THE EVALUATION OF THE ADDITIVE EFFECT OF ZINC AND COPPER SUPPLEMENTATION FROM ORGANIC AND INORGANIC SOURCES ON GROWTH PERFORMANCE OF NURSERY PIGS AND THE DIGESTIBILITY OF THOSE MINERALS

by

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(Under the Direction of C. Robert Dove)

ABSTRACT

In this study, experiment 1 examined the growth characteristics of pigs fed different levels of organic Zn coupled with $CuSO_4$ compared to those fed treatments containing inorganic Zn from ZnO and $CuSO_4$. Experiment 2 determined the digestibility of copper and zinc in nursery pigs fed diets containing 4 or 100 ppm Cu as $CuSO_4$ and 60 ppm Zn from ZnO or 75 ppm Zn as organic zinc. Data from experiment 1 indicated that pigs fed 75 ppm of organic Zn have performance similar to those fed 100 ppm ZnO and that the addition of 100 ppm organic Zn resulted in decreased performance compared to those pigs fed 100 ppm ZnO. Experiment 2 saw the benefit of 100 ppm added Cu on ADG and ADF compared to 4 ppm Cu yet, no differences in 60 ppm inorganic Zn and 75 ppm organic Zn on growth characteristics were observed. 75 ppm organic Zn increased intake 21% over 60 ppm inorganic Zn. 100 ppm Cu increased Ca and Cu intake, however 4 ppm Cu increased retention by 45%.

INDEX WORDS: Copper, Zinc, Nursery pigs

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CHAPTER 1

INTRODUCTION

The health status and well being of a nursery age pig can have a great impact on growth performance throughout the pig's lifespan (Carlson et al., 2004). Due to factors such as post-weaning stress, a weanling pig's body is more immunologically challenged during this age than any other (Hill et al., 2000). Research has been performed for a number of years on dietary ingredients that help alleviate these health problems. The minerals copper and zinc are involved in metabolic functions and have been added to weanling pig diets to improve performance and health status during the nursery stage (Carlson et al., 2004).

For some time, copper (Cu) has been shown to have antimycotic and antibacterial activities as well as growth-stimulating values (Hill et al., 2000). Copper sulfate ($CuSO_4$) has been recognized for its growth-promoting properties when added to weanling swine diets (Bunch et al., 1961). Adding $CuSO_4$ to weanling diets at levels of 125 and 250 ppm has been shown to stimulate growth in young swine (Edmonds and Baker, 1986; Cromwell et al., 1998). Research has proposed that adding zinc oxide (ZnO), at levels of 2,000 to 3,000 ppm, to the diets of weanling pigs may foster a similar growth-promotional response to that of $CuSO_4$ (LeMieux et al., 1995; McCully et al., 1995). The Zn requirement for nursery pigs given in the NRC (1998) is set at 100 ppm, however pharmacological levels of 2,000 to 3,000 ppm are commonly used in the swine industry. Hahn and Baker (1993) observed a significant increase in average daily gain (ADG) and average daily feed intake (ADFI) when weanling swine diets were formulated with 3,000 ppm Zn from ZnO. Smith et al. (1997) also showed that supplementing starter pigs with 3,000 ppm Zn from ZnO improved growth performance during the first 4 weeks post weaning.

The common industry practice of using pharmacological levels of inorganic Zn, coupled with high levels of Cu, as a growth promotants has raised some environmental concerns. Due to low bioavailability of ZnO, excess excretion of Zn into the environment is common (Case and Carlsen, 2002). Interest in using organic zinc in nursery pig diets has increased significantly since Ward et al. (1996) published data showing that the beneficial effects of feeding pharmacological levels of inorganic zinc could be achieved feeding lower levels of organic zinc (250 ppm Zn-methionine) with normal concentrations of inorganic zinc (160 ppm Zn-sulfate).

Smith et al. (1997) found that feeding 3,000 ppm Zn from ZnO improved the growth performance of pigs weaned at 12 days of age, but observed no additive responses between growth-promotional levels of zinc oxide (3,000 ppm) and copper sulfate (250 ppm). Research has shown that in rats, the metallothionien concentration increases when supplemental Zn is added to the diet (Klevey 1987). Metallothionien has a greater affinity for Cu and replaces Zn in normal binding sites, which leads to an increase in free Zn in the body (Ogiso et al. 1979). This competitive binding between the inorganic minerals may be the reason no additive effect is seen between Cu and Zn. The objective of this study was to determine if, by using lower levels of organic zinc coupled with growth promoting levels and NRC recommended levels of copper sulfate, an additive effect exists between the two minerals.

CHAPTER 2

LITERATURE REVIEW

In the swine industry, an increase in weight gain by weanling pigs in the nursery is known to translate into fewer days to market weight (Carlson et al., 2004). However, due to factors such as post-weaning stress and harmful microorganisms, a weanling pig's body is more immunologically suppressed during this time period than any other in its lifespan (Hill et al., 2000). For years, research has been performed investigating post-weaning growth depression in weanling pigs. Decreases in early pig growth have often been linked to stress at weaning and opportunistic microorganisms that often times cause diarrhea and other health issues (Hill et al., 2000). Despite the fact that several factors have been shown to decrease the health status of weanling swine, some dietary ingredients have been shown to counteract these effects (Smith et al., 1997).

Copper and Zinc

Both copper and zinc are minor components of a nursery pig's body composition (Mahan and Shields, 1998). The weanling pig's body has been shown to have a Cu content of 1.32 mg/kg and a Zn content of 15.28 mg/kg (Mahan and Shields, 1998). However, both minerals are highly involved in the metabolic functions of the body. Hart et. al (1928) discovered that both copper and iron are required for hemoglobin synthesis. In 1948, Holmberg and Lawrell found that ceruloplasm was a copper containing protein. Wainio (1959) then reported that cytochrome oxidase contained copper.

Zinc is an essential trace mineral required for growth, bone development, immune function, enzyme structure and function, and appetite in the pig (Walker and Black, 2004). Zinc is a cofactor for more than 300 metalloenzymes and is required for several enzymes such as carboxypeptidase, alkaline phosphatase, and lactic dehydrogenase (Mavromichalis et al., 2000; Wedekind et al., 1994). Rink and Kirchner (2000) documented that a deficiency in Zn proved to reduce immune system function. The presence of both elements, in sufficient amounts, is pivotal to ensuring good performance and health status of weanling pigs (Jondreville et al., 2003). Because of their importance, the NRC (1998) suggests that pigs from 8-28 kg consume diets containing 5-6 ppm Cu and 80-100 ppm Zn. However, dietary requirements of trace minerals to optimize immune function are commonly higher than the requirements for growth (Klasing, 2001). For example, Hahn and Baker (1993) observed improved gain and health status in weanling pigs fed 3,000 ppm Zn from ZnO.

Copper and Zinc as Growth Promotants

For some time, copper (Cu) has been shown to have antimycotic and antibacterial activities as well as growth-stimulating values (Edmonds and Baker, 1986; Hill et al., 2000). The growth promoting properties of Cu were first recognized in 1925 when McHargue found that copper improved the growth of rats. Copper sulfate ($CuSO_4$) was later recognized for its growth-promoting properties when added to weanling swine diets (Braude et al., 1955; Bunch et al., 1961). Adding Cu sulfate to weanling diets at levels of 125 and 250 ppm has been shown to stimulate growth in young swine (Edmonds and Baker, 1986; Cromwell et al., 1998). In another study, Dove and Hayden (1991) showed that by adding 250 ppm Cu from Cu sulfate, pigs weaned from 26 to 28 days of age had increased growth performance.

Cu has also been shown to have an additive effect with feed-grade antibiotics when the two compounds were coupled together in starter swine diets (Stahly et al., 1980). A potential mode of action of copper was discovered when Yen and Nienaber (1993) proved that added dietary Cu increased circulation by the portal-drained viscera. It has been documented that this increase in circulation may actually improve the absorptive capacity of the enterocytes in the small intestine which would lead to improved growth performance of the animal (Hill et al., 2000). In 1995, Dove used pigs weaned at 26 days of age to prove that feeding 250 ppm Cu increased the apparent digestibility of added fat in the diet. These results showed that added Cu, in excess of levels normally found in swine diets, plays an important role in the utilization of added dietary fat. The ability of weanling pigs to utilize dietary fat efficiently for the first 21 days after weaning provides producers a means of increasing energy levels in weanling diets, therefore increasing gain through the nursery phase (Dove, 1995).

The Zn requirement for nursery pigs given in the NRC (1998) is set at 100 ppm. Feed-grade ZnO is the most common form of Zn used in the animal feed industry. Pharmacological levels of 2,000 to 3,000 ppm are commonly used in the swine industry. Recently, research has proposed that adding zinc oxide (ZnO) to the diets of weanling pigs may foster a similar growth-stimulating response to that of $CuSO_4$ (LeMieux et al., 1995; McCully et al., 1995). Zinc has been shown to inhibit the activity of hemolysin resulting from *Serpulina hyodysenteriae,* which is a virulence factor in the pathogenesis of swine dysentery (Dupont et al., 1994). Kidd et al. (1994) reported that turkey poults fed Zn methionine had an improved clearance of *E. coli* from the blood. Poulsen (1989) proposed that pharmacological levels of ZnO may help control *E. coli* scours in pigs. Hill et al. (2000) suggested that diets containing high Zn, high Cu, or a combination of the two resulted in pigs with firmer stools than pigs fed adequate levels of Zn or

Cu. Hahn and Baker (1993) observed a significant increase in average daily gain (ADG) and average daily feed intake (ADFI) when weanling swine diets were formulated with 3,000 ppm Zn from ZnO. Smith et al. (1997) also showed that supplementing starter pigs with 3,000 ppm Zn from ZnO improved growth performance during the first four weeks after weaning. It was reported by Carlson et al. (1999) that high dietary levels of ZnO resulted in improved gut morphology. Hampson (1986) proposed that increased growth due to high Zn supplementation could be contributed to physiological changes in the gastrointestinal tract that may enhance nutrient absorption by altering the intestinal morphology. In 1996, Hill et al. saw comparable improvements in ADG from 3,000 ppm of Zn from ZnO as well as with 250 ppm Cu. However, in this study, no additive responses between Zn and Cu were recorded.

Klevey (1987) proposed that by increasing dietary levels of Zn, the mineral may actually inhibit the uptake of Cu through metallothionien, which is involved in maintaining Zn homeostasis (Richards and Cousins, 1975). Zn homeostasis is known to be controlled by intestinal mucosal cells regulating the amount of Zn transferred to plasma. Within the intestinal mucosal cell, metallothionien competes with intestinal ligands involved in the absorption of zinc and controls the amount of Zn that is transferred across the basolateral membrane into circulation (Richards and Cousins, 1975). Metallothionien binds dietary Zn in the intestine in order to regulate the amount of Zn absorbed (Cousins, 1985). Excess Zn-metallothionien complex is not absorbed, but rather sloughed off into epithelial cells (Cousins, 1985). Zn in the plasma is deposited into the liver, where metallothionien controls its release and storage. Tissue concentrations of metallothionien are stimulated by the amount of Zn in the diet (Blalock et al., 1988). Metallothionien has a greater affinity for Cu and replaces Zn in normal binding sites, which leads to an increase in free Zn in the body (Ogiso et al. 1979). A decrease in the amount of available Cu may lead to a Cu:Zn ratio imbalance which has been suggested by Klevay (1987) to inhibit lipid and carbohydrate metabolism. However, Carlson et al. (1995) documented a three week Cu x Zn nursery trial where pigs fed both 250 ppm Cu and 3,000 ppm Zn gained faster than those fed adequate concentrations of either mineral alone. Smith et al. (1997) later noted that pigs fed 3,000 ppm Zn from ZnO grew significantly faster from day 0 to 14 post-weaning compared to those fed 110 ppm Zn, with or without the addition of 250 ppm Cu from $CuSO_4$.

As a required nutrient, Cu presents problems to the body because it exists in two different redox states and can change states by accepting or donating electrons (Hill and Link, 2009). Therefore it must be bound to a protein to prevent the production of free radicals. The amount of Cu in the body must be tightly regulated and the delivery to cells must be carefully managed (Hill and Link, 2009). The absorption of Cu occurs primarily in the duodenum, when it is transferred across the brush border into the enterocytes (Cater and Mercer, 2005). However, the uptake across these specialized cells of the intestinal mucosa is not understood. Two proteins that may be involved are a high-affinity Cu transport protein and the divalent metal transporter protein in the brush border (Cater and Mercer, 2005).

Length of Supplementation

Although the use of added Cu and Zn generally yields growth stimulating results in weanling pigs (Smith et al. 1997), the question of what growth stages should be supplemented and why feeding the minerals during these stages of growth affects the body, often arises. An interesting study by Carlsen et al. (1999) found that feeding pharmacological levels of Zn during the first two weeks post-weaning produces an ADG comparable to pigs supplemented throughout the 28 day nursery period. However, if pigs are only supplemented with high levels of Zn the first week post-weaning, growth is not enhanced. This may be due to the fact that newly weaned pigs may not consume enough feed during the first week to stimulate metallothionien production. In fact, low daily feed intake during the first week post-weaning may contribute to the need for more Zn supplementation in a Phase I nursery diet. The study also noted that Zn supplementation during only the second and third weeks after weaning does not enhance growth performance due to the fact that early preloading of Zn is needed along with stimulation of metallothionien production, which would enhance Zn absorption in the small intestine.

