THE IMPORTANCE OF THE SUPPLEMENTATION OF ZINC IN

NURSERY PIG DIETS

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The undersigned, appointed by the Dean of the Graduate School, have examined the

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NURSERY PIG DIETS

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A candidate for the degree of MASTER OF SCIENCE

And hereby certify that in their opinion it is worthy of acceptance.

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ABSTRACT

Two experiments were conducted to investigate the effect of feeding pharmacological concentrations of Zn, from organic and inorganic sources, on growth performance and intestinal microbial population in nursery pigs. Furthermore, to determine the effect of feeding pharmacological concentrations of Zn as ZnO on the number of *Escherichia coli* and lactobacilli excreted per gram of wet feces of nursery pigs. In Exp. 1, 96 crossbred pigs (6.74 ± 0.25 kg; 19 ± 1 d of age) were weaned and allotted to one of four dietary treatments based on weight and ancestry (three pigs/pen and eight reps), for the 28-d study. In both experiments, Phase 1 (d 1 to 14) and Phase 2 (d 15 to 28) nursery diets were fed in meal form. Both dietary phases utilized four dietary treatments: (1) Basal diet contained 165 ppm Zn as $ZnSO_4$ which was supplied by the trace mineral premix, (2) Basal + 3,000 ppm Zn as inorganic ZnO, (3) Basal + 250 ppm Zn as organic Zn proteinate, (4) Basal + 250 ppm Zn as organic Zn polysaccharide. In Exp. 2, 40 crossbred pigs (7.53 ± 0.14 kg; 24 ± 0.5 d of age) were weaned and allotted to one of four treatments based on weight and ancestry (one pig/metabolism crate and 10 reps), for the duration of the 28-d study. Diets were: (1) Basal diet contained 165 ppm Zn as $ZnSO_4$ which was supplied by the trace mineral premix, (2) Basal + 750 ppm Zn as

ZnO, (3) Basal + 1,500 ppm Zn as ZnO, (4) Basal + 3,000 ppm Zn as ZnO. In Exp. 1 and

Exp. 2, body weights, feed disappearance, fecal swabs (Exp. 1) and fecal samples (Exp.

2) were collected weekly. In Exp. 1, nursery pigs fed diets containing 3,000 ppm Zn as

ZnO had greater average daily gain in week 2, 3, and overall ($P \leq 0.05$). In Exp. 2, there

was no effect of dietary Zn treatments on the average daily gain of nursery pigs during

week 1, 2, 3, 4, or overall ($P > 0.05$). In Exp. 1, during week 2, 3, and overall, pigs fed

3,000 ppm Zn as ZnO had greater feed intake than pigs fed the basal or organic Zn diets

($P \leq 0.05$). However, in Exp. 2 dietary Zn treatments did not affect feed intake during

week 1, 2, 3, 4, or overall ($P > 0.05$). Feed efficiency (G:F) was not affected by the

dietary Zn treatments throughout the 28-d study of both Exp. 1 and Exp. 2. In Exp. 1,

data showed that nursery pigs supplemented with inorganic Zn as ZnO exhibited higher

correlation values between the biochemical phenotypes of colonic microflora than pigs

fed the basal or organic Zn treatments for 28-d. Throughout the 28-d study nursery pigs

supplemented with pharmacological concentration of Zn (3,000 ppm) as ZnO or 250 ppm

Zn as Zn polysaccharide had a trend for higher ($P \leq 0.1$) fermentative capacity of fecal

flora compared to pigs fed either basal or 250 ppm Zn as Zn proteinate. In Exp 2, the

number of *Escherichia coli* and lactobacilli excreted per gram of wet feces was not

affected by the dietary Zn treatments ($P > 0.05$). However, the number of *Escherichia

coli* and lactobacilli changed over time ($P \leq 0.05$). These data indicate that supplementing

3,000 ppm Zn as ZnO in the nursery pigs diets improved growth performance throughout

the 28-d study. However, the positive growth performance improvement of feeding

pharmacological concentrations of Zn as inorganic ZnO had no effect under the

environmental condition of minimal stress, minimal pathogen challenge, and high health

status nursery pigs. Furthermore, this may be a reason why no difference was observed in fecal microflora among dietary treatments fed to nursery pigs in Exp. 2. The improvement in average daily gain observed in pigs fed 3,000 ppm Zn as ZnO in Exp. 1 could possibly be attributed to changes in substrate utilization of bacteria or increased fermentative capacity in the intestine.

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CHAPTER I

INTRODUCTON

Minerals play an important role in growth, health, and well being of the pig, even though constituting a small percentage of swine diet (NRC, 1998). Minerals can be classified into macrominerals and microminerals (Mateos et al., 2005).

Zinc (Zn) is an essential micromineral for swine and plays important roles in immunity, wound healing, normal growth and development, reproduction, and several metabolic processes (Ensminger, 1991).

The Zn requirement for nursery pigs (5 to 10 kg) given in the NRC (National Research Council), 1998 is set at 100 ppm Zn. However, research studies have shown that pharmacological supplementation of Zn (2,000 to 3,000 ppm Zn), usually as inorganic Zn oxide (ZnO), will decrease the incidence of post-weaning scouring, and increase average daily gain in nursery pigs (Poulsen, 1989; Hahn and Baker, 1993; Carlson et al.,1999; Hill et al., 2000; Case and Carlson, 2002).

The use of high concentrations of inorganic Zn has raised some environmental concerns due to low Zn retention rates and bioavailability of ZnO. Therefore, interest in using organic minerals has increased because organic Zn sources have been shown to be higher in bioavailability resulting in much less Zn supplement to achieve the same growth performance than from inorganic sources (Hahn and Baker, 1993).

High doses of dietary ZnO have been shown to be beneficial for maintaining the stability of the intestinal microflora, to support a large diversity of coliforms in weaned

piglets (Katouli et al., 1999), and to reduce the susceptibility of pigs to *Escherichia coli* (*E. coli*) infection, which is responsible for post-weaning diarrhea, reductions in growth performance, and higher mortality rates in the nursery (Mores et al., 1998).

Previous research in our laboratory has shown that nursery pigs fed diets supplemented with pharmacological concentrations of Zn as ZnO combined with 440 ppb of D-biotin had their microbial population altered (Wilt and Carlson, 2005).

Lactobacilli are among the earliest bacteria to colonize the gut (Servin, 2004). Studies have show that lactobacilli are considered to have beneficial effects on human and animal health (Fuller, 1992; Sanders, 1993) due to its antimicrobial activity against microbial pathogens (Servin, 2004).

The objectives of this research was to determine the efficacy of feeding pharmacological concentrations (3,000 ppm) of inorganic Zn as ZnO compared with lower level (250 ppm) of Zn from two organic sources of Zn (proteinate and polysaccharide) on growth performance of nursery pigs and intestinal microbial population in nursery pigs. Furthermore, to determine if the supplementation of pharmacological concentrations of Zn (750 to 3,000 ppm) as ZnO will modify the number of *E. coli* and lactobacilli excreted per gram of wet feces resulting in an improvement in nursery pig performance. Therefore, possibly providing a mechanism behind the observed enhancement in performance of newly weaned pigs fed pharmacological concentrations of Zn.

CHAPTER II

LITERATURE REVIEW

Minerals

Minerals constitute a small percentage of the swine diet, but they are very important for the health and well being of the pig. The increasing trends toward confinement of pigs, without access to soil or forage, increases the importance of meeting dietary mineral requirements (NRC, 1998).

Although mineral-deficiency diseases and actual death losses are relatively rare, inadequate supplies of minerals may result in poor gains, inefficient feed utilization, lowered reproduction, and decreased production of meat, and milk (NRC, 1998). To avoid deficiencies, minerals are usually provided in the quantities necessary (Table 2.1).

Minerals have been classified into two types: macrominerals and microminerals. Macrominerals that are commonly added to swine diets are calcium, phosphorus, sodium, and chloride. Microminerals, also called trace minerals, that are commonly added include copper, iron, iodine, manganese, selenium, and zinc (Mateos et al., 2005).

Calcium and Phosphorus

Calcium (Ca) and phosphorus (P) play important roles in the body such as, bone and teeth development and maintenance, and other physiologic functions (Kornegay, 1985). A Ca to P ratio between 1:1 and 1.25:1 is suggested when based on total P and 2:1 and 3:1 when based on available P. A wide Ca to P ratio reduces P absorption, especially

if the diet is marginal in P (Qian et al., 1996). Vitamin D is necessary for P assimilation and utilization as well as proper metabolism of Ca (Ensminger, 1991).

Calcium or P deficiency is characterized by depressed growth and poor bone mineralization, resulting in rickets in young pigs and osteomalacia in older swine (NRC, 1998). Excess concentrations of Ca and P may reduce performance of pigs, and the result is worse when the Ca:P ratio is increased since Ca reduces the utilization of Zn and P and decreases Zn absorption (Ensminger, 1991).

Common sources of Ca added into swine diets are: limestone (35% Ca), and mono/dicalcium phosphate (17 to 24% Ca). In addition, monocalcium or dicalcium phosphate provides P (18 to 21% P).

Sodium and Chloride

Salt is commonly added to diets as a source of sodium (Na) and chloride (Cl). These minerals are important to maintain osmotic pressure in body cells, upon which depends the transfer of nutrients to the cells and the removal of waste materials. Furthermore, sodium is associated with muscle contraction and is important in making bile, which aids in the digestion of fats and carbohydrates and chlorine is required for the formation of hydrochloric acid in the gastric juice so vital for protein digestion (Ensminger, 1991).

Sodium or Cl deficient pigs have their rate and efficiency of growth decreased (NRC, 1998). In contrast, salt toxicity is characterized by nervousness, weakness, staggering, epileptic seizures, paralysis and death. This condition is accentuated with restriction of water intake (Carson, 1986).

Usually the trace mineral premix contains salt, which is 39.5% Na and 59% Cl.

Copper

Copper (Cu) plays an important role in the formation of hemoglobin, along with iron and vitamin B-12, essential in enzyme systems, hair development and pigmentation, connective tissue development, bone development, myelination of the spinal cord, reproduction, and lactation (McDowell, 1992).

Copper deficiency leads to poor iron mobilization, keratinization and synthesis of collagen, elastin, and myelin, abnormal hemopoiesis, hypochromic anemia, lack of rigidity in the leg joints, spontaneous fractures, cardiovascular disorders, and depigmentation (Hill et al., 1983a).

The Cu requirement for nursery pigs given in the NRC, 1998 is set at 6 ppm Cu. However, research studies have shown that pharmacological supplementation of Cu (100 to 250 ppm), usually as Cu sulfate ($CuSO_4$) stimulates average daily gain in nursery pigs (Barber et al., 1955; Bunch et al., 1965; Hawbaker et al., 1961).

Diets containing more than 250 ppm of Cu may be toxic when fed for a long period of time. The signs of toxicity are depressed hemoglobin levels and jaundice which are the results of excessive Cu accumulation in the liver and other vital organs (NRC, 1998).

Usually, the trace mineral premix contains $CuSO_4$ (40% Cu) or Cu carbonate ($CuCO_3$; 50% Cu) as the source of Cu.

Iron

Iron (Fe) plays important roles as constituent of hemoglobin in red blood cells, in cellular oxidations, being a component of certain enzymes concerned with oxygen transfer (Ensminger, 1991).

Baby pigs born in confinement normally get a single intramuscular injection of 100 to 200 mg of Fe, in the form of Fe dextran since they have low stores of Fe at birth and sow's milk contains an average of only 1 mg of Fe per liter, which is insufficient to meet their daily requirement of 7 to 16 mg of Fe daily. Iron requirement in swine will decrease as the animal gets older because of a decrease in the blood volume per unit of body weight and increased feed consumption (McDowell, 1992).

Iron deficient animals will have a smaller number of red cells and a lower amount of hemoglobin than normal. This drop in hemoglobin suggests anemia which is characterized by poor growth, listlessness, rough hair coats, wrinkled skin, and paleness of mucous membranes (Zimmerman, 1980).

Excessive supplementation of Fe may cause Fe toxicity symptoms of which include reduced feed intake, growth rate, feed efficiency, shivering, incoordination, hyperpnea, and tetanic convulsions (McDowell, 1992).

Usually, the trace mineral premix contains ferrous sulfate ($FeSO_4$; 20 to 30% Fe) as the source of Fe.

Iodine

Iodine (I) is an important component of the hormones, thyroxine and tri-iodothyronine, produced by the thyroid gland. Those hormones are responsible for

regulating basal metabolic rates, cellular oxygen consumption, cellular integrity and enzyme synthesis (Hetzel and Clugston, 1999).

In pigs, a severe I deficiency causes enlargement of the thyroid gland (goiter) and the animals to be stunted and lethargic (Sihombing et al., 1974). Iodine deficient sows farrow weak or dead pigs that are hairless, and have an enlarged, hemorrhagic thyroid (Devilat and Skoknic, 1971).

Newton and Clawson (1974) reported that growing pigs receiving a diet containing 800 ppm of I had depressed growth, hemoglobin level, and liver iron concentration.

Usually, the trace mineral premix contains ethylene diaminedihydriodide ($C_2H_8N_2$2HI; 80% I) as the source of I.

Manganese

Manganese (Mn) is essential for normal bone formation, and for the activation of enzyme systems involved in the metabolism of carbohydrates, fats, proteins, and nucleic acids (Leach and Muenster, 1962).

Pigs fed diets containing 0.5 ppm of Mn for a long period of time show symptoms of Mn deficiency, such as, abnormal skeletal growth, increased fat deposition, irregular or absent estrous cycles, resorbed fetuses, small, weak pigs at birth, and reduced milk production (Plumlee et al., 1956).

Manganese toxicity was observed in pigs receiving a diet supplemented with 4,000 ppm of Mn. Their symptoms were depressed feed intake and reduced growth rates (Leibholz et al., 1962).

Usually, trace mineral premix contains Mn sulfate ($MnSO_4$) as the source of Mn.

Selenium

Selenium (Se) is a component of the enzyme glutathione peroxidase, which protects cellular and subcellular membranes against peroxide damage by detoxifying lipid peroxides (Rotruck et al., 1973). Selenium and vitamin E share the same function as protection for biological membranes from oxidative degeneration (McDowell, 1992).

The deficiency signs of Se are identical to those of vitamin E deficiency: nutritional muscular dystrophy, called white muscle disease; impaired reproduction; reduced milk production; and impaired immune response (Ensminger, 1991).

Selenium toxicity was observed in pigs fed diets supplemented with a range of 20 to 600 ppm of Se. The common signs of Se toxicity are: anorexia, weight loss, subnormal body temperature, hair loss, fatty infiltration of the liver, degenerative changes in the liver and kidney, edema, occasional separation of hoof and skin at the coronary band, and death (Herigstad et al., 1973).

Usually, the trace mineral premix contains sodium selenite (Na_2SeO_3; 45% Se) as the source of Se.

Zinc

Zinc (Zn) is known to play an important role in the immune system, from the barrier of the skin to gene regulation within lymphocytes, normal development and function of cells mediating nonspecific immunity and stabilization of membranes (Shankar and Prasad, 1998).

Zinc is a structural component of a great number of proteins, including enzymes of cellular signaling pathways, and transcription factors. It is essential for cell proliferation and differentiation, especially for the regulation of DNA synthesis and

mitosis. Zinc can modulate cellular signal recognition, second messenger metabolism, protein kinase, and protein phosphatase activities (Beyersmann and Haase, 2001).

Zinc deficient animals have suppressed immune responses, growth retardation, impaired taste and smell, and decreased spermatogenesis in males. In cases of severe Zn deficiency, severely depressed immune function, frequent infections, dermatitis, diarrhea, alopecia, and mental disturbances are observed (Walsh et al., 1994; Zalewski, 1996). A dietary deficiency of Zn can also reduce appetite (Chesters, 1983).

Cunnane (1988) observed that a moderate degree of Zn depletion given from 10 d post-weaning in rats for 10 weeks will decrease weight gain, liver weight, impair food conversion efficiency, and alter fatty acid composition of plasma and liver when compared with animals fed a control treatment or animals fed a Zn supplemented treatment.

In growing pigs, Zn deficiency is characterized by parakeratosis (Kernkamp and Ferrin, 1953; Tucker and Salmon, 1955). Gilts fed zinc-deficient diets during gestation and lactation produce fewer and smaller pigs, which have reduced serum and tissue Zn concentrations (Pond and Jones, 1964; Hoekstra et al., 1967; Hill et al., 1983a b c).

In contrast, the toxicity of Zn depends upon the source of Zn, its level in the diet, the duration of feeding, and the levels of other minerals in the diet.

Growing pigs fed a corn-soybean meal diet supplemented with 2,000 to 4,000 ppm Zn as Zn carbonate ($ZnCO_3$) showed symptoms of Zn toxicity such as growth depression, loss of appetite, gastritis arthritis, hemorrhage in axillary spaces, and death (Brink et al., 1959). However, Holm and Poulsen (1996) reported that 2,500 ppm of Zn as

ZnO is not toxic but that 4,000 pm of Zn as ZnO is mildly toxic to piglets when administrated for 2 weeks after weaning.

Pigs fed a diet containing 1,000 ppm of Zn as Zn lactate ($C_6H_{10}O_6Zn$) for two months, became lame and unthrifty (Grimmett et al., 1937). However, Hill et al. (1983b) reported that growing pigs did not show Zn toxicity symptoms when fed diets containing 2,000 to 4,000 of Zn as ZnO. Pigs fed diet supplemented with 1,000 ppm of Zn as Zn sulfate ($ZnSO_4$) for 7 months did not show Zn toxicity symptoms (Kulwich et al., 1953). Usually, the premix contains $ZnSO_4$ (35% Zn) as the source of Zn.

Pharmacological Concentration of Zinc: The nursery phase is a critical stage in swine production. Part of the stress of weaning is associated with the transition from maternal milk and dependency on the sow, to a physically and chemically different diet as well as different feeding regimens and environmental stress which are responsible for profound changes in the gastrointestinal tract of the piglets (Hedemann et al., 2003). The stress is mainly characterized by reduced feed intake, villus atrophy, and diarrhea, resulting in lower digestive and absorptive capacity and, ultimately, reduced weigh gain (Cera et al., 1988).

The Zn requirement for nursery pigs given in the NRC, 1998 is set at 100 ppm Zn. However, in order to ameliorate the problem due to the weaning, the swine industry often supplement pharmacological concentrations of Zn (2,000 to 3,000 ppm) usually as inorganic ZnO, in the diets of nursery pigs immediately following weaning because it has been shown to decrease the incidence of post-weaning scouring, and increase average daily gain in nursery pigs (Poulsen, 1989; Hahn and Baker, 1993; Carlson et al., 1999; Hill et al., 2000; Case and Carlson, 2002).

The mechanism behind the growth response of feeding 2,000 to 3,000 ppm of Zn as ZnO is not fully understood. Zinc oxide has been shown to improve the gastrointestinal tract function by increasing mucosal thickness, villi height, and width of the small intestine (Li et al., 2001). Nursery pigs fed diets supplemented with 3,000 ppm of Zn as ZnO showed alteration in the duodenum intestine, such as deeper crypts and greater total thickness, and increased intestinal metallothionein concentrations, which indicates that high concentrations of Zn may have an enteric effect on the growing pig (Carlson et al., 1998). Therefore, pharmacological doses of Zn as ZnO may act as an antimicrobial agent (Cromwell, 2001) or enterically on the intestinal microflora by reducing turnover of the intestinal cells and leaving more nutrients available for absorption (Fuller et al., 1960).

