

THE EFFECTS OF ZINC SOURCE AND SUPPLEMENTAL COPPER ON GROWTH  
PERFORMANCE, CARCASS CHARACTERISTICS, AND MORBIDITY AND MORTALITY  
OF GROWING-FINISHING PIGS RAISED UNDER COMMERCIAL CONDITIONS

BY

RACHEL LOREN SCHMITT

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Professor Michael Ellis

## ABSTRACT

Zinc hydroxychloride and tribasic copper chloride are relatively new mineral sources that are claimed to have improved bioavailability relative to commonly used zinc sources such as zinc oxide. Both sources were evaluated in two studies that were carried out to determine the effects of zinc source and supplemental copper on growth performance, carcass characteristics, and morbidity and mortality of growing-finishing pigs raised under commercial conditions. In both studies, the zinc sources were included at levels to marginally exceed the requirements of growing-finishing pigs suggested by NRC (2012); the copper source, which was only included in the first study, was included to provide pharmacological levels of copper. Study 1 was carried out using a randomized complete block design (blocking factor was date of start on test) to compare 4 treatments: Trt. 1: Control [zinc oxide (assuming 65% bioavailability) + supplemental copper (tribasic copper chloride at 150 ppm)]; Trt. 2: [zinc hydroxychloride (assuming 65% bioavailability) + supplemental copper (tribasic copper chloride at 150 ppm)]; Trt. 3: [zinc hydroxychloride (assuming 100% Bioavailability) + supplemental copper (tribasic copper chloride at 150 ppm)]; and Trt. 4: As Treatment 1 without supplemental copper. The pigs used for Study 2 had previously been allotted to additional experimental treatments that were independent of those used in the current study. Consequently, Study 2 used a split-plot design with the main plot being the additional experimental treatments and the subplot being the two zinc treatments: Trt. 1: Control (zinc oxide assuming 65% bioavailability) and Trt. 2: zinc hydroxychloride (assuming 100% bioavailability). A total of 2,040 (27 replicates) and 2,888 (66 replicates) commercial crossbred barrows and gilts (housed in single-sex groups of 22 at a floor space of 0.59 m<sup>2</sup>/pig) were used in Studies 1 and 2, respectively. Studies 1 and 2 were carried out from 47.0 ± 5.1 kg to 127.1 ± 3.9 kg body weight and from 41.9 ± 1.7 kg to 132.0 ± 4.4 kg

body weight, respectively. There were 5 dietary phases in Study 1 and 3 dietary phases in Study 2. Diets were formulated to a constant standardized ileal digestible lysine:ME ratio within phase and to meet or exceed nutrient requirements suggested by NRC (2012). Ractopamine hydrochloride (7.5 ppm) was included in the final dietary phase in all dietary treatments for both studies. Pen weights and pen feed intakes were collected every 2 and 3 weeks for Studies 1 and 2, respectively, and used to calculate ADG, ADFI and G:F. At the end of the study, pigs were sent to a commercial facility for harvest and collection of carcass measurements. For both studies, the pen of pigs was the experimental unit; data were analyzed using the PROC MIXED procedure of SAS (v. 9.2; SAS Inst. Inc., Cary, NC) with the model accounting for the fixed effects of treatment and the random effects of replicate. Results from Study 1 showed that Trt. 3 had greater ( $P = 0.04$ ) live weight ADG compared to Trt. 1, with Trt. 2 being intermediate and not different ( $P = 0.39$ ) than the other 2 treatments (0.97, 0.98, 0.99 kg for Trt. 1, 2, and 3 respectively; SEM 0.03). Treatment 3 also had greater ( $P = 0.03$ ) live weight G:F than Trt. 2, but not ( $P = 0.16$ ) Trt. 1 (0.362, 0.361, 0.366 for Trt. 1, 2, and 3 respectively; SEM 0.0024). Adding supplemental copper to the diet had no effect ( $P > 0.05$ ) on live weight ADG, ADFI, or G:F, however, carcass weight ADG was numerically increased ( $P = 0.07$ ) and carcass weight G:F was significantly improved ( $P = 0.03$ ) for Trt. 4 compared to Trt.1. In Study 2, pigs fed diets supplemented with zinc hydroxychloride (Trt. 2) had lower ( $P < 0.05$ ) overall ADG, on both a live and carcass weight basis (1.021 and 1.002, and 0.821 and 0.780, for Trt. 1 and 2, respectively), and ADFI, (2.62 and 2.59 for Trt. 1 and 2, respectively), but similar ( $P > 0.05$ ) live weight and carcass weight G:F compared to those fed diets containing zinc oxide (Trt. 1). There was no effect ( $P > 0.05$ ) of zinc source on carcass measurements or morbidity and mortality in either study. The results for Study 1 suggested comparable or small improvements in growth

performance from using zinc hydroxychloride (assumed bioavailability 100%; Trt. 3) compared to zinc oxide. However, the opposite was evident in Study 2. Study 1 also suggested small improvements in growth performance from feeding high levels of copper to growing-finishing pigs. Further research is needed to clearly establish the advantage, if any, of replacing zinc oxide with zinc hydroxychloride and of including high levels of copper as tribasic copper chloride in diets for growing-finishing pigs.

*To My Family*

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## CHAPTER 1: LITERATURE REVIEW

### *Introduction*

Minerals have been added to diets for swine for many years. The effects of trace minerals, specifically zinc, on animals were noticed as early as 1933 when Todd et. al. fed zinc deficient diets to rats causing growth retardation. O'Dell and Savage (1957) observed abnormal leg bone development in chicks which received no supplemental zinc. Additionally, the health benefits of zinc for animal agriculture have been widely recognized for some time. It was reported that zinc was a key mineral that prevented and cured hyperkeratinization of the skin, a condition known as parakeratosis (Tucker and Salmon, 1955; Hoekstra et al., 1956; Morgan et al., 1969). Zinc also plays a key role in many physiological functions within the body such as immune and cell development, tissue and bone development as well as protein, carbohydrate and lipid metabolism (NRC, 2012). Furthermore, adding supplemental copper in the diets of swine, specifically weaned pigs, has been shown to improve growth performance (Cromwell et al., 1998). Recent research has focused on alternative forms of minerals, especially organic forms, for use in swine diets. This review will focus on the effects of zinc and copper sources as well as levels used to meet the dietary requirement of the animal and levels to promote growth performance.

### *Determining Zinc Deficiency*

Nutrient deficiencies can be described as the result of failing to provide the level of a substance which is needed to meet physiological requirements of an animal (Miller et al., 1991). There are a number of common symptoms of zinc deficiency in swine. In NRC (2012), zinc deficiency symptoms included reduction in the rate and efficiency of growth, and reductions in

the serum levels of zinc alkaline phosphatase and albumin. Zinc deficiency is manifested as parakeratosis along with anorexia, depleted fat depots, serous atrophy of fat, atrophy of thymus, and keratinization of tongue, esophagus and cardia of the stomach (Miller et al., 1979). Other consequences of feeding a zinc deficient diet include prolonged duration of farrowing in sows, and poorer milk quality during lactation, as well as retarded development of young pigs (NRC 2012). The classic and most common sign of zinc deficiency in growing pigs is hyperkeratinization of the skin, a condition called parakeratosis (Kernkamp and Ferrin 1953; Tucker and Salmon 1955).

### *Zinc Requirements*

Much of the early research to determine zinc requirements was carried out with poultry after Todd et. al. (1934) discovered the effects that zinc deficient diets had on rats. Early studies conducted using chicks showed that they could obtain some zinc from galvanized battery cages in which they were housed if they were fed a Zn deficient diet; however, those fed zinc deficient diets showed symptoms of severe deficiency compared to chicks fed diets supplemented with zinc (O'Dell et al., 1958; Young et al., 1958). When deficient diets (15 ppm zinc) were supplemented with 40 ppm of zinc, not only were deficiency symptoms alleviated, but the growth performance of the chicks was also improved, suggesting that up to 40 ppm of supplemental zinc is required for normal development and growth (Young et al., 1958).

Historically, research was focused on determining the requirements of swine for zinc largely to cure and prevent a skin disease known as parakeratosis. Luecke et al. (1956) reviewed incidences of outbreaks of skin lesions dating back to 1941 and reported that Raper and Curtin (1953) observed an absence of lesions when cobalt and zinc were supplemented in the diet. Tucker and Salmon (1955) were the first to note that zinc could prevent and cure parakeratosis



which occurred more frequently in diets with high levels of calcium. According to the NRC (2012), high incidences of parakeratosis have been associated with high calcium levels in swine diets which were often provided by supplemental bone meal or calcium carbonate. This caused a mineral imbalance as well as an adverse effect on the availability of dietary zinc which has been shown in several studies (Hoekstra et al., 1956; Lewis et al., 1956, 1957a,b; Luecke et al., 1956, 1957; Stevenson and Earle, 1956). In NRC (2012), the negative effect of other components in the diet, such as plant phytates, copper, protein level and source, and calcium, were described. It is important, therefore, to take into account the potential interaction of zinc with other components of the diet when establishing the dietary level of zinc needed to meet the requirement.

Early studies evaluating the relationship between dietary zinc and calcium defined the zinc requirement in relation to dietary calcium inclusion level. Stevenson and Earle (1956) evaluated the relationship between calcium and zinc levels in the diets of weaned pigs and concluded that an inclusion level of 32 ppm of zinc as zinc oxide, which was considered the basal diet, in the presence of 0.48% dietary calcium level produced mild parakeratosis which intensified as the dietary calcium content increased to 0.67% and 1.03%. When the total level of zinc was increased to 44 ppm, there was a reduction in the signs of parakeratosis at the three calcium inclusion levels (0.48%, 0.67% and 1.03%). In addition, increasing the total dietary zinc content to 80 ppm prevented parakeratosis in diets containing up to 1.03% calcium. Therefore, in diets for growing pigs which contain up to 1% calcium, the minimum zinc content for prevention of parakeratosis was determined to be between 44 ppm and 80 ppm (Stevenson and Earle, 1956). These authors also determined that the addition of 48 ppm of zinc either as zinc

oxide or zinc sulfate, was effective in quickly restoring normal growth rate as well as restoring healthy skin condition.

