Zn is excreted and therefore supplementation should only be for two weeks. Plasma and tissue data would also support this statement due to the apparent mobilization of Zn stores during the 3 and 4 wk of the nursery phase to possibly rid the body of excess Zn stores.

In Exp. 3, hepatic Zn concentrations of ZnO fed pigs inoculated with E.

coli were decreased compared to sham inoculated pigs which is in contrast to reports of increased hepatic Zn concentration and decreased plasma Zn in human during infection and in chicks inoculated with E. coli (Tufft et al., 1988). This was also supported by findings from Whitenack et al. (1978) that pigs fed adequate Zn diets and infected with transmissible gastroenteritis had decreased plasma Zn concentrations and increased hepatic storage of Zn. In Exp. 2, pigs consuming the high ZnO diet had decreased hepatic Cu concentrations and increased renal Cu. The results are supported by finding by Hill et al. (1983) that reported decreased liver Cu stores when high dietary Zn was fed.

The Fe and Cu concentrations in bone were not different between dietary treatments in Exp. 2. Pigs fed the diet containing ZnO, retained a greater amount of Zn in bone than pigs fed control, ZnMet, or ZnAA diets, (P=0.0001). Our data suggests that the concentration of Zn in the bone was not different between pigs fed the control, ZnMet or ZnAA diets even though diets containing organic products were formulated to contain 250 ppm more supplemental Zn than the control diet. Actually, pigs consuming diets containing organic products numerically retained less zinc in bone than the control diet, although not significant (1026.35 μg/g, 984.65 μg/g, and 969.30 μg/g of zinc in bone from pigs fed control, ZnMet, or ZnAA, respectively).

Zinc concentration in muscle tissue in Exp. 2 were not different between dietary treatments. Schell and Kornegay (1996) reported that feeding pharmacological concentrations of ZnO to nursery pigs for 2 wk resulted in lower

concentration of Zn in muscle compared to pigs fed the control diet at 105 mg/kg.

IMPLICATIONS

The results of this study show that supplementation of Zn at or above 500 ppm from inorganic and organic sources during wk 3 of the nursery phase led to excess excretion of Zn beyond amount consumed. Supplementation during the 3 wk may lead to alterations in Zn metabolism by decreasing Zn absorption and ridding body of excess Zn. However the environmental impact of this excessive Zn in waste is unknown and warrants further research.

Table 1. Composition of Phase 1 (d 0-14 for Exp. 1 & 3 and d 0-20 for Exp. 2)

Ingredient	Exp.1 & 2 (%)	Exp. 3 (%)
Edible whey	23.57	19.27
Response fines	—	7.86
Oat groats, steam rolled	21.93	—
Corn, ground	15.0	—
Fat-whey blend	10.89	8.32
Fish meal	10.0	9.0
Soybean meal 48%	5.0	—
Animal plasma	4.5	4.5
Soy concentrate, extruded	3.3	—
Calcium carbonate	.64	.29
Antibiotic[1]	.5	.50
Oat flour	.5	45.32
Dicalcium phosphate	.05	.68
L-lysine	.01	.09
Flavor	.4	.4
CS blend	2.75	2.75
Copper lysine complex	.1	.1
Microingredients[2]	.86	.92
Calculated Nutrient Analysis		
Crude protein	22.5	22.5
Fat	10.0	10.0
Fiber	1.5	.82
Calcium	1.0	1.0
Phosphorus	.8	.8

[1]Antibiotic provided 100 g chlortetracycline, 100 g sulfathiazole and 50 g penicillin per ton of feed.

[2]Microingredients include swine vitamin and trace mineral premix, vitamin A 18.18 KIU/kg, vitamin D_3 2.18 KIU/kg, vitamin E 49.99 IU/kg, vitamin K 21.99 mg/kg, menadione 7.27 mg/kg, ascorbic acid 9.76 mg/kg, biotin 0.25 mg/kg, choline 234.20 mg/kg, 3 mg/kg, niacin 63.63 mg/kg, pantothenic acid 45.45 mg/kg, pyridoxine 10 mg/kg, riboflavin 10.91 mg/kg, thiamine 10.01 mg/kg, vitamin B_{12} 39.99 mcg/kg, iodine 1.58 mg/kg, iron 250 mg/kg, manganese 39.37 mg/kg, selenium 0.3 mg/kg, zinc 250 mg/kg.

Table 2. Composition of Phase 2 (d 14-33 in Exp. 1 and d 14-28 in Exp. 3)

Ingredient	Exp. 1 (%)	Exp. 3 (%)
Corn, ground	50.05	49.83
Soybean meal, 48%	31.21	33.02
Standard wheat midds	6.74	5.18
White grease	4.0	3.67
Edible dried whey	2.74	2.74
Fish meal	1.5	1.50
Dicalcium phosphate	1.38	1.36
Calcium carbonate	.73	.97
Antibiotic[1]	.5	.5
Salt	.29	.31
L-lysine	.1	.1
Copper lysine complex	.05	.05
Flavor	.2	.2
Microingredients[2]	.58	.57
Calculated Nutrient Analysis		
Crude protein	21.18	21.5
Fat	6.3	6.0
Fiber	3.15	2.76
Calcium	.8	.9
Phosphorus	.7	.7

[1]Antibiotic provided 100 g chlortetracycline, 100 g sulfathiazole and 50 g penicillin per ton of feed.