In contrast, Roselli et al. (2003) reported that ZnO may protect intestinal cell from *E. coli* infections by inhibiting the adhesion and internalization of bacteria, preventing the disruption of barrier integrity, and modulating cytokine gene expression, but not by a direct antibacterial effect.

High doses of dietary ZnO have been shown to be beneficial for maintaining the stability of the intestinal microflora, to support a large diversity of coliforms in weaned piglets (Katouli et al., 1999), and to reduce the susceptibility of pigs to *E. coli* infection (Mores et al., 1998).

Carlson et al. (1999) reported that the greatest response in growth performance associated with supplemental Zn to nursery pig diets occurs during the first 2 weeks post-weaning.

Kavanagh (1992) reported that nursery pigs increased their daily gain 16%, and decreased their mortality from 4.1% to 1.3% when 2,400 ppm of Zn as ZnO was added to post-weaning diets. Nursery pigs receiving diets supplemented with pharmacological concentrations of Zn as inorganic ZnO increased their average daily feed intake, therefore, increasing their average daily gain (Hollis et al., 2005; Hill et al., 2000, 2001; Carlson et al., 1999). However, improvements in feed efficiency in pigs have not been consistently demonstrated (Poulsen, 1995; Smith et al., 1997; Hollis et al., 2005).

Sources and Bioavailability of Zinc: Sources of Zn can be divided into: organic – zinc is organically bound to a ligand such as an amino acid chelate, amino acid complex, protein or a polysaccharide; and inorganic – zinc is generally bound to an inorganic salt such as chloride, sulfate, carbonate or oxide.

The use of amino acid complex minerals in mineral supplements compared to inorganic forms is still controversial (Rojas et al., 1995).

In organic Zn sources, the ligand encircles the metal atom to form a heterocyclic ring structure, which protects Zn from unwanted chemical reactions in the gastrointestinal tract, allows the passage of the mineral intact through the intestinal wall into the blood stream, and increases stability at low pH (Acda and Chae, 2002). However, in inorganic Zn sources, the mineral Zn is released during digestion and may re-combine with other dietary molecules, forming insoluble complexes and excreted, reducing their absorption across the small intestine (Mullan and Souza, 2005).

Spears (1989) found that when a deficient diet was fed, apparent absorption of Zn from Zn methionine (organic), or ZnO (inorganic) forms were similar, but Zn retention increased with Zn methionine suggesting different metabolism following absorption. Hill

et al. (1986), Swinkels et al. (1991), and Wedekind et al. (1994) demonstrated no differences in bioavailability among organic and inorganic Zn sources on studies conducted with growing pigs.

Organic Zn sources have been shown to be higher in biovailability resulting in much less Zn supplement to achieve the same growth performance than from inorganic sources (Hahn and Baker, 1993). Ward et al. (1996), Case and Carlson (2002), Buff et al. (2005) found that lower dietary concentrations of an organic Zn source were found to maintain growth performance compared with pharmacological concentrations of Zn (2,000 ppm to 3,000 ppm) as ZnO in nursery pigs. However, improvements in Zn availability and growth performance in nursery pigs have not been consistently demonstrated (Hill et al., 1986; Swinkels et al., 1996; Cheng et al., 1998; Carlson et al., 2004).

Hollis et al. (2005) reported that nursery pigs fed supplemental Zn at a concentration of 500 ppm, whether in the form of the oxide or in an organic form was not as efficacious for improved average daily gain as 2,000 to 2,500 ppm of Zn as ZnO.

Zinc Metabolism and Interactions: In monogastric species, Zn is absorbed principally throughout the small intestine with the greatest absorption in the duodenum (Naveh et al., 1988).

The intestinal absorption of Zn was proposed to occur in four phases: uptake by the intestinal cell, movement through the mucosa cell, transfer to the portal circulation, and secretion of endogenous Zn back into the intestinal cell (Cousins, 1982). Solomons and Cousins (1984) reported that the transfer of Zn from the lumen of the intestine into

the mucosal cell across the brush borders appears to be a carrier-mediated process that probably involves interaction with Zn in a chelated form.

Zinc absorption can be influenced by Zn intake, protein quantity, phytate and fiber, interaction among minerals, low-molecular-weight ligands and chelators, amino acids, and organic acids (Lonnerdal, 2000).

Zinc intake will influence Zn absorption since an increase of Zn in a diet will decrease the fractional Zn absorption (%). This reduction in fractional absorption of Zn at higher doses is due to saturation of the transport mechanisms for this mineral (Lonnerdal, 2000).

The amount of protein in a diet affects Zn absorption which will increase with increasing protein content (Sandstrom et al., 1980). Protein is the major source of dietary Zn that results in an increased Zn intake with increased protein content of the diet. Thus, in general, increased dietary protein leads to increased Zn intake and a higher bioavailability of the Zn provided (Lonnerdal, 2000).

Phytates are complex molecules that bind P and other minerals such as Ca, Cu, Fe and Zn for storage in seeds and grains, rendering the elements partially or totally unavailable for pigs, therefore, negatively affecting Zn absorption. The inhibitory effect is more pronounced when Ca intake is high due to the formation of zinc-calcium-phytate complexes in the lumen of the gastrointestinal tract (Lowe et al., 2002). Fiber is often implied as having a negative effect on Zn absorption due to the fact that most fiber-containing foods also contain phytate (Lonnerdal, 2000).

Interactions among different minerals also affect the absorption of Zn. Hill and Matrone (1970) proposed that elements with similar physical and chemical properties act

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antagonistically to each other in biological system. For instance, Bremner and Beattie (1995) reported that high dietary supplementation of Zn leads to inhibition of intestinal absorption, hepatic accumulation and placental transfer of Cu. High levels of Cu and Fe depress Zn absorption, and an excess tends to increase the requirement of the other minerals (Fairweather-Tait, 1995).

Citrate, picolinate, ethylenediaminetetraacetic acid (EDTA) and amino acids such as histidine and glutamic acid are low-molecular-weight binding ligands that have been shown to enhance mucosal uptake and absorption of Zn under experimental conditions (Hambidge et al., 1986).

Regulation of Zn absorption is made by metallothionein, a protein synthesized in response to high concentrations of divalent metals, which binds metal ions and limits their absorption (McAnena, 2005). Carlson et al. (1999) reported that nursery pigs fed 3,000 ppm Zn as ZnO increase production of metallothionein in liver, kidney, and intestinal mucosa.

The majority of bioavailable Zn when supplemented in relatively high concentrations is stored in body organs such as liver, spleen, kidney, and pancreas, with minor storage in bone, muscle, and skin (Ott et al., 1966). Case and Carlson (2002) reported an increased concentration of Zn in the liver and kidney of pigs supplemented with 3,000 ppm of Zn from ZnO but not in pigs supplemented with 500 ppm of Zn from ZnO.

Plasma Zn concentrations increase when Zn is supplemented to the animal, serving as an immediate source of stored Zn (Ott et al., 1966). Buff et al. (2005) found that plasma Zn concentration was greater for pigs fed a diet supplemented with 2,000

ppm of Zn as inorganic ZnO compared to animals fed diets supplemented with 150, 300, or 450 ppm of Zn as organic Zn polysaccharide. This is in agreement with other experiments where lower concentrations of Zn were provided as organic Zn compared with a pharmacological dose of Zn as ZnO (Case and Carlson, 2002; Carlson et al., 2004). Plasma Zn and average daily gain showed a positive relationship when plasma Zn concentrations were below 2.5 mg/L (Hahn and Baker, 1993; Poulsen, 1995; Carlson et al., 1999).

Case and Carlson (2002) reported that in excess of 99% of the Zn is excreted in the feces, regardless of source (ZnO or organic zinc) or dietary concentration (150, 500, or 3,000 ppm). Fecal excretion of Zn includes the excretion of unabsorbed dietary Zn, which normally accounts for a large proportion of the intake, and of endogenous Zn derived from gastrointestinal, pancreatic, and bilary secretions (Hambidge et al., 1986).

High dietary concentrations of Zn as ZnO result in large quantities of Zn excreted in manure (Poulsen and Larsen, 1995; Hoover et al., 1997; Carlson et al., 2004), which may result in a net accumulation of Zn in the soil when applied as a crop fertilizer (Jongbloed and Lenis, 1998). The quantity of Zn excreted in the feces by pigs is directly related to the dietary Zn concentration. Fecal Zn excretion was markedly decreased when lower concentrations of Zn from organic sources were fed to weanling pigs as a replacement for pharmacological doses of Zn as ZnO (Case and Carlson, 2002; Carlson et al., 2004; Buff et al., 2005).

Phene Plate Generalized Microplate: The gastrointestinal tract is a complex ecosystem that includes a resident microbiota and cells of various phenotypes lining the epithelial wall. The intestinal microbiota is very important for normal gut function and in

maintaining host health (Servin, 2004). Therefore, several research studies have been conducted to look at the composition of the intestinal floras of animals during different nutritional and pathophysiological states (Katouli et al., 1997a).

The PhenePlate^{TM} system is an automated system for simple and rapid subtyping of bacteria. Katouli et al. (1997a) reported that the Phene Plate generalized microplate (the PhP-48) is an efficient method in recognizing the differences as well as similarities among the coliform populations of different groups. The system consist of two sets of 46 substrate containing wells and two control wells without a carbon source in each set (Table 2.2).

The system is based on numerical analysis of biochemical reaction kinetics, also known as biochemical fingerprinting which is the characterization of bacterial strains based on quantitative measurements of reaction products formed by the bacterial metabolism of several different substrates (Mollby et al., 1993). Fermentative capacity which is defined as the capacity of a given flora to ferment different carbohydrates can be obtained from biochemical fingerprinting of mixed flora. The combination of fermentative capacity measurement and biochemical fingerprinting, can be used to study changes in the functional status of the gut flora (Katouli et al., 1997a,b).

Katouli et al. (1997b), using the Phene Plate generalized microplates, reported that the metabolic fingerprints obtained from fecal floras of sows and their litters were very similar at the early stage of the animal's lives indicating that sows were the initial source of the flora for piglets. However, this similarity was lost by week 2 post-weaning and piglets developed new types of flora which were similar among the litter-mates. The

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metabolic fingerprints of pig's floras during the post-weaning period also differed from those of the suckling period.

Previous research in our laboratory has shown that nursery pigs fed diets supplemented with pharmacological concentrations of Zn as ZnO combined with 440 ppb of D-biotin had their microbial population altered (Wilt and Carlson, 2005).

Escherichia coli

The gastrointestinal tract of the pig is sterile at birth, but within a few hours, it becomes colonized by microorganisms mainly acquired from maternal feces, skin, and teats (Arbuckle, 1968; Bertschinger et al., 1988). *Escherichia coli* cells, which are facultative anaerobic gram-negative rods, together with streptococci of Lancefield groups D and K and *Clostridium perfringens*, are among the earliest bacteria to colonize the gut in piglets (Drasar and Barrow, 1985).

All mammals and birds are colonized by *E. coli*, and these organisms become a permanent part of the normal microflora. However, certain *E. coli* strains have been associated with gastroenteritis, urogenital disease, septicemia, and pleural infections in both humans and animals (Oswald et al., 2000).

The most important cause of neonatal and post-weaning diarrhea in pigs has been associated with *E. coli*. Disease caused by *E. coli* in neonatal pigs can be fatal, with mortality reaching high levels during the first few days after birth (Tzipori, 1985; Bertschinger et al., 1992).

Disease in neonatal pigs includes the following serotypes of *E. coli* O8, 9, 20, 101, 141, 147, 149, and 157 (Blanco et al., 1991; Timoney et al., 1988). The major cause of diarrhea and death in neonatal and newly weaned pigs is associated with

Enterotoxigenic *E. coli* strains that express K88 (F4) frimbriae, which adhere to the small intestinal microvilli producing enterotoxins that act locally on enterocytes resulting in hypersecretion of water and electrolytes, and reducing absorption (Mackinnon, 1999; Nagy and Fekete, 1999; Fairbrother, 1999).

Under normal conditions, the intestinal flora is stable and composed of diverse groups of bacterial strains. This stability has a great impact on the regulation of the host's non specific resistance to infectious diseases, a phenomenon which is referred to as colonization resistance (Waiij and Verhoef, 1979). Therefore, in micro ecosystems such as the gastrointestinal tract, a high diversity of the bacterial flora enhances the stability of the microbial community and contributes to a high colonization resistance against invading pathogens (Kuhn et al., 1993).

High doses of dietary ZnO have been shown to be beneficial for the stability of the intestinal microflora, to support a large diversity of coliforms in weaned piglets (Katouli et al., 1999) and to reduce the susceptibility of pigs to *E. coli* infection (Mores et al., 1998; Holm, 1988; Poulsen, 1989).

In vitro studies show a reduction of the total number of *E. coli* when samples taken from the intestines of pigs were cultivated in agar supplemented with ZnO (Jensen, 1987). In addition, Sawai (2003) reported that ZnO inhibits the growth of *Staphylococcus aureus* and *E. coli* in experiments in vitro. However, Jensen-Waern et al. (1998), Katouli et al. (1999), and Li et al. (2001) reported no effect on the number of *E. coli* or enterococci bacteria excreted per gram of feces when weanling pigs were fed a pharmacological dose of Zn as ZnO.

Lactobacilli

Lactobacilli are gram-positive anaerobic or facultative aerobic rods, which can be isolated from the human and animal body, plants and material of plant origin, sewage and fermented products (Hammes and Vogel, 1995).

Studies have shown that populations of lactobacilli inhibit the proximal regions of the digestive tracts of pigs, fowl, and rodents. The surface of stratified squamous epithelium in the esophagus, crop, or stomach can be colonized by some gastrointestinal strains of lactobacilli which have the ability to adhere to the surface of organs. Other lactobacillus strains appear to be inhabitants of the gastrointestinal lumen (Fuller et al., 1978; Fuller and Turvey, 1971; Savage et al., 1968; Tannock et al., 1987; Tannock et al., 1982).

Fuller (1992) and Sanders (1993) reported that lactobacilli are considered to have beneficial effects on human and animal health. Studies in vitro and in animals have shown that lactobacilli may prevent *E. coli* from colonizing the jejunum and produce substances directed against the enterotoxins resulting in an inhibition of *E. coli*-induced enterotoxin reactions (Foster et al., 1980; Johnson and Calia, 1979; Mitchell and Kenworthy, 1976). In agreement, Conway (1989) and Chan et al. (1985) studying the concept of competitive exclusion of pathogenic *E. coli* by lactobacilli in the intestine and urinary tract in vitro, respectively, found that the colonization of the lactobacilli sterically hindered the adhesion of *E. coli* to the surface.

Lactobacilli are highly acid-resistant, with growth being possible at an initial pH of 5.0 (Hammes and Vogel, 1995). Most other intestinal bacteria are not able to grow at an acidic pH. Vandenbergh (1993) reported that among the mechanisms suggested by

which the selected lactobacillus strains may act against microbial pathogens, is the production of metabolites such as acetic and lactic acids, which will lower the pH, therefore, inhibiting the growth of bacterial pathogens.

A recent experiment has suggested that a non-specific interaction of lactobacilli with the immune system is an important mechanism by which lactobacilli may act against microbial pathogens (Blum and Schiffrin, 2003). Therefore, lactobacilli are considered to play a role in the formulation of well balanced indigenous microflora, and improve the colonization of the intestinal, respiratory and urogenital tracts.

Due to the beneficial effects on human and animal health, several species of lactobacilli are used as probiotics (Fuller, 1992; Sanders, 1993). Schrezenmeir and Vrese (2001) define probiotic as "a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and causes beneficial health effects in this host".

The most widespread use of probiotics in swine is for the control of bacterial gastrointestinal disease in young growing pigs, particularly *E. coli* and *Salmonellosis*. The bacteria most commonly used as probiotics are lactobacilli, but some work has been done using *Enterococcus faecium* and *Bifidobacterium sp*. Abe et al. (1995) conducted an experiment with nursery pigs and reported that Lactobacillus and Bifidobacteria increased weight gain and reduced mortality when used as a probiotic. Dritz et al. (1997) found an improvement in growth and a decrease in diarrhea when administrating *Lactobacillus casei* as a probiotic to nursery pigs.

Li et al. (2001) reported no effect of pharmacological concentrations of Zn as ZnO for nursery pigs with respect to the number of enterobacteriaceae, clostridia and lactobacilli in ileal digesta and feces. In contrast, Broom et al. (2003) and Jensen-Waern et al. (1998) found that pharmacological concentrations of Zn as ZnO reduce fecal counts of lactobacilli and enterococci during the post-weaning period of pigs, but only temporarily. In agreement, Hojberg et al. (2005) reported that feeding weaned piglets with 2,500 ppm of Zn as ZnO reduced the MRS counts (lactic acid bacteria) and Rogosa counts (lactobacilli) in all segments of the gastrointestinal tract.