Similarly, Smith et al. (1958) designed a study to determine if parakeratosis was a true deficiency and, if so, what quantitative level of zinc was required to prevent it as well as promote growth performance. In this study, the basal ration contained 16 ppm of zinc as zinc oxide and 7 zinc level treatments were applied; 16, 21, 26, 31, 36, 41, and 46 ppm total zinc. Dietary calcium level was 0.66% for all treatments. Within the first 8 weeks of the 10 week study, growth rate increased with each increasing increment of added zinc. During that same time, pigs fed the 16, 21, 26, 31, and 36 ppm zinc rations exhibited a gradual increase of skin lesions, presumably parakeratosis, as the study progressed. At the end of 8 weeks, treatment levels of 16, 21, 26, and 31 were discontinued leaving only the pigs receiving the 36, 41, and 46 level treatments on trial for two additional weeks. In those two weeks, growth rate continued to increase with increasing dietary zinc level. Pigs fed the diets containing 41, and 46 ppm of zinc had no parakeratosis throughout the entire study. During a 4 week recovery period following the end of the study, pigs from the low zinc level treatments were placed on a diet with 50 ppm of zinc and showed improvements in skin condition as well as in growth rate. Throughout the study period, there were no evident signs of parakeratosis in pigs fed the diet containing 41 ppm total zinc, thus, suggesting that 41 ppm of total zinc was the optimum dietary level for prevention of this disease. However, pigs fed the 46 ppm ration also failed to show any signs of parakeratosis, and showed additional improved growth over the 41 ppm dietary treatment (ADG 0.43 vs. 0.38 kg, respectively). Thus, Smith et al. (1958) concluded that increasing zinc to 46 ppm elicited the fastest growth rate as well as being effective at preventing parakeratosis compared to the other 6 treatment levels. Recommendations for total dietary zinc levels to maximize growth

performance as well as preventing parakeratotic symptoms from Smith et al. (1958) were 46 to 50 ppm.

Luecke et al. (1957) conducted 2 studies to evaluate the effect of zinc supplementation in the presence of high calcium and phosphorus levels on weaned pigs of 6 to 7 weeks of age. In the first study, two diets were compared; diet A contained 45 ppm zinc, 0.65% calcium, and 0.53% phosphorus and diet B, which was considered the high calcium and phosphorus diet, contained 40 ppm zinc, 1.25% calcium, and 0.95% phosphorus. Diet A, which was formulated with the calcium and phosphorus levels suggested by the NRC (1953), resulted in greater growth performance and a lower incidence of parakeratosis (10% vs. 100% for Diets A and B respectively) than Diet B. These results show that formulating the diets with high calcium and phosphorus levels will increase the incidence of parakeratosis, presumably because the availability of zinc in the diet was reduced. However, these results also show that even with the lower levels calcium and phosphorus in the diet, such as in diet A, there was still an incidence of this condition even with 45 ppm zinc present in the diet. Luecke et al. (1957) used an additional 2 dietary treatments in which Diets A and B were supplemented with 50 ppm zinc as zinc carbonate and found that this eliminated any indications of parakeratosis and, also, improved growth rates. These results suggest that at levels of calcium and phosphorus at requirement or higher, a total of least 90 ppm of zinc is needed in the diet to improve growth rate as well as prevent parakeratosis.

In the second study, Lueke et al. (1957) fed 6 diets that used additional limestone to increase the calcium content of the diets to determine the effects on growth rate. Diet C, which did not have any added limestone, contained 0.51% calcium, 0.61% phosphorus, and 32 ppm zinc, Diet D contained 1.21% calcium, 0.61% phosphorus and 29 ppm zinc; and Diet E

contained 1.90% calcium, 0.61% phosphorus, and 31 ppm zinc. All 3 diets had the same levels of phosphorus and similar levels of zinc. The other 3 diets were created by adding 50 ppm of zinc as zinc carbonate to Diets C, D, and E. Growth rates for C, D and E were much lower compared to the zinc supplemented diets. Additionally, there was a high incidence of parakeratosis in diets C, D, and E and no signs of the disease in the zinc supplemented diets. In both studies, adding supplemental zinc at 50 ppm (as zinc carbonate) increased growth rate and completely prevented symptoms of parakeratosis (Luecke et al. 1957). Therefore, results of these studies suggest that high levels of dietary calcium can have a negative effect on growth performance, but providing a diet with 90 to 100 ppm of total zinc to weaned pigs was effective in increasing growth and mitigating parakeratosis in the presence of elevated levels of calcium and phosphorus.

Lewis et al. (1957b) further investigated the relationship between calcium and zinc. In a previous study (Lewis et al., 1956) showed that feeding weaned pigs (13 to 18 kg BW) a basal diet containing 35 ppm zinc with 50 ppm of added zinc as zinc sulfate (for a total of 85 ppm zinc) in the presence of 0.8% calcium reduced parakeratotic symptoms but did not completely alleviate them. However, increasing the supplemental level of zinc to 100 ppm completely alleviated symptoms of parakeratosis. In a subsequent study, Lewis et al. (1957b) evaluated the effects of limiting dietary calcium levels compared to supplementing zinc on the incidence of parakeratosis. In that study, weaned pigs were fed 5 diets formulated with increasing calcium levels of 0.5%, 0.8% and 1.2%. Diets 1, 2 and 3 had a basal zinc level of 28 ppm and diets 4 and 5 were supplemented with 100 and 1,000 ppm of additional zinc as zinc sulfate, for a total of 128 and 1,028 ppm, respectively. Diets were formulated as follows: Diet 1: 0.5% calcium, 0.5% phosphorus, 28 ppm zinc; Diet 2: 0.8% calcium, 0.5% phosphorus, 28 ppm zinc; Diet 3: 1.2 %

calcium, 0.9% phosphorus, 28 ppm zinc; Diet 4: 0.8% calcium, 0.5% phosphorus, 100 ppm zinc; Diet 5: 0.8% calcium, 0.5% phosphorus, and 1,000 ppm zinc. Pigs fed Diet 1 grew significantly faster than pigs on Diets 2 and 3. Pigs fed Diet 2 with 0.8% calcium gained less than the pigs on Diet 1, but more than the pigs on Diet 3. A calcium level of 0.5% was below the NRC level (which was 0.65% for a 23 to 45 kg pig at the time; NRC, 1953), yet there was still evidence of parakeratosis. The authors concluded that it was more favorable to increase the amount of zinc present in the diet rather than decreasing dietary calcium levels. Feeding pigs 100 ppm of zinc with 0.8% calcium (Diet 4 and 5) significantly improved growth rate compared to diets containing 0.8% and 1.2% calcium without added zinc (Diet 2 and 3), but only marginally compared to diet 1 containing 0.5% calcium. Supplementing 100 ppm zinc in the diet also completely eradicated parakeratosis. Supplementing 1,000 ppm of zinc did not improve growth rate greater than what was achieved by supplementing 100 ppm of zinc. Findings by Lewis et al. (1957) confirmed findings by Luecke et al., (1957) that 100 ppm of supplemental zinc prevented parakeratosis as well as promoted growth performance in nursery pigs in the presence of high levels of calcium.

Collectively, the studies that were summarized above evaluated the relationship of dietary zinc and calcium and established a requirement for trace minerals such as zinc in relation to calcium levels in typical swine diets. Miller et al. (1991) cited NRC (1959) in which results from Tucker and Salmon (1955), Luecke et al. (1956, 1957), Lewis et al. (1956, 1957), and Stevenson and Earle (1956) were summarized resulting in the determination of the requirement of 50 ppm of zinc to be adequate for growth and prevention of parakeratosis in diets for growing pigs fed at or below the requirements for calcium and phosphorus. However, the authors cautioned that higher zinc levels may be needed if excess calcium is present in the diet.

More recent research by van Heugten et al. (2003) evaluated the use of organic forms of zinc on growth performance, at supplementation levels commonly used by the swine industry rather than at levels to meet requirements suggested by NRC (1998). The control treatment included the NRC (1998) suggested required level of 80 ppm supplemental zinc as zinc sulfate, provided by a mineral premix for a total level of 104 ppm zinc in the diet. Additional treatments included the control supplemented with 80 ppm zinc sulfate, or organic (zinc-methionine, zinc-lysine, zinc-methionine + zinc-lysine) forms of zinc, or 160 ppm zinc sulfate for a total zinc content of either 184 or 264 ppm and 6 dietary treatments. Diets were fed to weaned pigs for 5 weeks. Pigs that were fed diets supplemented with 160 ppm zinc as zinc sulfate had greater feed efficiency but similar ADG and ADFI to the control. Pigs fed diets supplemented with organic forms of zinc had similar ADG, ADFI and G:F compared to the control, however, there were no significant effects of supplementing diets with organic sources of zinc. Results from this study suggest that recommendations from the NRC (1998) of 80 to 100 ppm supplemental zinc provided in the diet to weanling pigs weighing 5 to 25 kg BW, respectively were sufficient for optimum growth performance. These results are a validation of both the 1998 and 2012 NRC suggested requirement for pigs from 11-25 kg BW.

The estimates of requirements in the current NRC (2012) recommendations reflect the requirements that were established from the work summarized in this section. Current requirements of zinc for different weights of pigs range from 100 ppm down to 50 ppm for pigs weighing 5 to 135 kg, respectively. These levels are generally associated with diets that typically include total calcium at levels ranging from 0.85% to 0.46% and total phosphorus at levels ranging from 0.70% to 0.43% for pigs weighing 5 to 135 kg, respectively (NRC 2012). The requirements for zinc suggested by NRC (2012) are shown in Table 1. Adding zinc in the

required amounts suggested by the NRC (2012) allows for normal growth performance as well as mitigation of deficiency conditions such as parakeratosis.

### *Industry Levels of Zinc*

In the industry, it is not uncommon for minerals to be added in the diet at levels greater than those suggested by NRC (2012). Commercial mineral inclusion levels can be double that which is recommended by NRC or greater (Flohr et al., 2016). Greater amounts of vitamins and minerals are fed in a commercial setting as insurance against loss of mineral efficacy due to interactions with other components of the diet, which can decrease availability of mineral sources (Oberleas et al., 1962). Zinc inclusion levels used by the US swine industry for finishing pigs from 23 kg to market weight are generally in the range of 98.8 to 112.5 ppm (Flohr et al., 2016). Whereas, the NRC (2012) recommendations decrease from 60 to 50 ppm over this weight range (25 to 135 kg BW).