[2]Microingredients include swine vitamin and trace mineral premix, vitamin a 11.36 KIU/kg, vitamin D_3 1.36 KIU/kg, vitamin E 50 IU/kg, vitamin K 13.75 mg/kg, menadione 4.54 mg/kg, biotin 0.1 mg/kg, folic acid 3 mg/kg, pantothenic acid 28.41mg/kg, riboflavin 6.81 mg/kg, vitamin B_{12} 24.99 mg/kg, iodine 1 mg/kg, iron 160 mg/kg, manganese 25 mg/kg, selenium 0.3 mg/kg, zinc 160 mg/kg.

Table 3. Growth performance of weanling pigs fed supplemental zinc from inorganic and organic forms (Exp. 1 and 2)

Supplemental Zn:								
Zn as ZnO	0	0	500	0	0	500	3000	
Zn as ZnAA	0	0	0	250	500	250	0	
Zn as ZnMet	0	250	0	0	0	0	0	SEM
Exp.1								
ADG, kg								
(0-14 d)	.269^{ab}	.281^{ab}	.318^{a}	.301^{a}	.268^{ab}	.301^{a}	.236^{b}	.018
(14-33 d)	.422	.456	.433	.427	.443	.415	.444	.024
(0-33 d)	.357	.382	.382	.373	.368	.366	.356	.018
ADFI, kg								
(0-14 d)	.302^{ab}	.302^{ab}	.346^{a}	.320^{a}	.303^{ab}	.331^{a}	.274^{b}	.016
(14-33 d)	.593^{ab}	.631^{ab}	.657^{a}	.587^{ab}	.611^{ab}	.555^{b}	.612^{ab}	.030
(0-33 d)	.470	.491	.521	.472	.480	.460	.469	.021
G:F								
(0-14 d)^{c}	.887^{ab}	.932^{a}	.918^{a}	.940^{a}	.885^{ab}	.904^{ab}	.855^{b}	.020
(14-33 d)	.712	.723	.658	.726	.726	.771	.724	.040
(0-33 d)	.760	.778	.734	.789	.769	.805	.757	.028
Exp. 2								
ADG, kg								
(0-20 d)	.220	.267		.291			.226	.026
ADFI, kg								
(0-20 d)	.265	.303		.342			.260	.026
G:F								
(0-20 d)	.831	.880		.853			.861	.038

[a,b]Means in the same row with uncommon superscripts differ (P < .05).
[c]Quadratic effect of ZnAA dietary treatments (P < .05).

Table 4. Growth performance of weanling pigs fed supplemental zinc and inoculated with *Escherichia coli* (Exp. 3)

Inoculation:	Sham	E.coli	Sham	E. Coli	Sham	E. Coli	
Supplemental Zn:							
Zn as ZnO	0	0	0	0	3000	3000	
Zn as ZnAA	0	0	250	250	0	0	SEM
ADG, kg							
(0-14 d)	.157	.164	.212	.163	.165	.074	.046
(14-28 d)	.493	.487	.451	.499	.531	.561	.055
(0-28 d)	.265	.267	.299	.275	.284	.240	.036
ADFI, kg							
(0-14 d)	.174	.173	.214	.191	.197	.111	.035
(14-28 d)	.607	.640	.587	.653	.628	.653	.080
(0-28 d)	.374	.388	.386	.404	.396	.361	.046
G:F							
(0-14 d)	.911	.949	.952	.855	.719	.515	.174
(14-28 d)	.820	.751	.772	.776	.892	.862	.076
(0-28 d)	.718	.686	.776	.688	.749	.657	.086

Table 5. Plasma mineral concentrations of weanling pigs fed supplemental zinc from inorganic and organic forms (Exp. 1 and 2)

Supplemental Zn:								
Zn as ZnO	0	0	500	0	0	500	3000	
Zn as ZnAA	0	0	0	250	500	250	0	
Zn as ZnMet	0	250	0	0	0	0	0	SEM
Exp. 1								
Plasma Zn, μg/ml								
Day 0	.81	.87	.79	.81	.79	.87	.74	.03
Day 33[e]	.64[bd]	.73[b]	.75[b]	.72[b]	.74[b]	.80[bc]	1.66[a]	.04
Plasma Cu, μg/ml								
Day 0	1.73	1.62	1.53	1.68	1.69	1.78	1.70	.06
Day 33[d]	1.39[bc]	1.59[a]	1.31[c]	1.50[ab]	1.48[b]	1.55[a]	1.46[b]	.05
Plasma Fe, μg/ml								
Day 0	2.44	2.14	2.71	2.35	3.09	2.45	1.88	.28
Day 33	1.65[a]	1.80[a]	1.91[a]	1.83[a]	2.05[ab]	2.46[ab]	2.96[b]	.34
Exp. 2								
Plasma Zn, μg/ml								
Day 0	.90	1.02		.81			.93	.11
Day 20	.92[b]	.96[b]		1.00[b]			3.03[a]	.08[f]
Plasma Cu, μg/ml								
Day 0	1.63	1.96		1.42			1.82	.12
Day 20	1.13	1.26		1.13			1.10	.10[f]
Plasma Fe, μg/ml								
Day 0	2.09	1.65		1.32			3.77	.82
Day 20	1.60	2.05		2.54			3.40	1.1