Table 2.1. Dietary mineral requirements of nursery pigs allowed feed ad libitum – 90% dry matter (NRC, 1998)

	Body Weight (Kg)					
	3 to 5	5 to 10	10 to 20	20 to 50	50 to 80	80 to 120
Average weight in range (Kg)	4	8	15	35	65	100
DE content of diet (Kcal/Kg)	3,400	3,400	3,400	3,400	3,400	3,400
ME content of diet (Kcal/Kg)	3,265	3,265	3,265	3,265	3,265	3,265
Estimated DE intake (Kcal/day)	855	1,690	3,400	6,305	8,760	10,450
Estimated ME intake (Kcal/day)	820	1,620	3,265	6,050	8,410	10,030
Estimated feed intake (g/day)	250	500	1,000	1,855	2,575	3,075
Requirements (% or amount/Kg of diet)						
Mineral elements						
Calcium (%)	0.90	0.80	0.70	0.60	0.50	0.45
Phosphorus, total (%)	0.70	0.65	0.60	0.50	0.45	0.40
Phosphorus, available (%)	0.55	0.40	0.32	0.23	0.19	0.15
Sodium (%)	0.25	0.20	0.15	0.10	0.10	0.10
Chlorine (%)	0.25	0.20	0.15	0.08	0.08	0.08
Magnesium (%)	0.04	0.04	0.04	0.04	0.04	0.04
Potassium (%)	0.30	0.28	0.26	0.23	0.19	0.17
Copper (mg)	6.00	6.00	5.00	4.00	3.50	3.00
Iodine (mg)	0.14	0.14	0.14	0.14	0.14	0.14
Iron (mg)	100	100	80	60	50	40
Manganese (mg)	4.00	4.00	3.00	2.00	2.00	2.00
Selenium (mg)	0.30	0.30	0.25	0.15	0.15	0.15
Zinc (mg)	100	100	80	60	50	50

Table 2.2. Reagents in the PhP-48 general plate

	1	2	3	4	5	6	7	8	9	10	11	12
A **E**	1 Mannonic acid lacton	2 L-Arabinose	3 D- Xylose	4 Galactose	5 Maltose	6 Cellobiose	7 Trehalose	8 Palatinose	9 Sucrose	10 Lactose	11 Melibiose	12 Lactulose
B **F**	13 Gentobiose	14 Melezitose	15 Raffinose	16 Inosine	17 Adonitol	18 Inositol	19 D-Arabitol	20 Glycerol	21 Maltitol	22 Sorbitol	23 Dulcitol	24 pH 7.4 Con.
C **G**	25 Sorbose	26 Deoxy-glucose	27 Deoxy-ribose	28 Rhamnose	29 D-Fucose	30 L-Fucose	31 Tagatose	32 Amygdalin	33 Arbutin	34 b -Methyl-glucoside	35 5- Keto-gluconate	36 Gluconate
D **H**	37 Melbionate	38 Galacturo-nic lacton	39 Salicine	40 pH 5.5 Con *	41 Citrate *	42 Fumarate *	43 Malinate *	44 Malonate *	45 Pyruvate *	46 L-Tartarate *	47 Urea *	48 Ornithine *

CHAPTER III

THE EFFECT OF FEEDING ORGANIC AND INORGANIC SOURCES OF ZINC ON GROWTH PERFORMANCE AND MICROBIAL POPULATION IN NURSERY PIGS

ABSTRACT

This experiment was conducted to evaluate the effect of feeding pharmacological concentrations of zinc (Zn), from organic and inorganic sources, on growth performance and intestinal microbial population in nursery pigs. Ninety-six crossbred pigs (6.74 ± 0.25 kg; 19 ± 1d of age) were weaned and allotted to one of four dietary treatments based on weight and ancestry (three pigs/pen and eight reps), for the 28-d study. Phase 1 (d 1 to 14) and Phase 2 (d 15 to 28) nursery diets were fed in meal form. Both dietary phases utilized four dietary treatments: (1) Basal diet contained 165 ppm Zn as $ZnSO_4$ which was supplied by the trace mineral premix, (2) Basal + 3,000 ppm Zn as inorganic ZnO, (3) Basal + 250 ppm Zn as organic Zn proteinate, (4) Basal + 250 ppm Zn as organic Zn polysaccharide. Body weights, feed disappearance, and fecal swabs were collected weekly. Fecal swabs were collected to determine the fermentative capacities and biochemical phenotypes of the pigs' colonic microflora over time and treatments. Nursery pigs fed diets containing 3,000 ppm Zn as ZnO had greater average daily gain in week 2, 3, and overall (P ≤ 0.05). During week 2, 3, and overall, pigs fed 3,000 ppm Zn

as ZnO had greater feed intake than pigs fed the basal or organic Zn diets ($P \leq 0.05$).

These results indicate that feeding 250 ppm Zn as organic Zn had no impact on growth

performance. However, supplementing 3,000 ppm Zn as ZnO in the nursery pigs diets

improved growth performance throughout the 28-d study. Data showed that nursery pigs

supplemented with pharmacological concentration (3,000 ppm) of inorganic Zn as ZnO

exhibited higher correlation values between the biochemical phenotypes of colonic

microflora than pigs fed either the basal or organic Zn treatments for 28-d. Nursery pigs

supplemented with pharmacological concentration of Zn (3,000 ppm) as ZnO or 250 ppm

Zn as Zn polysaccharide had a trend for higher ($P \leq 0.1$) fermentative capacity of fecal

flora compared to pigs fed either basal or 250 ppm of organic Zn proteinate throughout

the 28-d study. Therefore, these data indicate that the improvement in average daily gain

observed in pigs fed 3,000 ppm Zn as ZnO could possibly be attributed to changes in

substrate utilization of bacteria or increased fermentative capacity in the intestine.

INTRODUCTION

Currently, many swine producers supplement pharmacological concentration of

Zn (2,000 to 3,000 ppm) as inorganic ZnO in nursery pig diets for two weeks post-

weaning since it has been proven to ameliorate the problem due to stressful weaning by

decreasing the incidence of post-weaning scouring and increasing average daily gain in

nursery pigs (Poulsen, 1989; Hahn and Baker, 1993; Carlson et al., 1999; Hill et al.,

2000; Case and Carlson, 2002).

Zinc is an essential micromineral for swine and plays important roles in immunity, wound healing, normal growth and development, reproduction and metabolic processes (Ensminger, 1991). The Zn requirement for nursery pigs given in the NRC (1998) is set at 100 ppm Zn.

Sources of Zn can be divided into organic – zinc is organically bound to a ligand such as an amino acid chelate, amino acid complex, protein or a polysaccharide; and inorganic – zinc is generally bound to an inorganic salt such as chloride, sulfate, carbonate or oxide.

Acda and Chae (2002) reported that the body more readily absorbs organic Zn sources because it is chemically bound to a mixture of amino acids, protecting it from becoming unavailable and allowing it to be more readily absorbed in the small intestine. On the other hand, in inorganic Zn sources, the mineral Zn is released during digestion, allowing Zn to form insoluble complexes with dietary molecules, therefore, reducing its absorption across the small intestine (Mullan and Souza, 2005).

Ward et al. (1996), Case and Carlson (2002), Buff et al. (2005) reported that lower dietary concentrations of an organic Zn source were found to maintain growth performance of nursery pigs compared with pharmacological concentrations of Zn as ZnO. However, these findings are in contrast to those of Hollis et al. (2005), Hahn and Baker (1993), Carlson et al. (1999), and Hill et al. (2000), who reported that feeding pharmacological concentrations of inorganic Zn from ZnO improves the growth rate of weanling pigs compared with pigs receiving the control diet or lower dietary concentrations of added Zn from ZnO or any organic Zn source.

Currently, there is no valid explanation concerning the mode of action of ZnO in pigs, although its antibacterial properties are well established in human medicinal practice (Sordeberg et al., 1990). Li et al. (2001) reported that ZnO has been shown to improve the gastrointestinal tract function by increasing mucosal thickness, villi height and width of the small intestine. Carlson et al. (1998) proposed that high concentrations of Zn may have an enteric effect on the growing pig because nursery pigs fed diets supplemented with 3,000 ppm of Zn as ZnO showed alteration in the duodenum intestine, such as deeper crypts and greater total thickness, and increased intestinal metallothionein concentrations.

Roselli et al. (2003) reported that ZnO may protect intestinal cells from *E. coli* infection by inhibiting the adhesion and internalization of bacteria, preventing the disruption of barrier integrity, and modulating cytokine gene expression, but not by direct antibacterial effect.

High doses of dietary ZnO have been shown to be beneficial for maintaining the stability of the intestinal microflora, to support a large diversity of coliforms in weaned piglets (Katouli et al., 1999), and to reduce the susceptibility of pigs to *E. coli* infection (Mores et al., 1998).

The objectives of this research study are to investigate the effect of feeding pharmacological concentration of Zn, from organic and inorganic sources, on growth performance and intestinal microbial population in nursery pigs.

This research project was approved by the Animal Care and Use Committee of the University of Missouri-Columbia before initiation of the experiment (protocol # 4058).

Animals

A total of ninety-six crossbred (Monsanto Choice Genetics GPK 1 Landrace x GPK 4 Large White) pigs were weaned at an average age of 19 ± 1 day, and an initial average body weight of 6.74 ± 0.25 kg. Pigs were allotted to one of four dietary treatments based on weight and ancestry, for the 28-d study.

Health Status

On the first day of the experiment, blood samples were collected from one pig in each pen in order to verify their health status for M. Hyopneumoniae, PRRS, swine influenza H1N1, and swine influenza H3N2.

Diets

Pigs were fed typical Phase 1 (d 1 to 14) and Phase 2 (d 15 to 28) nursery diets in meal form (Table 3.1). Phase 1 diet contained 22.7% CP and 1.6% total lysine, and Phase 2 diet contained 20.4% CP and 1.3% total lysine. The basal diet contained 165 ppm of Zn as inorganic $ZnSO_4$, which was supplied by the trace mineral premix. The four dietary treatments utilized were: (1) Basal diet, (2) Basal diet + 3,000 ppm of Zn as inorganic ZnO; (3) Basal diet + 250 ppm of Zn as organic Zn proteinate; (4) Basal diet + 250 ppm of Zn as organic Zn polysaccharide. Both phase 1 and phase 2 of nursery diets were

based on corn and soybean meal. All nutrients met or exceed NRC (1998) recommendations for 5 to 20 kg nursery pigs.

General Husbandry

Pigs were housed in an environmentally controlled building with plastic flooring over a pit. Pens were 1.2 m^2, equipped with a stainless steel cup drinker and one stainless steel self-feeder. Pigs were penned in groups of three, utilizing 32 pens and eight replications per treatment, and allowed ad libitum access to feed and water for the duration of the 28-d study. The temperature in the nursery facility was maintained at approximately 30^oC for week 1, with a 2^oC decrease each week thereafter.

Growth Performance

Every week, pig weights (WT) and feed consumption was determined by pen in order to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F).

Biochemical fingerprinting and fermentative capacity of fecal flora

Fecal swabs were collected weekly from one pig per pen by inserting a sterile swab 3 cm into the rectum. Every week, the swabs were collected from the same pigs and washed in 10 mM phosphate buffer saline (PBS) solution (1 mL). Three dilutions, initial, 1 to 10 and 1 to 100 were made with the PBS solution.

Sterile MacConkey agar plates were prepared and using a sterile inoculation loop, the three dilutions were streaked out on the agar plates following the quadrant streak technique, which allows sequential dilution of the original material over the entire surface

of the plate, therefore, individual cells were isolated on the surface of the agar. Thereafter, plates were then incubated at 37°C in anaerobic chambers.

After incubating the plates overnight, approximately 3 mg wet weight of individual colonies were picked and suspended in 0.011% Bromothymol blue suspending medium which was made in our laboratory by mixing 100 ml of bromothymol blue stock solution with 1 L of PhP (Phene-plate) suspending medium. The pH of the solution was adjusted to 7.8 to 8.0. The methods for preparing 1 L of bromothymol stock solution and 1 L medium were: Bromothymol stock solution - 1) 1.1 g of bromothymol blue dissolved in 90 ml distilled water; 2) 10 ml of 1M NaOH and 900 ml of distilled water added to the solution prepared in step 1. 3) The concentration of the indicator solution was between 1.9 and 2.3 as checked at 620 nm. Medium – 1) 8 ml of proteose peptone dissolved in 900 ml of distilled water.

Suspending medium and colonies were allowed to equilibrate for 1 hr, and then 0.15 ml of the bacterial suspension was inoculated into each of the wells of the PhP-48 micro plates (Ph Plate, Stockholm AB, Sweden). The generalized PhP-48 micro plate with two sets of 46 substrate-containing wells (Figure 2.2) and two control wells without a carbon source in each set and which contained only buffers at pH 7.4 and 5.5 was used in this experiment. In order to create an anaerobic environment, paraffin oil was dropped into each well of the plate.

The PhP-48 plates were placed in an incubator set at 37°C for 48 hr. The absorbancies of each well was measured after 7, 24, and 48 hrs of incubation in Tecan microplate reader A_{620}. The indicator Bromothymol blue changes color as bacterias utilize the different carbon sources and produce or consume acid in the PhP-48. The color

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indicator (Bromothymol blue) at neutral and alkaline pH is blue, and at acidic pH is yellow.

Absorbancies values were entered into Phene Plate software which was responsible for converting the absorbance data to PhP data, determining colonic fecal biochemical fingerprinting, fermentative capacities of pig's fecal flora, and producing dendograms.

The metabolic fingerprint was calculated by getting the mean value of all readings for each well. The metabolic fingerprint values ranges from 0 to 30 for each sample, where low values mean acidic reactions (yellow), and high values mean alkaline reactions (deep blue).

Metabolic fingerprints were compared to each other in pairwise fashion and the similarity between each pair was calculated as correlation coefficient which may range from +1 (similar biochemical fingerprints, and most probably similar floras) and -1 (different biochemical fingerprints). The correlation coefficients, which were clustered according to the unweighted pair group method with arithmetic averages (UPGMA) are presented in the form of dendograms (Figure 3.5 to Figure 3.14) where the horizontal line represents each sample and vertical lines connects one sample to another at the similarity level between them. Correlation values are considered low when ranging from 0.60 to 0.69, moderate from 0.70 to 0.79, high from 0.80 to 0.89 and very high from 0.90 to 1.00.

The ability of the each bacterial population in a fecal sample to utilize various carbohydrate sources is known as fermentative capacity (FC). The fermentative capacity values range from 0 (low metabolic activity of the flora) to 1 (high metabolic activity of the flora).

Statistical Analysis

Data were analyzed as a completely randomized design (RCD) using the Mixed Models of SAS (SAS Inst. Inc., Cary, NC) as described by Littell et al. (1998). The statistical model included the effects of treatment (diet), time (week), and the interaction between treatment and time. Differences were determined using Fisher's Least Significant Difference (LSD) and were considered significant at $P \leq 0.05$. Trends are reported at $P > 0.60$ up to 0.10. Data were analyzed using pen as the experimental unit.

RESULTS

Health Status

Pigs tested negative for M. Hyopneumoniae, PRRS, swine influenza H1N1, and swine influenza H3N2.

Growth Performance

Average daily gain was affected by dietary Zn treatments, and time and there was a treatment*time interaction (Table 3.2). Nursery pigs fed 3,000 ppm of Zn as inorganic ZnO had higher ADG in week 2, 3, and overall ($P \leq 0.05$) than pigs fed the basal or organic Zn diets (Table 3.3 and 3.4). In addition, pigs fed 3,000 ppm Zn as ZnO averaged 16% higher gain throughout the 28-d study (Figure 3.1; $P \leq 0.05$). Thus, pigs fed 3,000 ppm Zn as ZnO were 1.9 kgs heavier (10%) at the end of the 28-d study than pigs fed the basal or organic Zn diets ($P \leq 0.05$).

Average daily feed intake was also affected by dietary Zn treatments, and time, and there was a treatment*time interaction (Table 3.2). Feed intake was greater during

week 2, 3, and overall in pigs fed 3,000 ppm Zn as ZnO than in pigs fed either basal or organic Zn diets ($P \leq 0.05$; Table 3.3 and 3.5). Overall, nursery pigs fed 3,000 ppm Zn had a 23% greater feed intake ($P \leq 0.05$) than pigs fed basal or organic Zn treatments (Figure 3.2).

Feed efficiency (gain/feed) was affected by time but not by dietary treatment and there was no treatment by time interaction (Table 3.2 and Table 3.6). Gain to feed was similar when pigs were fed either basal or Zn diets ($P > 0.05$) as shown on Table 3.6. Figure 3.3 shows that dietary treatment had no effect on G:F throughout the 28-d study ($P > 0.05$).

Fermentative Capacity

Fermentative capacity (FC) was not affected by dietary Zn treatments ($P > 0.05$) as shown on Table 3.2. However, nursery pigs supplemented with pharmacological concentration of Zn (3,000 ppm) as ZnO or 250 ppm Zn as Zn polysaccharide had a trend for higher ($P \leq 0.1$) FC of fecal flora compared to pigs fed either basal or 250 ppm of organic Zn proteinate (Table 3.3 and Figure 3.4). Overall, pigs fed 3,000 ppm Zn as ZnO had 12% higher FC than pigs fed the basal or organic Zn treatments. Fermentative capacity was affected by time (Table 3.2, Table 3.7 and Figure 3.4) as pigs had the lowest FC at weaning which increased during week 1 post-weaning and essentially stayed constant throughout the nursery period.

Biochemical Fingerprinting of Fecal Flora

Biochemical fingerprinting of the fecal flora indicated a moderate to very high correlation at weaning (Figure 3.5; Figure 3.6). During week 1, pigs receiving control,

ZnO and Zn proteinate diets had their correlation coefficients ranging from low to very

high and pigs supplemented with Zn polysaccharide diet showed a correlation value more

uniform ranging from high to very high (Figure 3.7). The correlation values of

biochemical fingerprinting among dietary treatments of nursery pigs 1 week post-

weaning are shown on Figure 3.8.

During week 2 post-weaning nursery pigs fed either basal or Zn polysaccharide

diets had a correlation value of biochemical fingerprinting of the fecal flora ranging from

moderate to very high. On the other hand, pigs supplemented with either 3,000 ppm Zn as

ZnO or 250 ppm Zn as Zn proteinate showed a correlation value ranging from very low

to very high (Figure 3.9). The correlation values among dietary treatments for nursery

pigs 2 weeks post-weaning are shown on Figure 3.10.

During week 3, pigs from basal, ZnO or Zn polysaccharide treatments showed a

correlation value of the biochemical fingerprinting of the fecal flora ranging from

moderate to very high correlated. The correlation values of metabolic fingerprints of the

fecal flora of pigs supplemented with 250 ppm of Zn as Zn proteinate ranged from very

low to very high (Figure 3.11). Figure 3.12 shows the correlation values among dietary

treatments for nursery pigs 3 weeks post-weaning.

By week 4 post-weaning, the biochemical fingerprinting of the fecal flora of pigs

from ZnO treatment were more correlated (high to very high) compared to pigs receiving

basal (very low to very high), 250 ppm Zn as Zn proteinte (very low to very high) or 250

ppm Zn as Zn polysaccharide diets (moderate to very high) shown in Figure 3.13.

Correlation values among treatments are shown on Figure 3.14.

DISCUSSION

In the present study, feeding 3,000 ppm of Zn as inorganic ZnO improved growth rate of nursery pigs during week 2, 3, and overall. These data agree with previous studies in which supplementation of pharmacological levels of Zn (2,000 to 3,000 ppm) as ZnO enhanced growth in weanling pigs (Poulsen, 1989; Hahn and Baker, 1993; Carlson et al., 1999; Hill et al., 2000; Case and Carlson, 2002).

Results of this experiment indicated that feeding 250 ppm Zn as organic Zn has no impact on growth performance. These data supports the findings of Hill et al. (1986), Swinkels et al. (1996), Cheng et al. (1998) and Carlson et al. (2004), who reported no improvement on growth performance when organic Zn (Zn proteinate, Zn polysaccharide, Zn methionine, Zn amino acid chelate or Zn lysine complex) was supplemented from 9 to 800 ppm in nursery pig diets. In contrast to these data, dietary supplementation of organic Zn has been shown to improve growth performance at lower dietary concentrations than pharmacological concentration of Zn (2,000 ppm to 3,000 ppm) as ZnO in nursery pigs (Ward et al., 1996; Case and Carlson, 2002; Buff et al., 2005).

In the current study, nursery pigs fed diet supplemented with 3,000 ppm Zn as ZnO had greater feed intake than pigs fed either basal or organic Zn diets during week 2, 3 and overall. In agreement to these data, pharmacological concentrations of Zn as ZnO increased average daily feed intake and average daily gain in nursery pigs (Kavanagh, 1992; Hollis et al., 2005; Hill et al., 2000, 2001; Carlson et al., 1999). However, these findings are in contrast to those of Poulsen (1995) and Smith et al. (1997), who found no

improvement in feed intake, although reporting an increase in growth rate for weanling pigs fed pharmacological concentrations of Zn (2,000 or 3,000 ppm) as ZnO.

Over the entire 28-d nursery period, there was no effect of dietary Zn, regardless of Zn source, on feed efficiency (G:F), similar to other experiments where no improvement in G:F from Zn were reported (Poulsen, 1995; Smith et al., 1997; Hollis et al., 2005).