### *Pharmacological Levels of Zinc*

In the early 1990's much interest was placed in evaluating the response of newly-weaned pigs to feeding pharmacological levels of minerals, specifically zinc. Several studies have shown that feeding pharmacological levels of zinc to newly-weaned pigs improves growth rate (Smith et al., 1995; Carlson et al., 1999; Hill et al., 2000), reduces the usage of antibiotics (Hill et al., 2001), as well as reduces post-weaning scours (Poulsen, 1998; Hill et al., 2000). Feeding of pharmacological levels of zinc began when Poulsen (1995) discovered that high concentrations of dietary zinc from zinc oxide decreased post-weaning scours. Additionally, there have been many reports of growth performance improvements from feeding pharmacological levels of zinc (Hahn and Baker, 1993; Poulsen, 1995). Hill et al. (2001) observed increased growth performance when zinc was added to the diet at up to 3,000 ppm zinc

as zinc oxide until 21 d post weaning. Carlson et al. (1999) suggested that the greatest responses in ADG to pharmacological doses of zinc were provided by 3,000 ppm zinc as zinc oxide fed during the first 2 to 4 weeks post weaning. Perez-Mendoza (2008) reviewed studies that reported improved growth rate from feeding pharmacological levels of zinc for 2 to 5 weeks post-weaning and found 5 studies that suggested supplementing 2,000 ppm zinc as zinc oxide improved ADG by 15%, 10 studies that suggested supplementing 3,000 ppm zinc as zinc oxide improved ADG by 22% and 2 studies that failed to see any growth rate improvement from supplementing zinc at 2,000 and 3,000 ppm. Feeding diets with added zinc at 2,000 ppm for the first 5 weeks post-weaning has also been shown to improve ADFI by an average 3.6% and G:F by an average of 5.3% (Hill et al., 2001; Buff et al., 2005). When zinc was added at 3,000 ppm as zinc oxide in diets fed for 4 weeks post-weaning, ADFI was increased by an average of 14.5% while G:F was increased by an average of 5.3% (Hill et al., 2000; Mavromichalis et al., 2000; Case and Carlson, 2002). Poulsen (1995) reported increased ADG when 2,500 ppm of zinc was added to diets as zinc oxide, however, ADFI and G:F were not affected. Additionally, supplementation of zinc to the diet at 4,000 ppm as zinc oxide improved diarrhea in weaned pigs compared to pigs that did not receive supplemental zinc, however, ADG was decreased by 7% compared to 2,500 ppm zinc. Thus, research results suggested that responses to supplemental zinc were greatest when the levels included were between 2,000 and 3,000 ppm (Perez-Mendoza, 2008). Furthermore, Case and Carlson (2002) suggested that dietary levels of 2,000 to 3,000 ppm of supplemental zinc as zinc oxide is a common recommendation for promotion of growth performance.

Additionally, there have been some suggestions that supplementing zinc at pharmacological levels could provide health benefits to newly-weaned pigs. Trace minerals, especially zinc, are considered to be a key part of immune development and function (Richards



et al., 2010). As previously mentioned, supplemental zinc is an effective method used to prevent post-weaning diarrhea (Poulsen, 1995). However, the method by which feeding pharmacological levels of zinc reduces post-weaning diarrhea is not fully understood. It has been suggested that zinc can stabilize the intestinal microbiota, reduce bacterial populations, and promote growth in the absence of antibiotics (Perez-Mendoza, 2008). There is very limited evidence of the impact of pharmacological levels of zinc on morbidity and mortality levels in newly-weaned pigs. Tokach et al. (2000) reported that feeding newly-weaned pigs in a commercial nursery with diets containing 2,000 ppm zinc compared to a control diet with no added zinc diet reduced mortality from 8.0% to 0.96%. Further research would be required to determine the mechanism(s) by which supplemental zinc improves morbidity and mortality.

Although zinc oxide is the most common source of zinc that has been used at pharmacological levels (Hahn and Baker, 1993), there are other zinc sources that could be used. However, NRC (2012) cited a study Brink et al. (1959) that feeding diets with 2,000 ppm of added zinc as zinc carbonate caused signs of toxicity including lethargy, arthritis, and even death. Therefore, NRC (2012) suggested that the maximum tolerable level of added zinc for swine was 1,000 ppm for sources other than zinc oxide, which could be included at higher levels (NRC, 2012).

In summary, feeding pharmacological levels of zinc to weaned pigs offers an effective strategy to improve growth performance and control scours in the first 2 to 4 weeks post weaning; however, it is important to consider the zinc source used.

#### *Zinc Sources: Organic and Inorganic*

There are several sources of zinc used in swine diets with the most commonly used ones including inorganic forms, particularly zinc oxide and zinc sulfate (Schell and Kornegay, 1996;

Edwards and Baker, 1999; Richards et al., 2010; Hill et al., 2014), and organic forms such as zinc-methionine, zinc-polysaccharide, zinc-lysine, and zinc amino acid chelate (Cao et al., 2000; Case and Carlson, 2002; van Heugten et al., 2003; Hollis et al., 2005). Inorganic trace mineral use in swine diets has evolved considerably since Tucker and Salmon (1955) first reported the benefits of inorganic trace minerals in the diet of rats. Zinc oxide is the most extensively used inorganic source used in the commercial feed industry (Baker, 2001). Previously summarized research illustrated the use of inorganic sources of zinc in swine diets dating back to the 1950's. Inorganic trace minerals in the form of sulfates and oxides are most commonly used to provide supplemental zinc in swine diets, but can be susceptible to dietary antagonism such as dissociation in low pH environments which in turn allows for interactions with other minerals or phytic acid, ultimately leading to a reduction in the availability of the mineral (Richards et al., 2010). More recently, inorganic mineral research has focused on supplementation at pharmacological levels which involves adding zinc in the diet in amounts much greater than the requirement (Wedekind et al., 1992; Hahn and Baker, 1993). As previously summarized, there are significant growth benefits to adding pharmacological levels of zinc in the diet of weaned pigs, however, there is concern with soil contamination due to excretion of excessive amounts of zinc.

Organic trace minerals, which are minerals chelated or bound to an organic substance or ligand, often amino acids (Owen et al., 1973; Herrick, 1993; Manangi et al., 2012) can be more efficiently utilized due to increased stability and higher availability (Hynes and Kelly, 1995; Power and Horgan, 2000; Mateo et al., 2007). Using chelated or organic trace minerals has been of interest since 1973 when Owen et al. (1973) evaluated chelated trace mineral supplementation effects on growing-finishing swine due to the suggestion the chelation could

improve utilization of trace minerals. Results described by Owen et al. (1973) suggested that chelated minerals had the same potential as inorganic trace minerals if they were supplemented at levels required by the pig. The concept of decreasing the amount inorganic of minerals added in the diets by way of organic mineral supplementation has also been researched (Hollis et al., 2005; Manangi et al., 2012; Gowanlock et al., 2013) in an effort to increase the efficacy of mineral supplementation as well as reduce mineral excretion. Supplementation of zinc oxide has been shown to improve growth performance in many studies (Hill et al., 2000, 2001; Case and Carlson, 2002), but some studies have found that some organic zinc supplements were equally as effective at lower levels than zinc oxide (Ward et al., 1996, Case and Carlson 2002). There are variable results on the effects of supplementation of organic and inorganic sources of zinc and the following review summarizes some of those research findings.

Hahn and Baker (1993) carried out 3 studies to evaluate the growth and plasma zinc responses in piglets fed up to 3,000 ppm of added zinc as either zinc oxide, zinc sulfate, zinc-lysine, or zinc-methionine complex and found that the results were inconsistent across studies. In the initial study, supplemental zinc as either zinc oxide, zinc sulfate, or zinc-lysine complex were titrated in amounts of 0, 250, 500, 1,000, 1,500, 2,500, 3,000 and 5,000 ppm. Pigs fed diets containing >1,000 ppm of supplemental zinc had higher plasma zinc concentration than pigs fed diets  $\leq$ 1,000 ppm of added zinc for all three sources, but there was no effect of supplemental zinc source or zinc level on ADG, ADFI, G:F, or blood hemoglobin levels. In the second study, the control diet (125 ppm zinc) was supplemented with 3,000 or 5,000 ppm zinc as zinc oxide or zinc sulfate. There was a significant zinc source  $\times$  level interaction for growth performance; both levels of 3,000 and 5,000 ppm zinc as zinc oxide improved growth rate and feed intake, however, only 3,000 ppm zinc as zinc sulfate produced a similar response. Adding supplemental

zinc as zinc sulfate at 5,000 ppm decreased ADG, ADFI, and G:F by 18%, 16% and 2%, respectively compared to pigs fed diets with at 3,000 ppm supplemental zinc as zinc sulfate. Feeding diets with 3,000 ppm added zinc (as zinc oxide or zinc sulfate) compared to unsupplemented diets increased ADG and ADFI, by an average of 16% and 12%, respectively. In a third study, feeding zinc at 3,000 ppm in the forms of either zinc sulfate, zinc lysine, or zinc methionine increased plasma zinc concentration when compared to zinc oxide, but did not improve growth performance. Zinc added to the diet as zinc oxide at 3,000 ppm increased ADG and ADFI by 12% compared to the control in study 3. Overall, in all 3 studies, zinc added to the diet as zinc oxide at 3,000 ppm increased ADG by 17% and ADFI by 14% compared to the control (Hahn and Baker, 1993). These studies illustrate some differences in the efficacy of zinc sources.

Case and Carlson (2002) conducted 3 experiments evaluating performance results from feeding pharmacological levels of both organic and inorganic forms of zinc. A basal nursery diet which included 100 ppm of zinc as zinc sulfate was fed as the control for 28 days in studies 1 and 2 and for 15 days in study 3. Two dietary phases were fed, from day 0 to 14 and day 15 to 28, respectively. For all three studies the treatments consisted of the basal diet supplemented with the following forms of zinc: 150 ppm zinc oxide, 500 ppm zinc oxide, 500 ppm zinc-amino acid complex, 500 ppm zinc as a zinc-polysaccharide complex, and 3,000 ppm zinc as zinc oxide. In study 1, over the entire study period pigs fed diets supplemented with 3,000 ppm zinc as zinc oxide as well as pigs fed the zinc-polysaccharide complex at 500 ppm had improved ADG compared to the control (0.46 and 0.44 vs. 0.35 kg/day, respectively). However, there was no effect of zinc supplementation on ADFI or G:F in this study. In contrast, in a second study, pigs fed diets supplemented with 3,000 ppm zinc as zinc oxide had significantly greater ADG

and ADFI than pigs supplemented with zinc as 150 ppm zinc oxide, 500 ppm zinc oxide, 500 ppm zinc-amino acid complex, or 500 ppm zinc-polysaccharide complex. Specifically, pigs fed diets supplemented with 3,000 ppm of zinc as zinc oxide had increased ADG and ADFI of 14% and 16%, respectively, compared to pigs fed the diet supplemented with zinc-polysaccharide complex at 500 ppm, with no differences in G:F. However, the results of the third study were similar to the first; from day 0 to 15 pigs fed diets containing 3,000 ppm of supplemental zinc as zinc oxide had the highest growth rate and feed intake, which were 51% and 12%, greater than the control, respectively. Pigs fed supplemental zinc as zinc-polysaccharide complex at 500 ppm had similar ADG and ADFI to those fed diets supplemented with 3,000 ppm zinc as zinc oxide. In summary, providing zinc as 3,000 ppm zinc oxide gave the greatest growth rate in all 3 studies, however, pigs fed diets supplemented with zinc as 500 ppm zinc-polysaccharide complex had similar growth rates and feed intake to pigs that received diets supplemented with 3,000 ppm zinc as zinc oxide in two of the studies but not the other. These results support the concept that substituting inorganic sources of zinc with organic alternatives may provide similar performance at a lower zinc level, but the results were inconsistent. Moreover, it was concluded by Case and Carlson (2002) that feeding 3,000 ppm of zinc oxide provided the greatest growth performance improvements in weaned pigs.