Environmental Concern

The common industry practice of using pharmacological levels (2,000 to 3,000 ppm) of inorganic Zn coupled with high levels of Cu, as growth stimulants, has raised some environmental concerns. Due to low the bioavailability of ZnO (39%), excess excretion of Zn (2,157 mg/d average from a 3,000 ppm diet) into the environment is common (Case and Carlsen, 2002). Consequently, high levels of Cu and Zn are often found in swine manure, which may concentrate in top soil and cause toxicity to plants and microorganisms (Case and Carlsen, 2002). Chaney (1993) documented that Zn accumulation in the soil actually reduced plant growth. This becomes a greater concern in areas of intensive pig farming. Reducing the dietary supply of these elements is the main approach to preventing this environmental risk. The annual accumulation of these elements could be reduced by 35% if Cu and Zn concentrations could be reduced in swine diets from 100 to 20 ppm and from 250 to 100 ppm, respectively (Jondreville, 2003).

When pig manure is applied on arable land, most of the Cu and Zn are accumulated in the top soil (Case and Carlsen, 2002). Crop uptake and leaching of these elements usually does not exceed 10% of the supply (McGrath, 1980), leading to a high concentration of the metals in the soil. Because of erosion, Cu and Zn from manure may concentrate in sediments, as well as in sea flora and fauna (Jondreville, 2003).

Organic Mineral Sources

In the last several years, organically bound sources of minerals have been introduced as a supplement for feeding livestock (Jondreville et al., 2003). These organically bound sources result from complexing a soluble metal with an organic molecule (Kirchgessner and Grassman, 1970). The organic molecules can be a polysaccharide, an amino acid, or a partially hydrolyzed protein (Kirchgessner and Grassman, 1970). Chelates are metal complexes in which the metal is held by more than one point of attachment (Kirchgessner and Grassman, 1970). Chelating a mineral enhances its availability to monogastric animals by limiting the detrimental effects of antagonists naturally present in the digestive tract, as well as by its absorption and metabolic utilization (Kirchgessner and Grassman, 1970).

Hahn and Baker (1993) reported that organic minerals potentially have a 20% higher bioavailability than inorganic mineral sources. Interest in using organic zinc in nursery pig diets has increased significantly since Ward et al. (1996) published data showing that the beneficial effects of feeding pharmacological levels of inorganic zinc could be achieved feeding lower levels of organic zinc (250 ppm Zn-methionine) with normal concentrations of inorganic zinc (160 ppm Zn-sulfate). Wedekind et al. (1994) found that Zn from a Zn-methionine complex had an availability of 206% compared to a $ZnSO_4$ control whereas ZnO provided only 61% bioavailable Zn compared to the control. Creech et al. (2004) noted that in the nursery phase, pigs fed 50% of their supplemental Zn, Cu, Fe, and Mn from chelated metal proteinates gained more efficiently than pigs fed similar concentrations from inorganic sulfate forms.

Implication

The health and growth benefits of adding zinc and copper to weanling swine diets has been well documented (Hahn and Baker, 1993; Case and Carlsen, 2002). A common industry practice includes the addition of ZnO and $CuSO_4$ to achieve dietary concentrations as great as 2,000 to 3,000 ppm and 250 ppm, respectively, even though the NRC (1998) recommendation is set at only 100 ppm for Zn and 6-8 ppm for Cu. Using pharmacological levels of inorganic zinc and copper has raised some environmental concerns due to high levels of both minerals found in swine manure (Case and Carlsen, 2002). These high levels may concentrate in top soil and cause toxicity to plants and microorganisms, which becomes a great concern in areas of intensive swine farming (Case and Carlsen, 2002). However, more research on the digestibility of Cu and organic Zn sources in weanling pigs is needed. The objective of this research is to compare the combinations of recommended and higher levels of $CuSO_4$ with varying low levels of organic Zn. Data from this research will be used to determine whether these combinations of minerals have a significant impact upon growth in nursery swine, as well as to determine the digestibility of low levels of organic Zn in diets. This research should help determine whether or not low levels of organic Zn can have a significant impact on growth while lowering the amount of mineral excretion in swine feces. Moreover, producers will be able to formulate diets with mineral levels sustainable enough to increase growth while low enough to decrease pollution of the soil in intensely farmed areas.

Literature Cited

Blalock, T. L., M. A. Dunn, and R. J. Cousins. 1988. Metallothionein gene expression in rats: tissue-specific regulation by dietary copper and zinc. J. Nutr. 118:222-228.

Bunch, R. J., V. C. Speer, V. W. Hays, J. H. Hawbaker, and D. C. Catron. 1961. Effects of copper sulfate, copper oxide and chlortetracycline on baby pig performance. J. Anim. Sci. 20:723.

Carlson, M. S., G. M. Hill, and J. E. Link. 1999. Early and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: Effect on metallothionein and mineral concentrations. J. Anim. Sci. 77:1199-1207.

Carlson, M. S., G. M. Hill, J. E. Link, G. A. McCully, D. W. Rozeboom, and R. L. Weavers. 1995. Impact of zinc oxide and copper sulfate supplementation on the newly weaned pig. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

Case, C. L., and M. S. Carlsen. 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J. Anim. Sci. 80:1917-1924.

Chaney, R. L. 1993. Zinc phytotoxicity. In: Zinc in Soils and Plants. Pp 135-150. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cousins, R. J. 1985. Absorption, transport and hepatic metabolism of copper and zinc: Special reference to metallothionien and ceruloplasm. Physiol. Rev. 65:238-309.

Creech, B. L., J. W. Spears, W. L. Flowers, G. M. Hill, K. E. Lloyd, T. A. Armstrong, and T. E. Engle. 2004. Effect of dieary trace mineral concentration and source (inorganic vs. chelated) on performance, mineral status, and fecal mineral excretion in pigs from weaning through finishing. J. Anim. Sci. 82:2140-2147.

Cromwell, G. L., M. D. Lindemann, H. J. Monegue, D. D. Hall, and D. E. Orr, Jr. 1998. Tribasic copper chloride and copper sulfate as copper sources for weanling pigs. J. Anim. Sci. 76: 118-123.

Dove, C. R. 1995. The effect of copper level on nutrient utilization of weanling pigs. J. Anim. Sci. 73:166-171.

Dove, C. R., and K. D. Haydon. 1991. The effect of copper addition to diets with various iron levels on the performance and hematology of weanling swine. J. Anim. Sci. 69:2013.

Dupont, D. P., G. E. Duhamel, M. P. Carlson, and M. R. Mathiesen. 1994. Effect of divalent cations on hemolysin synthesis by *Serpulina (Treponema) hyodysenteriae*: inhibition induced by zinc and copper. Vet. Microbiol. 41:63-73.

Edmonds, M. S., and D. H. Baker. 1986. Toxic effects of supplemental copper and roxarsone when fed alone or in combination to young pigs. J. Anim. Sci. 63:533-537.

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

Hampson, D. J. 1986. Alterations in piglet small intestinal structure at weaning. Res. Vet. Sci. 40:32-40.

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

Jondreville, C. P. S. Revy, and J. Y. Dourmad. 2003. Dietary means to better control the environmental impact of copper and zinc by pigs from weaning to slaughter. Livestock Prod. Sci. 84:2. 147(10).

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

Kirchgessner, M. and E. Grassmann. 1970. The dynamics of copper absorption. In: C. F. Mills, Editor, Trace Elements Metabolism in Animals. 277-287.

Klasing, K. C. 1992. Nutrition and immunity. What is known about feeding animals for optimum immunocompetence? Large. Anim. Vet. 47:16.

Klevey, L. M. 1987. Dietary copper: A powerful determinant of chloesterolemia. Med. Hypoth. 24:111.

LeMieux, F. M., L. V. Ellison, T. L. Ward. L. L. Southern, and T. D. Bidner. 1995. Excess dietary zinc for pigs weaned at 28 days. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

Mahan, D. C., and R. G. Shields, Jr. 1998. Macro- and micromineral composition of of pigs from birth to 145 kilograms of body weight. J. Anim. Sci. 76:506-512.

McCully, G. A., G. M. Hill, J. E. Link, R. L. Weavers, M. S. Carlsen, and D. W. Rozeboom. 1995. Evaluation of zinc sources for the newly weaned pig. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

McGrath, M. C. 1980. Implications of applying copper rich pig slurry to grassland; effects on plant and soil. In: P. L'Hermite and J. Dehandtschutter, Editors, *Copper in Animal Wastes and Sewage Sludge*. 144-153.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.

Ogiso, T., N. Ogawa, and T. Miura. 1979. Effect of high dietary zinc on copper absorption in rats. II. Binding of copper and zinc to cytosol proteins in the intestinal mucosa. Chem. Pharm. Bull. 27:515.

Poulsen, H. D. 1989. Zinkoxid til grise I fravaenningsperioden [Zinc oxide for pigs during weaning]. Meddelelse. (English summary). No. 746. Statens Husdrybrugsforsoeq (Denmark).

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

Smith, J. W., II, M. D. Tokach, R. D. Goodband, J. L. Nelssen, and B. T. Richert. 1997. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. J. Anim. Sci. 75:1861-1866.

Stahly, T. S., G. L. Cromwell and H. J. Monegue. 1980. Effects of the dietary inclusion of copper and(or) antibiotics on the performance of weanling pigs. J. Anim. Sci. 51:1347.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CABI Publishing, New York, NY.

Walker, C. F. and R. E. Black. 2004. Zinc and the risk for infectious disease. Annu. Rev. Nutr. 24:235-275.

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

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.

Yen, J. T., and J. A. Nienaber. 1993. Effects of high-copper feeding on portal ammonia absorption and on oxygen consumption by portal vein-drained organs and by the whole animal in growing pigs. J. Anim. Sci. 71:2157.

CHAPTER 3

THE EVALUATION OF THE ADDITIVE EFFECT OF ZINC AND COPPER SUPPLEMENTATION FROM ORGANIC AND INORGANIC SOURCES ON GROWTH PERFORMANCE OF NURSERY PIGS

[1]Marchant, M. R., T. C. Tsai, P. M. Cline, M. J. Azain, and C. R. Dove. To be submitted to The Journal of Animal Science.

Abstract

In this study three hundred crossbred pigs (150 gilts and 150 barrows, 3 trials of 100 pigs) were weaned at 21 d ± 2 (initial Wt. 6.27 kg) and housed 5 per pen. Pigs were blocked by weight and equalized by gender, resulting in 6 replicates per treatment. Pigs within a pen were assigned using a RCB design to one of ten dietary treatments. Dietary treatments were arranged in a 2 X 4 factorial with 15 or 250 ppm Cu, as $CuSO_4$ and 25, 50, 75, or 100 ppm Zn as organic Zn (Bioplex Zinc, Altech Inc., Lexington, KY). A positive control diet containing either 15 or 250 ppm Cu and 100 ppm ZnO was included in the study. All diets met or exceeded NRC requirements. Diet phases were changed and pigs were weighed, feed intake recorded and a blood sample collected on d 0, 7, 21, and 35 of the 35 d study. Pigs were housed in an environmentally controlled building with ad libitum access to feed and water. Over the 35 d trial, there were no Cu by Zn interactions ($P > 0.1$) detected. The addition of 250 ppm Cu increased ADG (291 vs 342 g/d for 15 and 250 ppm Cu respectively), ADFI and gain:feed ratio ($P < .01$) over the 35 d trial compared to the 15 ppm Cu diet. The addition of increasing levels of organic Zn increased ADG (312, 310, 334, 292 g/d for 25, 50, 75, and 100 ppm organic Zn, respectively) cubically ($P < .05$) with the addition of 75 ppm organic Zn having the highest ADG. The ADG of pigs fed 75 ppm organic Zn was similar ($P < .01$) to the ADG of pigs fed 100 ppm ZnO (334 vs 336 g/d). The addition of organic Zn tended ($P < .08$) to increase ADFI (522, 526, 541, 492, g/d for 25, 50, 75 and 100 ppm organic Zn respectively) in a manner similar to the increase in ADG. Feed intake of pigs fed 75 ppm organic Zn was similar (541 vs 543) to the intake of pigs fed 100 ppm ZnO. The addition of organic Zn to the diets had no effect on gain:feed ratio ($P > .10$). The addition of Cu or Zn had no effect ($P > .10$) on serum Cu, Fe or Zn. Data from this study indicates that pigs fed 75 ppm of organic Zn have performance similar

to those fed 100 ppm ZnO and that the addition of 100 ppm organic Zn to the diet resulted in decreased performance compared to those pigs fed 100 ppm ZnO.

Introduction

The health status and well being of a nursery age pig can have a great impact on growth performance throughout the pig's lifespan (Carlson et al., 2004). Due to factors such as post-weaning stress and harmful microorganisms, a weanling pig's body is more immunologically challenged during this age than any other (Hill et al., 2000). Research has been performed for a number of years on dietary ingredients that help alleviate these health problems. The minerals copper and zinc are highly involved in metabolic functions and have been added to weanling pig diets to improve performance and health status during the nursery stage (Carlson et al., 2004).