Biochemical fingerprinting of the fecal flora of pigs at weaning had higher correlation values than those obtained during the post-weaning period. These findings supports Katouli et al. (1997b, and 1999) who reported that metabolic fingerprints of pigs fecal floras during the suckling period were more similar to each other than those obtained during the post-weaning period.

Limited research studies are available to document the effect of supplementing pharmacological concentration of Zn on fermentative capacity and metabolic fingerprinting of fecal flora of nursery pigs.

In the present study, nursery pigs fed diet supplemented with 3,000 ppm Zn as ZnO indicated higher correlation values than pigs fed either basal or 250 ppm Zn as organic Zn diets during 4 weeks post-weaning. In agreement to these data, dietary supplementation of ZnO has been reported to help to maintain the stability of the intestinal flora in weaned pigs and thus, preserves the protective ability of the flora which would otherwise be lost due to weaning. In addition, it supports a large diversity of coliforms which may compete for colonization sites with diarrhoegenic strains (Kuhn et al., 1993; Katouli et al., 1999; Katouli et al., 1997).

In the current study, FC values of fecal floras from nursery pigs fed diet containing 3,000 ppm Zn as ZnO did not differ from FC values from pigs receiving either basal or 250 ppm Zn as organic Zn during the 28-d study. However, FC values from pigs supplemented with 3,000 ppm Zn as ZnO tended to be higher when compared to FC values from pigs fed either basal or 250 ppm Zn as organic Zn proteinate dietary treatments. Katouli et al. (1997b) reported that nursery pigs receiving pharmacological supplementation of Zn as ZnO had higher FC values than basal pigs during the first 2 weeks of the study.

Results of this experiment suggest that substrate utilization of bacteria in the intestine or increased fermentative capacity may be a possible mechanism for the observed improvement in growth performance of nursery pigs. Higher FC values and greater correlations of biochemical fingerprinting of fecal floras observed in nursery pigs fed 3,000 ppm Zn as ZnO, which had improved overall growth performance of nursery pigs, compared to FC values and biochemical fingerprints from pigs fed either the basal or 250 ppm Zn as organic Zn proteinate dietary treatments support this hypothesis.

This study suggests that pharmacological concentration of Zn as ZnO improves growth performance of nursery pigs post-weaning. The mechanism for the observed improvement in growth performance of nursery pigs could possibly be attributed to changes in substrate utilization of bacteria or increased fermentative capacity in the intestine.

IMPLICATIONS

Data from this experiment indicate that feeding nursery pigs with pharmacological concentrations of Zn (3,000 ppm) as ZnO improves growth performance throughout the 28-d post-weaning study. However, the supplementation of 250 ppm of organic Zn as either Zn polysaccharide or Zn proteinate has no impact on growth performance of nursery pigs under the environmental conditions of this study. These data indicate that the improvement in average daily gain observed in pigs fed 3,000 ppm Zn as ZnO could possibly be attributed to changes in substrate utilization of bacteria or increased fermentative capacity in the intestine. This hypothesis is supported by the fact that, nursery pigs fed 3,000 ppm Zn as ZnO had higher FC values and greater correlations of biochemical fingerprinting of fecal floras, when compared to FC values and biochemical fingerprints from pigs fed either the basal or 250 ppm Zn as organic Zn proteinate dietary treatments.

Table 3.1. Composition of basal diets (%, as-fed basis) [a]

Ingredient	Phase 1 [b]	Phase 2 [b]
Yellow dent corn	35.20	51.00
Soybean meal (48%)	25.00	27.40
Dried whey	25.00	10.00
Spray-dried animal plasma	6.30	2.50
Choice white grease	5.00	5.00
Dical phosphate, 21%	1.62	2.22
Limestone	0.75	0.80
Vitamin premix [c]	0.50	0.50
Mineral premix [d]	0.15	0.15
Salt NaCl	0.20	0.20
L-Lysine HCl	0.15	0.15
DL- Methionine	0.13	0.08
Zn [e]	-	-
Calculated composition		
Crude protein, %	22.73	20.40
Lysine, %	1.61	1.33
Calcium, %	1.04	1.10
Available phosphorus, %	0.62	0.56
Metabolizable energy, kcal/kg	3,244.09	3,387.07

[a] Formulated to contain at least 0.90% Ca and 0.55% available P.

[b] Phase 1 diets were formulated to contain 1.6% total lysine and 22.5% CP, and Phase 2 diets contained 1.25% lysine and 19.4% CP. Phase 1 fed d 1 to 14 of experiment. Phase 2 fed d 15 to 28 of experiment.

[c] Supplied per kilogram of diet: retinyl acetate, 11,000 IU; cholecalciferol, 1,100 IU; DL-α-tocopheryl acetate, 44.1 IU; menadione Na dimethylpyrimidinol bisulfate, 4.0 mg; vitamin B_{12}, 30.3 µg; riboflavin, 8.3 mg; D-Ca-pantothenate, 28.1 mg; nicotinamide, 33.1 mg; choline chloride, 551.3 mg; D-biotin, 0.22 mg; folic acid, 1.65 mg.

[d] Supplied per kilogram of diet: Zn, 165 mg ($ZnSO_4$); Fe, 165 mg ($FeSO_4H_2O$); Cu, 16.5 mg ($CuSO_45H_2O$); Mn, 33 mg ($MnSO_4$); I, 0.3 mg $Ca(IO_3)_2$; Se, 0.3 mg (Na_2SeO_3).

[e] Zinc additions, replacing corn, were made based on zinc concentrations of source.

Table 3.2. Type 3 tests of fixed effects of nursery pigs fed supplemental Zn

	Pr > F				
Sources	Weight	ADG	AFI	G:F	FC
Treatment	0.0003	< 0.0001	0.0030	0.1044	0.1007
Time	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment * Time	0.1615	0.0496	0.0002	0.6648	0.2600

Table 3.3. Overall effect of Zn supplementation on nursery pigs[1]

Zn Source:	Basal	ZnO	Zn Proteinate	Zn Polysaccharide
Added Zn, ppm:	0	3,000	250	250
Treatment No.:	1	2	3	4
WT (Kg)	10.37^a	11.24^b	10.44^a	10.16^a
ADG (g/d)	358.60^a	425.30^b	367.10^a	346.40^a
ADFI (g/d)	379.90^a	483.60^b	384.10^a	349.90^a
G:F (g/g)	1.07	0.92	1.11	1.18
FC	31.94^c	38.38^d	32.68^c	36.76^{cd}

[1] Data are LS Means of eight replicate pens of three pigs

[a, b] Means within a row lacking common superscript differ ($P \leq 0.05$)

[c, d] Means within a row lacking common superscript differ ($P \leq 0.10$)

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Table 3.4. Effect of Zn supplementation on average daily gain of nursery pigs[1]

Zn Source	Basal	ZnO	Zn Proteinate	Zn Polysaccharide
Zn Concentration (ppm)	0	3,000	250	250
Treatment No.	1	2	3	4
Weight (Kg)				
Initial weight	6.8	6.7	6.7	6.7
Final weight	16.8[a]	18.6[b]	17.0[a]	16.4[a]
Average Daily Gain (g/d)				
Week 1	79.5	70.8	67.9	65.5
Week 2	215.5[a]	362.2[b]	245.5[a]	204.8[a]
Week 3	470.2[a]	588.1[b]	473.5[a]	480.4[a]
Week 4	669.3	680.1	681.6	635.1

[1] Data are LS Means of eight replicate pens of three pigs with SEM for ADG = 22

[a, b] Means within a row lacking common superscript differ (P ≤ 0.05)
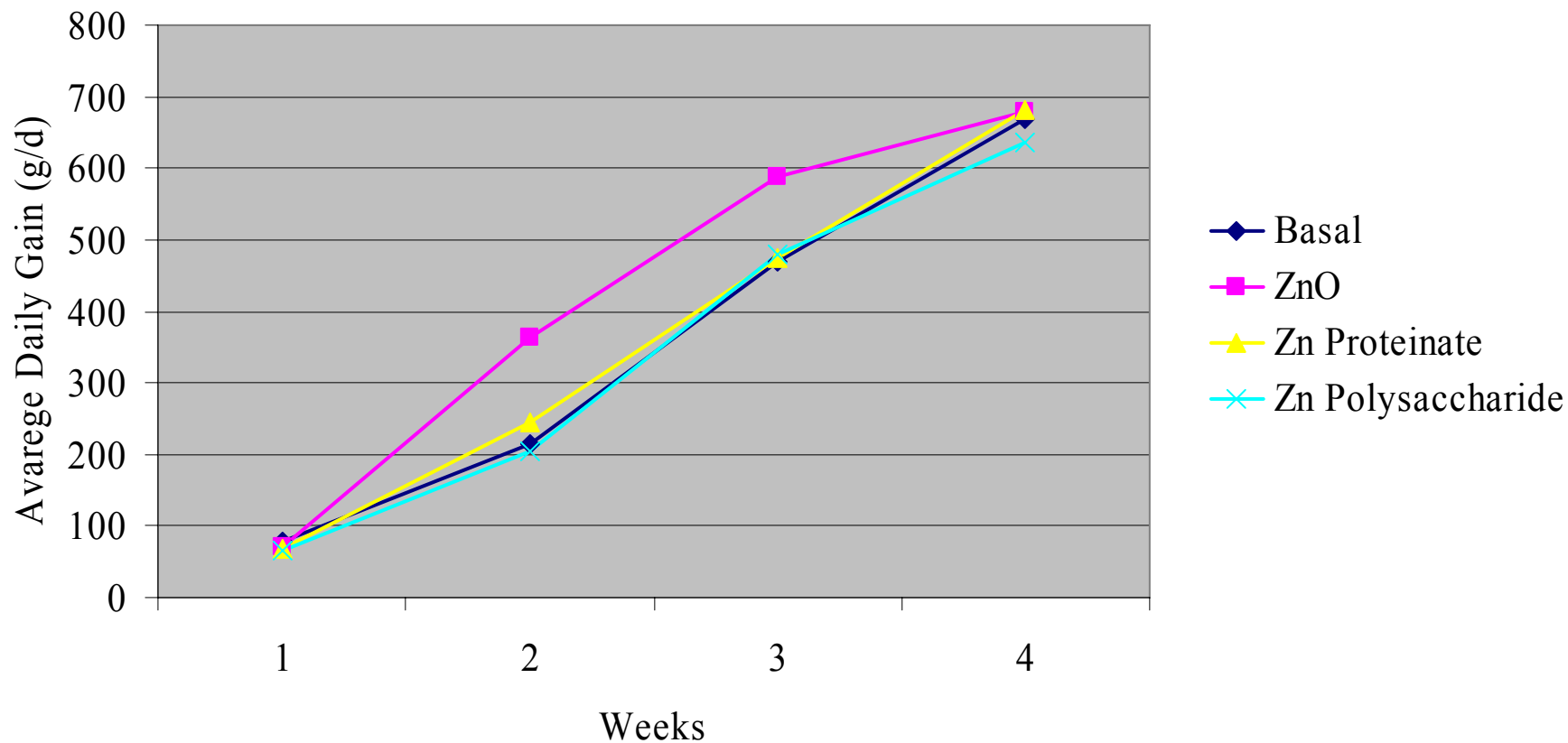
Figure 3.1. Effect of dietary treatment on average daily gain over time: Nursery pigs fed 3,000 ppm of Zn as inorganic ZnO had higher ADG in week 2, 3, and overall (P ≤ 0.05). In addition, pigs fed 3,000 ppm Zn as ZnO had improved gain throughout the 28-d study (P ≤ 0.05). Data are LS Means of eight replicate pens of three pigs.

Table 3.5. Effect of Zn supplementation on average daily feed intake of nursery pigs[1]

Zn Source:	Basal	ZnO	Zn Proteinate	Zn Polysaccharide	
Added Zn, ppm:	0	3,000	250	250	
Treatment No.:	1	2	3	4	Avg/wk
Feed Intake (g/d)					
Week 1	112.3	110.4	104.2	86.0	103.2[w]
Week 2	149.1[a]	263.7[b]	145.2[a]	108.6[a]	166.7[x]
Week 3	466.1[a]	687.5[b]	475.3[a]	453.6[a]	520.6[y]
Week 4	792.0	872.9	811.6	751.2	806.9[z]

[1] Data are LS Means of eight replicate pens of three pigs with SEM = 30.9

[a, b] Means within a row lacking common superscript differ (P ≤ 0.05)

[w, x, y, z] Means within a column lacking common superscript differ (P ≤ 0.05)
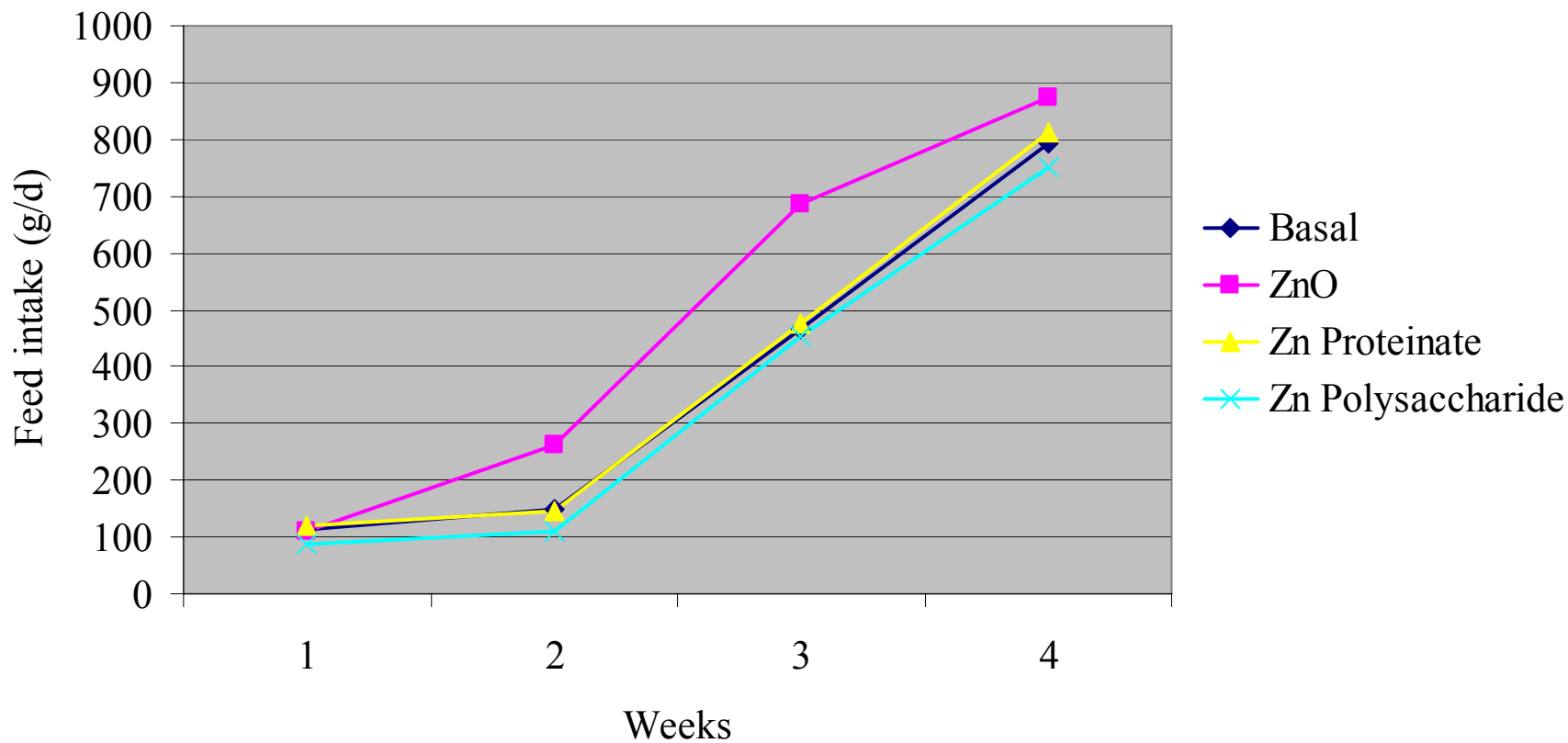
Figure 3.2. Effect of dietary treatment on feed intake over time: Feed intake was greater during week 2, 3, and overall in pigs fed with 3,000 ppm Zn as ZnO than pigs fed either basal or organic Zn diets ($P \leq 0.05$). Pigs fed 3,000 ppm Zn had increased feed intake overtime ($P \leq 0.05$) than pigs fed basal or organic Zn treatments. Data are LS Means of eight replicate pens of three pigs.

Table 3.6. Effect of Zn supplementation on feed efficiency of nursery pigs[1]

	Basal	ZnO	Zn Proteinate	Zn Polysaccharide	
Zn Source:	Basal	ZnO	Zn Proteinate	Zn Polysaccharide	
Added Zn, ppm:	0	3,000	250	250	Avg/wk
Treatment No.:	1	2	3	4	
Gain:Feed (g/g)					
Week 1	0.73	0.56	0.67	0.74	0.68^a
Week 2	1.69	1.46	1.89	2.06	1.77^b
Week 3	1.03	0.86	1.02	1.09	1.00^c
Week 4	0.85	0.80	0.85	0.85	0.84^{ac}

[1] Data are LS Means of eight replicate pens of three pigs

a, b, c Means within a column lacking common superscript differ (P ≤ 0.05)
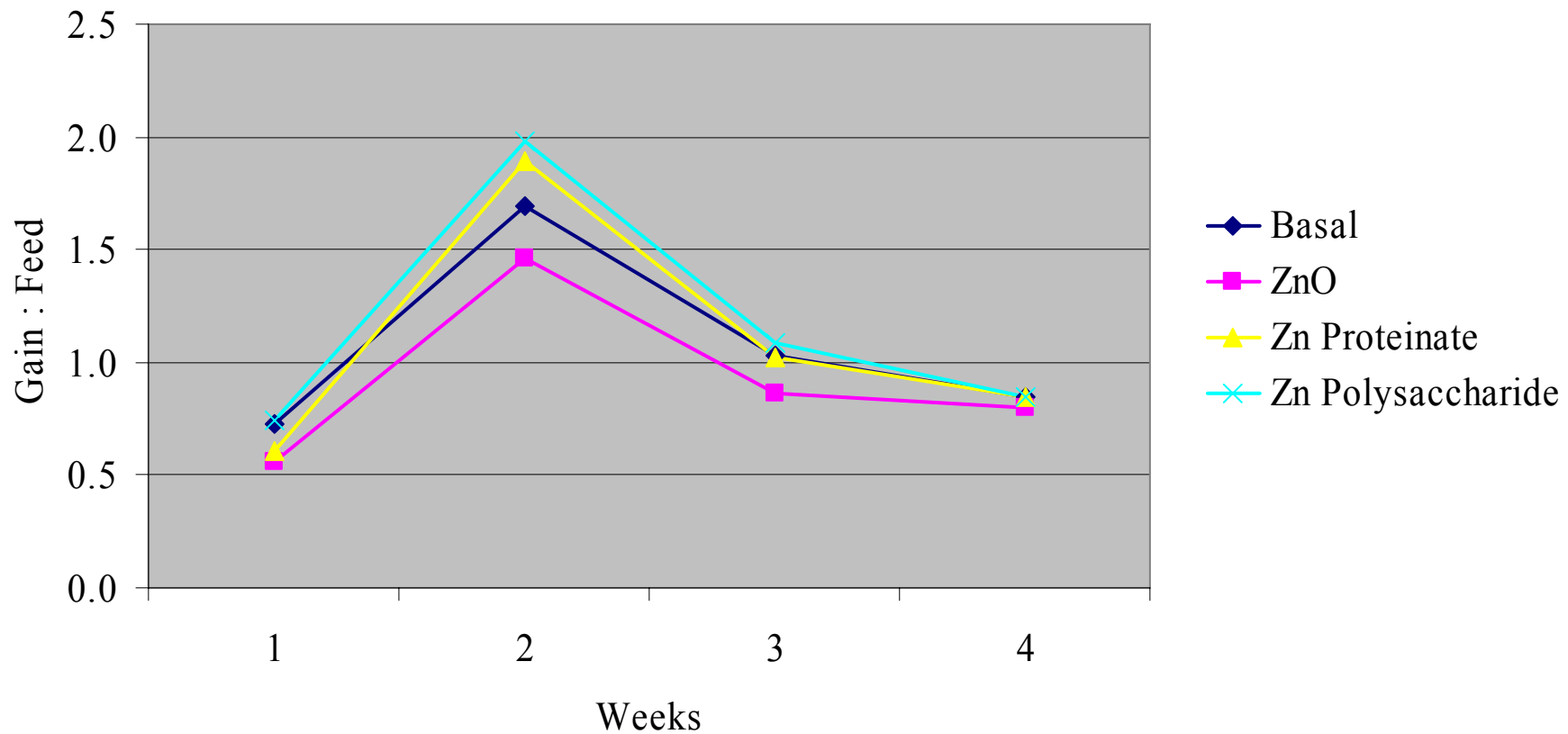
Figure 3.3. Effect of dietary treatment on feed efficiency over time: Feed efficiency (gain/feed) was similar when pigs were fed either basal or Zn diets. Dietary treatment had no effect on G:F throughout the 28-d study ($P > 0.05$). Data are LS Means of eight replicate pens of three pigs.