Hollis et al. (2005) evaluated the effects of replacing zinc oxide with lower levels of organic zinc sources. In two 28 day studies, pigs fed a diet containing 2,500 ppm of supplemental zinc from zinc oxide were compared to pigs fed diets supplemented with 125, 250, or 500 ppm of zinc from zinc-methionine and a control treatment containing 125 ppm zinc from a trace mineral premix. In the first experiment, pigs fed diets with supplemental zinc gained faster than the unsupplemented control diet fed pigs, with the diet containing 2,500 ppm supplemental zinc as

zinc oxide providing the greatest overall improvements in ADG, ADFI, and G:F over the control of 11%, 9% and 1%, respectively. In this experiment, pigs fed diets supplemented with zinc methionine had significantly greater ADG and ADFI than the control with improvements of 6% and 5%, respectively. However, during the 28 day study period, pigs fed diets with 2,500 ppm zinc as zinc oxide had increased ADG and ADFI of 5% and 4%, respectively, compared to pigs fed 500 ppm zinc as zinc methionine, with no differences in G:F. Thus, feeding pigs diets supplemented with 2,500 ppm zinc as zinc oxide resulted in the greatest improvements in growth performance. In the second trial, the effects of feeding pigs diets containing 500 or 2,000 ppm added zinc from zinc oxide, 500 ppm of zinc from one of the following organic sources: zinc-polysaccharide complex, zinc-proteinates, zinc-amino acid complex, zinc-amino acid chelate, or zinc-methionine, or a basal control diet containing 140 ppm zinc as zinc oxide were evaluated. Overall, pigs that received diets supplemented with 2,000 ppm zinc as zinc oxide had greater ADG and ADFI (352 vs. 322 and 553 vs. 523 g/d, respectively) than the pigs supplemented with 500 ppm of zinc as zinc oxide or any of the organic zinc sources. Pigs fed diets supplemented with 2,000 ppm zinc as zinc oxide had the greatest improvements in growth performance over the control of 10%, 5%, and 4% for ADG, ADFI and G:F, respectively. Hollis et al. (2005) concluded that organic sources of zinc supplemented in the diet at 500 ppm did not significantly improve overall growth performance beyond what was achieved by feeding the control diet. Therefore, in these two studies, it was shown that only growth performance benefits from feeding at least 2,000 ppm zinc oxide were achievable and repeatable.

Manangi et al. (2012) supplemented broilers with organic and inorganic minerals for 54 days in two separate studies to evaluate the performance effects of feeding chelated trace minerals at lower levels than those commonly used in industry for inorganic mineral sources. In

the first study, dietary treatments consisted of zinc added at 100 ppm as zinc sulfate (to provide the inorganic source) or an organic source of zinc {zinc bis(-2-hydroxy-4-methylthio)butanoic acid [Zn-(HMTBA)<sub>2</sub>]} included at 30 ppm. Overall, there were no significant treatment differences in end of test body weight, ADFI, or feed conversion ratio (3.26 vs 3.24 kg; 6.36 vs. 6.28 kg; 2.03 vs 2.03 kg, respectively) of feeding broilers 30 ppm zinc HMTBA compared to 100 ppm from zinc oxide. Additionally, birds fed diets supplemented with 30 ppm zinc as zinc HMTBA saw a 27% improvement in footpad condition. In the second study, inorganic minerals were added to the diets by supplementing the following levels and sources; 100 ppm zinc from zinc sulfate, 125 ppm of supplemental copper as copper sulfate or 90 ppm of supplemental manganese as manganese sulfate. Organic or chelated minerals were supplemented in the diet as 32 ppm of zinc as, Zn bis(-2-hydroxy-4-methylthio) butanoic acid [Zn-(HMTBA)<sub>2</sub>], 8 ppm of supplemental copper as Cu-(HMTBA)<sub>2</sub>, or 32 ppm of supplemental manganese as Mn-(HMTBA)<sub>2</sub>. Supplementing the diets with reduced levels of HMTBA chelated trace minerals significantly improved footpad health and reduced trace mineral concentrations in the litter. Moreover, there were no effects of feeding lower levels of the organic sources on end of test body weight, ADFI, or feed conversion ratio (3.28 vs 3.31 kg; 6.56 vs. 6.55 kg; 2.00 vs 1.99 kg, respectively) in comparison to birds fed the inorganic supplemented diets (Manangi et al., 2012).

In summary, results reported by Manangi et al. (2012) are similar to results reported by Ward et al., (1996), which suggested that feeding diets to pigs with 250 ppm of zinc as zinc-methionine produced the same growth performance results as diets with 2,000 ppm zinc from zinc oxide. Additionally, Case and Carlson, (2002) found that zinc supplemented at 500 ppm in the form of zinc polysaccharide complex produced growth performance similar to that elicited by supplementation of zinc at 3,000 ppm as zinc oxide. These results are contrary to results

reported by Hahn and Baker, (1993) as well as by Hollis et al. (2005) that suggested that feeding supplemental levels of zinc as zinc oxide gave a greater growth response compared to pigs fed organic zinc sources. Research on feeding organic zinc sources to pigs have generally shown that responses were lower and less repeatable than with inorganic forms. Much of the published literature shows that dietary supplementation with inorganic forms of zinc at pharmacological levels has given the most consistent responses for enhancing growth performance in weaned pigs.

### *Hydroxy Trace Minerals*

Although inorganic and some organic sources of zinc are most commonly used in the swine industry, a new form of inorganic minerals known as hydroxy trace minerals has recently been a topic of research. Cohen and Steward (2014) described hydroxy minerals as, “metal salts partially reacted with alkali to produce hydrolyzed inorganic metal complexes”. Caramalac et al. (2017) found that weaned calves had a greater preferential intake of hydroxy zinc than organic or inorganic zinc sources and suggested that this was because the organic and inorganic sources dissolve more quickly in water, causing a metallic taste. Similarly, Coble et al. (2014) discovered that when given the choice between a diet fortified with 150 ppm of either copper sulfate or a hydroxy form of copper, finishing pigs ate significantly more feed that contained the hydroxy trace mineral. These results can be explained by the fact that hydroxy forms of minerals are less soluble in water at neutral pH due to their low hygroscopicity and crystal structure and, therefore, they are very stable and less reactive with water and other ingredients (Cromwell et al., 1998). Chemical form as well as degree of solubility can greatly impact the utilization of supplemental sources of minerals (Ammerman et al., 2005). A hydroxy form of copper known as tribasic copper chloride was introduced into the animal feed industry in 1995 (Cohen and



Steward, 2014) followed by a hydroxy form of zinc in 2014, known as zinc hydroxychloride. There has been limited research focused on zinc hydroxychloride, however, some studies have suggested beneficial effects of feeding zinc hydroxychloride compared to traditionally used inorganic sources of zinc.

Carpenter et al. (2016) evaluated the effects on growth performance and carcass characteristics of feeding finishing pigs diets with increasing levels of zinc as either zinc sulfate or zinc hydroxychloride. Two dietary treatments were compared in a  $2 \times 3$  factorial arrangement; the treatments were zinc source (zinc sulfate or zinc hydroxychloride), and zinc inclusion level (50, 100, or 150 ppm added zinc). There were no source  $\times$  level interactions for any of the variables. There were no effects of zinc source on growth performance over the 103 day study period. Feeding 100 ppm of zinc from either source maximized overall live weight ADG, body weight at the end of the study period, and hot carcass weight. Also, carcass yield increased linearly when pigs were fed increasing levels of either source of zinc. Additionally pigs fed zinc hydroxychloride had significantly increased hot carcass weight compared to pigs fed zinc sulfate. These results suggest that feeding finishing pigs zinc hydroxychloride could potentially improve hot carcass weight, however, no data was provided in the abstract to support this conclusion.

Cemin et al. (2018) evaluated the effects of increasing the level of zinc in the diets of growing-finishing swine with zinc hydroxychloride at levels of 50, 87.5, 125, 162.5 and 200 ppm. Diets were fortified with a trace mineral premix that did not include zinc; 5 dietary phases were fed over a 113 day test period. There was no effect of added zinc on overall live weight ADG. However, increasing the level of supplemental zinc in the diet produced trends ( $P < 0.10$ ) for quadratic responses in live weight ADFI and G:F. Feeding pigs diets supplemented with 87.5

and 125 ppm zinc as zinc hydroxychloride, produced the lowest ADFI and greatest G:F. Additionally, there were no treatment differences in carcass characteristics. Based on this study, there is some limited evidence that increasing the amount of supplemental zinc in the form of zinc hydroxychloride in swine diets increased feed intake and improved feed efficiency of growing-finishing pigs. However, this study was conducted without a positive control treatment, which is necessary to truly quantify the effects of zinc hydroxychloride.

Published research evaluating the effects of zinc hydroxychloride is limited. Although there is some evidence of improvements in, feed intake, feed efficiency, and hot carcass weight from supplementing swine diets with zinc in the form of zinc hydroxychloride, comparison of hydroxy zinc to inorganic forms of zinc is warranted.

### *Bioavailability*

Feeding pharmacological levels of inorganic and some organic sources of zinc to nursery pigs has been successfully used to improve nursery growth performance (Hahn and Baker, 1993; Smith et al., 1997; Hill et al., 2000, 2001; Case and Carlson 2002). As was previously summarized, different sources of zinc do not elicit the same response in the pig which can be partially explained by method of absorption and the bioavailability of each source. Ammerman et al. (2005) suggested that bioavailability estimates of minerals can be provided by measuring absorption, however, that is not always accurate. Despite high absorption rates, availability of the mineral once absorbed can be decreased due to either difficulty or ease of dissociation or interference from other metals (Perez-Mendoza, 2008). After absorption from the gastrointestinal tract, inorganic zinc enters the enterocytes and can remain in those cells where it can either be bound to a cysteine-rich protein known as metallothionein, or can pass through the basolateral membrane into the plasma (Lewis and Southern, 2001). Typically, absorption of

organic forms of zinc is via peptide or amino acid transport systems, usually resulting in higher digestibility and availability of organic mineral source compared to inorganic sources (Nitrayova et al., 2012). Ammerman et al. (1995) defined bioavailability as "the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal". It has been estimated that that absorbed and retained zinc is usually around 50% of zinc intake (NRC, 2012). Bioavailability values are often expressed as a percentage which is representative of the amount of a mineral that is available to be utilized by the animal after it is absorbed (Ammerman et al., 1995). Bioavailability values are measured relative to a standard, usually zinc sulfate (100% bioavailability) for zinc, resulting in a 'relative bioavailability value' (RBV) (Ammerman et al., 1995).