For some time, copper (Cu) has been shown to have antimycotic and antibacterial activities as well as growth-stimulating values (Hill et al., 2000). Copper sulfate ($CuSO_4$) has been recognized for its growth-promoting properties when added to weanling swine diets (Bunch et al., 1961). Adding $CuSO_4$ to weanling diets at levels of 125 and 250 ppm has been shown to stimulate growth in young swine (Edmonds and Baker, 1986; Cromwell et al., 1998). Research has proposed that adding zinc oxide (ZnO), at levels of 2,000 to 3,000 ppm, to the diets of weanling pigs may foster a similar growth-promotional response to that of $CuSO_4$ (LeMieux et al., 1995; McCully et al., 1995). The Zn requirement for nursery pigs given in the NRC (1998) is set at 100 ppm, however pharmacological levels of 2,000 to 3,000 ppm are commonly used in the swine industry. Hahn and Baker (1993) observed a significant increase in average daily gain (ADG) and average daily feed intake (ADFI) when weanling swine diets were formulated with 3,000 ppm Zn from ZnO. Smith et al. (1997) also showed that supplementing starter pigs with

3,000 ppm Zn from ZnO improved growth performance during the first 4 weeks after weaning. The common industry practice of using pharmacological levels of inorganic Zn, coupled with high levels of Cu, as a growth promotants has raised some environmental concerns. Due to low bioavailability of ZnO, excess excretion of Zn into the environment is common (Case and Carlsen, 2002). Interest in using organic zinc in nursery pig diets has increased significantly since Ward et al. (1996) published data showing that the beneficial effects of feeding pharmacological levels of inorganic zinc could be achieved feeding lower levels of organic zinc (250 ppm Zn-methionine) with normal concentrations of inorganic zinc (160 ppm Zn-sulfate).

Smith et al. (1997) found that feeding 3,000 ppm Zn from ZnO improved the growth performance of pigs weaned at 12 days of age, but observed no additive responses between growth-promotional levels of zinc oxide (3,000 ppm) and copper sulfate (250 ppm). Research has shown that in rats, the metallothionien concentration increases when supplemental Zn is added to the diet (Klevey 1987). Metallothionien has a greater affinity for Cu and replaces Zn in normal binding sites, which leads to an increase in free Zn in the body (Ogiso et al. 1979). This competitive binding between the inorganic minerals may be the reason no additive effect is seen between Cu and Zn. The objective of this study was to determine if, by using lower levels of organic zinc coupled with growth promoting levels and NRC recommended levels of copper sulfate, an additive effect exists between the two minerals.

Materials and Methods

General Procedures

All experimental procedures were approved by the University of Georgia Laboratory Animal Care Advisory Committee (AUP# A2007-10205-m1). Three Hundred crossbred pigs

(150 females and 150 castrated males) from the University of Georgia Swine Research Farm were used in the experiment. Pigs (initially 6.27 kg) were weaned at 21 ± 2, days of age into an environmentally controlled growth room inside the University of Georgia's Large Animal Research Unit. Pigs were housed five per pen (1.8m x 0.9m) on woven wire flooring. Each pen was equipped with a self-feeder and one nipple waterer to allow ad libitum consumption of feed and water. Temperature of the room was 31°C initially and was lowered 1-2°C each week thereafter. Body weights and feed intakes were determined weekly throughout the study to allow calculation of average daily gain, average daily feed intake, and gain/feed ratio. Pigs were bled via orbital sinus at days 0, 7, 21 and 35. Following centrifugation, blood serum was collected from heparinized tubes and frozen for later analysis of trace minerals.

Pigs were allotted to ten treatment groups by sex and weight, resulting in 6 replicates per treatment. Pigs were allotted randomly within blocks to pens and treatment diets. A complex Phase I diet formulated to contain 1.5% lysine and 22.7% crude protein was fed days 1-7, followed by a Phase II diet containing 1.3% lysine and 22.9% crude protein days 7-21, and followed then by a Phase III diet containing 1.2% lysine and 20.9% crude protein days 21-35 (Table 1). Individual diets within each experiment were made from a single batch of basal diet mixed with a mineral premix custom made for each treatment. All diets were formulated to meet or exceed the nutrient requirements of NRC (1998) except Zn. The ten dietary treatments composed a 2 x 4 factorial arrangement with either 15 or 250 ppm Cu from $CuSO_4$ and 25, 50, 75, or 100 ppm Zn from organic Zn (Bioplex Zinc, Alltech Inc., Lexington, KY). Two positive control diets contained either 15 or 250 ppm Cu from $CuSO_4$ and 100 ppm Zn from ZnO.

Sample Analysis

Diets were sampled at mixing and stored for later analysis. The diet samples were ground through a 1mm screen, mixed, and analyzed for crude protein by Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI), gross energy by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL), and Zn, Cu, Fe, and Ca by flame atomic absorption spectrophotometry (AAnalyst 400, PerkinElmer Inc., Shelton, CT). Prior to reading on the atomic absorption spectrophotometer, the samples were digested in nitric and perchloric acids (Hill et al., 1983b). P levels of samples were determined calorimetrically (AOAC, 1984).

Blood serum samples were diluted 1:10 with TCA solution, centrifuged, and diluted 1:10 with distilled water, and concentrations of Zn, Cu, and Fe were determined by flame atomic absorption spectrophotometry (AAnalyst 400, PerkinElmer Inc., Shelton, CT). Appropriate external standards were used for instrument standardization and quality control.

Statistical Analysis

The performance data for each week and the overall performance, fecal, and blood data were analyzed as a randomized complete block design (Steel and Torrie, 1980) using the PROC GLM procedure of SAS (1982). Dietary treatments composed a 2 x 4 factorial design. Orthogonal comparisons were made comparing the treatments to the positive control diets. Pigs were blocked by initial weight and equalized for sex. Pen was considered the experimental unit, and replications and treatments were considered fixed effects. Each set of six pens that were allotted to treatments was considered a replication.

Results

Growth Performance

The results of the growth performance are shown in tables 3-3, 3-4, and 3-5.

In phase I, (d 0-7), the addition of high copper (250 ppm $CuSO_4$) increased ADG ($p < 0.001$) compared to the 15 ppm Cu diet (105.6 vs 65.6 g/d). A Cu effect was observed for ADF ($p < 0.001$) between the 250 ppm Cu diet (186.5 g/d) and the 15 ppm Cu diet (147.1 g/d). There was no Zn effect ($p > 0.10$) or Cu by Zn interaction ($p > 0.10$) detected for ADG or ADF during the first week of the study. Gain:feed was increased ($p < 0.01$) during phase I for pigs fed 250 ppm Cu compared to controls. G:F ratio tended ($p = 0.09$) to increase as levels of organic Zn increased from 25 ppm to 75 ppm.

In phase II of the trial (d7-21), a Cu effect ($p < 0.001$) for ADG was observed for the 250 ppm Cu diet compared to the diet supplemented with 15 ppm Cu (Figure 3-1). The addition of organic Zn also had a quadratic effect on ADG (Figure 3-1). The ADG of pigs fed 75 ppm organic Zn was similar to the ADG of pigs fed 100 ppm ZnO (287 vs 293 g/d). Cu ($p < 0.01$) and Zn ($p < 0.02$) effects were also observed on ADF during this phase. The 250 ppm Cu diet had an average ADF of 458.4 g/d compared to 394.5 g/d of the 15 ppm Cu diet. The effects of Zn upon ADF values is shown in Figure 3-2. Just as in ADG, the pigs fed 75 ppm organic Zn had a similar ADF compared to the pigs fed 100 ppm ZnO (441 vs 447 g/d). No Cu by Zn interactions occurred in either ADG or ADF during phase II. A G:F effect occurred between 250 ppm Cu (0.664) and 15 ppm Cu (0.609), however no Zn or Cu by Zn interaction was observed.

During phase III of the nursery trial (d21-35), a copper effect ($p < 0.01$) on ADG was seen between to two dietary levels (499.2 vs 453.8 g/d). There was a trend for a Zn effect ($p = 0.08$) for 100 ppm organic zinc. The 75 ppm organic Zn diet yielded pigs with an average daily gain similar to pigs fed 100 ppm ZnO (499 vs 494 g/d). Cu also had an effect upon ADF ($p < 0.01$) with the 250 ppm diets averaging 835.9 g/d while the 15 ppm Cu diets averaged 768.9 g/d. There was no effect of Zn ($p = 0.29$) observed on ADF of the nursery phase III. There was also no effect of either Cu ($p = 0.51$) or Zn ($p = 0.68$) on the G:F ratio during this phase of growth. No Cu x Zn interactions were observed for ADG ($p = 0.43$), ADF ($p = 0.59$), or G:F ($p = 0.61$).

Over the entire 35 d trial, no Cu by Zn interactions on ADG ($p = 0.91$), ADF ($p = 0.86$), or gain:feed ratio ($p = 0.64$) were detected. The addition of high copper levels (250 ppm) increased ADG (342.4 vs 290.8 g/d, $P < 0.01$), ADF (555.0 vs 494.8 g/d, $P < 0.01$), G:F (0.621 vs 0.589, $p < 0.01$) over the 35 d trial compared to the diets containing 15 ppm Cu. The addition of increasing levels of organic Zn increased ADG (312, 310, 334, and 292 g/d for 25, 50, 75, and 100 ppm organic Zn, respectively) cubically ($p < 0.05$). The addition of 75 ppm organic Zn had the highest ADG and was similar to the ADG of pigs fed 100 ppm ZnO (334 vs 336 g/d). The addition of organic Zn tended ($p < 0.08$) to increase ADF (522, 526, 541, and 492 g/d for 25, 50, 75 and 100 ppm, respectively) cubically. The ADF intake of pigs fed 75 ppm organic Zn was similar to the intake of pigs fed 100 ppm ZnO (541 vs 543 g/d). The addition of organic Zn to diets had no effect on gain:feed ratio ($p > 0.10$).

Serum Cu, Zn, and Fe

The results of serum Cu, Zn, and Fe levels are shown in table 3-6.

In our study, there was no effect of bleed day (day 0, 7, 21, or 35) on serum Cu, Fe, or Zn concentrations. The addition of either organic Zn or ZnO (positive control) to the diets of pigs in the nursery trial, had no effect on serum Cu ($p = 0.63$), Fe ($p = 0.32$), or Zn ($p = 0.34$). Added Cu also had no effect on serum Cu ($p = 0.45$) or serum Zn ($p = 0.67$). However, added Cu had an effect ($p < 0.01$) on serum Fe levels. As the levels of organic Zn increased in the treatments, a trend ($p = 0.08$) was observed for a linear organic Zn interaction in Zn serum concentrations at days 0-35.

Discussion

During the first week of the nursery study, the addition of 250 ppm Cu increased ADG ($p < 0.01$) when compared to 15 ppm Cu. A Cu effect was also found in feed intake ($p < 0.01$) and efficiency ($p < 0.01$) when comparing the two levels of supplementation. The results were similar to the findings of Edmonds et al. (1985) who found that supplementing 250 ppm Cu in the diet markedly and consistently increased rate and efficiency of gain in weanling pigs, particularly during the first week post-weaning. However, no Zn effect or Cu by Zn interaction was observed for ADG or ADF during the first seven days, and only a trend was seen for organic Zn to improve feed efficiency. The ADG data contradicts data reported by Carlson et al. (2004) who reported 125 ppm organic Zn improved ADG during the first week post-weaning. However the dietary organic zinc concentration was lower in this study compared to Carlson et al. (2004). The ADF and feed efficiency data from Carlson et al. (2004) was similar to the results of this study.

The addition of 250 ppm Cu significantly improved ADG, ADF, and feed efficiency from days 7-21 of the study as compared to the 15 ppm supplemented diets. The improvement in growth performance from feeding 250 ppm Cu from $CuSO_4$ is similar to the response seen by Cromwell et al. (1989). Dove (1995) saw a 15.7% increase in feed intake by pigs fed 250 ppm Cu compared to those fed 15 ppm Cu, the same two levels used in this study. A significant Zn response was seen between the varying levels of organic Zn on ADG and ADF. Carlson et al. (1995) reported a 3 week nursery trial where pigs fed both 250 ppm Cu and varying levels of inorganic Zn gained faster than those fed adequate levels of each mineral. The 75 ppm organic Zn supplemented diet was similar to the positive control which contained 100 ppm Zn from ZnO. Case and Carlson (1992) noted that the plasma, tissue, urine, and fecal Zn concentrations of pigs fed 500 ppm added Zn from organic or inorganic sources was similar and indicated a similar bioavailability exists between the 2 types.

Throughout phase III of the study, a significant increase in ADG and ADF was observed in pigs fed diets containing 250 ppm Cu compared to 15 ppm Cu. Hill et al. (2000) documented that pigs fed high concentrations of Zn, Cu, and Zn + Cu consumed significantly more feed and acknowledged higher gains. In this study, only a slight trend for an increase in ADG was seen from the increase in Zn levels in the diets. Smith et al. (1997) found that added inorganic Zn at 110 ppm increased growth in pigs for 28 day post-weaning. These results were similar to this study, as an increase in growth was seen from day 0-21 and a trend for an increase was then observed from day 21-35.

Over the 35 day trial, growth responses were seen by increasing Cu and Zn levels, yet no Cu by Zn interactions were observed in ADG, ADF, or in feed efficiency. Creech et al. (2004) documented that pigs fed diets containing levels of 5 ppm Cu and 25 ppm Zn from organic and inorganic sources had similar ADG and AGF to those fed diets containing 25 ppm Cu and 150 ppm Zn from inorganic sources. Creech et al. (2004) observed no additive responses between the two minerals. The lack of additive response between the minerals may be due to increasing levels of dietary Zn inhibiting the uptake of Cu through metallothionien. Metallothionien concentration has been shown to increase when Zn is supplemented in diets. Metallothionien displaces Zn from normal binding sites with Cu. A decrease in the amount of available Cu may cause an imbalance of Cu and Zn which inhibits nutrient metabolism and may explain the lack of interaction between the two minerals (Klevay, 1987).