Table 3.7. Effect of Zn supplementation on fermentative capacity of fecal flora in nursery pigs[1]

Zn Source	Basal	ZnO	Zn Proteinate	Zn Polysaccharide	
Zn Concentration (ppm)	0	3,000	250	250	
Treatment No.	1	2	3	4	Avg/wk
Fermentative Capacity (FC)					
Week 0	0.27	0.20	0.25	0.30	0.25^a
Week 1	0.36	0.46	0.39	0.41	0.40^b
Week 2	0.25	0.39	0.27	0.36	0.32^c
Week 3	0.38	0.46	0.36	0.40	0.40^b
Week 4	0.33	0.42	0.36	0.37	0.37^b

[1] Data are LS Means of eight replicate pens of three pigs

[a, b, c] Means within a column lacking common superscript differ ($P \leq 0.05$)
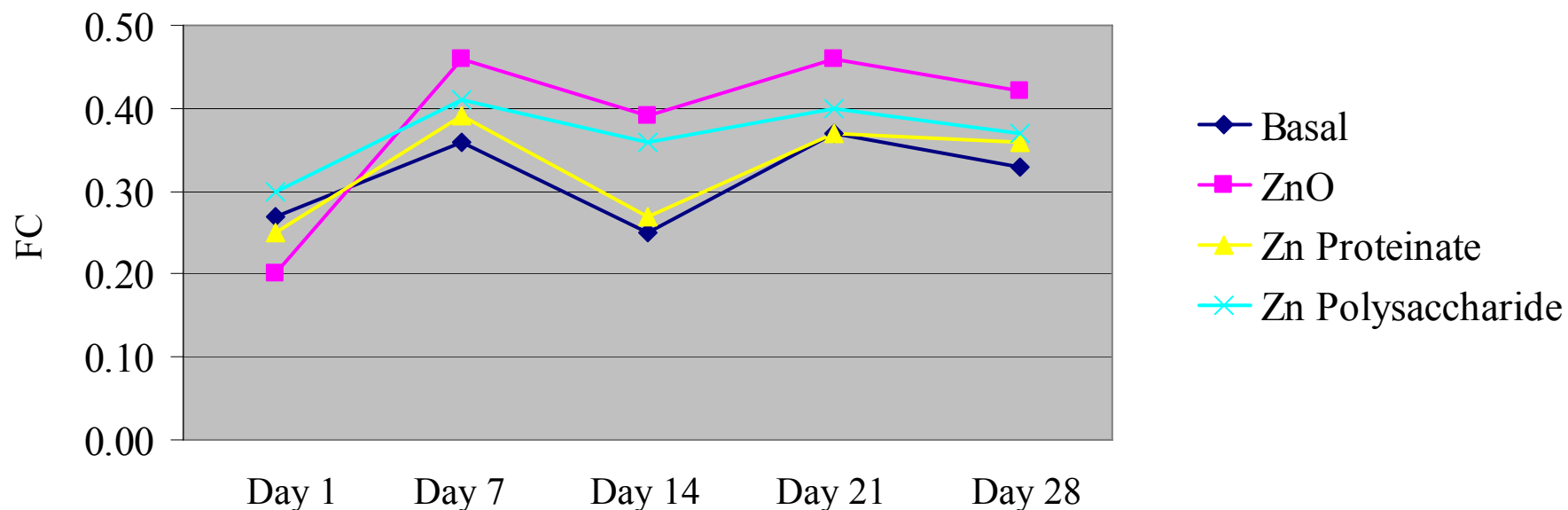
Figure 3.4. Effect of dietary treatment on fermentative capacity of fecal flora in nursery pigs over time: Fermentative capacity of fecal flora of nursery pigs did not differ among the dietary Zn treatments over time (P > 0.05). However, nursery pigs supplemented with pharmacological concentration of Zn (3,000 ppm) as ZnO had a trend for higher (P ≤ 0.1) FC compared to pigs fed either basal or 250 ppm of organic Zn proteinate. Data are LS Means of eight replicate pens of three pigs.

Figure 3.5. Correlation coefficients for the fecal flora's biochemical fingerprint of nursery pigs by dietary treatment at weaning [a]
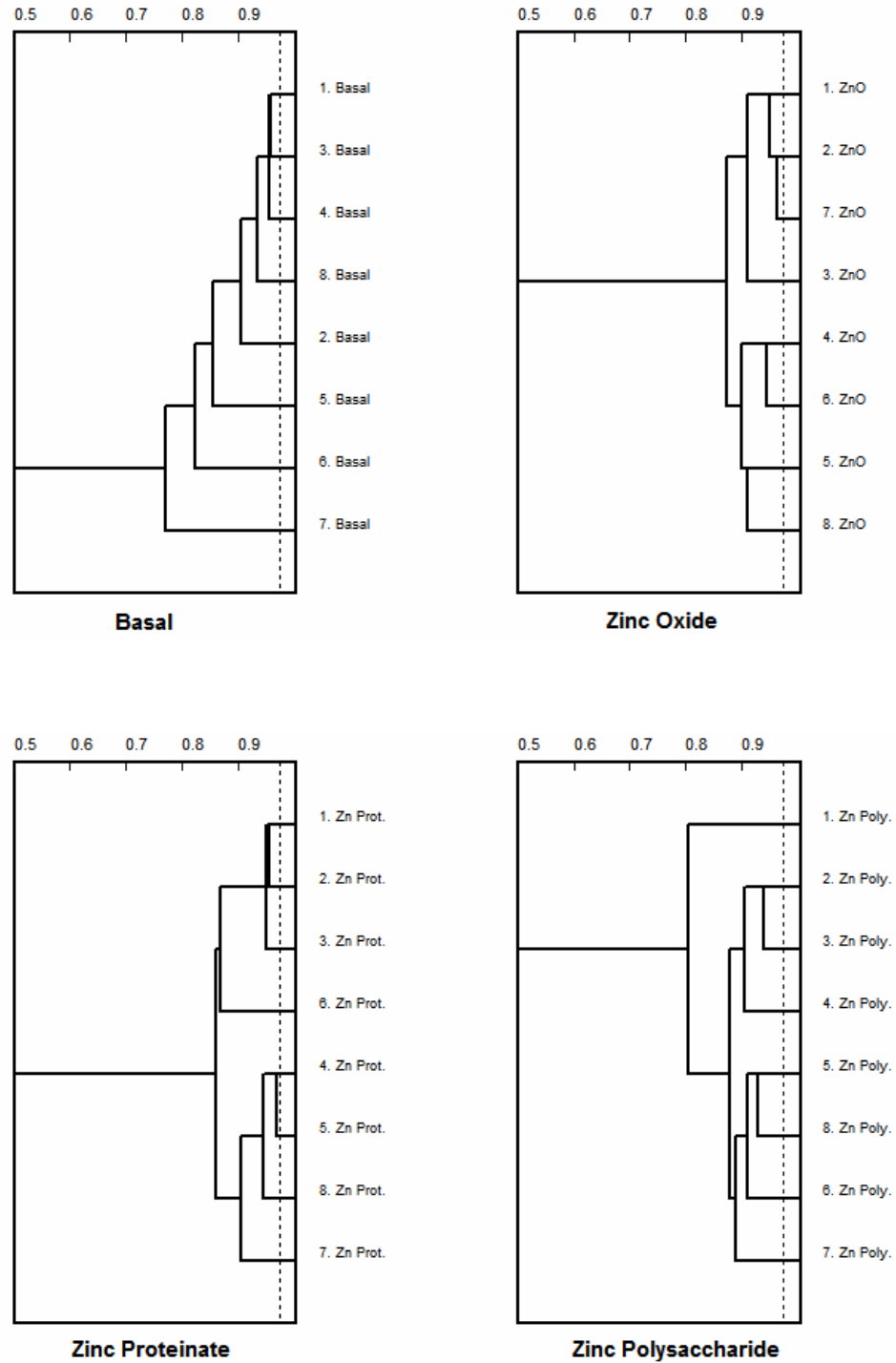
Figure 3.6. Correlation coefficients for the fecal flora's biochemical fingerprint per

dietary treatment of nursery pigs at weaning [a]
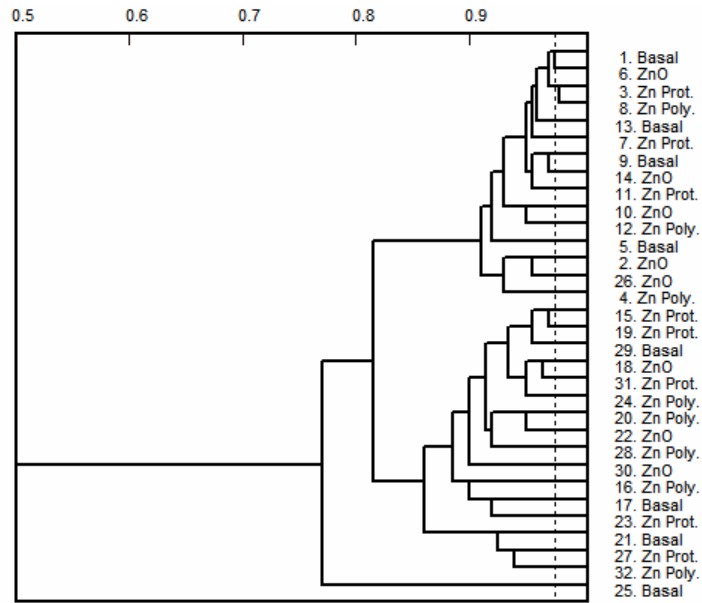
[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.7. Correlation coefficients for the fecal flora's biochemical fingerprint of nursery pigs by dietary treatment during week 1 post-weaning [a]

[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.8. Correlation coefficients for the fecal flora's biochemical fingerprint per

dietary treatment of nursery pigs 1 week post-weaning

[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.9. Correlation coefficients for the fecal flora's biochemical fingerprint of
nursery pigs by dietary treatment during week 2 post-weaning [a]

[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.10. Correlation coefficients for the fecal flora's biochemical fingerprint per

dietary treatment of nursery pigs 2 weeks post-weaning [a]

[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.11. Correlation coefficients for the fecal flora's biochemical fingerprint of
nursery pigs by dietary treatment during week 3 post-weaning [a]

[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.12. Correlation coefficients for the fecal flora's biochemical fingerprint per