As early as 1958, research was conducted using chicks to evaluate the bioavailability of various supplemental zinc sources and this work suggested that zinc in the form of salts, oxides, and carbonates were all relatively available (Ammerman and Miller, 1972). The relative bioavailability of zinc can be determined by measuring the zinc content in bones when dietary zinc is depleted and by zinc accumulation in plasma, zinc uptake in the liver, and metallothioneine synthesis when dietary zinc is supplemented at pharmacological levels. The two methods have been found to give similar results (Wedekind et al., 1992; Perez-Mendoza, 2008).

In a review of bioavailability studies, Owens et al. (2009) reported 4 studies in which chicks fed organic zinc deposited more available zinc that could be utilized by the animal than either zinc oxide or feed-grade zinc sulfate. Available zinc was defined as the amount of a mineral that has been absorbed, transported, and is present at the "site of action" to be utilized (Owens et al., 2009). In the same review, 3 studies were also cited that found no differences in zinc bioavailability between organic and inorganic sources in pigs. Wedekind et al. (1992)

reported that for chicks, zinc from zinc methionine complex was 206% bioavailable compared to the standard (zinc sulfate; 100%) and zinc oxide was only 61% bioavailable. Cao et al. (2000) reported bioavailability estimates from measuring zinc levels in liver, kidney and pancreas of chicks and determined bioavailability values for zinc proteinate, zinc amino acid complex, and zinc methionine of 130, 110, and 133%, respectively, relative to zinc sulfate (100%). Case and Carlson (2002) found no difference in zinc concentrations in plasma, tissue, urine or feces from feeding pigs diets supplemented with 500 ppm zinc as zinc oxide, zinc-amino acid complex, or zinc-polysaccharide. Owens et al. (2009) noted that it is important to consider the production process of organic, chelated minerals as that can have a direct impact on the quality of the trace mineral product and, therefore, can impact bioavailability.

Although feed-grade zinc oxide is the most commonly used supplemental zinc source in North America, it also has the lowest bioavailability (Baker, 2001). It has been reported that zinc oxide has bioavailability values around 40-60% of that of zinc sulfate (Wedekind and Baker, 1990; Wedekind et al., 1992; Hahn and Baker, 1993; Cao et al., 2000). Other estimates of bioavailability values for commonly used zinc supplements are 100%, >100%, and 50% for zinc-lysine, zinc-methionine, zinc oxide, respectively, when compared to a standard of zinc sulfate (100% bioavailable) (Baker, 2001). NRC (2012) reported that estimates of the bioavailability of zinc oxide ranged from 50% to 80% (average 65%). However, despite the variation in reported estimated bioavailability values of zinc oxide in relation to zinc sulfate, zinc oxide is more efficacious when used as a growth promoter. Perez-Mendoza (2008) conducted a review of studies comparing differences in bioavailability as well as efficacy of zinc sulfate compared to zinc oxide. In this review, it was noted that supplementing 3,000 ppm zinc in the diet as zinc sulfate or zinc oxide resulted in an increase in ADG of 17% and 15% respectively. Despite

higher bioavailability of zinc sulfate, this study was the only one out of 5 publications that found that it was a more effective growth promoter than zinc oxide. Thus, bioavailability is not always an indicator of efficacy of zinc sources for growth promotion. However, the low bioavailability of zinc oxide results in the need to use relatively high levels of this source to achieve maximum growth performance responses in pigs.

Hydroxy trace minerals can be strongly bound by covalent bonds, which protects them from deleterious interactions with other minerals and enzymes. Consequently, hydroxy trace minerals have been reported to have bioavailability values greater than 100% (Cohen and Steward, 2014). Zinc Hydroxychloride is a relatively new zinc source which has been claimed to have improved bioavailability compared to commonly used zinc sources such as zinc sulfate (Micronutrients, Inc., personal communication). It has been claimed that the average bioavailability of zinc hydroxychloride, relative to zinc sulfate as indicated by the slope ratio of the linear response of weight gain and tibia zinc content in the chick, was 155% (Micronutrients, Inc., personal communication). However, the bioavailability of zinc in zinc hydroxychloride has not been widely studied in swine. Additionally, there are few studies that have evaluated the effects of zinc hydroxychloride compared to common inorganic zinc sources on growth performance. Thus, growth trials comparing performance of pigs fed diets supplemented with an inorganic form of zinc as well as zinc hydroxychloride are warranted.

#### *Copper as a Growth Promoter*

Much like zinc, copper has been added to diets for growing pigs to promote growth. High dietary levels of copper have produced improvements in growth rate, feed intake and feed efficiency in weaned pigs when fed at 125 to 250 ppm (Braude, 1967; Cromwell et al., 1998; NRC, 2012) as well as in grow-finish pigs (Davis et al., 2002; Coble et al., 2017). The suggested

requirement for copper for different weights of pigs ranges from 6 ppm down to 3 ppm for pigs weighing 5 to 135 kg, respectively (Table 1) (NRC, 2012). Levels of dietary copper fed in excess of 250 ppm could become toxic if fed for prolonged periods of time, thus, the maximum level tolerable by pigs is 250 ppm (NRC, 2012). Similar to zinc, copper has often been added at pharmacological levels in the form of an inorganic salt, typically as copper sulfate (Cromwell et al., 1989). It has been reported that copper sulfate has 25% copper, is greater than 99% soluble in water and the copper is highly available (Pereze-Mendoza, 2008). Cromwell et al. (1998) summarized 22 studies evaluating the effects of adding copper in the diets of weaning pigs. In this review, it was reported that the supplementing the diets of weaned pigs with 250 ppm of copper as copper sulfate increased ADG, ADFI and improved G:F by on average 12%, 8%, and 5%, respectively, compared to pigs fed unsupplemented diets. Thus, copper supplementation at pharmacological levels was considered an effective strategy to promote growth performance, however, differences in efficacy amongst copper sources exists (Perez-Mendoza, 2008). Much like zinc sources that were previously discussed in this review, research evaluating the use of organic forms of copper as well as tribasic copper chloride has been conducted.

Several forms of organic copper have been used at pharmacological levels in weaned pig diets in an effort to improve growth performance. In a review of 7 studies (Bunch et al., 1965; Stansbury et al., 1990; Coffey et al., 1994; Zhou et al., 1994; Apgar et al., 1995; Apgar and Kornegay, 1996; Veum et al., 2004), supplementing diets with 200 ppm copper as organic forms of copper increased ADG, ADFI, and G:F in weaned pigs by on average 14.6%, 12.0%, and 3.1%, respectively, compared to unsupplemented control diets. Thus, growth performance can be improved by supplementing swine diets with organic forms of copper. The majority of the studies have concluded that growth performance improvements were similar for copper sulfate

and organic forms of supplemental copper (Bunch et al., 1965; Stansbury et al., 1990; Apgar et al., 1995; Apgar and Kornegay, 1996). However, 2 studies concluded that organic forms of copper improved growth performance to a greater extent than copper sulfate. Coffey et al. (1994) concluded that 100 ppm added copper as copper-lysine was as efficacious at improving growth rate and feed intake as copper sulfate supplemented at 200 ppm. In this study, feeding diets with 100 ppm copper as copper-lysine resulted in an increase over the unsupplemented control diets for ADG and ADFI of 16.8% and 14.1%, respectively, compared to increases of 11.5% and 8.7%, respectively, for diets containing 200 ppm copper sulfate. Additionally, Zhou et al. (1994b) reported that supplementing the diets of nursery pigs with 200 ppm copper as copper-lysine resulted in increases over the unsupplemented control diets for ADG and ADFI and G:F by 27.3%, 19.3%, and 2.0%, respectively compared to increases of 7.0% and 12.4% for ADG and G:F, respectively for diets containing 200 ppm copper sulfate.

Thus, there is evidence that supplementing diets with organic forms of copper can provide similar or greater growth performance benefits to those elicited by higher levels of copper sulfate.

A hydroxy form of copper known as tribasic copper chloride was introduced into the animal feed industry in 1995 (Cohen and Steward, 2014). Similar to zinc hydroxychloride, less than 1% of tribasic copper chloride is soluble in water making it more stable and less destructive of vitamins and other organic compounds in the diet (Cromwell et al., 1998). Tribasic copper chloride has 58% copper and a relative bioavailability value of 100% (Perez-Mendoza, 2008). It has been shown that there is a positive relationship between the bioavailability of copper and its growth promoting impacts (NRC, 2012).

Studies evaluating the growth performance effects of copper hydroxychloride or tribasic copper chloride have been conducted. For example, Espinosa et al. (2017) conducted two studies, each lasting 28 days, evaluating the effects of feeding added copper as copper hydroxychloride on growth performance of weaned pigs. In the first study, feeding newly-weaned pigs a diet with 150 ppm copper from tribasic copper chloride increased overall ADG, ADFI, G:F, and final body weight compared to the unsupplemented control. However, the increase was much greater in barrows than gilts (ADG, + 107g and +12g; ADFI, + 104g and 15g; G:F, + 0.09 and + 0.008; final body weight, 3.3kg and 0.23kg for barrows and gilts, respectively). Nevertheless, there were no significant sex by diet interactions for overall growth performance.

In the second experiment, 3 treatments were compared: unsupplemented control diet, and diets with 100 ppm or 200 ppm copper from copper hydroxychloride. There was a greater response in ADG, ADFI, and G:F from feeding 200 ppm copper hydroxychloride compared to feeding 100 ppm or an unsupplemented control diet. Differences between the control and the test diet for ADG, ADFI, and G:F were 10.9%, 8.2%; and 7.0% , respectively, for the 200 ppm treatment and 4.6%, 3.5% vs. 1.5%, respectively, for the 100 ppm treatment for the 28 day study period. However, overall, none of the differences in growth performance were statistically significant. There was, however, a statistically significant increase in body weight at the end of the 28-day test period which was on average 7.4% for the 100 and 200ppm tribasic copper chloride treatments compared to the control. Therefore, this study suggested that supplementing copper from copper hydroxychloride at 100 or 200 ppm numerically improved growth performance and significantly improved body weight at the end of test.



In one of the first studies conducted comparing commonly used copper sulfate and tribasic copper chloride, also known as copper hydroxychloride, Cromwell et al. (1998) found that supplementing 200 ppm of copper as tribasic copper chloride in the diets of weaned pigs improved ADG, ADFI and G:F by 8%, 5%, and 4%, respectively, compared to pigs fed a control diet without supplemental copper. These improvements were comparable to those produced with 200 ppm copper as copper sulfate which were 10%, 8% and 2% for ADG, ADFI, and G:F, respectively, compared to pigs fed a control diet without supplemental copper. Thus, it was concluded that tribasic copper chloride is as effective as commonly used copper sulfate at improving growth performance in newly weaned pigs. In contrast, other research has suggested that lower levels of tribasic copper chloride can be as effective at improving growth performance of nurse piglets as greater levels of copper sulfate. For example, Perez-Mendoza (2008) summarized a study by Dove (1995) that found both levels of 100 and 200 ppm supplemental copper as tribasic copper chloride were as effective as 250 ppm of copper as copper sulfate at improving growth performance. Based on results of these studies, including copper sulfate or tribasic copper chloride in diets for nursery pigs have both been shown to improve growth performance. However, it is unclear if the growth response to these sources differs and further research is required to address this question.