The addition of Zn in the diets had no significant effect on serum Cu, Fe, or Zn. Added Cu in the test diets had a significant effect upon serum Fe concentrations ($p < 0.01$) while posing no significant impact upon serum Cu or Zn levels. Data from Hill et al. (2000) found that when Cu is fed in adequate concentrations, regardless of the Zn concentration, plasma Fe concentrations will be higher than with concentrations of 250 ppm Cu and adequate Zn. Our results show that 15 ppm added Cu in the diets had a greater impact on serum Fe concentrations than did 250 ppm Cu. The data from Hill et al. (2000) reported that a synergistic effect on plasma Fe and Zn was observed when added levels of Zn and Cu were fed. This could explain why Cu exhibited an effect on serum Fe and also why a trend was observed for a linear effect of organic Zn upon serum Zn levels. Carlson et al. (1997) documented that metallothionien was increased in the duodenum of pigs fed pharmacological levels of Zn. Since metallothionien preferentially binds Cu, it is believed to cause a Cu deficiency when high levels of Zn are fed (Hill et al. 1983a). No significant differences in serum Cu were noted in this study which may be due to coupling high levels of Cu with Zn in levels of 100 ppm or less.

Implications

The significant Cu effect of the 250 ppm $CuSO_4$ diet on ADG and ADFI over phase 1, 2, and 3 confirms copper sulfate's growth-stimulating properties when added to weanling pig diets. The addition of 75 ppm organic Zn had similar ADG and ADFI to the positive control (100 ppm ZnO). The cubic effect of the four levels of organic Zn on ADG suggests that levels of 25 and 50 ppm organic Zn are too low for optimal intake and growth while 100 ppm organic Zn may have been too high and resulted in a negative effect on growth performance. The lack of Cu x Zn interaction is similar to the results of Hill et al. (1996).

Literature Cited

AOAC. 1984. Official Methods of Analysis (14th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Bunch, R. J., V. C. Speer, V. W. Hays, J. H. Hawbaker, and D. C. Catron. 1961. Effects of copper sulfate, copper oxide and chlortetracycline on baby pig performance. J. Anim. Sci. 20:723.

Carlson, M. S., C. A. Boren, C. Wu, C. E. Huntington, D. W. Bollinger, and T. L. Veum. 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. J. Anim. Sci. 82:1359-1366.

Carlson, M. S., G. M. Hill, and J. E. Link. 1999. Early and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: Effect on metallothionein and mineral concentrations. J. Anim. Sci. 77:1199-1207.

Carlson, M. S., G. M. Hill, J. E. Link, G. A. McCully, D. W. Rozeboom, and R. L. Weavers. 1995. Impact of zinc oxide and copper sulfate supplementation on the newly weaned pig. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

Case, C. L., and M. S. Carlsen. 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J. Anim. Sci. 80:1917-1924.

Creech, B. L., J. W. Spears, W. L. Flowers, G. M. Hill, K. E. Lloyd, T. A. Armstrong, and T. E. Engle. 2004. Effect of dieary trace mineral concentration and source (inorganic vs. chelated) on performance, mineral status, and fecal mineral excretion in pigs from weaning through finishing. J. Anim. Sci. 82:2140-2147.

Cromwell, G. L., M. D. Lindemann, H. J. Monegue, D. D. Hall, and D. E. Orr, Jr. 1998. Tribasic copper chloride and copper sulfate as copper sources for weanling pigs. J. Anim. Sci. 76: 118-123.

Cromwell, G. L., T. S. Stahly, and H. J. Monegue. 1989. Effects of source and level of copper on performance and liver copper stores in weanling pigs. J. Anim. Sci. 67:2996.

Dove, C. R. 1995. The effect of copper level on nutrient utilization of weanling pigs. J. Anim. Sci. 73:166-171.

Dove, C. R., and K. D. Haydon. 1991. The effect of copper addition to diets with various iron levels on the performance and hematology of weanling swine. J. Anim. Sci. 69:2013.

Edmonds, M. S., and D. H. Baker. 1986. Toxic effects of supplemental copper and roxarsone when fed alone or in combination to young pigs. J. Anim. Sci. 63:533-537.

Edmonds, M. S., O. A. Izquierdo, and D. H. Baker. 1985. Feed additive studies with newly weaned pigs: Efficacy of supplemental copper, antibiotics and organic acids. J. Anim. Sci. 60:462.

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

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

Hill, G. M., P. K. Ku, E. R. Miller, D. E. Ullrey, T. A. Losty, and B. L. O'Dell. 1983a. A copper deficiency in neonatal pigs induced by a high zinc maternal diet. J. Nutr. 113:867-872.

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

Klevey, L. M. 1987. Dietary copper: A powerful determinant of chloesterolemia. Med. Hypoth. 24:111.

LeMieux, F. M., L. V. Ellison, T. L. Ward. L. L. Southern, and T. D. Bidner. 1995. Excess dietary zinc for pigs weaned at 28 days. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

McCully, G. A., G. M. Hill, J. E. Link, R. L. Weavers, M. S. Carlsen, and D. W. Rozeboom. 1995. Evaluation of zinc sources for the newly weaned pig. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

NRC. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.

Ogiso, T., N. Ogawa, and T. Miura. 1979. Effect of high dietary zinc on copper absorption in rats. II. Binding of copper and zinc to cytosol proteins in the intestinal mucosa. Chem. Pharm. Bull. 27:515.

SAS. 1985. SAS User’s Guide: Statistics (version 5 Ed.). SAS Inst. Inc., Cary, NC.

Smith, J. W., II, M. D. Tokach, R. D. Goodband, J. L. Nelssen, and B. T. Richert. 1997. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. J. Anim. Sci. 75:1861-1866.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Publishing Co., New York.

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

Table 3-1. Treatment Design

Treatment	Copper ($CuSO_4$)	Zinc
1	15 ppm	25 ppm organic Zn
2		50 ppm organic Zn
3		75 ppm organic Zn
4		100 ppm organic Zn
5		100 ppm ZnO
6	250 ppm	25 ppm organic Zn
7		50 ppm organic Zn
8		75 ppm organic Zn
9		100 ppm organic Zn
10		100 ppm ZnO

Zinc requirement for 8-28 kg pigs is 80-100 ppm (NRC, 1998).
Copper requirement for 8-28 kg pigs is 4-10 ppm (NRC, 1998).
Minerals were premixed with ground corn.

Table 3-2. Diet Composition

Ingredient	Phase 1	Phase 2	Phase 3
Corn	32.05	53.45	60.00
Soybean Meal 49%	20.00	25.70	26.41
Dehydrated Whey	20.00	8.00	-
Lactose	10.00	-	-
Spray Dried Plasma	6.00	-	-
Blood Cells	-	2.00	-
Menhaden Meal	5.00	4.77	4.48
Soybean Oil	3.00	2.90	6.00
Dicalcium Phosphate	2.16	1.50	1.27
Limestone	1.00	1.00	1.00
Common Salt	0.35	0.16	0.25
Vitamin Premix[a]	0.15	0.15	0.15
Custom Mineral Premix[b]	0.15	0.25	0.25
DL-Methionine	0.11	0.12	0.06
L-Lysine HCl	0.03	-	0.13
Calculated Analysis:			
ME[c], mcal/kg	3.14	3.31	3.56
Crude Protein, %	22.72	22.92	20.95
Lysine, %	1.50	1.3	1.20
Calcium, %	1.36	1.10	0.96
Total P, %	1.02	0.80	0.70
Available P, %	0.78	0.56	0.49

[a] Supplied per kg of diet: vitamin A 660 IU; vitamin D 99,000 IU; vitamin E 2,640 IU; vitamin K 264 IU; riboflavin 594 mg; niacin 3,300 mg; vitamin B12 2,640 micrograms.

[b] Supplied per kg of diet: iron 150 mg; manganese 6.0 mg; iodine 0.21 mg; selenium 0.3 mg; copper and zinc varied according to treatment.

[c] Metabolizable Energy.

Table 3-3. Analyzed Diet Composition

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10
Phase 1										
Dry Matter, %	88.02	87.69	87.92	88.35	87.78	88.14	88.26	87.96	87.75	88.03
Ash, %	5.92	5.99	5.83	5.79	5.66	5.69	5.75	5.70	5.68	5.82
Gross Energy, mcal/kg	3.72	3.75	3.72	3.78	3.73	3.73	3.80	3.79	3.76	3.72
Crude Protein, %	22.32	22.45	22.36	22.42	22.01	22.23	22.31	22.30	22.24	22.13
Ca, %	1.31	1.30	1.34	1.34	1.34	1.36	1.34	1.33	1.36	1.34
P, %	0.77	0.75	0.75	0.73	0.74	0.74	0.78	0.77	0.78	0.76
Zn, mg/kg	33	57	81	111	109	32	59	83	109	112
Cu, mg/kg	16	17	16	18	17	260	259	255	252	260
Fe, mg/kg	353	359	372	365	362	371	382	371	366	359
Phase 2										
Dry Matter, %	88.11	88.04	87.65	88.10	87.89	87.93	88.05	87.91	87.86	88.11
Ash, %	5.86	5.84	5.78	5.64	5.69	5.85	5.91	5.96	5.75	5.86
Gross Energy, mcal/kg	3.96	3.94	3.99	4.02	4.01	3.98	4.11	4.08	4.09	3.98
Crude Protein, %	22.45	22.52	22.41	22.48	22.32	22.39	22.45	22.41	22.42	22.39
Ca, %	1.10	1.14	1.12	1.12	1.13	1.09	1.14	1.08	1.11	1.10
P, %	0.61	0.60	0.61	0.62	0.59	0.60	0.62	0.63	0.62	0.61
Zn, mg/kg	34	59	84	116	111	34	62	85	114	111
Cu, mg/kg	18	18	19	20	19	263	260	258	259	261
Fe, mg/kg	377	368	375	382	365	369	389	385	379	369
Phase 3										
Dry Matter, %	88.08	87.72	87.82	88.14	87.85	88.06	88.14	88.35	87.86	88.08
Ash, %	5.83	5.45	5.56	5.54	5.74	5.86	5.71	5.82	5.67	5.90
Gross Energy, mcal/kg	4.15	4.18	4.26	4.03	4.21	4.19	4.16	4.09	4.15	4.32
Crude Protein, %	20.07	20.14	20.25	20.24	20.14	20.21	20.03	20.42	20.15	20.32
Ca, %	0.98	0.96	1.01	1.00	1.04	0.98	0.99	1.01	1.01	1.03
P, %	0.51	0.55	0.51	0.53	0.52	0.52	0.52	0.53	0.56	0.55
Zn, mg/kg	31	58	83	108	114	33	61	84	112	115
Cu, mg/kg	20	21	21	22	23	265	266	263	262	260
Fe, mg/kg	357	365	366	374	371	382	399	387	386	379

Table 3-4. Growth Performance: Average Daily Gain

	15 ppm Cu (from $CuSO_4$)						250 ppm Cu (from $CuSO_4$)						P-value		
	25 ppm OrZn	50 ppm OrZn	75 ppm OrZn	100 ppm OrZn	100 ppm ZnO	SEM	25 ppm OrZn	50 ppm OrZn	75 ppm OrZn	100 ppm OrZn	100 ppm ZnO	SEM	Cu	Zn	Cu x Zn
Day 0-7	51.1	69.2	69.8	59.4	80.1	15.0	94.4	94.9	127.2	88.3	125.4	15.2	<0.01	0.21	0.81
Day 7-21	230.3	235.0	244.1	228.6	264.2	13.5	292.4	300.5	330.6	278.9	322.2	13.6	<0.01	0.02	0.74
Day 21-35	469.2	428.3	484.1	400.6	488.5	21.3	497.5	504.1	513.2	481.1	501.6	22.2	<0.01	0.08	0.43
Day 0-35	290.6	279.8	305.2	263.1	317.5	14.2	334.5	341.2	362.7	321.0	354.9	14.6	<0.01	0.02	0.91

Table 3-5. Growth Performance: Average Daily Feed Intake

	15 ppm Cu (from $CuSO_4$)						250 ppm Cu (from $CuSO_4$)						P-value		
	25 ppm OrZn	50 ppm OrZn	75 ppm OrZn	100 ppm OrZn	100 ppm ZnO	SEM	25 ppm OrZn	50 ppm OrZn	75 ppm OrZn	100 ppm OrZn	100 ppm ZnO	SEM	Cu	Zn	Cu x Zn
Day 0-7	142.2	155.1	144.8	142.1	151.1	12.5	186.2	181.1	200.6	166.1	198.2	12.68	<0.01	0.51	0.65
Day 7-21	379.9	399.1	409.6	368.6	415.3	15.1	457.9	449.6	482.1	423.1	479.0	15.3	<0.01	0.01	0.88
Day 21-35	784.4	786.1	784.1	690.5	799.5	33.02	824.8	828.9	856.8	823.6	845.4	33.3	<0.01	0.29	0.59
Day 0-35	494.2	505.1	506.4	452.1	516.1	18.6	550.4	547.6	575.7	531.9	569.4	18.8	<0.01	0.07	0.86