[a,b,c]Means in the same row with uncommon superscripts differ (P < .05).
[d]250 ZnAA dietary treatment effect (P < .05).
[e]500 ZnO dietary treatment effect (P < .05).
[f]data that was log transformed prior to analysis indicated by logSEM but actual means are presented.

Table 6. Tissue mineral concentrations of weanling pigs fed supplemental zinc from inorganic and organic forms (Exp. 1)

Supplemental Zn:								
Zn as ZnO	0	0	500	0	0	500	3000	
Zn as ZnAA	0	0	0	250	500	250	0	
Zn as ZnMet	0	250	0	0	0	0	0	SEM
Liver, μg/g of wet tissue								
Zn[efgh]	52.8[b]	59.5[bc]	62.5[bcd]	77.5[d]	67.8[cd]	65.1[bcd]	580.8[a]	.07[k]
Cu	9.58	8.40	9.55	9.51	9.95	9.21	8.41	.12[k]
Fe	8.63[ab]	10.37[ab]	8.28[b]	10.72[a]	8.12[ab]	8.48[ab]	8.33[ab]	.13[k]
Kidney, μg/g of wet tissue								
Zn	24.6[b]	24.6[b]	24.8[b]	23.7[b]	23.5[b]	24.3[b]	86.2[a]	.05[k]
Cu	7.87[b]	7.88[b]	7.15[b]	8.33[b]	6.27[b]	6.85[b]	42.1[a]	.13[k]
Fe	19.9[b]	23.5[b]	21.3[b]	31.8[b]	20.5[b]	19.9[b]	45.8[a]	.12[k]
Metacarpal Bone, μg/g of DM								
Zn	76.7[b]	83.6[b]	81.6[b]	75.4[b]	81.5[b]	90.4[b]	152.2[a]	7.3
Cu	.57	.53	.51	.54	.61	.64	.57	.08[k]
Fe	47.2[b]	61.4[ab]	53.5[ab]	58.0[ab]	57.8[ab]	54.2[ab]	65.0[a]	.1[k]

[a,b,c,d]Means in the same row with uncommon superscripts differ (P < .05).
[e]250 ZnAA dietary treatment effect (P < .05).
[f]250 ZnAA * 500 ZnO dietary treatment effect (P < .05).
[g]Linear ZnAA dietary treatment effect (P < .05).
[h]Quadratic ZnAA dietary treatment effect (P < .05).
[k]data was transformed prior to analysis but, actual means are presented and the logSEM.

Table 7. Tissue mineral concentrations of weanling pigs fed supplemental zinc from inorganic and organic forms (Exp. 2)

Supplemental Zn:					
Zn as ZnO	0	0	0	3000	
Zn as ZnAA	0	0	250	0	
Zn as ZnMet	0	250	0	0	SEM
Liver, μg/g of wet tissue					
Zn	107.1[b]	122.3[bc]	155.2[c]	1166.2[a]	.1[k]
Cu	43.2	30.9	45.0	19.4	11.7
Fe	135.0	140.2	133.5	123.0	15.2
Kidney, μg/g of wet tissue					
Zn	31.7[b]	31.2[b]	34.4[b]	231.8[a]	.15[k]
Cu	18.8[b]	12.9[b]	13.8[b]	68.9[a]	.19[k]
Fe	16.0	16.4	16.4	25.1	4.6
Longissimus Muscle, μg/g of DM					
Zn	50.0	111.3	71.1	68.5	31.1
Cu	23.4	95.1	31.0	52.9	47.5
Fe	44.0	43.9	41.5	43.3	2.9
Metacarpal Bone, μg/g of DM					
Zn	1026.3[b]	984.6[b]	969.3[b]	2805.2[a]	.06[k]
Cu	3 .25	3.1	3.6	3.11	.21[k]
Fe	274.1	222.1	195.2	279.4	51.2

[a,b,c,d]Means in the same row with uncommon superscripts differ (P < .05).
[k]data was transformed prior to analysis but, actual means are presented and the logSEM.

Table 8. Hepatic mineral concentrations of weanling pigs fed supplemental zinc and inoculated with Escherichia coli (Exp. 3)

Inoculation:	Sham	E.coli	Sham	E. Coli	Sham	E. Coli	
Supplemental Zn:							
Zn as ZnO	0	0	0	0	3000	3000	
Zn as ZnAA	0	0	250	250	0	0	SEM
Liver, μg/g of wet tissue							
Zn	54.5^{c}	65.9^{c}	53.1^{c}	55.2^{c}	611.4^{a}	458.9^{b}	37.9
Cu	10.3^{b}	10.8^{b}	18.1^{a}	12.3	10.5^{b}	11.1	2.13
Fe	148.5^{b}	141.9^{b}	131.8^{b}	131.5^{b}	305.2^{a}	327.4^{a}	25.3

^{a,b,c}Means in the same row with uncommon superscripts differ ($P < .05$).