#### *Copper Supplementation in Grow-Finish Swine Diets*

Adding pharmacological levels of copper to nurse pig diets has been proven to improve growth performance, however, supplementing the diets of grow-finish swine with pharmacological levels of copper has been shown to produce varied results. Some studies have shown a positive effect of diet supplementation with copper on growth performance of grow-finish pigs. For example, Coble et al. (2017) carried out a grow-finish study, over a feeding

period of 111 days, using diets supplemented with 75 or 150 ppm copper as either copper sulfate or tribasic copper chloride. There were no significant copper source by level interactions or differences between copper sources for any of the response criteria. Overall, increasing the amount of supplemental copper in either form had no effect on live weight G:F; however, live weight ADG, ADFI, and body weight at the end of the test period increased linearly by a total of 3%, 4%, and 4%, respectively, compared to the unsupplemented control. There were no effects of copper source on carcass characteristics, however, increasing copper level linearly increased hot carcass weight and loin depth. In contrast, Carpenter et al. (2017) fed grow-finish swine over a period of 105 days with diets containing either copper sulfate at levels of 70 and 130, or a 50/50 mixture of copper sulfate plus copper-amino acid complex at levels of 70, 100, and 130 ppm, compared to a control containing 17 ppm copper as copper sulfate. Neither source nor level of supplemental copper had a significant effect on overall growth performance or carcass characteristics. Coble et al. (2017) reviewed literature relating to the effect of added copper in the finishing phase and reported that that copper supplementation is most efficacious in the early finishing period, with little to no response in late finishing. In the same review, there was one study that found improvements in ADG and G:F from feeding diets with 125 ppm copper from copper sulfate during late finishing (Coble et al., 2017).

In summary, much like other minerals, studies in the literature show variability in responses to feeding different sources and levels of supplemental copper. Supplementing copper as copper sulfate in the diets of newly-weaned pigs has been shown to improve growth performance (Cromwell et al., 1998). Additionally, organic forms of copper have been shown to be as efficacious as inorganic sources (Zhou et al., 1994b) sometimes at lower inclusion levels (Coffey et al., 1994). Using a hydroxy form of copper as a growth promoter has been proven to

improve growth performance in nursery swine (Espinosa et al., 2017). Moreover, while copper supplementation was an effective method of improving growth performance in newly-weaned pigs, there were inconsistent results for the effects of supplemental copper on growth performance in grow-finish swine.

### *Zinc and Copper Supplementation*

It has been well documented that independently, both zinc and copper have the ability to improve growth performance, particularly in nursery pigs (Hahn and Baker, 1993; Case and Carlson, 2002; Cromwell et al., 1998). A number of studies have investigated the impact of including both copper and zinc at pharmacological levels in the diets of pigs and, in general, these have produced variable results. Smith et al. (1997) reported that feeding 3,000 ppm zinc from zinc oxide improved nursery pig performance, however, there was no additional response observed when 250 ppm copper from copper sulfate was added. Similarly, Hill et al. (2001) reported on a regional study that was carried out at a number of research centers that evaluated feeding 3,000 ppm zinc and 250 ppm copper, individually and in combination. Compared to the unsupplemented control treatment, ADG, ADFI, and G:F were significantly improved by adding either 3000 ppm zinc (12.5%, 8.3%, 4.3%, respectively) or 250 ppm copper (9.1% 5.3% and 4.3% for ADG, ADFI, and G:F, respectively). However, there was no extra improvement in growth performance from including both copper and zinc in the diet. In contrast, Shelton et al. (2011) reported additive effects of including both 3,000 ppm zinc and also 125 ppm of copper in diets of weaned pigs. Compared to the unsupplemented control treatment, supplementing the diet with 3,000 ppm zinc improved ADG, ADFI, G:F, and final BW by + 32 g/d, + 30 g/d, + 0.02, and + 0.7 kg, respectively; equivalent improvements from adding 125 ppm copper were + 50 g/d, + 24 g/d, + 0.07, and + 1.0 kg, respectively. Feeding both 3000 ppm zinc and 125 ppm

copper resulted in improvements in ADG, ADFI, and final BW of + 90 g/d, + 87 g/d, and + 2.2 kg, respectively, compared to the unsupplemented controls, however, there was no evidence of additive effects of zinc and copper treatments on G:F. Additionally, in a second study, Shelton et al. (2011) reported that supplementing diets with 3,000 ppm zinc from zinc oxide and 150 ppm copper from tribasic copper chloride produced overall growth improvements both individually and in combination. Feeding 3,000 ppm zinc improved ADG, ADFI, and final BW by +60 g/d, + 81 g/d, and + 1.7 kg, respectively with no differences in G:F. Equivalent improvements of adding 150 ppm copper were + 51 g/d, + 58 g/d, + 0.02, and + 1.4 kg, for ADG, ADFI, GF, and final BW, respectively. Feeding 3,000 ppm zinc and 150 ppm copper improved ADG, ADFI, and final BW by + 77 g/d, + 100 g/d, and + 2.5 kg, respectively, compared to the unsupplemented control, however, there was no additive effects of zinc and copper treatments on G:F. These results suggested advantages of adding both zinc and copper to the diets of weaned pigs for 28 days. Similarly, Perez-Mendoza (2008) reported additive improvements in growth performance for nursery pigs fed diets supplemented with 100 ppm copper from copper-amino acid complex and 3,000 ppm supplemental zinc. Compared to the unsupplemented control treatment, supplementing the diet with 3,000 ppm zinc improved ADG, ADFI, and final BW by + 50 g/d, + 58 g/d, and + 2.0 kg, respectively; equivalent improvements from adding 100 ppm copper-amino acid complex were + 33 g/d, + 44 g/d, and + 1.3 kg, for ADG, ADFI, and final BW, respectively, with no effect on G:F. Feeding 3000 ppm zinc and 100 ppm copper-amino acid complex resulted in improvements in ADG, ADFI, G:F, and final BW of + 65 g/d, + 80 g/d, + 0.008, and + 2.2 kg, respectively, compared to the unsupplemented control.

Recently, Cemin et al. (2017) evaluated the effects of adding pharmacological levels of a relatively new zinc source, zinc hydroxychloride, compared to zinc oxide, and tribasic copper

chloride on the growth performance of nursery pigs. Zinc was supplemented at levels of 2,000 ppm and 3,000 ppm from zinc oxide or 1,000 ppm from zinc hydroxychloride. Copper was supplemented in the diet at 200 ppm from tribasic copper chloride. Overall, results were inconsistent and will not be discussed in this review. It is important to note that research evaluating the effects of zinc hydroxychloride and tribasic copper chloride is relevant and needed.

In summary, supplementation of diets for nursery pigs with pharmacological levels of zinc and copper individually have been shown to improve growth performance. However, results relating to the effects on growth performance of feeding both of these minerals in diets have been variable. Moreover, levels of zinc in the studies in this thesis were fed to meet the dietary requirement of the animal; copper was supplemented at pharmacological levels. Thus it is important to acknowledge the relationship between the two minerals, but interaction was not anticipated due to study design. Validation of growth performance and or carcass characteristics improvements made by copper in the diets of growing finishing swine is warranted. Additionally, very little research has been conducted to evaluate the efficacy of zinc hydroxychloride, especially compared to zinc oxide at levels fed to meet the requirement of the animal.

## TABLE

**Table 1.** Dietary zinc requirements of growing pigs allowed feed ad libitum (90% dry matter).

Item	Body Weight Range (kg) <sup>1</sup>						
	5-7	7-11	11-25	25-50	50-75	75-100	100-135
Zinc (mg/kg)	100.00	100.00	80.00	60.00	50.00	50.00	50.00
Copper (mg/kg)	6.00	6.00	5.00	4.00	3.50	3.00	3.00
Phosphorus (%) <sup>2</sup>	0.70	0.65	0.60	0.56	0.52	0.47	0.43

<sup>1</sup>Body weight ranges and requirements are suggested by NRC (2012).

<sup>2</sup>Values represent total phosphorus requirements of growing pigs (mixed gender).

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## **CHAPTER 2: THE EFFECTS OF ZINC SOURCE AND SUPPLEMENTAL COPPER ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND MORBIDITY AND MORTALITY OF GROWING-FINISHING PIGS RAISED UNDER COMMERCIAL CONDITIONS**

### *Introduction*

Zinc and copper are key minerals used in swine diets to meet dietary requirements of the animals and can also be used to improve growth performance. Zinc supplements are typically included in swine diets to meet dietary requirements and mitigate deficiencies (NRC, 2012). It has been documented that zinc deficiency is typically manifested as a skin condition known as parakeratosis (Tucker and Salmon, 1955), and also results in growth retardation (O'Dell and Savage, 1957). Current estimates for the requirements for zinc range from 100 ppm to 50 ppm for pigs weighing 5 to 135 kg, respectively (NRC, 2012). Despite the requirements suggested by NRC (2012), it has become common industry practice to add vitamins and trace minerals to swine diets at levels in excess of the requirement as a safety margin to account for variation in the diet manufacturing process (Flohr et al., 2016). These increased levels are still used to meet the requirement of the animal. Various sources of zinc can be used in swine diets (Hahn and Baker, 1993; Case and Carlson, 2002; Hollis et al., 2005).

Copper is also required in the diets of swine at trace mineral levels, however, studies have shown advantages from adding levels much greater than the requirement, known as pharmacological levels, to the diet for growth promotion benefits (Braude, 1967; Cromwell et al., 1998, NRC, 2012).

Typically, mineral supplements used in swine diets are in either organic or inorganic forms. Recently, hydroxy forms of inorganic trace minerals have become available. These are

less soluble in water at neutral pH due to their low hygroscopicity and crystal structure which makes them very stable and less reactive with water and other dietary ingredients (Cromwell et al., 1998). There is some evidence of improvements in growth performance from supplementing swine diets with zinc in the form of zinc hydroxychloride compared to zinc oxide (Carpenter et al., 2016; Cemin et al., 2018). However, there is limited research evaluating the effects of zinc hydroxychloride compared to common zinc sources such as zinc oxide when fed at levels to meet dietary requirements of the animal. Additionally, while adding supplemental copper in the form of tribasic copper chloride to the diets of weaned improved growth performance (Cromwell et al., 1998), results have varied when supplemental copper was added to the diets of grow-finish swine (Carpenter et al., 2017; Coble et al., 2017). Thus, research is necessary to evaluate the efficacy of zinc hydroxychloride compared to zinc oxide when fed at levels to meet dietary requirements of the animal, as well as the effects of pharmacological levels of tribasic copper chloride on the growth performance of grow-finish swine.