Table 3-6. Growth Performance: Gain:Feed

	15 ppm Cu (from $CuSO_4$)						250 ppm Cu (from $CuSO_4$)						P-value		
	25 ppm OrZn	50 ppm OrZn	75 ppm OrZn	100 ppm OrZn	100 ppm ZnO	SEM	25 ppm OrZn	50 ppm OrZn	75 ppm OrZn	100 ppm OrZn	100 ppm ZnO	SEM	Cu	Zn	Cu x Zn
Day 0-7	0.36	0.45	0.48	0.42	0.53	0.07	0.51	0.52	0.63	0.53	0.63	0.07	<0.01	0.09	0.98
Day 7-21	0.61	0.59	0.60	0.62	0.64	0.02	0.64	0.67	0.69	0.66	0.67	0.02	<0.01	0.59	0.72
Day 21-35	0.60	0.54	0.62	0.58	0.61	0.02	0.60	0.61	0.60	0.58	0.59	0.02	0.51	0.68	0.61
Day 0-35	0.59	0.55	0.60	0.58	0.62	0.01	0.61	0.62	0.63	0.60	0.62	0.01	0.01	0.37	0.64

Table 3-7. Main Effects of Cu or Zn on Hematocrit (PCV %) [a] and Serum Zn, Fe, and Cu (ppm) [b], day 35

	15 ppm $CuSO_4$	250 ppm $CuSO_4$	SEM	25 ppm Organic Zn	50 ppm Organic Zn	75 ppm Organic Zn	100 ppm Organic Zn	100 ppm ZnO	SEM	Cu P-value	Organic Zn linear P-value	Organic Zn Quadratic P-value	Organic Zn vs. ZnO P-value
Hematocrit	43.25	44.05	1.42	44.15	43.68	43.82	43.03	44.04	1.55	0.52	0.33	0.41	0.39
Serum Zn	1.75	1.71	0.25	1.64	1.70	1.83	1.80	1.66	0.18	0.67	0.08	0.59	0.34
Serum Fe	6.82	6.48	0.19	6.58	6.84	6.64	6.42	6.78	0.31	0.01	0.26	0.11	0.32
Serum Cu	3.32	3.26	0.61	3.24	3.33	3.30	3.28	3.25	0.63	0.45	0.74	0.48	0.63

[a] Hematocrit values ran only on trial 2 and 3.
[b] No effects of day of bleed on serum concentrations were observed.

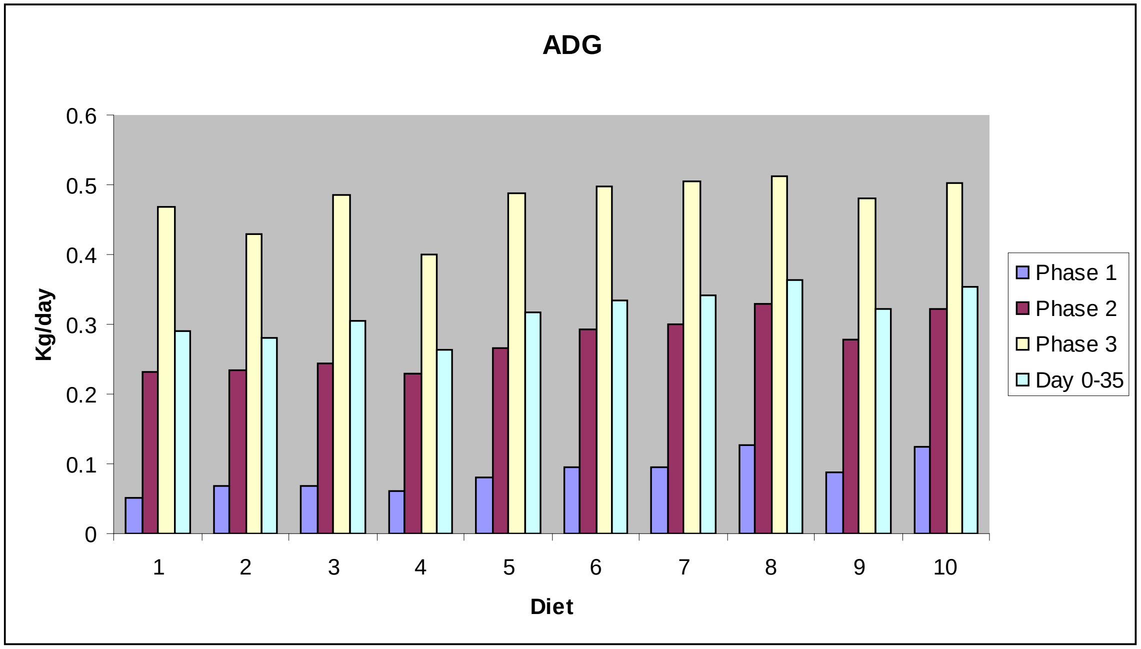

Figure 3-1. Growth Performance: Average Daily Gain

Diet #1 15 ppm $CuSO_4$ + 25 ppm Bioplex Zn
Diet #2 15 ppm $CuSO_4$ + 50 ppm Bioplex Zn
Diet #3 15 ppm $CuSO_4$ + 75 ppm Bioplex Zn
Diet #4 15 ppm $CuSO_4$ + 100 ppm Bioplex Zn
Diet #5 15 ppm $CuSO_4$ + 100 ppm ZnO
Diet #6 250 ppm $CuSO_4$ + 25 ppm Bioplex Zn
Diet #7 250 ppm $CuSO_4$ + 50 ppm Bioplex Zn
Diet #8 250 ppm $CuSO_4$ + 75 ppm Bioplex Zn
Diet #9 250 ppm $CuSO_4$ + 100 ppm Bioplex Zn
Diet #10 250 ppm $CuSO_4$ + 100 ppm ZnO

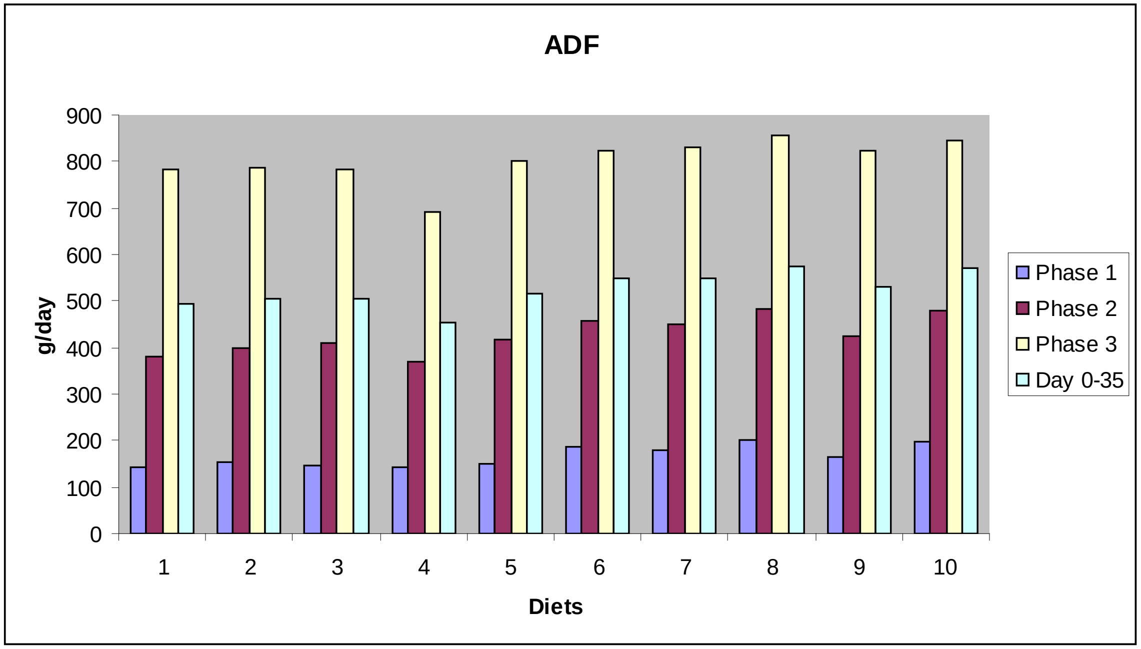

Figure 3-2. Growth Performance: Average Daily Feed Intake

Diet #1 15 ppm $CuSO_4$ + 25 ppm Bioplex Zn
Diet #2 15 ppm $CuSO_4$ + 50 ppm Bioplex Zn
Diet #3 15 ppm $CuSO_4$ + 75 ppm Bioplex Zn
Diet #4 15 ppm $CuSO_4$ + 100 ppm Bioplex Zn
Diet #5 15 ppm $CuSO_4$ + 100 ppm ZnO
Diet #6 250 ppm $CuSO_4$ + 25 ppm Bioplex Zn
Diet #7 250 ppm $CuSO_4$ + 50 ppm Bioplex Zn
Diet #8 250 ppm $CuSO_4$ + 75 ppm Bioplex Zn
Diet #9 250 ppm $CuSO_4$ + 100 ppm Bioplex Zn
Diet #10 250 ppm $CuSO_4$ + 100 ppm ZnO

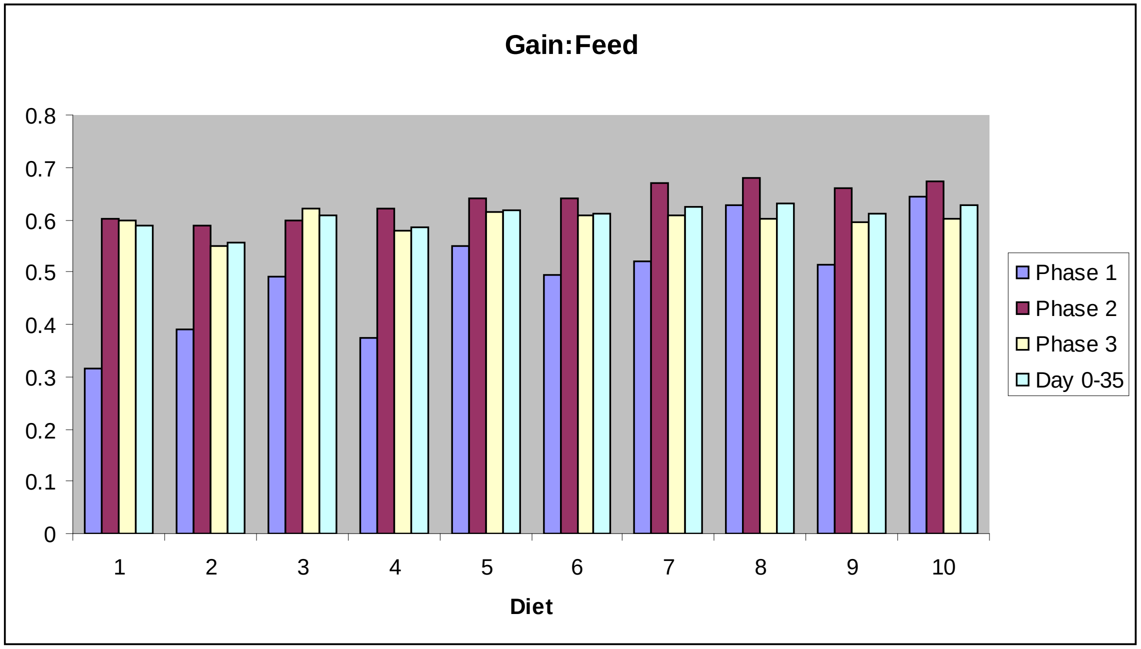

Figure 3-3. Growth Performance: Gain:Feed

Diet #1 15 ppm $CuSO_4$ + 25 ppm Bioplex Zn
Diet #2 15 ppm $CuSO_4$ + 50 ppm Bioplex Zn
Diet #3 15 ppm $CuSO_4$ + 75 ppm Bioplex Zn
Diet #4 15 ppm $CuSO_4$ + 100 ppm Bioplex Zn
Diet #5 15 ppm $CuSO_4$ + 100 ppm ZnO
Diet #6 250 ppm $CuSO_4$ + 25 ppm Bioplex Zn
Diet #7 250 ppm $CuSO_4$ + 50 ppm Bioplex Zn
Diet #8 250 ppm $CuSO_4$ + 75 ppm Bioplex Zn
Diet #9 250 ppm $CuSO_4$ + 100 ppm Bioplex Zn
Diet #10 250 ppm $CuSO_4$ + 100 ppm ZnO

CHAPTER 4

THE DIGESTIBILITY OF COPPER AND ZINC IN NURSERY PIGS FED VARYING LEVELS OF COPPER SULFATE AND ORGANIC ZINC

[1]Marchant, M. R., T. C. Tsai, P. M. Cline, M. J. Azain, and C. R. Dove. To be submitted to The Journal of Animal Science.