Table 9. Urine and feces mineral concentrations of weanling pigs fed supplemental zinc from inorganic and organic forms (Exp. 2)

Supplemental Zn:					
Zn as ZnO	0	0	0	3000	
Zn as ZnAA	0	0	250	0	
Zn as ZnMet	0	250	0	0	SEM
Urine, mg/d					
Zn	.48[c]	.86[b]	1.10[b]	3.01[a]	.14[d]
Cu	.23[c]	.26[abc]	.32[abc]	.35[ab]	.14[d]
Fe	.36[bc]	.46[ab]	.53[a]	.28[c]	.16[d]
Feces, mg/d					
Zn	108.3[c]	210.0[b]	221.0[b]	1022.1[a]	.07[d]
Cu	47.3	52.0	47.1	48.9	3.7
Fe	128.8[c]	157[ab]	132.9[bc]	165.7[a]	.07[d]

[a,b,c]Means in the same row with uncommom superscripts differ (P < .05).
[d]data was transformed prior to analysis but, actual means and logSEM are presented.

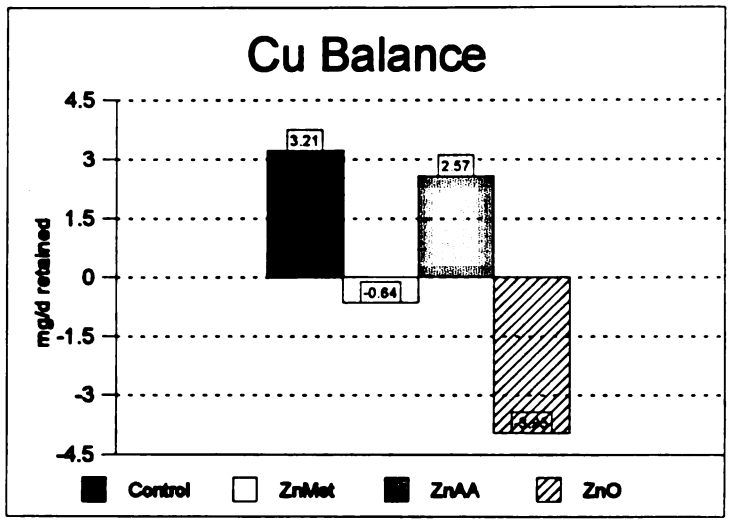

Figure 3. Effect of Zn forms on Cu balance of nursery pigs (d 16-20). Cu balance did not differ significantly between dietary treatments (P > .05).

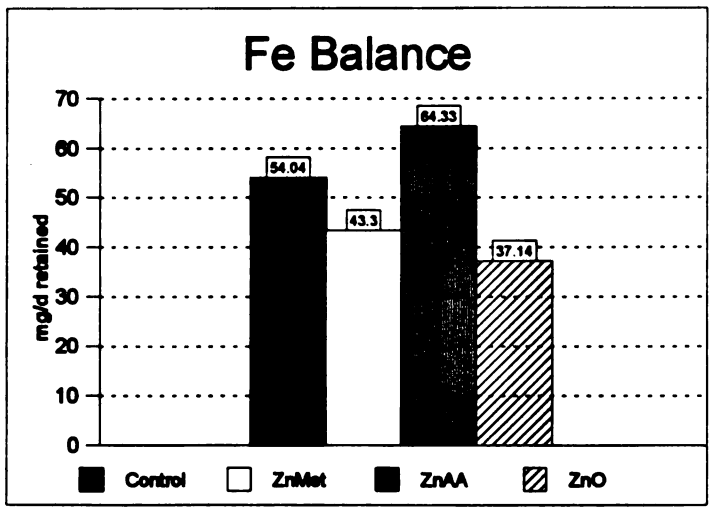

Figure 4. Effect of Zn forms on Fe balance of nursery pigs (d 16-20). Fe balance did not differ significantly between dietary treatments (P > .05).

APPENDIX A

THE EFFECTS OF ZINC SUPPLEMENTATION ON METALLOTHIONEIN AND GUT MORPHOLOGY OF PIGS INOCULATED WITH *ESCHERICHIA COLI*

MATERIALS AND METHODS

Seventy-two crossbred pigs (Duroc x (Yorkshire x Landrace), non creep fed, were weaned at an average age of 16 d and average weight of 4.86 kg and allotted by sex and litter to a 28 d trial with a 3 x 2 factorial arrangement of treatments. Six separate temperature controlled rooms were used with three pens per room and four pigs per pen with ad libitum access to feed and water. Each dietary treatment was represented by three replicates of *E. coli* inoculated and sham inoculated pigs. The dietary treatments across the two health statuses were as follows: basal (control), basal + 250 ppm Zn as ZnAA, and basal + 3000 ppm Zn as ZnO. Phase 1 (d 0-14) and phase 2 (d 14-28) basal diets were the same as those in Exp. 1. All diets (Table 1 and Table 2) were pelleted and met or exceeded NRC nutrient recommendations (NRC, 1988).