### *Materials and Methods*

Two studies were conducted at the Mach 9 Research Center of the Maschhoffs LLC, located near Beardstown, IL, which is a standard commercial wean-to-finish facility that is equipped to collect data on growth performance and feed intake under typical commercial conditions. The experimental protocol was approved by the University of Illinois Institutional Animal Care and Use Committee.

### **Experimental Design and Treatments**

#### **Study 1:**

Study 1 used a randomized complete block design (blocking factor was date of start of test). There were 4 treatments that involved a combination of different sources (zinc oxide or hydroxychloride) and inclusion levels of zinc, and different levels of supplemental copper (0 or 150 ppm). The treatments were as follows:

1: Control with added copper [0.16 kg zinc as zinc oxide /1,000 kg of feed + 0.24 kg tri-basic copper chloride /1,000 kg of feed]

2: Zinc hydroxychloride (65% Bioavailable) [0.20 kg zinc as zinc hydroxychloride /1,000 kg of feed + 0.24 kg tri-basic copper chloride /1,000 kg of feed]

3: Zinc hydroxychloride (100% Bioavailable) [0.13 kg zinc as zinc hydroxychloride /1,000 kg of feed + 0.24 kg tri-basic copper chloride /1,000 kg of feed]

4: Control without added copper [0.16 kg zinc as zinc oxide /1,000 kg of feed; no added copper]

The target for all treatments was to have the same amount (ppm) of bioavailable zinc in each phase based on weight and age of the pig. For the treatments that included tri-basic copper chloride (Treatments 1, 2, and 3), the target was to have 150 ppm of added copper. For Treatments 1 and 4, that included zinc oxide, the assumed bioavailability was 65% (NRC, 2012). For Treatment 2, zinc hydroxychloride was included as the supplemental zinc source based on an assumed bioavailability of 65%. For Treatment 3, the assumed bioavailability of zinc hydroxychloride was 100%. Treatments 1, 2, and 3 were supplemented with 150 ppm copper as tribasic copper chloride which has an assumed bioavailability of 100% (NRC, 1998). Treatment 4 did not include any supplemental copper beyond that contained in the mineral premix (12 ppm).

## **Study 2:**



The pigs used for Study 2 had previously been allotted to additional experimental treatments that were independent of those used in the current study. Consequently, Study 2 used a split-plot design with the main plot being the additional experimental treatments and the subplot being the two treatments that compared two different sources of zinc. The treatments were as follows:

1: Control [0.16 kg zinc as zinc oxide /1,000 kg of feed]

2: Zinc hydroxychloride [0.13 kg zinc as zinc hydroxychloride /1,000 kg of feed]

The assumed bioavailability of zinc oxide and zinc hydroxychloride were 65% (NRC, 2012) and 100%, respectively.

Pen was used as the experimental unit and there were 4 pens/replicate in Study 1 and 2 pens/replicate in Study 2

## **Animals and Allotments**

### **Study 1:**

Allotment was carried out when pigs reached  $47.0 \pm 5.1$  kg. A total of 2,040 animals, which were progeny of TML sires mated to commercial dams, were used. Prior to allotment, these pigs had previously been on a nursery study and had been housed in single-gender pens of 44 pigs. The allotment was carried out within weaning day and previous nursery-study treatment. Pens that had been weaned on the same day and had been on the same previous nursery study treatment were weighed and sorted into groups of 2 pens of the same gender, with similar BW and similar group sizes. Each pen within the group of 2 pens was split into 2 groups of 22 pigs with similar mean live weight to form a replicate of 4 single-gender pens. Within a

replicate, pens were randomly allotted to one of the four dietary treatments. This process was repeated until there was a total of 27 pens per treatment.

### **Study 2:**

Allotment was carried out when pigs reached  $41.9 \pm 1.7$  kg. A total of 2,888 animals, which were progeny of TML sires mated to commercial dams, were used. Prior to allotment, these pigs had previously been on a nursery study and had been housed in single-gender pens of pens of 44 pigs. The allotment for the current study was carried out within weaning day and previous nursery-study treatment. During the nursery trial, pigs had been in groups of 44 pigs. For allotment to the current study, pens were split into 2 equal sized groups with similar BW and group size. If needed, additional pigs that were from the same weaning day and previous treatment, and of the appropriate gender were added to the 2 pens to standardize the number of pigs to 22 pigs per pen. Both pens were weighed and if necessary, pigs were exchanged between the two pens, until the average pig weight of the pens was within  $\pm 0.45$  kg. The two pens were randomly allotted to one of the two dietary treatments with the process being repeated until there were 66 pens per treatment.

## **Diets**

### **Diet Formulation**

For both studies, diets were formulated on a total zinc basis to exceed the requirements of growing-finishing proposed by NRC (2012). According to the National Swine Nutrition Guide Fact Sheet, in commercial swine production, diets are formulated with consideration of NRC requirements, with a safety margin of minerals added to the diet, sometimes double the amount

of the suggested requirement (Reese and Hill, 2010; Flohr et al., 2016). Therefore, levels of total zinc (ppm) in the studies reported in this thesis are greater than NRC recommendations.

In Study 1, the trace mineral premix used to manufacture the experimental diets was devoid of zinc, thus, all of the supplementary zinc was from either zinc oxide or zinc hydroxychloride depending on treatment. In Study 2, the zinc source was included in a specially manufactured trace mineral premix for each of the dietary treatments.

Some zinc was also present in other dietary ingredients. Corn, corn germ meal and soybean meal have reported zinc levels of 16 ppm, 133 ppm, and 48 ppm of zinc, respectively (NRC, 2012). Nutrient analysis of ingredients used in Study 1 found levels of zinc for corn, corn germ meal, dried distiller's grains with solubles, and soybean meal of 16.5 ppm, 91.9 ppm, 59.6 ppm, and 55 ppm, respectively. Similar zinc levels were found for the ingredients used in Study 2. Therefore, including such ingredients in the diet will contribute zinc to total levels of zinc in the diets as seen in the formulated and analyzed values in Tables 12 and 13.

In Study 1 and 2, inclusion levels for the zinc sources were based on assumed zinc bioavailability for the zinc sources. Thus, inclusion levels were adjusted by treatment to ensure that all treatments were formulated with equivalent bioavailable zinc. The source of copper used in Study 1 (tribasic copper chloride) is reported to have a bioavailability of 100% (NRC 1998). Copper was added to provide a level of additional copper of 150 ppm for Trt. 1, 2, and 3.

The final dietary phase of both studies, which included ractopamine hydrochloride, included an additional amount of zinc. Some studies have suggested that additional zinc in the presence of ractopamine hydrochloride can improve growth performance (Patience et al., 2011; Fry et al., 2013; Paulk et al., 2015).

Diets were formulated to meet or exceed NRC (2012) recommendations for nutrient requirements and feed and water were available on an *ad libitum* basis during both studies. In Study 1, there were 5 dietary phases and diets were fed according to the following feed budget:

Barrows:

Phase 1, feed budget 27.7 kg feed/pig (approximate BW range fed 43.1 – 55.8 kg)

Phase 2, feed budget 29.9 kg feed/pig (approximate BW range fed 56.2 – 68.5 kg)

Phase 3, feed budget 59.9 kg/ feed pig (approximate BW range fed 69.0 – 90.3 kg)

Phase 4, feed budget 70.8 kg feed/pig (approximate BW range fed 90.7 – 112.5 kg)

Phase 5, feed budget 59.4 kg feed/pig (approximate BW range fed 113.0 – 129.3 kg)

Gilts:

Phase 1, feed budget 38.1 kg feed/pig (approximate BW range fed 44.0 – 61.2 kg)

Phase 2, feed budget 31.3 kg feed/pig (approximate BW range fed 61.7 – 74.4 kg)

Phase 3, feed budget 73.5 kg feed/pig (approximate BW range fed 74.8 – 101.1 kg)

Phase 4, feed budget 34.0 kg feed/pig (approximate BW range fed 101.6 – 112.5 kg)

Phase 5, feed budget 54.4 kg feed/pig (approximate BW range fed 112.9 – 129.3 kg)

In Study 2, there were 3 dietary phases and diets were fed according to the following feed budget:

Barrows:

Phase 1, feed budget 117.9 kg feed/pig (approximate BW range fed 40.8 – 88.0 kg)

Phase 2, feed budget 65.3 kg feed/pig (approximate BW range fed 88.4 – 108.9 kg)

Phase 3, feed budget 72.1 kg/ feed pig (approximate BW range fed 109.3 – 129.3 kg)

#### Gilts:

Phase 1, feed budget 121.1 kg feed/pig (approximate BW range fed 40.8 – 90.7 kg)

Phase 2, feed budget 58.5 kg feed/pig (approximate BW range fed 91.2 – 110.7 kg)

Phase 3, feed budget 60.3 kg/ feed pig (approximate BW range fed 111.1 – 129.3 kg)

Feed samples were collected every 2 weeks during the study period. Samples of each dietary treatment were collected from a minimum of 4 randomly selected feeders and these were formed into a composite sample that was used for analysis. Samples were also collected every time that a new dietary phase was introduced. Samples were stored in a freezer (-18° C) prior to proximate and mineral, analysis. Proximate and mineral analyses were performed at Midwest Laboratories (Omaha, NE) and the results are presented in Tables 2 to 9. Diets were analyzed for each phase during the experiment. Diets were analyzed for moisture (method 930.15; AOAC Int., 2007), crude protein (method 990.03; AOAC Int., 2007), crude fat (method 2003.05; AOAC Int., 2007), acid detergent fiber (MWL FD 026 method), crude fiber (method AOCS BA 6a-05), neutral detergent fiber (MWL FD 022 method), ash (method 942.05; AOAC Int., 2007) and minerals were analyzed by inductively coupled plasma (ICP) optical emissions spectrometry (method 985.01 A, B, and C; AOAC Int., 2007).

#### **Housing**

The same two buildings were used for the two studies; a tunnel-ventilated wean-to-finish building that had 2 identical rooms, and an insulated, negative pressure, mechanically ventilated

barn that had a single room. Both buildings had fully slatted concrete flooring. Pen divisions and gates were of horizontal steel rods; there was an adjustment gate at the back of each pen which was moved if pigs were removed during the study to maintain the same floor space allowance per pig. The floor space allowance for both studies for the entire study period was 0.59 m<sup>2</sup> per pig. Each pen contained a 2-space wet/dry box feeder mounted in the fence line that gave a trough space of 3.30 cm/pig and a cup-type water drinker located on the side of the pen.

Throughout the study period for both studies, the room temperature was maintained with thermostatically-controlled heaters and fan ventilation. The thermostat in all 3 rooms was set to a standard commercial temperature curve to provide a gradual reduction in room temperature as the pigs grew. The thermostat was set at 27.2°C on day 1 of pig placement and the temperature setting was decreased gradually over time until it reached 18.3°C by day 180 of the study period. Each room was equipped with water sprinklers located above each pen which were activated when the temperature of the rooms reached 27.2°C.