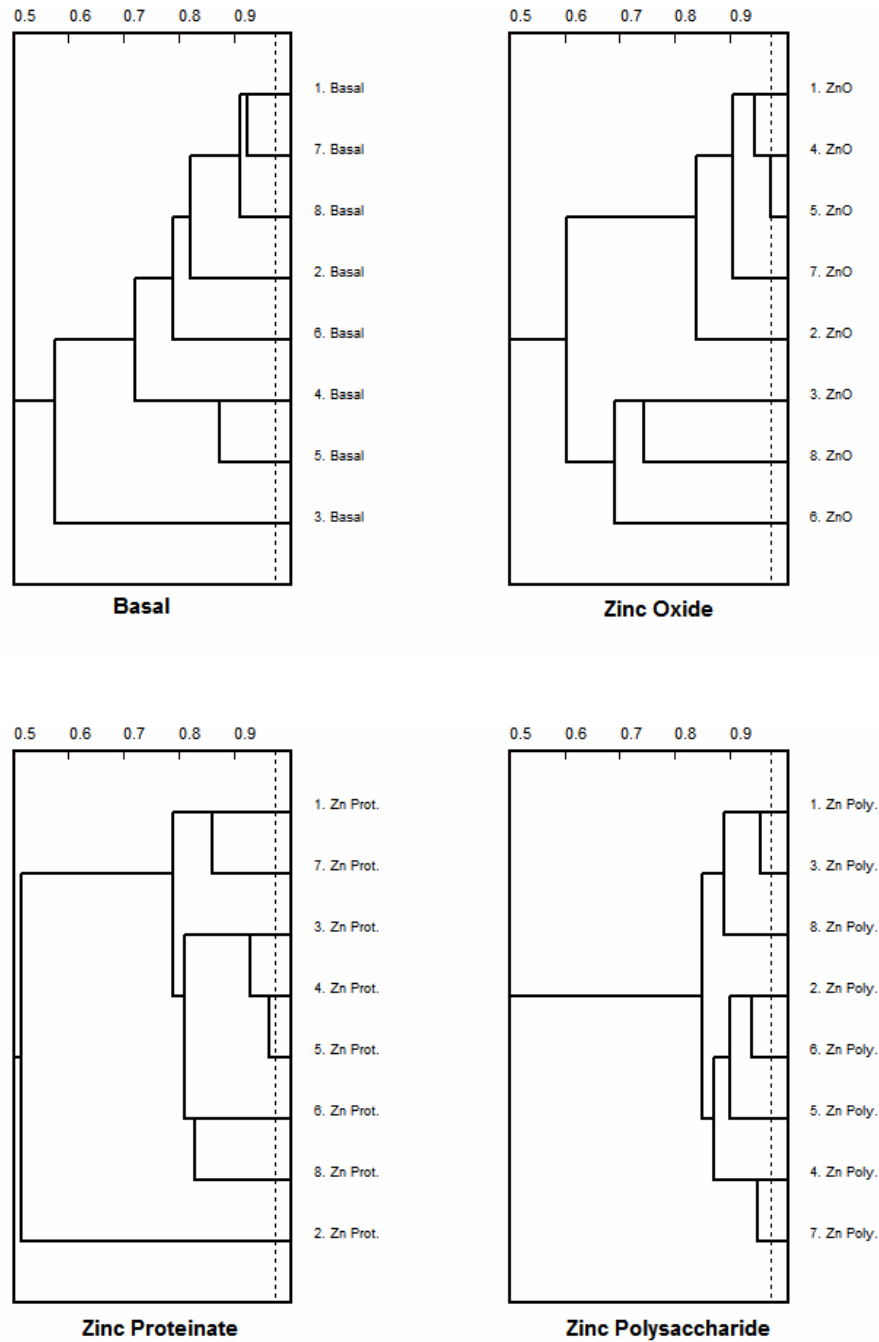dietary treatment of nursery pigs 3 weeks post-weaning [a]
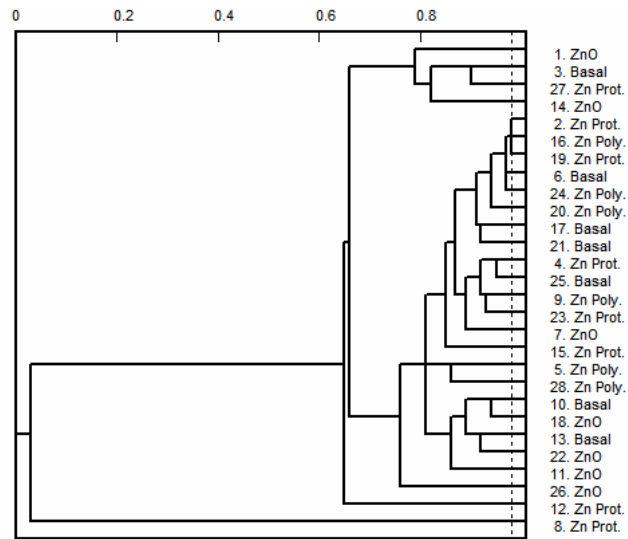
[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.13. Correlation coefficients for the fecal flora's biochemical fingerprint of nursery pigs by dietary treatment during week 4 post-weaning [a]
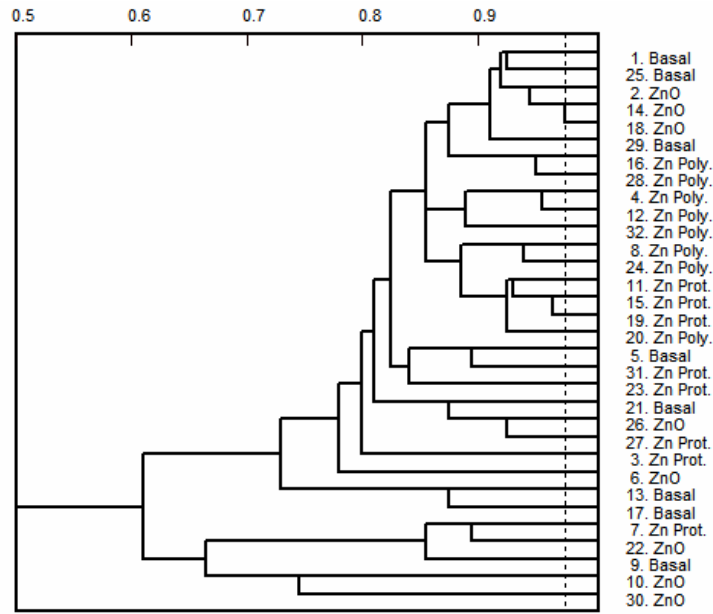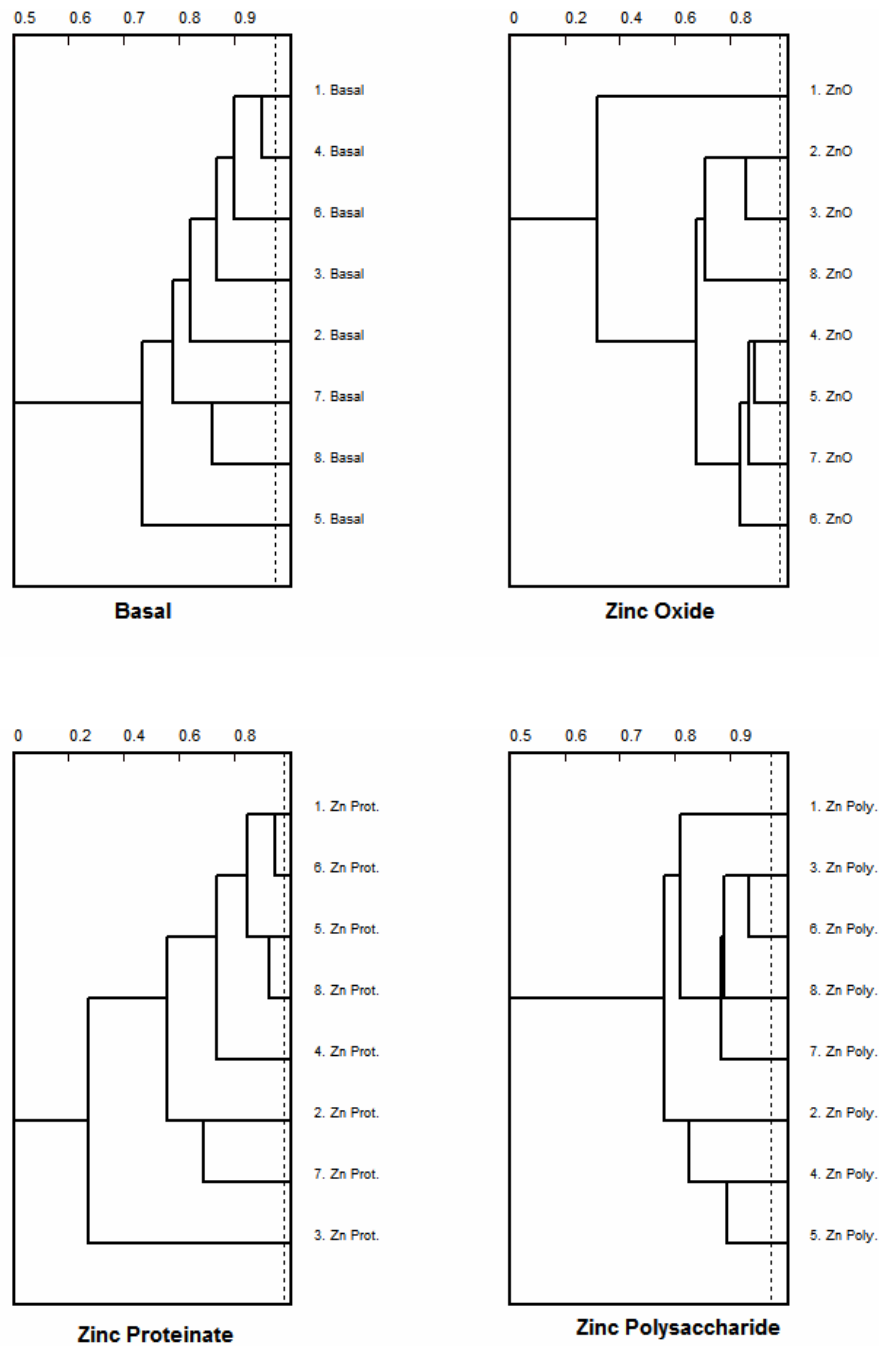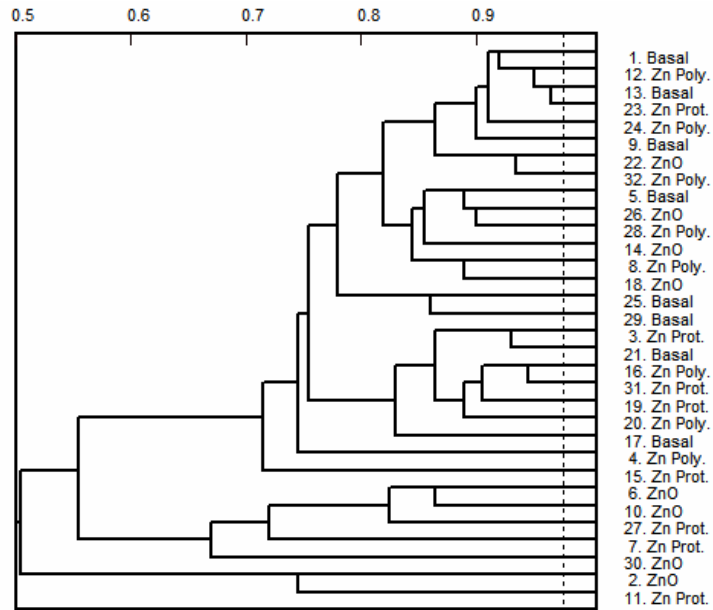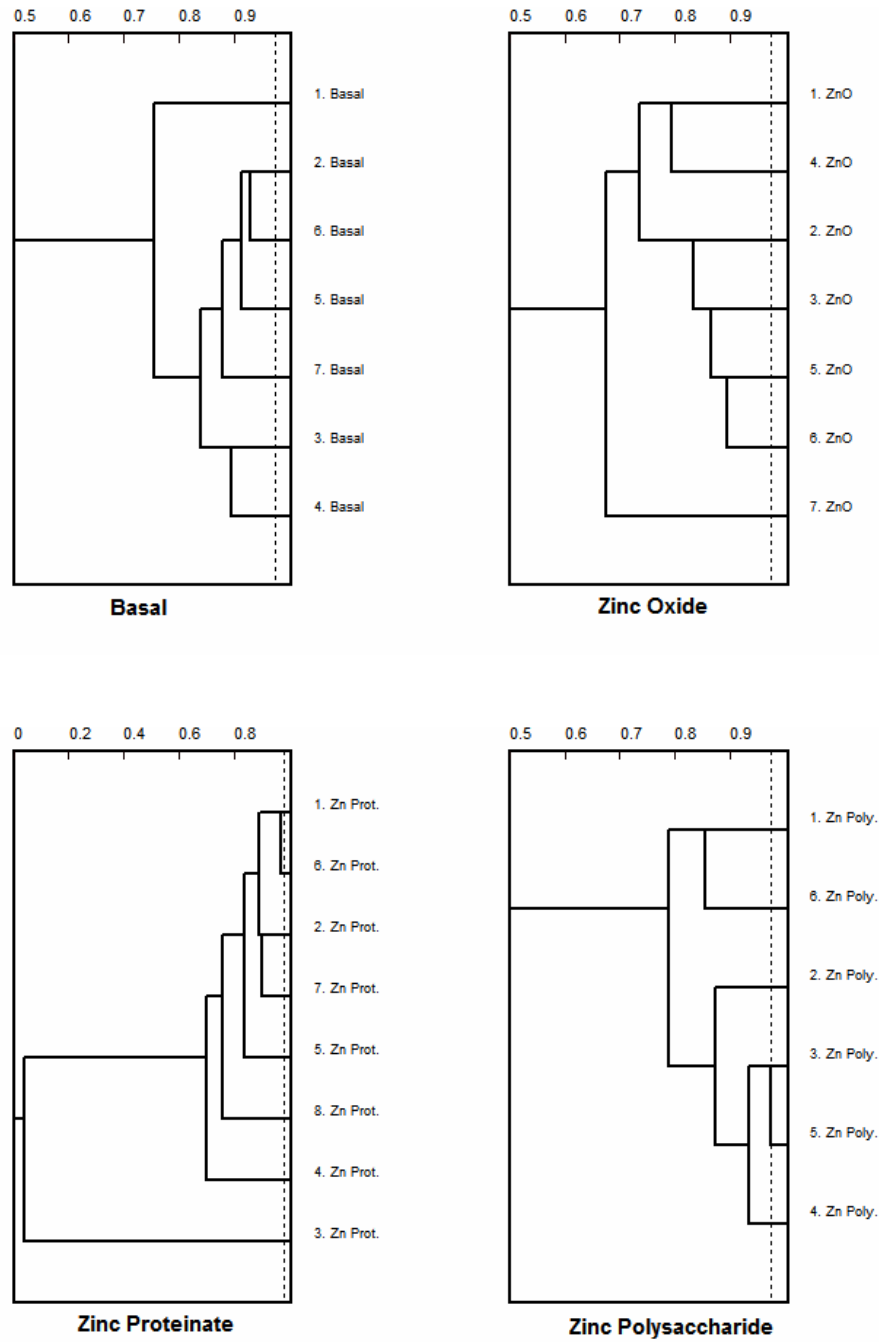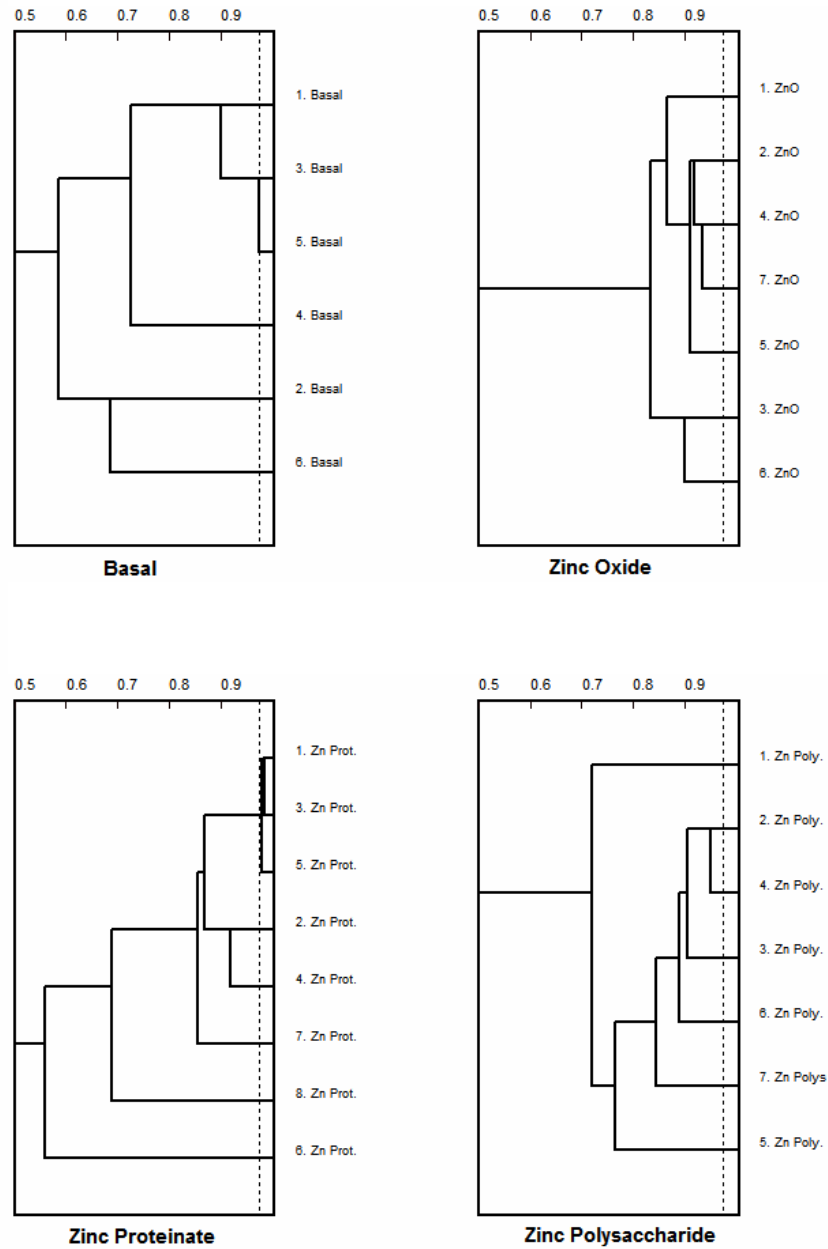

[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

Figure 3.14. Correlation coefficients for the fecal flora's biochemical fingerprint per

dietary treatment of nursery pigs 4 weeks post-weaning [a]
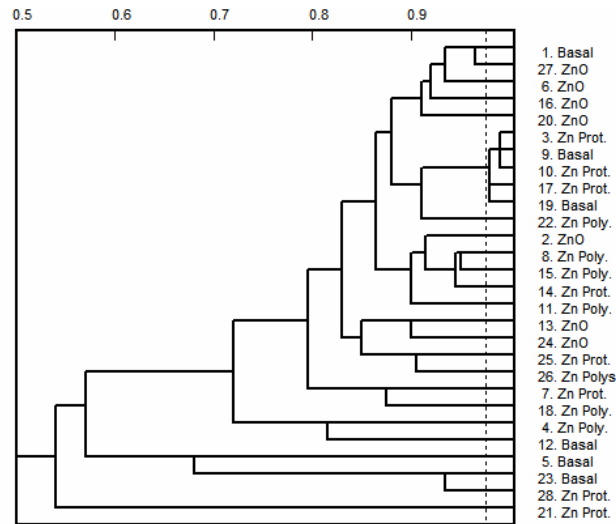
[a] Low correlation (0.60 to 0.69), moderate (0.70 to 0.79), high (0.80 to 0.89) and very high (0.90 to 1.00)

CHAPTER IV

THE EFFECT OF FEEDING PHARMACOLOGICAL CONCENTRATIONS OF ZINC OXIDE ON GROWTH PERFORMANCE AND FECAL MICROFLORA IN NURSERY PIGS

ABSTRACT

The experiment was conducted to evaluate the effect of feeding pharmacological levels of zinc oxide (ZnO) on growth performance and fecal microflora in nursery pigs. Forty crossbred pigs (7.53 ± 0.14 kg; 24 ± 0.5 d of age) were weaned and allotted to one of four treatments based on weight and ancestry (one pig/metabolism crate and 10 reps), for the duration of the 28-d study. Phase 1 (d 1 to 14) and Phase 2 (d 15 to 28) nursery diets were fed in meal form. Both dietary phases utilized four dietary treatments: (1) Basal diet contained 165 ppm Zn as $ZnSO_4$ which was supplied by the trace mineral premix, (2) Basal + 750 ppm Zn as ZnO, (3) Basal + 1,500 ppm Zn as ZnO, (4) Basal + 3,000 ppm Zn as ZnO. Body weight, and feed disappearance data, and fecal samples were collected weekly. Fecal samples were collected to determine the number of *E. coli* and lactobacilli excreted. There was no effect of dietary Zn treatments on average daily gain, feed intake and feed efficiency (gain/feed) during week 1, 2, 3, 4, or overall ($P > 0.05$). The number of *E. coli* and lactobacilli excreted per gram of wet feces was not affected by the dietary treatment ($P > 0.05$). However, the number of *E. coli* and lactobacilli excreted

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changed over time (P ≤ 0.05). These results indicate that the positive performance improvements of feeding pharmacological concentrations of Zn as inorganic ZnO has no effect under conditions of minimal stress, minimal pathogen challenge, and high health status nursery pigs. Therefore, this may be a reason why no difference was observed in fecal microflora among dietary treatments.

INTRODUCTION

Currently, many swine producers supplement pharmacological concentration of Zn (2,000 to 3,000 ppm) as inorganic ZnO in nursery pig diets for two weeks post-weaning since it has been proven to ameliorate the problem due to stressful weaning by decreasing the incidence of post-weaning scouring and increasing average daily gain in nursery pigs (Poulsen, 1989; Hahn and Baker, 1993; Carlson et al., 1999; Hill et al., 2000; Case and Carlson, 2002).

The mechanism behind the growth response of feeding 2,000 to 3,000 ppm of Zn as ZnO is not fully understood. Zinc oxide has been shown to improve gastrointestinal tract function by increasing mucosal thickness, villi height, and width of the small intestine (Li et al., 2001). Nursery pigs fed diets supplemented with 3,000 ppm of Zn as ZnO showed alteration in the duodenum intestine, such as deeper crypts and greater total thickness, and increased intestinal metallothionein concentrations, which indicates that high concentrations of Zn may have an enteric effect on the growing pig (Carlson et al., 1998). However, Roselli et al. (2003) reported that ZnO may protect intestinal cell from *E. coli* infections by inhibiting the adhesion and internalization of bacteria, preventing the

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disruption of barrier integrity, and modulating cytokine gene expression, but not by direct antibacterial effect.

High doses of dietary ZnO have been shown to be beneficial for maintaining the stability of the intestinal microflora, to support a large diversity of coliforms in weaned piglets (Katouli et al., 1999), and to reduce the susceptibility of pigs to *E. coli* infection (Mores et al., 1998). *Escherichia coli* infection has been associated with the most important cause of neonatal and post-weaning diarrhea in pigs with mortality reaching high levels during the first few days after birth (Tzipori, 1985; Bertschinger et al., 1992).

Lactobacilli (gram-positive anaerobic or facultative aerobic rods) are considered to have beneficial effects on human and animal health. Studies in vitro and in animals have shown that lactobacilli may prevent *E. coli* from colonizing the jejunum and producing substances directed against the enterotoxins resulting in an inhibition of *E. coli*-induced enterotoxin reactions (Foster et al., 1980; Johnson and Calia, 1979; Mitchell and Kenworthy, 1976).

Because Exp. 1 showed that pigs fed 3,000 ppm Zn as ZnO had 12% higher FC and greater correlations between substrate utilization of bacteria in the intestine compared to pigs fed basal or other Zn treatments, a second experiment was conducted to investigate the effect of feeding pharmacological levels of ZnO on growth performance and to determine changes in *E. coli* and lactobacilli excreted per gram of wet feces of nursery pigs.

MATERIALS AND METHODS

This research was approved by the Animal Care and Use Committee of the University of Missouri-Columbia before initiation of the experiment (protocol # 4058).

Animals

A total of forty crossbred (PIC C22 x TF4) pigs weaned at an average age of 25 ± 0.5 d, and initial average body weight of 7.53 ± 0.14 kg were allotted to one of four dietary treatment based on weight and ancestry, for the 28-d study.

Diets

Pigs were fed typical Phase 1 (d 1 to 14) and Phase 2 (d 15 to 28) nursery diets in meal form (Table 4.1). The Phase 1 diet contained 22.74% CP and 1.62% total lysine, and the Phase 2 diet contained 20.41% CP and 1.34% total lysine. The basal diet contained 165 ppm of Zn as inorganic $ZnSO_4$, which was supplied by the trace mineral premix. The four dietary treatments utilized were: (1) Basal diet, (2) Basal diet + 750 ppm Zn as ZnO; (3) Basal diet + 1,500 ppm Zn as ZnO; (4) Basal diet + 3,000 ppm Zn as ZnO. Both phases of nursery diets were corn and soybean meal based. All nutrients met or exceed NRC (1998) recommendations for 5 to 20 kg nursery pigs.

General Husbandry

Pigs were housed in individual, solid-walled, stainless steel metabolism crates with stainless steel nipple drinkers, feeders, and woven wire or slotted floors, with 10 replications per treatment. Pigs were allowed ad libitum access to feed and water, and feed was added to the feeders twice daily (0800 and 1600) for the duration of the 28-d

study. The temperature in the nursery facility was maintained at approximately 30°C for week 1, with a 2°C decrease each week thereafter.

Growth Performance

Every week, pig weights (WT) and feed consumption was determined by pen in order to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F).