At weaning, three rooms of pigs received an oral dose of 5 ml of enterotoxigenic *Escherichia coli* (serotype 0149:k88 + non motile) inoculum (1.3 x 10^{-10} CFU/ml), in peptone saline. A sham of peptone saline was orally administered to pigs in the remaining three rooms. On d 5, pigs that had

previously received *E. coli* were given another 5 ml of *E. coli* inoculum (3.2×10^{10} CFU/ml). On d 7, one pig per replicate group (n=18) was killed. The remaining pigs (n=54) continued on test for 21 d. On d 28, pigs were killed by injection of penibarbitol at ml for every 4.5 kg. Immediately, tissue specimens for histological study were collected from the duodenum and jejunum. One gram of duodenal mucosa was scraped with a glass slide, weighed, combined with a homogenate buffer with protease inhibitors at a 1:4 ratio. Sample was homogenized with a Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Germany). Homogenate buffer contained 0.2 mMol/L of PMSF, 0.6 mg/L of leupeptin, 0.9 mg/L of pepstatin A, 10 mMol/L of Tris HCl, 154 mMol/L of NaCl and 0.2 g/L of NaN_3. Samples were then frozen at -80°C for later determination of metallothionein. A second duodenum sample (10-cm) was taken adjacent the first segment. A mid jejunal sample was also obtained and both the duodenal and jejunal samples were fixed in 10% phosphate-buffered formalin. Histological measurements were completed according to Coussement et al. (1982) and Moxley et al. (1989). Samples were stained with hematoxylin and eosin. The length of the villi (from the tip of the villus to villus/crypt junction) and depth of crypt (junction of villus/crypt to base of crypt), villus:crypt ratio, brunner's gland, circular muscle, longitudinal muscle, and total thickness were measured with an ocular micrometer on a minimum of seven different places per small intestinal segment.

Liver samples were frozen at -80°C. Metallothionein (MT) concentrations in the intestine and liver were determined by the non-radioactive silver binding assay according to Lee et al. (1991) with an assay volume of 1 ml. Liver samples were combined with glycine buffer in a 1:4 ratio and homogenized. Samples were boiled for 2 min at 100°C and then centrifuged (microcentrifuge, Fischer Scientific, Pittsburg, PA) for 2 min to separate supernant fraction. Intestinal and liver homogenates were combined separately with glycine buffer at a pH of 8 and .3 ml of silver to reach a volume of 1 ml. Samples were mixed and incubated for 10 min to saturate the metal binding sites of MT. The unbound metals (including excess silver) were removed by adding .1 ml of hemolysate from rat red blood cells followed by boiling in water for 2 min. Samples were centrifuged to separate the supernant fraction and silver concentration was determined with flame atomic absorption spectrophotometery (Smith-Hieftje 4000, Thermo Jarrell Ash Corporation, Franklin, MA). The amount of silver in the supernant fraction was assumed to proportional to the amount of MT present. MT was expressed in micrograms of MT per gram of wet tissue.

The herd from which pigs were obtained were determined to be exposed to the TGE (transmissible gastroenteritis virus) prior to weaning of the pigs in this study. No pigs in this study died, however vomiting in some pens did occur for one to two days.

Statistical Analysis. Data for this experiment was analyzed using SAS software for GLM procedure and least squares of means by treatment (SAS

manual, 1994). The mean differences between the treatments were detected by comparison of least square means. Differences were considered significant at $P < .05$. Individual pig was the experimental unit and treatment and room were part of the model statement.

RESULTS AND DISCUSSION

The effects of Zn supplementation on MT concentrations in the intestine and liver of pigs inoculated with sham or E. coli are given in Table 10. Intestinal and liver MT followed the same pattern in that pigs fed the ZnO diet in the sham and E. coli inoculated pigs had greater ($P < .05$) concentrations of MT than pigs fed the control or ZnAA supplemented diet. These results are similar to those reported by Carlson et al. (1997). They concluded that pigs supplemented with high concentrations of ZnO stimulated the synthesis of a greater amount of MT than the control or organic supplemented diets. However, contrary to results also reported by Carlson et al. (1997), the control fed pigs did not stimulate the synthesis of a greater amount of MT in the intestine than organic supplemented pigs. Concentrations of MT in control and ZnAA supplemented diets from sham and E. coli inoculated pigs in the intestine and liver where different ($P < .05$). Proliferation of E. Coli in the intestines of the pigs in this study was minimal as determined by morphological evaluation. The diets the pigs were consuming contained an antibiotic (CPS 250) which may have effected the ability of the

E. coli to proliferate. Mild signs of scouring were seen only after the second dose of *E. Coli* and lasted for two days. Vomiting was exhibited and as stated previously, TGE may have been prevalent in some rooms. Differences in gut morphology are given in Table 11. Minimal differences were seen and do not appear to reflect the dietary treatment.