Abstract

The objective of this research is to determine the digestibility of copper and zinc in nursery pigs fed diets containing varying levels of $CuSO_4$ and organic zinc. Eight dietary treatments composed a 2 x 2 x 2 factorial arrangement, where diets contained either 4 or 100 ppm Cu as $CuSO_4$; 60 ppm Zn from ZnO or 75 ppm organic Zn; and either 1000 ppm of chromic oxide or 1000 ppm of titanium dioxide. Chromic oxide and Titanium dioxide were added to the diets as indigestible markers to compare the digestibility of the marker. The study involved forty-eight crossbred pigs (24 females and 24 castrated males) with pigs housed in metabolism cages. Over the entire 14 day study, pigs fed diets containing 100 ppm $CuSO_4$ had a significantly higher average daily gain (584.1 vs 532.4 g/day, $p = 0.04$) compared to pigs fed the low Cu diet (4 ppm $CuSO_4$). The high Cu diet also tended to increase average daily feed intake (699.6 vs 659.5 g/day, $p = 0.09$) when compared to the low Cu diet. Zn had no effect on ADG or ADF over the entire 14 day period. No significant interactions or trends were observed by Cu, Zn, or Cu by Zn on gain:feed ratio. The intake and retention of phosphorous and iron were not affected ($p > 0.1$) by the different levels of Cu and Zn in the diets. The retention percentage of calcium was also not affected ($p > 0.1$) by the varying levels of Cu and Zn, however a significant copper interaction ($p = 0.02$) was observed in calcium intake of the pigs fed 100 ppm Cu compared to 4 ppm Cu. Pigs fed the 100 ppm Cu diets had calcium intakes 13% greater than the intakes of those fed the 4 ppm Cu diets. A significant increase in Zn intake ($p < 0.01$) was seen in pigs fed 75 ppm organic Zn compared to those on diets with 60 ppm inorganic Zn. Pigs fed the diets containing organic Zn had a 21% higher intake of Zn compared to those fed inorganic Zn. Zn intake also tended to be affected by a Cu by Zn interaction ($p = 0.06$). Despite the increases in Zn intake, no significant responses were seen in Zn retention. A significant increase in Cu intake (p

< 0.01) was observed in pigs fed 100 ppm Cu compared to 4 ppm Cu. However, a significant increase in Cu retention ($p < 0.01$) was seen in pigs fed 4 ppm Cu rather than 100 ppm Cu. Pigs on the 4 ppm Cu diet actually retained 45% more Cu than did those fed 100 ppm. In addition, a trend for a Cu by Zn interaction ($p = 0.08$) was observed for the retention percentage of Cu. No significant effects of Cu or Zn were observed for fecal freeze dry percentage or fecal oven dry percentage. There were also no effects of Cu or Zn on the digestibility of energy or crude protein. No effects of Cu or Zn were observed on digestibility of Ca, P, Zn, Cu, or Fe when evaluated by total collection (ATTD) or by marker (ATTDM). However, a Cu x marker effect ($p < 0.01$) was observed for Cu digestibility when the apparent total digestibility was evaluated using indigestible markers (ATTDM). A trend for a Zn x marker effect ($p = 0.09$) was observed for Fe digestibility when digestibility was evaluated by ATTDM. When comparing total collection to marker collection, Zn digestibility from total collection of pigs was higher ($p = 0.04$) than Zn digestibility from marker collection of pigs. The Ca digestibility tended ($p = 0.09$) to be greater from total collection than mineral collection as well. However, the Fe digestibility from marker collection was greater ($p = 0.02$) than total collection.

Introduction

The health and growth benefits of adding zinc and copper to weanling swine diets have been well documented (Hahn and Baker, 1993; Case and Carlsen, 2002). The addition of 2000-3000 ppm ZnO and 250 ppm $CuSO_4$ to nursery diets has become a common industry practice, even though the NRC (1998) recommendation is set at only 100 ppm for Zn and 5-6 ppm for Cu. The use of pharmacological levels of inorganic zinc and copper has raised some environmental concerns due to high levels of Cu and Zn found in swine manure. These high levels of Cu and Zn may concentrate in top soil and cause toxicity to plants and microorganisms, which becomes a great concern in areas of intensive pig farming (Case and Carlsen, 2002). However, very little is known about the digestibility of organic zinc sources in weanling pigs. Little research has been published evaluating the effect of organic zinc on copper or copper on organic zinc. The objective of this research is to determine the digestibility of copper and zinc in nursery pigs fed diets containing 4 or 100 ppm Cu as $CuSO_4$ and 60 ppm Zn from ZnO or 75 ppm Zn as organic zinc. Data from this study will be used to determine the most efficient combinations of dietary copper and zinc, as well as help producers formulate diets that will provide optimal growth efficiency.

Materials and Methods

General Procedures

All experimental procedures were approved by the University of Georgia animal care and use committee (AUP# A2007-10205-m1). Forty-eight crossbred pigs (24 females and 24 castrated males) from the University of Georgia Swine Research Farm were used in the experiment. Pigs (initially 10.5 kg) were randomly selected at 42 ± 2 days of age, and brought into an environmentally controlled growth room inside the University of Georgia's Large Animal Research Unit. Pigs used for this experiment were weaned at 21 ± 3 days of age and fed a common starter ration for three weeks before the study began. The temperature of the room was set at 24°C and a 12-hour light/dark cycle was set. The pigs were trained to meal feeding twice daily at approximately 0800 and 1600 hours at the rate of 3-4% of body weight per day. The allowed feeding time was 50-60 minutes per feeding. The total feed consumption was recorded after each meal as well as feed refusals and spillage. Pigs were weighed at day 0 and housed individually in stainless steel metabolism cages (0.71m x 0.81m). Each cage was equipped with a water nipple, to allow ad libitum consumption of water, a feeding bowl holder, and plastic-coated expanded metal flooring. Pigs were given a 10 day adaptation period, and on day 10 the pigs were weighed, pens were cleaned, and cages were set up for the four day collection period. During the four day collection period, the total fecal excretion was collected and recorded from each cage twice daily. Urine was also collected twice daily into containers with 25 ml of 3N Hydrochloric acid. The total urine volume was recorded twice daily, and a 10% aliquot of the total urine collection was stored in a 1L bottle. Fecal and urine samples were stored at -20°C until further analysis. The screens and trays from the metabolism cages were cleaned and washed after each collection.

All pigs were fed a standard starter diet for the three weeks between weaning and the initiation of the study. During the trial, a corn-soybean meal basal diet was fed with each treatment meeting or exceeding NRC (1998) recommendations except for Zn. Pigs were allotted to eight treatment groups by weight and equalized by sex, resulting in 3 replicates per treatment. Pigs were allotted randomly within blocks to cages and treatments. A complex Phase III diet was fed for the entire 14 d of the study (Table 1.1). Individual diets within each experiment were made from a single batch of basal diet mixed with a mineral premix custom made per treatment. The eight dietary treatments, which composed a 2 x 2 x 2 factorial arrangement, where diets contained either 4 or 100 ppm Cu as $CuSO_4$; 60 ppm Zn from ZnO or 75 ppm organic Zn; and either 1000 ppm of chromic oxide or 1000 ppm of titanium dioxide. Chromic oxide and Titanium dioxide were added to the diets as indigestible markers to compare the digestibility of total collection and marker.

Sample Analysis

Fecal samples collected from each pig over the entire study were thawed and mixed by blender. The total sample weight from each pig was recorded. Two fecal sub-samples were prepared. One of the fecal sub-samples was oven dried at 65 °C for 72 hours, and the dry matter weight was recorded. Another sub-sample was freeze dried and the freeze-dry matter weight was recorded. Freeze dried samples were ground in a Wiley mill to pass through a 1.0mm screen, and were used for mineral, crude protein, and gross energy determination. Diet and fecal CP were analyzed using a Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Diet and fecal gross energy were determined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL). The diet and fecal sub-samples were then digested in nitric and perchloric acids (Hill et al., 1983b), and analyzed for Zn, Cu, Fe, and Ca by flame atomic absorption spectrophotometry (AAnalyst 400, PerkinElmer Inc., Shelton, CT). Appropriate external standards were used for instrument standardization and quality control. Cr, Ti, and P levels of fecal and diet sub-samples were determined colorimetrically (AOAC, 1984).

Digestibility

Apparent nutrient digestibility was calculated using total collection and the external marker. Apparent total tract digestibility of nutrients was determined by the following equation:

$$\text{ATTD} = \frac{([Nuti.]_{intake} - [Nuti.]_{feces})}{[Nuti.]_{intake}} \text{ x } 100$$

Apparent total digestibility of nutrient by marker was calculated by the following equation:

$$\text{ATTDM} = 1 - \frac{([\text{marker}]_{feed} \text{ x } [Nuti.]_{feces})}{([\text{marker}]_{feces} \text{ x } [\text{Nuti}]_{feed})}$$

Statistical Analysis

All data were analyzed using PROC GLM procedure of SAS (1982) as a 2 x 2 x 2 design with main effects of level of Cu (4 or 100), level of Zn (60 or 75), and marker (chromic oxide or titanium dioxide). Pigs were equalized for sex. Each pig was used as the experimental unit in ANOVA.

Results

Growth Performance

The results of growth performance are shown in Table 4-3.

During the acclimation period (day 0-10), the addition of 100 ppm copper (from $CuSO_4$) significantly increased average daily gain (652.7 vs 591.6 g/day, $p = 0.03$) compared to diets containing 4 ppm Cu. However, no significant copper effects were observed on average daily feed intake or gain:feed ratio during the first ten test days. The addition of either 60 ppm inorganic Zn or 75 ppm organic Zn produced no significant Zn effects on ADG, ADF, or G:F. Also, no Cu by Zn interactions were observed on day 0-10.

During the collection period (day 11-14), a trend for a Cu by Zn interaction ($p = 0.06$) was observed for ADG. The ADG tended to increase as the level of Cu in the diet increased (from 4 ppm to 100 ppm) along with the level of Zn (from 60 ppm ZnO to 75 ppm organic Zn). Diets formulated with 100 ppm Cu tended to increase average daily feed intake (803.5 vs 751.0 g/day, $p = 0.08$) when compared to the 4 ppm Cu diet during days 10-14. There were no significant Cu, Zn, or Cu by Zn interactions observed for gain:feed ratio during this four day period of the study.

Over the entire 14 day study, pigs fed diets containing 100 ppm $CuSO_4$ had a higher average daily gain (584.1 vs 532.4 g/day, $p = 0.04$) compared to pigs fed the low Cu diet (4 ppm $CuSO_4$). The high Cu diet also tended to increase average daily feed intake (699.6 vs 659.5 g/day, $p = 0.09$) when compared to the low Cu diet. Zn had no effect on ADG or ADF over the entire 14 day period. No significant interactions or trends were observed by Cu, Zn, or Cu by Zn on gain:feed ratio.

Intake and Retention

The results of mineral intake and retention are shown in table 4-4.

The intake and retention of phosphorous and iron were not affected ($p > 0.1$) by the different levels of Cu and Zn in the diets. Calcium retention was not affected ($p > 0.1$) by the varying levels of Cu and Zn, however a copper effect ($p = 0.02$) was observed in calcium intake of the pigs fed 100 ppm Cu compared to 4 ppm Cu. Pigs fed the 100 ppm Cu diets had calcium intakes 13% greater than the intakes of those fed the 4 ppm Cu diets. A significant increase in Zn intake ($p < 0.01$) was seen in pigs fed 75 ppm organic Zn compared to those on diets with 60 ppm inorganic Zn. Pigs fed the diets containing organic Zn had a 21% higher intake of Zn compared to those fed inorganic Zn. Zn intake also tended to be affected by a Cu by Zn interaction ($p = 0.06$). Despite the increases in Zn intake, no significant responses were seen in Zn retention ($p > 0.10$). An increase in Cu intake ($p < 0.01$) was observed in pigs fed 100 ppm Cu compared to 4 ppm Cu. However, an increase in Cu retention ($p < 0.01$) was seen in pigs fed 4 ppm Cu rather than 100 ppm Cu. Pigs on the 4 ppm Cu diet actually retained 45% more Cu than did those fed 100 ppm. In addition, a trend for a Cu by Zn interaction ($p = 0.08$) was observed for the retention percentage of Cu.

Digestibility of Minerals

The results of mineral digestibility are shown in tables 4-5 and 4-6.

No effects of Cu or Zn were observed on digestibility of Ca, P, Zn, Cu, or Fe when evaluated by total collection (ATTD) or by marker (ATTDM). However, a Cu x marker effect ($p < 0.01$) was observed for Cu digestibility when the apparent total digestibility was evaluated using indigestible markers (ATTDM). Pigs fed treatments containing titanium dioxide as a marker had 11% higher Cu digestibility than did those fed treatments containing chromic oxide. A trend for a Zn x marker effect ($p = 0.09$) was observed for Fe digestibility when digestibility was evaluated by ATTDM. In this case, pigs fed treatments containing chromic oxide tended to have a greater Fe digestibility than did those fed titanium dioxide. No differences in markers (chromic oxide x titanium dioxide) were observed when digestibility was evaluated by ATTDM. When comparing total collection to marker collection, Zn digestibility from total collection of pigs was higher ($p = 0.04$) than Zn digestibility from marker collection of pigs. The Ca digestibility tended ($p = 0.09$) to be greater from total collection than mineral collection as well. However, the Fe digestibility from marker collection was greater ($p = 0.02$) than total collection.

Fecal Dry Matter, Energy, and Crude Protein

No effects of Cu or Zn were observed for fecal freeze dry percentage or fecal oven dry percentage. There were also no effects of Cu or Zn on the digestibility of energy or crude protein. No effects of sex were observed on digestibility or retention of minerals.

Discussion

The effects of added dietary copper and zinc on the growth performance of nursery pigs have been thoroughly documented (Bunch et al., 1961; Hahn and Baker, 1993; LeMieux et al. 1995; Case and Carlsen, 2002). Historically, pharmacological levels of Cu from $CuSO_4$ and inorganic Zn from ZnO has been used achieve these increases in growth performance (Hahn and Baker, 1993; Smith et al. 1997). The addition of Cu sulfate provided at 125 to 250 ppm Cu has been shown to stimulate growth performance (Apgar et al., 1995; Cromwell et al., 1998). In this study, Zn was provided at levels of 75 ppm from organic Zn or 60 ppm from ZnO. The 75 ppm organic Zn level was used because in the previous study this level yielded the best growth performance data and was most similar to 100 ppm ZnO. 60 ppm ZnO was used as a comparison to growth performance and digestibility of the organic Zn. When $CuSO_4$ was supplemented at 100 ppm in our 14 day trial, average daily gain (P = 0.04) was improved and average daily feed intake (P = 0.09) tended to improve compared to diets supplemented at only 4 ppm. Despite the differences in ADG and ADF, the different levels of added Cu had no significant effects on feed efficiency. Our results in feed efficiency are in contrast to the results of Hill et al. (2000) who showed a significant improvement in gain:feed ratio of pigs fed high Cu compared to adequate levels. However, pigs in this study were only supplemented 14 days, beginning 21 days after weaning. Also, high Cu diets in this study contained 100 ppm Cu while diets of Hill et al. (2000) contained 250 ppm Cu.