Determination of the number of E. coli excreted per gram of wet feces

Fecal grab samples were collected weekly from each pig in order to prepare and plate serial dilutions. Eight serial decimal dilutions were prepared. For the first dilution, 10 g of each fresh fecal sample was weighted and diluted in 90 ml of buffered medium (10 fold dilution – 10^{-1}), which was made in our laboratory. To prepare 1 L of this buffered medium the following steps were followed: 1) 25 g of modified biopro buffered peptone water was weighed and diluted in 1 L of deionized water; 2) 0.5 g of cysteine HCl and 1 ml of Tween 80 were measured and added to the solution prepared in step 1; 3) The pH was adjusted to 6.5; 4) Medium was autoclaved at 121°C for 15 min (Hartemink et al., 1997). This first dilution was mixed in the Stomacher blender for 1 min at high speed.

For the following dilutions (10^{-2} to 10^{-8}), the diluent used was buffered dilution water, which consisted of 0.5 g of neutralized peptone, 8.0 g of NaCl, and 0.5 g cysteine HCl per litter of deionized water. The pH was adjusted to 6.7 before autoclaving at 121°C for 15 min (Hartemink et al., 1997). Nine ml of the buffered dilution water was pipetted into each of the seven sterile tubes. Each dilution was prepared by pipetting 1 ml from a

previous dilution into 9 ml of buffered dilution water and then vortexing to mix prior to the next dilution step. For instance, to prepare the second dilution, 1 ml of the first dilution was added to 9 ml of buffered dilution water (10^{-2}).

The 3M Petrifilm test E. coli/Coliform count plate (3M Microbiology, St. Paul, MN), a sample-ready-culture-medium system, was used to determine the number of *E. coli* excreted per gram of wet feces. In order to plate the dilutions, the top film of the plate was lifted and 1 ml of the dilution was pipetted onto the center bottom of the plate. Thereafter, the top film was rolled down onto the sample and a plastic spreader was used to distribute sample evenly. All the plating was done in duplicates in order to get accurate data.

Plates were placed in the incubator set at $37^{o}C$ for 24 hr. After incubating the plates overnight, the number of *E. coli* colonies per plate was counted. Blue and red colonies associated with gas (small bubble) were identified as *E. coli* colonies. The fermentation of lactose in the medium by *E. coli* forms gas, which appears as a small bubble associated with the colony on the plate.

During the study the dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were plated. Plates with colony forming units (cfu) between 20 and 250 were counted, however, plates containing fewer than 20 colonies and more than 250 colonies on a plate were not counted because they might not be representative of the sample and are likely to produce colonies too close to each other to be distinguished as distinct cfu. Duplicate's average was taken when the cfu from duplicates did not differ more than 10% between them. When the difference between duplicates was higher than 10% the highest cfu was considered.

For calculating the number of *E. coli* per gram of wet fecal samples, the following

formula was used: Number of *E. coli* colonies (cfu) x Dilution factor.

amount plated (1 ml)

Determination of the number of excreted lactobacilli per gram of wet feces

A medium to isolate lactobacilli colonies was prepared in our laboratory. The

medium consisted of three different solutions: solution A, B and C. In order to prepare

800 ml of the medium the following recipe was used: *Solution A* – 41.76 g of MRS broth,

0.2 g of Cysteine – HCl, 0.02 g of Bromocresol green and 400 ml of deionized water. The

pH of this solution was adjusted to 5.0 with 4 M HCl before autoclaving at 121°C for 15

min; *Solution B* – 16 g of agar and 400 ml of deionized water. Solution B was sterilized

at 121°C for 15 min after being prepared; *Solution C* – 0.16 g of vancomycin

hydrochloride was diluted in 8 ml of deionized water. Thereafter, this solution was

sterilized by filtration using a 0.2-μm filter and kept at 0-4°C (Hartemink et al., 1997).

After sterilization, solution A was cooled to room temperature and solution B was

placed in a water bath in order to cool to 50°C. Before plating the dilutions, solution C

was added to solution A and solution B was added to the mixture of solution A + C. The

prepared medium was kept in a water bath until be used.

The pour plate technique was used to determine the number of lactobacilli/g of

wet feces. In order to plate the dilutions, the following steps were followed: 1) petri dish

was inoculated with 1 ml of the dilution; 2) Approximately 15 ml of melted agar medium

was poured into the inoculated petri dish; 3) The sample and agar were mixed thoroughly

by rotating the plate several times, clockwise, then counterclockwise; 4) Inoculated petri

dish with agar was placed on a flat surface for about 5 min to allow the agar to

completely gel; 5) Plates were placed upside down in an anaerobic chamber and incubated at 37°C for 48 hrs; 6) Lactobacilli colonies, which in most cases were green and, sometimes white, were counted.

During the entire study the dilutions 10^{-6}, 10^{-7} and 10^{-8} were plated. All the plating was done in duplicate to get accurate data. Plates with colony forming units (cfu) between 20 and 250 were counted. Duplicate's average was taken when the cfu from duplicates did not differ more than 10% between them. When the difference between duplicates was higher than 10% the highest cfu was considered.

For calculating the number of lactobacilli per gram of wet fecal samples, the following formula was used: Number of lactobacilli colonies (cfu) x Dilution factor.

amount plated (1 ml)

Diarrhea occurrence

In order to verify if diarrhea was associated with the dietary Zn treatments, scours occurrence was recorded every day throughout the 28-d study.

Statistical Analysis

Data were analyzed as a completely randomized design (RCD) using the Mixed Models of SAS (SAS Inst. Inc., Cary, NC) as described by Littell et al. (1998). All bacterial counts were transformed to $\log_{10}$ values. The statistical model included the effects of treatment (diet), time (week), and the interaction between treatment and time. Differences were determined using Fisher's Least Significant Difference (LSD) and were considered significant at $P \leq 0.05$. Response values for treatment effects on growth performance (ADG, ADFI and G:F, number of *E. coli* colonies, and lactobacilli colonies)

were compared using polynomial contrasts (linear, quadratic and cubic) using one degree of freedom F test. To determine if the occurrence of diarrhea was associated with dietary treatments, the GENMOD procedure (modeling procedures of SAS) based on differences in odds and binomial distribution was used. Data were analyzed using pen as the experimental unit and differences were considered significant at $P \leq 0.05$.

RESULTS

Growth Performance

Average daily gain, ADFI and G:F were not affected by dietary Zn treatments during week 1, 2, 3, 4, and overall (Table 4.2 and 4.3). Nursery pigs had an average of 719 g/d gain, 975 g/d feed intake and 0.74 g/g feed efficiency throughout the 28-d study. Average daily gain (Table 4.4; Figure 4.1), ADFI (Table 4.5; Figure 4.2) and G:F (Table 4.6; Figure 4.3) were affected by time. During the nursery study, pigs increased ADG from 452 g/d during week 1 to 959.8 g/d during week 4. Feed intake increased from 562.2 g to 1,406.1 g during week 1 and 4, respectively.

Number of E. coli and lactobacilli colonies excreted per gram of wet feces

The number of excreted *E. coli* and lactobacilli colonies was not affected by the dietary Zn treatments ($P > 0.05$) as shown on Table 4.2 and 4.3. Nursery pigs had an average of 5.77 $\log_{10}$ *E. coli*/g of wet feces and 8.43 $\log_{10}$ lactobacilli/g of wet feces for the 28-d study. However, the number of colonies of *E. coli* (Table 4.7; Figure 4.4) and lactobacilli (Table 4.8; Figure 4.5) per gram of wet feces was affected by time ($P \leq 0.05$). *Escherichia coli* colonies excreted from nursery pigs were the highest ($P \leq 0.05$) during

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week 1 and 4 compared to week 2 and 3 of the experiment (Table 4.7; 6.14 $\log_{10}$ vs. 5.4 $\log_{10}$ *E. coli*/g of wet feces, respectively). Nursery pigs had decreasing concentrations of lactobacilli colonies during the 28-d study ($P \leq 0.05$). Pigs averaged 8.97 $\log_{10}$ lactobacilli/g of wet feces during week 1 and 7.9 $\log_{10}$ lactobacilli/g of wet feces during week 4.

Diarrhea occurrence

The occurrence of diarrhea was affected by the dietary Zn treatments ($P \leq 0.05$). Nursery pigs fed diet containing either basal or 750 ppm Zn as ZnO were 16 or 20 times more likely to have diarrhea than compared to nursery pigs supplemented with 1,500 or 3,000 ppm Zn as ZnO ($P \leq 0.05$) during the first 14-d post-weaning and the second 14-d post-weaning as shown on Table 4.9 and 4.10, respectively.

DISCUSSION

Results of this experiment indicate that growth rate was not improved when nursery pigs were fed diet containing 750 ppm, 1,500 ppm or 3,000 ppm Zn as inorganic ZnO compared to animals fed basal diet under the environmental conditions of this study. These data disagree with previous studies in which nursery pigs supplemented with pharmacological concentrations of Zn (2,000 to 3,000 ppm) as inorganic ZnO had an improvement in growth (Poulsen, 1989; Poulsen 1995; Kavanagh, 1992; Hahn and Baker, 1993; Carlson et al., 1999; Hill et al., 2000; Case and Carlson, 2002).

Some possible explanations for not observing an improvement in growth performance in nursery pigs supplemented with pharmacological concentrations of Zn as

inorganic ZnO in this experiment are: 1) animals were housed in individual metabolism crates, therefore did not have direct contact with other animals; 2) the experiment was conducted in an isolated room on the University of Missouri campus, which did not have the environmental conditions usually present on most swine operations or at the University of Missouri swine farm; 3) nursery pigs were slightly older and had heavier body weights at weaning. Nursery pigs older than 21 d of age and heavier than 7.00 kg, have been shown to have an affect in growth performance when fed pharmacological concentrations of ZnO (Carlson, 2005). These conclusions are supported by the gain:feed performance.

In the present study, feeding pharmacological concentration of Zn as ZnO did not increase average daily feed intake in nursery pigs when compared to feeding the basal diet. These data supports Poulsen (1995) and Smith et al. (1997), who found no improvement in feed intake when pharmacological concentrations of Zn (2,000 or 3,000 ppm) as ZnO were fed to nursery pigs. However, these findings are in contrast to those of Kavanagh (1992), Hollis et al. (2005), Hill et al. (2000, 2001) and Carlson et al. (1999) who found that pharmacological levels of Zn as ZnO increased average daily feed intake and average daily gain in nursery pigs. These contradictions in performance results are more than likely due to the physiological and environmental conditions of the experiments.

Over the entire 28-d nursery period, there was no effect of dietary Zn on feed efficiency (G:F), similar to other experiments where no improvement in G:F from Zn was reported (Poulsen, 1995; Smith et al., 1997; Hollis et al., 2005).

In the current study increasing dietary Zn, results in a reduction of incidence in diarrhea of post-weaning pigs. However, the likely hood of reduction in diarrhea in weaned pigs does not further increase when feeding concentrations of ZnO above 1,500 ppm. These data support the findings of Poulsen (1995), Holm (1988) who reported lower incidence of diarrhea when nursery pigs were fed pharmacological concentration of Zn (2,500 to 4,000 ppm) as ZnO. In contrast to these data, dietary supplementation of inorganic Zn as ZnO has been shown to not ameliorate diarrhea post-weaning (Fryer et al., 1992; Tokach et al., 1992).

Limited research studies are available reporting the effect of supplementing pharmacological concentration of ZnO on the number of *E. coli* and lactobacilli excreted per gram of wet feces.

Results of this experiment indicate that dietary concentration of Zn as ZnO does not affect the number of *E. coli* excreted per gram of wet feces compared to pigs fed a basal diet. These results are similar to other experiments where no effect on the number of *E. coli* or enterococci bacteria excreted per gram of wet feces were observed when weanling pigs were fed a pharmacological dose of Zn as ZnO (Jensen-Waern et al., 1998; Katouli et al., 1999; Li et al., 2001).

In the present study, dietary ZnO treatments did not affect the number of lactobacilli excreted per gram of feces. These findings supports Li et al. (2001) who reported no effect of pharmacological concentrations of Zn as ZnO supplementation for nursery pigs on the number of enterobacteriaceae, clostridia and lactobacilli in ileal digesta and feces. In contrast, Broom et al. (2003) and Jensen-Waern et al. (1998) found that pharmacological concentrations of Zn as ZnO reduce fecal counts of lactobacilli and

enterococci during the post-weaning period of pigs, but only temporarily. In agreement, Hojberg et al. (2005) reported that feeding weaned piglets 2,500 ppm of Zn as ZnO reduced the MRS counts (lactic acid bacteria) and Rogosa counts (lactobacilli) for all segments of the gastrointestinal tract.

Data from this experiment show that the number of lactobacilli excreted decreases per gram of wet feces over time. In agreement to these data, Mulder et al. (1997) reported that after weaning there is a decrease in the population of lactic acid bacteria and an increase in coliform bacteria.

IMPLICATIONS

Data from this experiment indicate that feeding nursery pigs with pharmacological concentrations of Zn (750 ppm to 3,000 ppm) as ZnO did not improve growth performance, feed intake or gain:feed throughout the 28-d post-weaning study. Nursery pigs fed diet supplemented with either 1,500 ppm or 3,000 ppm Zn as ZnO had less incidence of diarrhea of post-weaning pigs ($P \leq 0.05$) when compared to pigs fed basal or 750 ppm Zn as ZnO. The number of *E. coli* and lactobacilli excreted per gram of wet feces was not affected by the dietary Zn treatments throughout the 28-d study. Therefore, these data indicate that the positive growth performance improvement of feeding pharmacological concentrations of Zn as inorganic ZnO had no effect under the environmental conditions of this study (conditions of minimal stress, minimal pathogen challenge, and high health status). Therefore, this may be a reason why no difference was observed in fecal microflora among dietary treatments.

Table 4.1. Composition of basal diets (%, as-fed basis) [a]

Ingredient	Phase 1 [b]	Phase 2 [b]
Yellow dent corn	35.20	51.00
Soybean meal (48%)	25.00	27.40
Dried whey	25.00	10.00
Spray-dried animal plasma	6.30	2.50
Choice white grease	5.00	5.00
Dical phosphate, 21%	1.62	2.22
Limestone	0.75	0.80
Vitamin premix [c]	0.50	0.50
Mineral premix [d]	0.15	0.15
Salt NaCl	0.20	0.20
L-Lysine HCl	0.15	0.15
DL- Methionine	0.13	0.08
Zn [e]	-	-
Calculated composition		
Crude protein, %	22.73	20.40
Lysine, %	1.61	1.33
Calcium, %	1.04	1.10
Available phosphorus, %	0.62	0.57
Metabolizable energy, kcal/kg	3,244.09	3,387.07

[a] Formulated to contain at least 0.90% Ca and 0.55% available P.

[b] Phase 1 diets were formulated to contain 1.6% total lysine and 22.5% CP, and Phase 2 diets contained 1.25% lysine and 19.4% CP. Phase 1 fed d 1 to 21 of experiment. Phase 2 fed d 22 to 28 of experiment.

[c] Supplied per kilogram of diet: retinyl acetate, 11,000 IU; cholecalciferol, 1,100 IU; DL-α-tocopheryl acetate, 44.1 IU; menadione Na dimethylpyrimidinol bisulfate, 4.0 mg; vitamin B_{12}, 30.3 μg; riboflavin, 8.3 mg; D-Ca-pantothenate, 28.1 mg; nicotinamide, 33.1 mg; choline chloride, 551.3 mg; D-biotin, 0.22 mg; folic acid, 1.65 mg.

[d] Supplied per kilogram of diet: Zn, 165 mg ($ZnSO_4$); Fe, 165 mg ($FeSO_4H_2O$); Cu, 16.5 mg ($CuSO_45H_2O$); Mn, 33 mg ($MnSO_4$); I, 0.3 mg $Ca(IO_3)_2$; Se, 0.3 mg (Na_2SeO_3).

[e] Zinc additions, replacing corn, were made based on zinc concentrations of source.

Table 4.2. Type 3 tests of fixed effects of nursery pigs fed supplemental Zn

			Pr > F		
Sources	ADG	AFI	G:F	*E. coli* colonies	Lactobacilli colonies
Treatment	0.1794	0.5848	0.0880	0.7691	0.2445
Time	< 0.0001	< 0.0001	< 0.0001	0.0061	< 0.0001
Treatment * Time	0.3586	0.7016	0.5771	0.2706	0.9518

Table 4.3. Overall effect of Zn supplementation on nursery pigs[1]

	Zn Source:	Basal	ZnO	ZnO	ZnO
	Added Zn, ppm:	0	750	1,500	3,000
	Treatment No.:	1	2	3	4
WT (Kg)		15.84	16.86	16.42	16.22
ADG (g/d)		693.60	753.90	713.00	715.50
ADFI (g/d)		966.20	1011.40	968.30	953.80
G:F (g/g)		0.71	0.76	0.75	0.77
E. coli colonies ($\log_{10}$/g)		6.02	5.75	5.61	5.70
Lactobacilli colonies ($\log_{10}$/g)		8.55	8.55	8.37	8.26

[1] Data are LS Means of 10 replicate pens of one pig

Table 4.4. Effect of Zn supplementation on nursery pig average daily gain [1]

	Added ZnO, ppm	0	750	1,500	3,000	
	Treatment No.	1	2	3	4	Avg/wk
		Average Daily Gain (g/d)				
Week 1		414.3	510.0	432.9	451.4	452.1[a]
Week 2		520.0	656.4	598.6	590.0	591.3[b]
Week 3		832.1	854.3	912.9	892.1	872.9[c]
Week 4		1007.9	995.0	907.9	928.6	959.8[d]

[1] Data are LS Means of 10 replicate pens of one pig

[a, b, c, d] Means within a column lacking common superscript differ ($P \leq 0.05$)

Figure 4.1. Effect of dietary treatment on average daily gain: Average daily gain was not affected by dietary Zn treatments during week 1, 2, 3, 4, and overall ($P > 0.05$). However, ADG was affected by time ($P \leq 0.05$). Pigs increased ADG from 452 g/d during week 1 to 959.8 g/d during week 4.