Table 10. Concentration of metallothionein (MT) in the intestine and liver of weanling pigs fed supplemental zinc and inoculated with Escherichia coli (Exp. 3)

Inoculation:	Sham	E.coli	Sham	E. Coli	Sham	E. Coli	
Supplemental Zn:							
Zn as ZnO	0	0	0	0	3000	3000	
Zn as ZnAA	0	0	250	250	0	0	C.V.
Intestinal MT, μg/g							
d 28	17.5[b]	31.5[b]	15.9[b]	24.8[b]	324.5[a]	352.7[a]	70.3
Liver MT, μg/g							
d 28	274.4[b]	528.4[b]	308.5[b]	284.0[b]	2972[a]	2566[a]	56.5

[a,b,c]Means in the same row with uncommon superscripts differ (P < .05).

Table 11. Morphological measurments of duodenal and jejunal segments of intestine from weanling pigs fed supplemental zinc and inoculated with Escherichia coli (Exp. 3)

Inoculation:	Sham	E.coli	Sham	E. Coli	Sham	E. Coli	
Supplemental Zn:							
Zn as ZnO	0	0	0	0	3000	3000	
Zn as ZnAA	0	0	250	250	0	0	C.V.
Duodenum (microns)							
vilus length	671[a]	661[ac]	538[b]	588[ac]	524[bc]	593[ac]	18.1
crypt depth	292	301	302	316	312	360	22.6
villus:crypt	2.38[a]	2.23[ab]	1.85[ab]	2.01[ab]	1.76[b]	1.73[b]	28.1
brunner's gland	120[ab]	103[ab]	110[ab]	111[ab]	140[a]	87[b]	34.0
circular muscle	105	88	101	100	107	77	33.8
longitudinal muscle	57[ab]	53[ab]	55[ab]	53[ab]	60[a]	38[b]	39.4
total thickness	1355	1327	1236	1297	1298	1250	12.3
Jejunum (microns)							
villus length	551	568	533	510	567	532	19.7
crypt depth	197[b]	236[ab]	238[ab]	226[ab]	239[ab]	264[a]	20.1
villus:crypt depth	2.87[a]	2.46[ab]	2.25[b]	2.38[ab]	2.47[ab]	2.06[b]	24.8
circular muscle	82[ab]	91[ab]	76[b]	104[ab]	77[b]	118[a]	43.5
longitudinal muscle	47[ab]	57[a]	37[b]	45[ab]	38[ab]	48[ab]	41.3
total thickness	978	1103	1003	1023	1028	1087	13.1

[a,b,c]Means in the same row with uncommon superscripts differ ($P < .05$).

LITERATURE CITED

Andrews, G. K. 1990. Regulation of metallothionein gene expression. Prog. Food Nutr. Sci. 14:193-258.

AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Carlson, M. S., G. M. Hill, and J. E. Link. 1996. The impact of dietary copper and(or) zinc supplementation on hepatic and intestinal cells in growing pigs. J. Anim. Sci. 74(Suppl. 1):304 (Abstr.).

Carlson, M. S., S. L. Hoover, G. M. Hill, J. E. Link, and T. L. Ward. 1997. The impact of organic and inorganic sources of zinc supplementation on intestinal metallothionein concentration in the nursery pig. J. Anim. Sci. 75(Suppl. 1):206 (Abstr.).

Chirase, N. K., D. P. Hutcheson, and G. B. Thompson. 1991. Feed intake, rectal temperature, and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci. 69:4137-4145.

Cousins, R. J. 1979. Regulation of zinc absorption: role of intracellular ligands. Am. J. Clin. Nutr. 32:339-345.

Coussement, W., R. Ducatelle, P. Debouck, and J. Hoorens. 1982. Pathology of experimental CV777 coronavirus enteritis in piglets. Vet. Pathol. 19:46-56.

Crooks, A. C., H. S. Hurd, D. A. Dargatz, and G. W. Hill. 1993. Economic cost of preweaning mortality: A report of the NAHMS nation swine survey. Swine Health Prod. 1(3):15-21.

Dean-Nystrom, E. A., and J. E. Samuel. 1994. Age-related resistance to 987P fimbria-mediated colonization correlates with specific glycolipid receptors in intestinal mucus in swine. Inf. Imm. 62(11):4789-4794.

Evans, G. W., C. I. Grace, and H. J. Votava. 1975. A proposed mechanism for zinc absorption in the rat. Am. J. Physiol. 228(2):501-505.

Evans, G. W., E. C. Johnson, and P. E. Johnson. 1979. Zinc absorption in the rat determined by radioisotope dilution. J. Nutr. 109:1258-1264.

Flanagan, P. R., J. Haist, and L. S. Valberg. 1983. Zinc absorption, intraluminal zinc and intestinal metallothionein levels in zinc-deficient and zinc-replete rodents. J. Nutr. 113:962-972.

Fryer, A., E. R. Miller, P. K. Ku, and D. E. Ullrey. 1992. Effect of elevated dietary zinc on growth performance of weanling swine. Mich. State Res. Rep. pp.128-132.

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson, and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J. Anim. Sci. 66:1818-1823.