In this study, the addition 75 ppm organic Zn failed to yield a significant difference in ADG, ADF, or feed efficiency when compared to added inorganic Zn (from ZnO) in levels of 60 ppm. A study by Carlson et al. (1995) noted an increase in growth performance from Zn diets, however it compared pharmacological levels of Zn (3,000 ppm) to adequate levels while this study compared 75 ppm from an organic source to 60 ppm from an inorganic source. Smith et al. (1997) reported a significant increase in growth from pharmacological levels of Zn fed on day 0-14 post-weaning, yet this study evaluated growth performance from dietary additions during days 21-35 post-weaning.

The varying levels of Cu and Zn in the diets had no effect on the intake or retention of phosphorous and iron. The retention of calcium was also unaffected, however the pigs fed 100 ppm Cu had a 13% greater Ca intake (p = 0.02) compared to those fed adequate levels. Dove (1995) also noted a significant effect of high Cu (250 ppm) on calcium intake compared to adequate Cu (15 ppm). Dove's (1995) research also coincided with this study by showing no significant effect of Cu on Ca retention, P intake, or P retention. Apgar and Kornegay (1996) found that absorption and retention percentages of Fe in pigs were similar across different treatment levels of Cu as well. This study also showed that pigs fed 75 ppm organic Zn had a 21% higher Zn intake ($p < 0.01$) than those fed 60 ppm inorganic Zn. These results contrast research by van Heughten et al. (2003) and Creech et al. (2004) which showed an intake of organic Zn similar to the intake of organic Zn. A trend for a Cu by Zn interaction ($p = 0.06$) was also seen for Zn intake. Despite the previously mentioned effects on intake, no significant differences were seen in Zn retention. Our findings compare with research by Wedekind et al. (1994) who reported that neither inorganic Zn as ZnO nor organic Zn as Zn methionine or Zn lysine provided more bioavailable Zn than Zn sulfate when three different concentrations of each source were fed to 25- to 90-kg pigs. Wedekind observed that high dietary Ca accentuates the effect of phytate on Zn bioavailability; therefore the availability of organic Zn sources, relative to inorganic sources, may depend on amounts of antagonistic factors such as Ca, phytate, and fiber. Case and Carlson (2002) also found that urine and fecal Zn concentrations of pigs fed 500 ppm added Zn as either organic or inorganic sources were similar, indicating no difference in Zn retention. However, it has been speculated that organic Zn enhances growth through a systemic effect within the body rather than an enteric effect in the intestinal tract, which would make organic sources more bioavailable than inorganic sources (Case and Carlson, 2002). This speculation directly contrasts results from this study. A significant increase in Cu intake ($p < 0.01$) was observed in pigs fed 100 ppm Cu compared to 4 ppm Cu. This effect was due to the sheer difference in dietary Cu levels in the test diets. However, pigs on the 4 ppm Cu diet actually retained 45% more Cu ($p < 0.01$) than did those fed 100 ppm. These results contrast those of Apgar and Kornegay (1996) who found that pigs fed elevated Cu absorbed more than those fed control diets. Moore et al. (1986) showed that younger pigs may absorb higher levels of dietary Cu than older pigs. In this study, it is uncertain but pigs may have better digested the lower levels of Cu in the diets due to a response to the requirement in the body.

Pigs fed treatments containing titanium dioxide as a marker had 11% higher Cu digestibility than did those fed treatments containing chromic oxide. These results are consistent with those of Jagger et al. (1992) who found that titanium dioxide, used as an inert marker at 1 ppm, had a higher recovery than did chromic oxide. However, pigs fed treatments containing chromic oxide tended to have a greater Fe digestibility than did those fed titanium dioxide, which disputes this data. This research also contrasts results from Jagger et al. (1992) by displaying no marker effects between chromic oxide and titanium dioxide when digestibility was evaluated by ATTDM. Results from this study suggest that no difference in digestibility exists between 60 ppm inorganic Zn and 75 ppm organic Zn. Since differences existed between total collection (ATTD) and marker collection (ATTDM), this research shows that total collection may be a more efficient method to evaluate the digestibility of Zn and Ca in nursery age swine.

Treatments were equalized for gender, however no effects of sex were observed on digestibility or retention of minerals. This suggests that digestibility of minerals does not significantly vary by gender in nursery aged pigs.

Implication

The benefit of 100 ppm added Cu on ADG and ADF was observed. No differences in 60 ppm inorganic Zn and 75 ppm organic Zn on growth performance were observed. 75 ppm organic Zn increased 21% over 60 ppm inorganic Zn. 100 ppm Cu increased Ca and Cu intake, however 4 ppm Cu increased retention by 45%. Further studies should be done to examine the effect of low levels of Cu upon mineral retention. Since differences existed between total collection (ATTD) and marker collection (ATTDM), this research shows that either method may be efficiently used to evaluate the digestibility of nutrients but total collection may result in higher values for certain minerals.

Literature Cited

AOAC. 1984. Official Methods of Analysis (14th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Apgar, G. A., E. T. Kornegay, M. D. Lindemann, and D. R. Notter. 1995. Evaluation of copper sulfate and a copper lysine complex as growth promoters for weanling swine. J. Anim. Sci. 73:2640-2646.

Apgar, G. A. and E. T. Kornegay. 1996. Mineral balance of finishing pigs fed copper sulfate or a copper-lysine complex at growth-stimulating levels. J. Anim. Sci. 74(7):1594-1600.

Bunch, R. J., V. C. Speer, V. W. Hays, J. H. Hawbaker, and D. C. Catron. 1961. Effects of copper sulfate, copper oxide and chlortetracycline on baby pig performance. J. Anim. Sci. 20:723.

Carlson, M. S., G. M. Hill, J. E. Link, G. A. McCully, D. W. Rozeboom, and R. L. Weavers. 1995. Impact of zinc oxide and copper sulfate supplementation on the newly weaned pig. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

Case, C. L., and M. S. Carlsen. 2002. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J. Anim. Sci. 80:1917-1924.

Creech, B. L., J. W. Spears, W. L. Flowers, G. M. Hill, K. E. Lloyd, T. A. Armstrong, and T. E. Engle. 2004. Effect of dieary trace mineral concentration and source (inorganic vs. chelated) on performance, mineral status, and fecal mineral excretion in pigs from weaning through finishing. J. Anim. Sci. 82:2140-2147.

Cromwell, G. L., M. D. Lindemann, H. J. Monegue, D. D. Hall, and D. E. Orr, Jr. 1998. Tribasic copper chloride and copper sulfate as copper sources for weanling pigs. J. Anim. Sci. 76: 118-123.

Dove, C. R. 1995. The effect of copper level on nutrient utilization of weanling pigs. J. Anim. Sci. 73:166-171.

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

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

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

Jagger, S., J. Wiseman, D. J. Cole, and J. Craigon. 1992. Evaluation of inert markers for the determination of ileal and fecal apparent digestibility values in the pig. Br. J. Nutr. 68(3):729-39.

LeMieux, F. M., L. V. Ellison, T. L. Ward. L. L. Southern, and T. D. Bidner. 1995. Excess dietary zinc for pigs weaned at 28 days. J. Anim. Sci. 73(Suppl. 1):72(Abstr.).

NRC. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.

SAS. 1985. SAS User’s Guide: Statistics (version 5 Ed.). SAS Inst. Inc., Cary, NC.

Smith, J. W., II, M. D. Tokach, R. D. Goodband, J. L. Nelssen, and B. T. Richert. 1997. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early-weaned pigs. J. Anim. Sci. 75:1861-1866.

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.

Table 4-1. The 2 x 2 x 2 Design Metabolism Study

Treatment	Marker	$CuSO_4$	Zinc
1	1000 ppm Chromic oxide (Cr_2O_3)	100 ppm	75 ppm Organic Zn
2		100 ppm	60 ppm ZnO
3		4 ppm	75 ppm Organic Zn
4		4 ppm	60 ppm ZnO
5	1000 ppm Titanium dioxide (TiO_2)	100 ppm	75 ppm Organic Zn
6		100 ppm	60 ppm ZnO
7		4 ppm	75 ppm Organic Zn
8		4 ppm	60 ppm ZnO

Zinc requirement for 8-28 kg pigs is 80-100 ppm (NRC, 1998).
Copper requirement for 8-28 kg pigs is 4-10 ppm (NRC, 1998).
Minerals were premixed with ground corn.

Table 4-2. Diet Composition

Ingredient	% in Diet
Corn	63.84
Soybean Meal 49%	29.41
Fat	2.98
Dical Phosphate	1.93
Limestone	0.64
Salt	0.35
Lysine HCl	0.20
Vitamin Premix[a]	0.25
DL-Methionine	0.05
Mineral Premix[b]	0.25
Marker (Cr_2O_3 or TiO_2)[c]	0.1
Calculated Analysis:	
ME[d], mcal/kg	3.52
Crude Protein, %	20.20
Lysine, %	1.20
Calcium, %	0.96
Total P, %	0.70
Available P, %	0.49

[a] Supplied per kg of diet: vitamin A 660 IU; vitamin D 99,000 IU; vitamin E 2,640 IU; vitamin K 264 IU; riboflavin 594 mg; niacin 3,300 mg; vitamin B12 2,640 micrograms.
[b] Supplied per kg of diet: iron 150 mg; manganese 6.0 mg; iodine 0.21 mg; selenium 0.3 mg; copper and zinc varied according to treatment.
[c] Chromic oxide or Titanium dioxide used as indigestible markers.
[d] Metabolizable Energy.

Table 4-3. Analyzed Diet Composition

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Dry Matter, %	88.96	88.45	88.56	88.68	88.54	88.34	88.68	88.52
Ash, %	5.78	5.81	5.86	5.92	5.75	5.79	5.85	5.74
Gross Energy, mcal/kg	3.97	3.92	3.96	4.01	4.11	3.89	3.96	3.95
Crude Protein, %	20.12	20.50	20.31	20.14	20.10	20.13	20.14	20.07
Ca, %	0.99	0.98	1.01	1.04	0.99	0.97	0.98	0.98
P, %	0.56	0.51	0.52	0.52	0.49	0.54	0.57	0.57
Zn, mg/kg	82	69	84	71	84	70	81	68
Cu, mg/kg	107	109	8	7	109	110	8	8
Fe, mg/kg	371	373	378	365	369	377	369	378

Table 4-4. Growth Performance

Diet									P-Value			
Marker	1000 ppm Chromic Oxide				1000 ppm Titanium Dioxide							
Copper (ppm)*	4	4	100	100	4	4	100	100				
Zinc (ppm)**	60	75	60	75	60	75	60	75	SEM	Cu	Zn	Cu x Zn
ADG, g/d												
Day 0-10	512.84	639.45	636.58	704.01	577.70	636.73	630.38	639.98	24.91	0.03	0.93	0.21
Day 10-14	307.06	436.5	379.81	454.45	382.65	410.99	393.99	422.33	22.94	0.29	0.98	0.06
Day 0-14	454.05	581.47	563.22	632.70	521.97	572.23	562.84	577.79	21.26	0.04	0.94	0.11
ADF, g/d												
Day 0-10	575.42	648.22	637.51	701.98	616.36	646.31	630.86	662.01	23.49	0.12	0.57	0.14
Day 10-14	666.31	779.79	812.42	824.93	774.50	783.46	752.11	824.54	29.79	0.08	0.87	0.11
Day 0-14	601.39	685.81	687.49	737.11	661.54	685.50	665.51	708.45	23.83	0.09	0.73	0.11
Gain:Feed												
Day 0-10	0.89	0.98	0.98	0.98	0.93	0.98	0.98	0.96	0.12	0.34	0.99	0.83
Day 10-14	0.46	0.56	0.47	0.55	0.50	0.51	0.48	0.54	0.14	0.95	0.97	0.26
Day 0-14	0.76	0.83	0.81	0.85	0.66	0.66	0.62	0.68	0.13	0.54	0.91	0.73

*Cu from $CuSO_4$;**Zn at 60 ppm from ZnO; Zn at 75 ppm from organic Zn.