Table 4.5. Effect of Zn supplementation on nursery pig feed intake[1]

Added ZnO, ppm	0	750	1,500	3,000	
Treatment No.	1	2	3	4	Avg/wk
Average Feed Intake (g/d)					
Week 1	560.7	597.7	545.3	545.0	562.2[a]
Week 2	823.4	960.5	882.4	848.4	878.7[b]
Week 3	1,050.3	1,041.7	1,059.7	1059.1	1052.7[c]
Week 4	1,430.3	1,445.7	1385.9	1362.6	1406.1[d]

[1] Data are LS Means of 10 replicate pens of one pig

[a, b, c, d] Means within a column lacking common superscript differ (P ≤ 0.05)

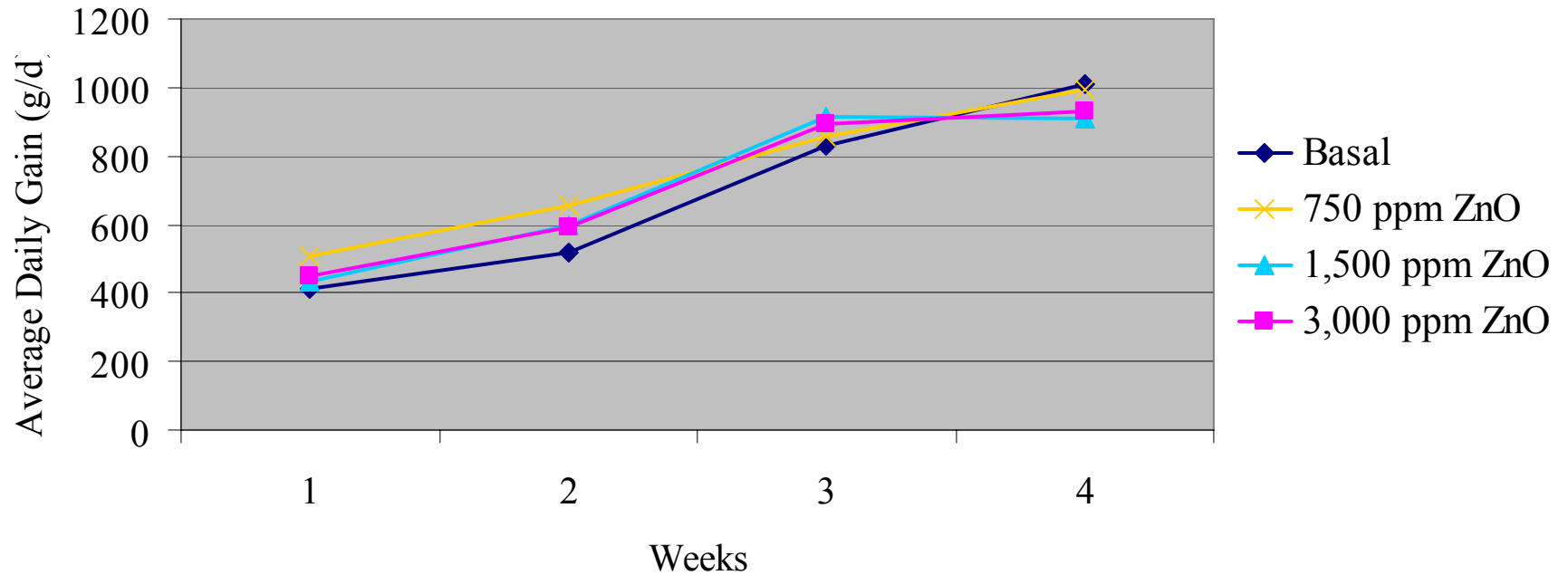*Figure 4.2. The effect of Zn supplementation on nursery pig feed intake:* Average daily feed intake was not affected by dietary Zn treatments during week 1, 2, 3, 4, and overall (P > 0.05). However, ADFI was affected by time (P ≤ 0.05). Feed intake increased from 562.2 g/d to 1,406.1 g/d during week 1 and week 4, respectively.

Table 4.6. Effect of Zn supplementation on nursery pig feed efficiency[1]

	Added ZnO, ppm	0	750	1,500	3,000	
	Treatment No.	1	2	3	4	Avg/wk
			Gain:Feed (g/g)			
Week 1		0.73	0.86	0.80	0.83	0.80[a]
Week 2		0.61	0.69	0.68	0.70	0.66[b]
Week 3		0.80	0.83	0.86	0.84	0.83[a]
Week 4		0.71	0.69	0.65	0.69	0.69[b]

[1] Data are LS Means of 10 replicate pens of one pig

[a, b] Means within a column lacking common superscript differ (P ≤ 0.05)

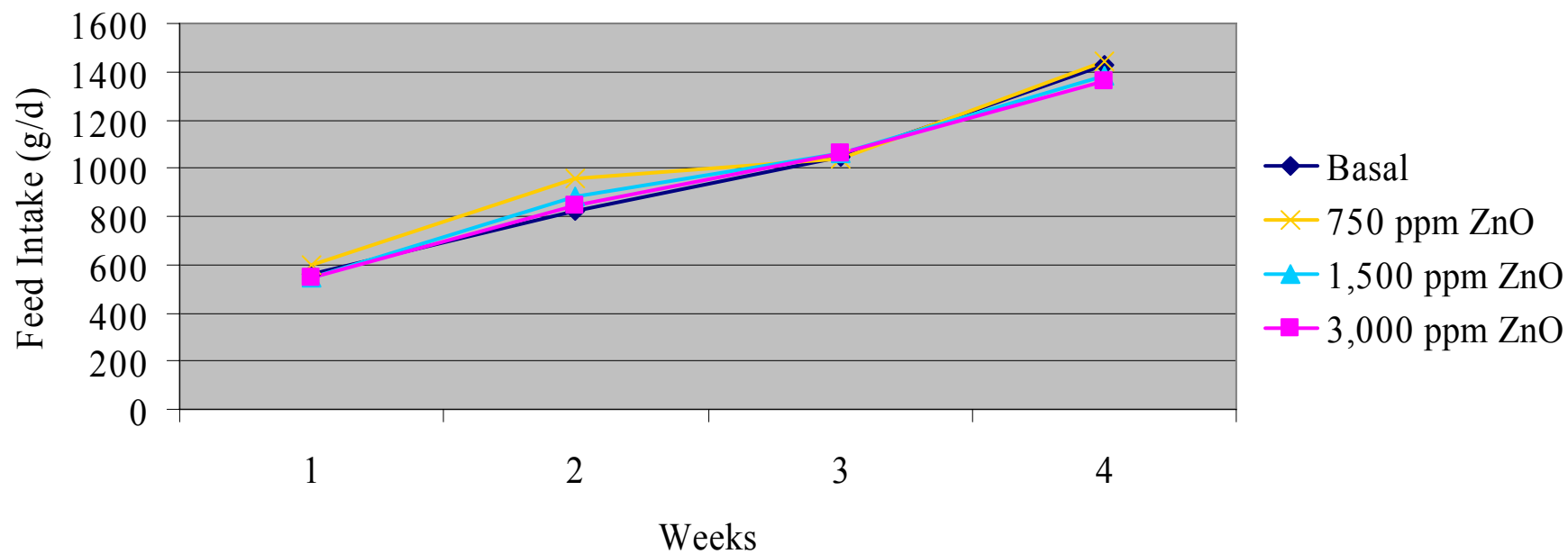*Figure 4.3. The effect of Zn supplementation on nursery pig feed efficiency:* Feed efficiency was not affected by dietary Zn treatments throughout the 28-d study ($P > 0.05$). However, G:F was affected by time ($P \leq 0.05$).

Table 4.7. Effect of Zn supplementation on the number of excreted *E. coli* per gram of wet feces in nursery pigs[1]

	Added ZnO, ppm	0	750	1,500	3,000	
	Treatment No.	1	2	3	4	Avg/wk
		Number of *E. coli* colonies ($\log_{10}$/g)				
Week 1		6.20	6.33	5.60	6.54	6.17[a]
Week 2		5.37	5.12	5.29	5.82	5.41[b]
Week 3		6.06	5.00	5.51	5.00	5.39[b]
Week 4		6.46	6.53	6.06	5.44	6.11[a]

[1] Data are LS Means of 10 replicate pens of one pig

[a, b] Means within a column lacking common superscript differ ($P \leq 0.05$)

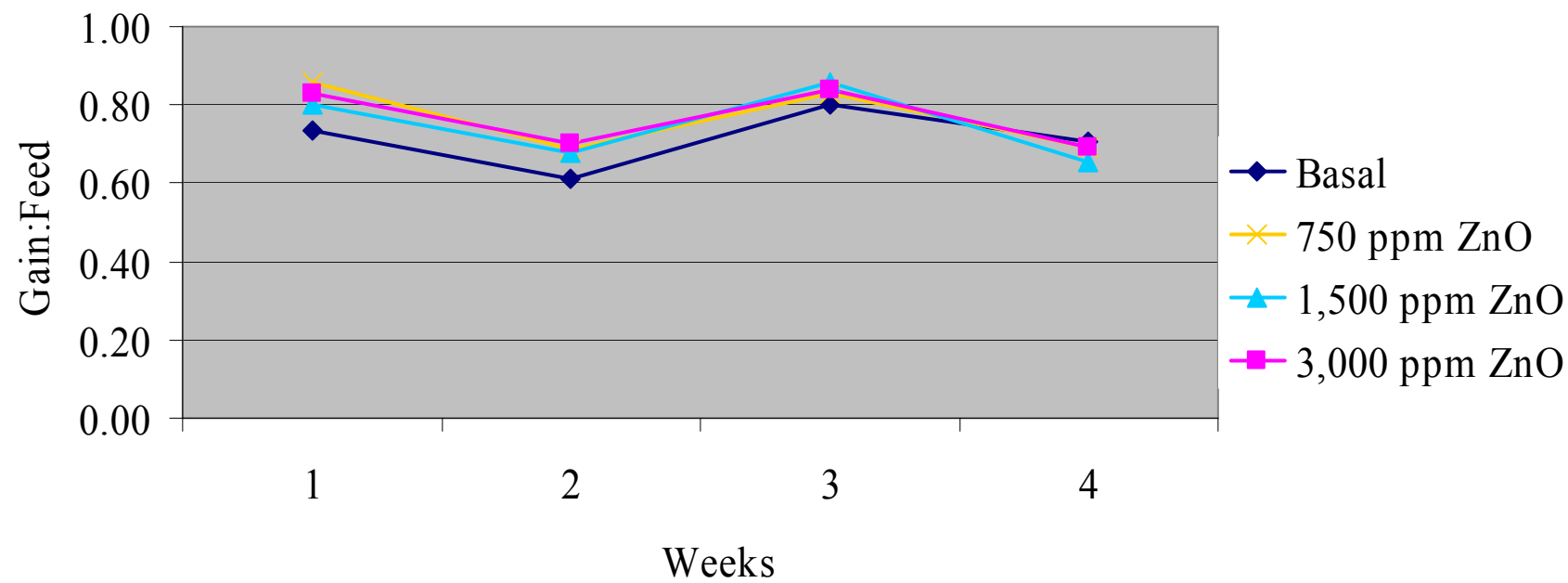*Figure 4.4. The effect of Zn supplementation on the number of excreted E. coli per gram of wet feces in nursery pigs:* The number of excreted *E. coli* colonies was not affected by the dietary Zn treatments ($P > 0.05$). However the number of colonies of *E. coli* per gram of wet feces was affected by time ($P \leq 0.05$). *Escherichia coli* colonies excreted from nursery pig were the highest ($P \leq 0.05$) during week 1 and 4 compared to week 2 and 3.

Table 4.8. Effect of Zn supplementation on the number of excreted lactobacilli per gram of wet feces in nursery pigs[1]

	Added ZnO, ppm	0	750	1,500	3,000	
	Treatment No.	1	2	3	4	Avg/wk
		Number of Lactobacilli colonies ($\log_{10}$/g)				
Week 1		9.11	9.04	8.87	8.86	8.97[a]
Week 2		8.65	8.60	8.28	8.29	8.46[b]
Week 3		8.55	8.57	8.30	8.20	8.41[b]
Week 4		7.90	7.97	8.04	7.70	7.90[c]

[1] Data are LS Means of 10 replicate pens of one pig

[a, b, c] Means within a column lacking common superscript differ ($P \leq 0.05$)

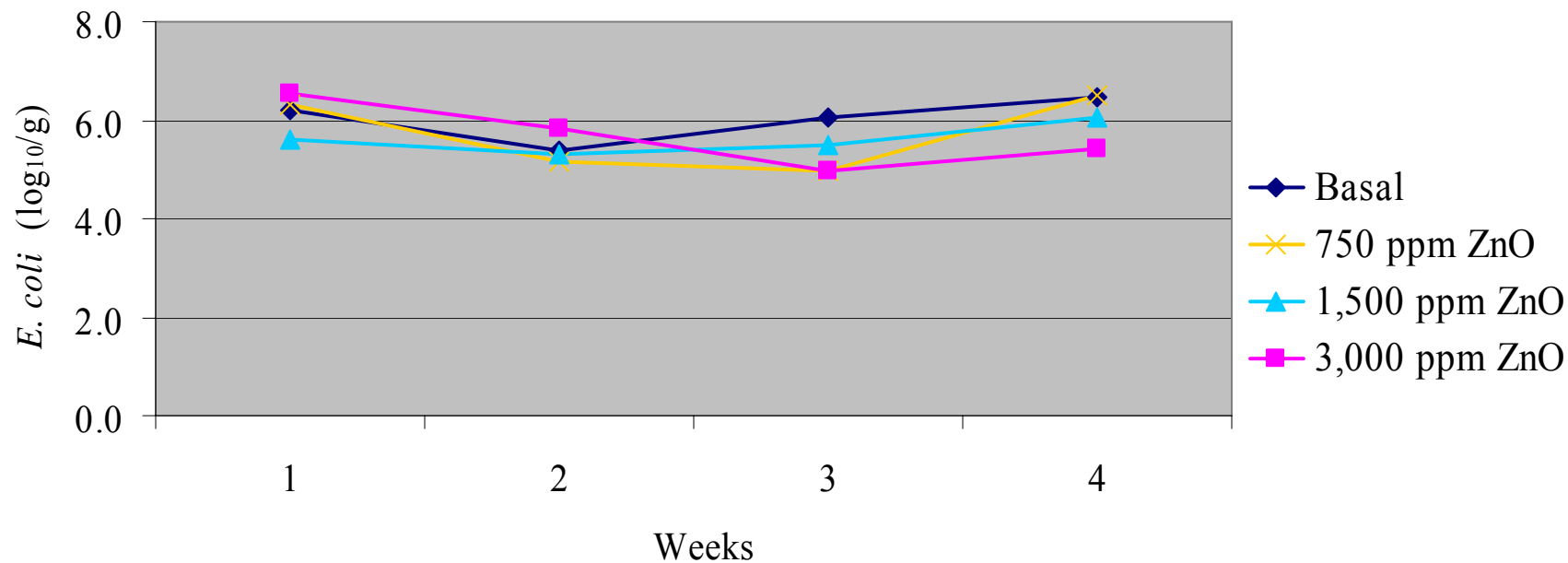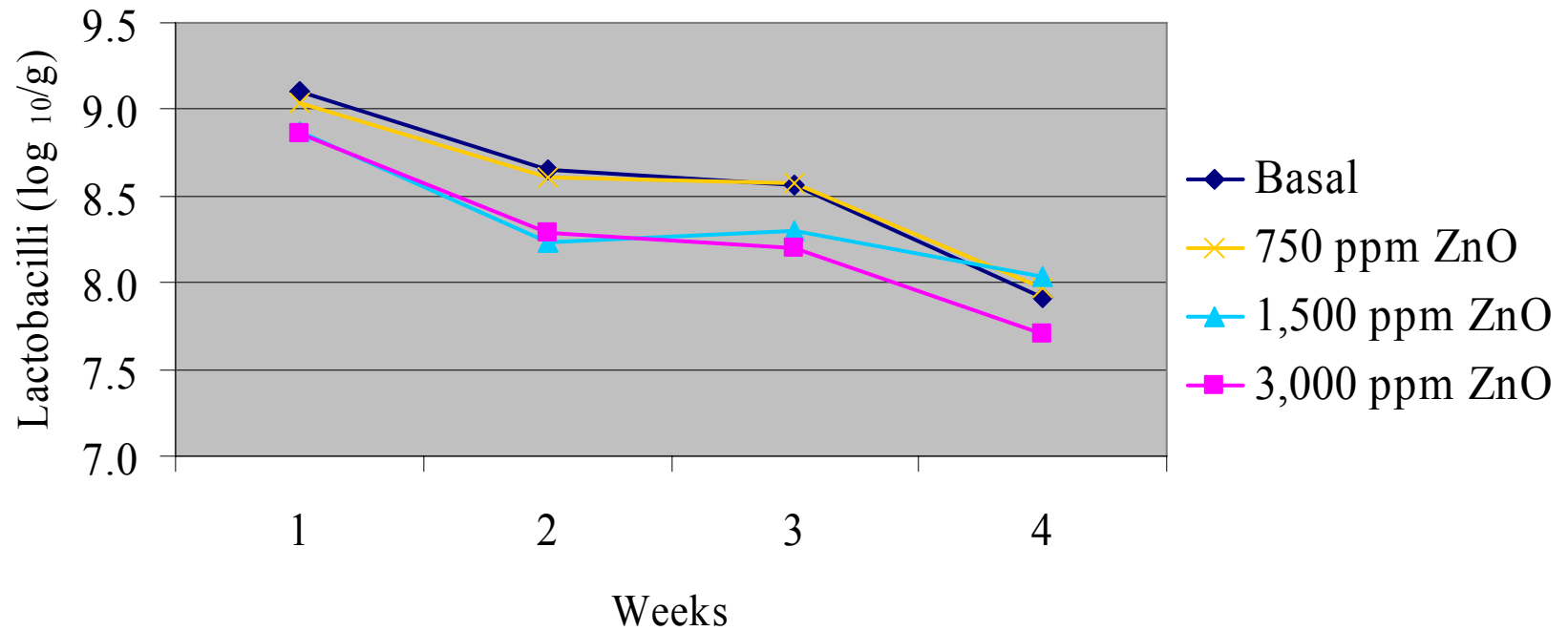*Figure 4.5. The effect of Zn supplementation on the number of excreted lactobacilli per gram of wet feces in nursery pigs:* The number of excreted lactobacilli colonies was not affected by dietary Zn treatment ($P > 0.05$). However, the number of lactobacilli per gram of wet feces was affected by time ($P \leq 0.05$). Nursery pigs had decreasing concentrations of lactobacilli colonies during the 28-d study ($P \leq 0.05$).

Table 4.9. Effect of dietary ZnO supplementation to weaned pigs on the incidence of diarrhea for the first 14-d post-weaning

Treatments	Estimate	Odds Ratio[1]	P > ChiSq
750 ppm ZnO vs Basal	0.6955	2.00	0.1909
1,500 ppm ZnO vs Basal	1.9617	7.11	0.0010
3,000 ppm ZnO vs Basal	1.5198	4.57	0.0045
1,500 ppm ZnO vs 750 ppm ZnO	1.2662	3.54	0.0126
3,000 ppm ZnO vs 750 ppm ZnO	0.8243	2.28	0.0593
3,000 ppm ZnO vs 750 ppm ZnO	0.4418	1.55	0.3876
1,500 and 3,000 ppm ZnO vs Basal and 750 ppm ZnO	2.7860	16.21	0.0002

[1] Odds ratios are the anti log of the parameter estimate obtained in the logistic regression analysis. An odd is defined as the probability of an event occurring (p) divided by 1-p.

Table 4.10. Effect of dietary ZnO supplementation to weaned pigs on the incidence of diarrhea for the second 14-d post-weaning

Treatments	Estimate	Odds Ratio[1]	Pr > ChiSq
750 ppm ZnO vs Basal	0.6902	1.99	0.1142
1,5000 ppm ZnO vs Basal	1.7469	5.74	0.0034
3,000 ppm ZnO vs Basal	1.9658	7.14	0.0019
1,500 ppm ZnO vs 750 ppm ZnO	1.0567	2.88	0.0511
3,000 ppm ZnO vs 750 ppm ZnO	1.2755	3.58	0.0280
3,000 ppm ZnO vs 1,500 ppm ZnO	0.2189	1.24	0.7574
1,500 and 3,000 ppm ZnO vs Basal and 750 ppm ZnO	3.0225	20.54	0.0003

[1] Odds ratios are the anti log of the parameter estimate obtained in the logistic regression analysis. An odd is defined as the probability of an event occurring (p) divided by 1-p

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