Hahn, J. D., and D. H. Baker. 1993. Growth and plasma zinc responses of young pigs fed pharmacological levels of zinc. J. Anim. Sci. 71:3020-3024.

Hampson, D. J. 1986. Influence of creep feeding and dietary intake after weaning on malabsorption and occurrence of diarrhoea in the newly weaned pig. Res. Vet. Sci. 41:63-69.

Hempe, J. M., and R. J. Cousins. 1989. Effect of EDTA and zinc-methionine complex on zinc absorption by rat intestine. J. Nutr. 119(8):1179-1187.

Hill, G. M., E. R. Miller, P. A. Whetter, and D. E. Ullrey. 1983. Concentration of minerals in tissues of pigs from dams fed different levels of dietary zinc. J. Anim. Sci. 57(1):130-138.

Hill, G. M., G. J. Brewer, A. S. Prasad, C. R. Hydrick, and D. E. Hartmann. 1987. Treatment of Wilson's disease with zinc. I. Oral zinc therapy regimens. Hepat. 7(3):522-528.

Hill, G. M., G. L. Cromwell, T. D. Crenshaw, R. C. Ewan, E. A. Knabe, A. J. Lewis, D. C. Mahan, G. C. Shurson, L. L. Southern, and T. L. Veum. Impact of pharmacological intakes of zinc and(or) copper on performance of weanling pigs. J. Anim. Sci. 74(Suppl. 1):300.

Holland, R. E. 1990. Some infectious causes of diarrhea in young farm animals. Clin. Microbiol. Rev. 3(4):345-375.

Hurley, T., S. Chudhary, J. Kliebenstein, J. McKean, and S. Westercamp. 1995. Cost of pigs scours. Iowa State Res. Rep. pp. 152-153.

Jackson, M. J., D. A. Jones, and R. H. T. Edwards. 1981. Zinc absorption in the rat. Br. J. Nutr. 46:15-27.

Johnson, P. E., J. R. Hunt, and N. V. C. Ralston. 1988. The effect of past and current dietary Zn intake on Zn absorption and endogenous excretion in the rat. J. Nutr. 118:1205-1209.

Katouli, M., A. Lund, P. Wallgren, I. Kuhn, O. Soderlind, and R. Mollby. 1995. Phenotypic characterization of intestinal Escherichia coli of pigs during suckling, postweaning, and fattening periods. App. Env. Microbiol. 61(2):778-783.

Kegley, E. B., and J. W. Spears. 1995. Immune response and performance of sheep fed supplemental zinc as zinc oxide or zinc methionine. Sheep Goat Res. J. 11(3):127-131.

Kidd, M. T., N. B. Anthony, and S. R. Lee. 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poult. Sci. 71:1201-1206.

Kidd, M. T., M. A. Qureshi, P. R. Ferket, and L. N. Thomas. 1994. Blood clearance of Escherichia coli and evaluation of mononuclear-phagocytic system as influenced by supplemental dietary zinc methionine in young turkeys. Poult. Sci. 73:1381-1389.

Kienholz, E. W., R. E. Moreng, and J. D. Flinchum. 1992. Zinc methionine for stressed laying hens. Poult. Sci. 71:829-832.

Kirchgessner, M., and E. Weigand. 1983. Zinc absorption and excretion in relation to nutrition. In: H. Sigel (Ed.) Metal Ions in Biological Systems. Vol. 15. Marcel Dekker, Inc., New York, NY.

Lee, D., G. J. Brewer, and Y. Wang. 1989. Treatment of Wilson's disease with zinc. VII. Protection of the liver from copper toxicity by zinc -induced metallothionein in a rat model. J. Lab Clin. Med. 114:639-646.

McDowell, L. R. 1992. Minerals in Animal and Human Nutrition. Academic Press, San Diego, CA.

Milne, D., B. Canfield, W. K. Mahalko, J. R. Sandstead, and H. Harold. 1983. Effect of dietary zinc on whole body surface loss of zinc:Impact on estimation of zinc retention by balance method. Am. J. Clin. Nutr. 38(2):181-186.

Moreng, R. E., D. Balnave, and D. Zhang. 1992. Dietary zinc methionine effect on eggshell quality of hens drinking saline water. Poult. Sci. 71:1163-1167.

Moxley, R. A., and L. D. Olson. 1989. Lesions of transmissible gastroenteritis virus infection in experimentally inoculated pigs suckling immunized sows. Am. J. Vet. Res. 50(5):708-716.

Nagy, B., T. A. Casey, S. C. Whipp, and H. W. Moon. 1992. Susceptibility of Porcine intestinal to pili-mediated adhesion by some isolates of pilated enterotoxigenic Escherichia coli increases with age. Inf. Imm. 60(4):1285-1294.

Nockels, C. F., J. DeBonis, and J. Torrent. 1993. Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. J. Anim. Sci. 71:2539-2545.

NRC. 1988. Nutrient Requirements of Swine (9th Ed.). National Academy Press, Washington, DC.

O'Dell, B. L. 1984. Bioavailability of trace elements. Nutr. Rev. 42:301-308.