Table 4-5. Mineral Retention

Diet									P-Value			
Marker	1000 ppm Chromic Oxide				1000 ppm Titanium Dioxide							
Copper (ppm)*	4	4	100	100	4	4	100	100				
Zinc (ppm)**	60	75	60	75	60	75	60	75	SEM	Cu	Zn	Cu x Zn
P intake, g/d	1.30	1.35	0.99	1.29	1.83	1.55	1.73	1.75	1.07	0.50	0.84	0.22
P retention, %	67.26	63.76	60.14	62.23	70.63	68.02	70.14	72.76	3.02	0.86	0.92	0.31
Ca intake, g/d	1.25	1.32	1.13	0.96	1.27	1.09	1.08	1.17	0.06	0.02	0.44	0.89
Ca retention, %	70.09	65.23	68.47	59.27	68.19	67.33	64.21	68.28	3.27	0.35	0.63	0.89
Zn intake, mg/d	67.47	49.89	60.17	57.40	65.93	51.19	60.62	51.49	2.71	0.65	<0.01	0.06
Zn retention, %	60.21	64.41	63.43	55.22	67.71	66.42	65.62	62.28	4.79	0.52	0.65	0.45
Cu intake, mg/d	36.79	34.41	1.02	1.59	27.46	26.77	2.86	2.43	4.64	<0.01	0.18	0.18
Cu retention, %	38.07	31.96	40.26	39.52	33.11	35.36	58.94	62.65	6.55	<0.01	0.26	0.08
Fe intake, mg/d	28.34	17.88	33.54	10.72	16.99	15.87	11.41	25.53	5.19	0.75	0.45	0.16
Fe retention, %	13.51	36.41	51.73	13.68	39.01	34.14	29.57	24.01	11.13	0.92	0.56	0.17

*Cu from $CuSO_4$

** Zn at 60 ppm from ZnO; Zn at 75 ppm from organic Zn.

Table 4-5 Apparent Total Tract Digestibility of Nutrient by Marker (ATTDM), %

Diet									P-Value			
Marker	1000 ppm Chromic Oxide				1000 ppm Titanium Dioxide							
Copper (ppm)*	4	4	100	100	4	4	100	100				
Zinc (ppm)**	60	75	60	75	60	75	60	75	SE	Cu x Marker	Zn x Marker	Cr_2O_3 x TiO_2
Ca	65.8	67.6	69.6	66.6	57.8	72.4	64.0	63.2	2.1	0.69	0.38	0.29
P	54.4	56.1	64.1	61.5	63.9	70.8	71.5	72.3	2.5	0.66	0.61	0.11
Zn	66.9	65.5	69.4	66.0	57.7	71.3	65.0	66.9	3.9	0.59	0.72	0.88
Cu	33.8	30.2	35.6	38.1	32.2	34.6	40.1	45.6	3.5	<0.01	0.66	0.70
Fe	47.2	39.4	45.8	40.3	41.0	47.9	37.6	40.9	1.4	0.76	0.09	0.24

*Cu from $CuSO_4$

** Zn at 60 ppm from ZnO; Zn at 75 ppm from organic Zn.

Table 4-6 Apparent Total Tract Digestibility of Nutrients (ATTD), %

Diet									P-Value			
Marker	1000 ppm Chromic Oxide				1000 ppm Titanium Dioxide							
Copper (ppm)*	4	4	100	100	4	4	100	100				
Zinc (ppm)**	60	75	60	75	60	75	60	75	SE	Cu	Zn	Total x Marker
Ca	73.3	65.7	67.7	67.4	71.6	73.8	68.9	67.5	3.4	0.44	0.56	0.09
P	69.9	59.6	52.4	64.6	71.0	74.5	74.2	72.1	3.2	0.59	0.81	0.24
Zn	73.8	65.0	69.0	68.4	70.8	73.8	69.9	69.5	3.8	0.78	0.72	0.04
Cu	24.7	27.3	22.3	30.8	28.5	28.2	29.7	33.0	3.5	0.28	0.14	0.11
Fe	45.1	35.3	43.7	38.2	38.9	45.8	35.5	38.7	2.6	0.76	0.79	0.02

*Cu from $CuSO_4$

** Zn at 60 ppm from ZnO; Zn at 75 ppm from organic Zn.

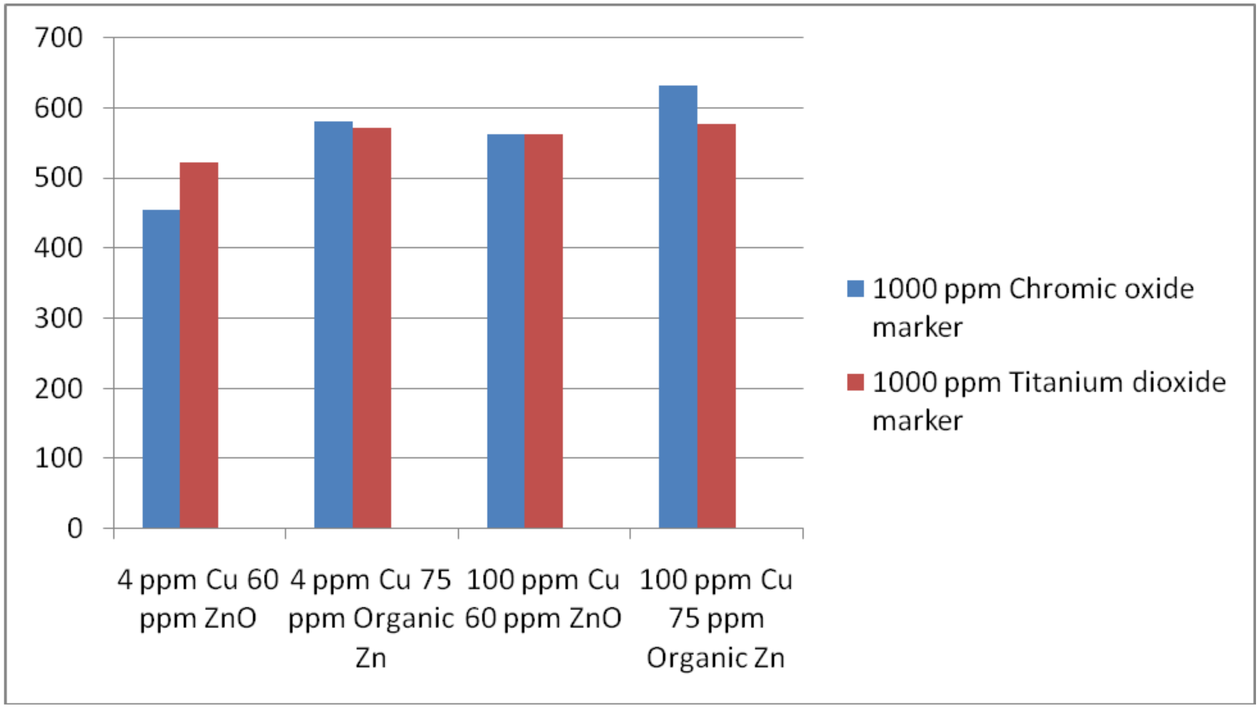

Figure 4-1. Average Daily Gain

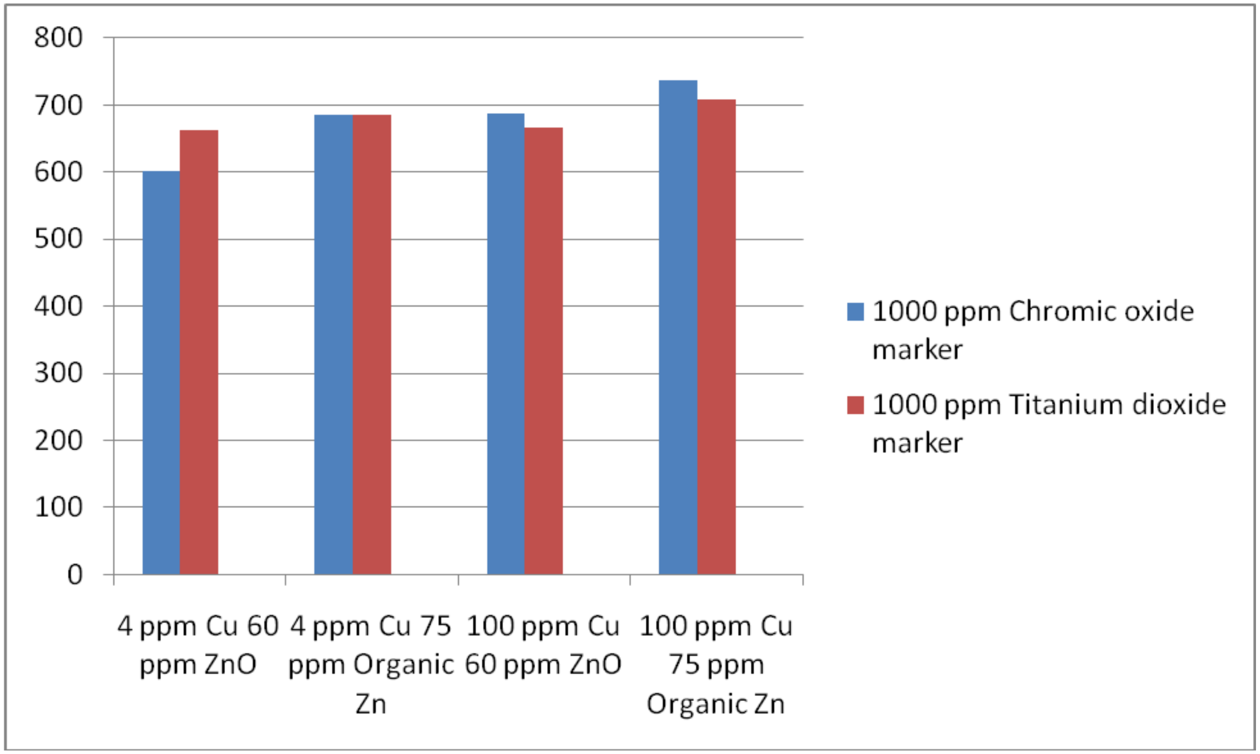

Figure 4-2. Average Daily Feed Intake

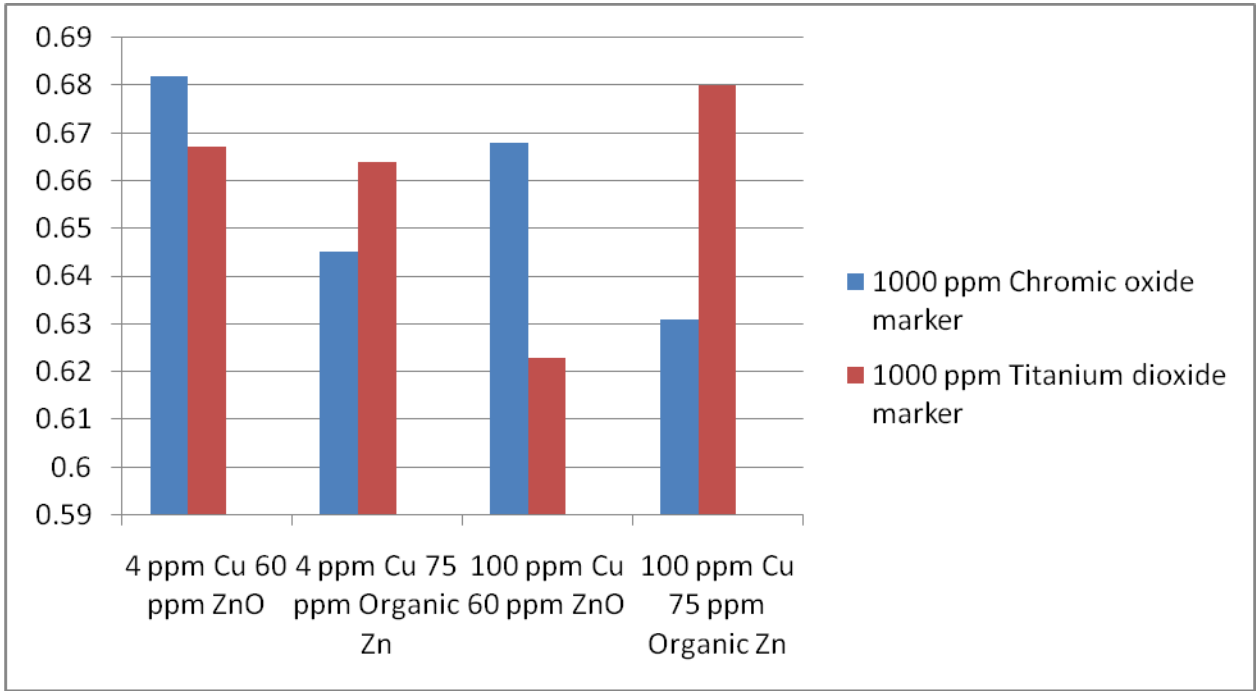

Figure 4-3. Gain:Feed

CHAPTER 5

CONCLUSION

The significant Cu effect of the 250 ppm $CuSO_4$ diet on ADG and ADFI over all 3 phases of experiment 1 confirms copper sulfate's growth-stimulating properties when added to weanling pig diets. The addition of 75 ppm organic Zn had similar ADG and ADFI to the positive control (100 ppm ZnO). The cubic effect of the four levels of organic Zn on ADG suggests that levels of 25 and 50 ppm organic Zn are too low for optimal intake and growth while 100 ppm organic Zn may have been too high and resulted in a negative effect on growth performance. The lack of Cu x Zn interaction is similar to previously reported results.

The benefit on growth performance of 100 ppm added $CuSO_4$ in the diets of nursery pigs was observed in experiment 2. No differences between 60 ppm inorganic Zn and 75 ppm organic Zn were observed upon growth characteristics. 75 ppm organic Zn increased 21% over 60 ppm inorganic Zn. 100 ppm Cu increased Ca and Cu intake, however 4 ppm Cu increased retention by 45%. Further studies should be done to examine the effect of low levels of Cu upon mineral retention. Since differences existed between total collection (ATTD) and marker collection (ATTDM), this research shows that either method may be efficiently used to evaluate the digestibility of nutrients but total collection may result in higher values for certain minerals.