Pekas, J. C. 1966. Zinc 65 metabolism: gastrointestinal secretion by the pig. Am. J. Physiol. 211(2):407-413.

Pimental, J. L., Me. E. Cook, and J. L. Greger. 1991. Research note: Bioavailability of zinc-methionine for chicks. Poult. Sci. 70:1637-1639.

Pond, W. G. 1985. Advances in swine nutrition. Cor. Vet. 75:201-220.

Poulsen, H. D. 1995. Zinc oxide for weanling piglets. Acta. Agric. Scand. 45:159-167.

Richards, M. P., and R. J. Cousins. 1975. Mammalian zinc homeostasis: Requirement for RNA and metallothionein synthesis. Biochem. Biophy. Res. 64(4):1215-1223.

Richards, M. P., and R. J. Cousins. 1976. Metallothionein and its relationship to the metabolism of dietary zinc in rats. J. Nutr. 106:1591-1599.

Rojas, L. X., L. R. McDowell, R. J. Cousins, F. G. Martin, N. S. Wilkinson, A. B. Johnson, and J. B. Velasquez. 1995. Relative bioavailability of two organic and two inorganic sources fed to sheep. J. Anim. Sci. 73:1202-1207.

Sarmiento, J. I., T. A. Casey, and H. W. Moon. 1988. Postweaning diarrhea in swine: Experimental model of enterotoxigenic Escherichia coli infection. Am. J. Vet. Res. 49(7):1154-1159.

Sarmiento, J. I., E. A. Dean, and H. W. Moon. 1988. Effects of weaning on diarrhea caused by enterotoxigenic Escherichia coli in three-week-old pigs. Am. J. Vet. Res. 49(12):2030-2033.

Sarmiento, J. I., P. L. Runnels, and H. W. Moon. 1990. Effects of preweaning exposure to a starter diet on enterotoxigenic Escherichia coli-induced postweaning diarrhea in swine. Am. J. Vet. Res. 51(8):1180-1183.

SAS Institute. 1985. SAS User's Guide: Basics. 5th Edition. Cary, NC.

Schell, T. C., and E. T. Kornegay. 1996. Zinc concentration in tissues and performance of weanling pigs fed pharmacological levels of zinc from ZnO, Zn-methionine, Zn-lysine, or $ZnSO_4$. J. Anim. Sci. 74:1584-1593.

Sojka, W. J. 1965. Escherichia Coli in Domestic Animals and Poultry. Commonwealth Agricultural Bureaux. Farnham Royal, Great Britain.

Spears, J. W. 1989. Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. J. Anim. Sci. 67:835-843.

Spears, J. W., R. W. Harvey , and T. T., Jr., Brown. 1991. Effects of zinc methionine and zinc oxide on performance, blood characteristics, and antibody titer response to viral vaccination in stressed feeder calves. J. Am. Vet. Med. Assoc. 199(11):1731-1733.

Starcher, B. C., J. G. Glaubner, and J. G. Madaras. 1980. Zinc absorption and its relationship to intestinal metallothionein. J. Nutr. 110:1391-1397.

Tucker, H. F., and W. D. Salmon. 1955. Parakeratosis or zinc deficiency disease in the pig. Proc. Soc. Exp. Biol. Med. 88:613-616.

Tufft, L. S., C. F. Nockels, and M. J. Fettman. 1988. Effects of escherichia coli on iron, copper, and zinc metabolism in chicks. Avian Dis. 32:779-786.

Underwood, E. J. 1977. Trace Elements in Human and Animal Nutrition (4th Ed.). Academic Press, New York, NY.

Underwood, E. J. 1981. The Mineral Nutrition of Livestock (2nd Ed.) Commonwealth Agricultural Bureaux, Slough, United Kingdom.

Wang, Z. S., A. Atkinson, R. F. P. Bertolo, S. Polberger, and B. Lonnerdal. 1993. Alterations in intestinal uptake and compartmentalization of zinc in response to short-term dexamethasone therapy or excess dietary zinc in piglets. Ped. Res. 33:118-124.

Ward, T. L., G. A. Asche, G. F. Louis, and D. S. Pollmann. 1996. Zinc-methionine improves growth performance of starter pigs. J. Anim. Sci. 74(Suppl. 1):303 (Abstr.).

Wedekind, K. J., and D. H. Baker. 1990. Zinc bioavailability in feed-grade sources of zinc. J. Anim. Sci. 68:684-689.

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

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

Weigand, E., and M. Kirchgessner. 1980. Total true efficiency of zinc utilization: Determination and homeostatic dependence upon the zinc supply status in young rats. J. Nutr. 110:469-480.

Whitenack, D. L., C. K. Whitehair, and E. R. Miller. 1978. Influence of enteric infection on zinc utilization and clinical signs and lesions of zinc deficiency in young swine. Am. J. Vet. Med. 39(9):1447-1454.

Wilson, R. A., and D. H. Francis. 1986. Fimbriae and enterotoxins associated with Escherichia coli serogroups isolated from pigs with colibacillosis. Am. J. Vet. Res. 47(2):213-217.

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