### **Growth Measurements**

For Study 1, pen BW was collected at the beginning of the study and every two weeks during the study period. All feed additions to the feeders were recorded using the computerized feed system and the feed remaining in the feeder at the time of pig weighing was measured. These measurements were used to calculate ADG, ADFI, and G:F.

For Study 2, pen BW was collected at the beginning of the study and every three weeks during the study period. All feed additions to the feeders were recorded and the feed remaining in the feeder at the time of pig weighing were recorded. These measurements were used to calculate ADG, ADFI, and G:F.

## **End of Test and Harvest**

For both studies, the timing of the end of the study and the shipping of the pigs for harvest was linked to the start of the feeding of the final dietary phase which contained ractopamine.

The start of the feeding of the final dietary phase was at a mean pen BW of 113.4 kg and 111.4 kg for Study 1 and 2, respectively. The timing of the removal of pigs from test and shipping for harvest was as follows;

Harvest Group 1: the heaviest 50% of the pen were removed and sent for harvest  $14 \pm 1$  days after the start of feeding of the final dietary phase.

Harvest Group 2: the remaining pigs in the pen were removed and sent for harvest  $28 \pm 1$  days after the start of feeding of the final dietary phase.

For both studies, on the day prior to shipping the pigs for harvest, the pen was weighed at which time all pigs to be sent for harvest received a slap tattoo on the left loin or the left ham with a unique number corresponding to their pen of origin. Pigs in the respective harvest group were considered off-test after the final pen weight was collected. They were held overnight at the farm in pens with access to feed and water and, on the following day, were transported on a standard trailer (with loads of ~ 165 pigs/each) to the JBS plant in Beardstown, IL.

## **Carcass Measurements**

Carcass measurements were taken on the slaughter line after harvest prior to the carcasses entering the chiller. Hot carcass weight and Fat-o-Meater measurements (backfat and *Longissimus* muscle depth at the 10<sup>th</sup> rib) were measured on each carcass.

## **Statistical Analysis**

All data were tested for normality and homogeneity of variance using the PROC UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Morbidity and mortality data were not normally distributed and were analyzed using a Chi-square rank-based test using the PROC FREQ procedure of SAS. Data meeting the criteria for normality were analyzed using the PROC MIXED procedure of SAS. For Study 1, data were analyzed as a randomized complete block design with the model accounting for the fixed effects of treatment and the random effect of block (day of start of test) and replicate. For Study 2, data were analyzed as a split-plot design with the model accounting for fixed effects of main plot treatment (additional experimental treatments that were independent of those used in the current study), zinc treatment, and main plot treatment  $\times$  zinc treatment interaction, and the random effects of block (date of start of test) and block  $\times$  main plot treatment. For both studies, pen was used as the experimental unit for all measurements. Least-squares means were compared using the PDIFF option of SAS.

### *Results and Discussion*

#### **Diet analysis:**

Calculated and analyzed composition for each dietary phase is presented in Tables 2 to 9. Diets were formulated to a constant standardized ileal digestible lysine:ME ratio within phase and to meet or exceed the nutrient requirements suggested by NRC (2012). Diets were formulated on a total zinc basis which included zinc supplemented from zinc oxide and zinc hydroxychloride for the study treatments, as well as, zinc from other dietary ingredients (Tables 10 and 11). Formulated and analyzed values for total zinc and copper (Study 1 only) levels for all treatments and dietary phases for Studies 1 and 2 are presented in Tables 12 and 13,



respectively. Differences between formulated and analyzed values for total dietary zinc content are most likely due to variation in diet manufacturing and/or to analytical variation. In general, formulated and analyzed values for zinc were relatively similar for both studies (Tables 12 and 13). The exception to this was for Trt. 4 in Study 1 for the Grow-Finish 5 phase where the analyzed values (50 ppm) were considerably below formulated values (193 ppm; Table 12). There is no obvious explanation for this discrepancy. Formulated and analyzed values for dietary zinc levels for Study 2 (Table 13) were also relatively similar. Likewise, formulated and analyzed values for total copper were relatively similar (Table 12). The differences between formulated and analyzed values should not influence the interpretation of the results.

**Effect of zinc source on growth performance, carcass characteristics, and morbidity and mortality:**

Results for the effect of zinc source on growth performance, carcass measurements, and morbidity and mortality for Study 1 and 2 are summarized in Tables 14 and 15, respectively.

In Study 1, there was no difference ( $P > 0.05$ ) in overall live weight ADG, ADFI, and G:F between pigs fed diets containing the same level of zinc from either zinc oxide and zinc hydroxychloride (assumed bioavailability of 65%) (Trt. 1 compared to Trt. 2; Table 14). This result is not surprising given that, as already discussed, the total dietary zinc concentrations for the diets for these two treatments were similar (Table 12).

The results relating to feeding the lower level of zinc hydroxychloride, which was based on an assumed bioavailability of 100%, were inconsistent across the two studies (Tables 14 and 15). In Study 1, the two treatments that included zinc hydroxychloride (Trt. 2 and 3) had similar ( $P > 0.05$ ) ADG (on both a live weight and carcass weight basis), and ADFI (Table 14);

however, live weight G:F was greater ( $P < 0.05$ ) for Trt. 3 than Trt. 2. Carcass G:F was numerically greater for Trt. 3 than Trt. 2 but this difference was not statistically significant ( $P > 0.05$ ; Table 14). Compared to Trt.1, pigs on Trt. 3 had greater ( $P < 0.05$ ) ADG, on a live weight, but not carcass weight basis, and had similar ( $P > 0.05$ ) ADFI and G:F (live weight and carcass weight) than those on Trt. 1. Collectively, the results from Study 1 suggest that the performance of pig fed diets with the lower level of zinc hydroxychloride (Trt. 3) was as good as those fed either zinc oxide (Trt. 1) or zinc hydroxychloride at the higher level (Trt. 2). There has been limited research carried out comparing the effect of feeding alternative zinc sources when included in diets at levels to meet animal requirements. There have been a number of studies comparing alternative forms of zinc such as zinc-amino acid complex, zinc-polysaccharide complex, and zinc-methionine when included in diets at pharmacological levels. Some studies have suggested that some of these alternative sources can result in similar growth performance when included at lower levels than zinc oxide (Ward et al., 1996; Case and Carlson, 2002). However, some studies reported that supplementing the diets of weaned pigs with zinc oxide (at 2,500 ppm) gave a greater growth response than including zinc-methionine at 500 ppm (Hahn and Baker, 1993; Hollis et al., 2005).

In contrast to the results of Study 1, pigs fed diets containing zinc hydroxychloride in Study 2 (Table 15) had lower ( $P < 0.05$ ) overall ADG, on both a live and carcass weight basis (1.021 vs 1.002 and 0.821 vs. 0.780, for Trt. 1 and 2, respectively), and ADFI, (2.62 vs. 2.59 for Trt. 1 and 2, respectively), but similar ( $P > 0.05$ ) G:F compared to those fed diets containing zinc oxide (Table 15). In Study 2, the assumed bioavailability of zinc hydroxychloride was 100%, therefore, the level of total zinc in the diets for that treatment was similar to that of Trt. 3 in Study 1. This is illustrated by comparing the zinc levels in the results for the diet analysis

(Tables 12 and 13) which were similar for the comparable treatments on the two studies. There is limited published research relating to zinc hydroxychloride. Only two published studies evaluated the effect of feeding zinc hydroxychloride to grow-finish pigs at levels to meet animal requirements. Neither of these studies included a control treatment based on zinc oxide to allow comparison of the efficacy of the two zinc sources (Carpenter et al., 2016; Cemin et al., 2018).

Therefore, the results of Study 1 indicate that the bioavailability of zinc in zinc hydroxychloride could be greater than that of zinc in zinc oxide, and may be close to 100%, whereas the results of Study 2 indicate that the bioavailability of zinc in zinc hydroxychloride is less than 100%. There has been limited published research on zinc hydroxychloride so it is not possible to compare our results directly with others. Other zinc sources with high bioavailability have not always produced an improvement in growth performance compared to sources with lower bioavailability. For example, zinc sulfate has a bioavailability of 100% compared to that of zinc oxide of 65% (NRC, 2012). However, the literature reviewed as part of this thesis (Chapter 1) showed that only 1 out of 5 publications reported an improvement in growth performance for zinc sulfate compared to zinc oxide (Perez-Mendoza, 2008). This highlights the fact that bioavailability is not a good indicator of the efficacy of zinc sources. There have been no published studies that have directly measured the bioavailability of zinc in zinc hydroxychloride. In contrast, a substantial number of studies have measured the bioavailability of zinc in zinc oxide (NRC, 2012) and, therefore, confidence in the NRC (2012) estimate of bioavailability is high. It is important to carry out research to establish the bioavailability of mineral sources to be used in swine diets.

There was no effect of zinc source on carcass measurements in either study (Tables 14 and 15). In general, most research evaluating the effect of including zinc supplements in diets

for growing pigs, even at pharmacological levels, has shown little if any effect on carcass measurements (Carpenter et al., 2016; Cemin et al., 2018). Therefore, the results of the present study that showed that including zinc sources in diets for growing pigs at levels that, in general, should have been adequate to meet the animal's requirement had no effect on carcass measurements are similar to previous research.

There was no effect ( $P > 0.05$ ) of zinc source on morbidity and mortality in either study. Feeding pharmacological levels of zinc can reduce morbidity and mortality, particularly in newly-weaned pigs (Tokach et al., 2000; Cromwell, 2002). However, the levels of zinc used in the current study were to meet requirements and no negative control treatment with a zinc-deficient diet was used. Consequently, it is not surprising that feeding diets that should have had adequate levels of zinc to meet requirements did not impact morbidity and mortality levels.

### **Effects of copper supplementation on growth performance, carcass characteristics, and morbidity and mortality:**

Study 1 included 3 treatments (Trt. 1, 2, and 3) that used diets with pharmacological levels of copper (150 ppm) supplied from tribasic copper chloride and 1 treatment that contained no supplemental copper (Trt. 4), and these results are presented in Table 15. The important comparison is Trt. 4 with Trt. 1 as both of these treatments had the same level of zinc oxide but different levels of copper. There was no effect ( $P > 0.05$ ) of adding supplemental copper to the diet (Trt. 1) on ADG (live or carcass weight) or ADFI compared to the treatment with no added copper (Trt. 4; Table 15). However, including 150 compared to 0 ppm of added copper (Trt. 1 vs. Trt 4) increased ( $P < 0.05$ ) G:F when measured on a carcass weight basis (0.272 vs. 0.277, respectively). Live weight G:F, carcass ADG, and carcass yield were numerically greater for

Trt. 1 compared to Trt. 4, however, these treatment differences were not statistically significant ( $P > 0.05$ ).

A number of studies that were discussed in the literature review (Chapter 1) showed improvements in ADG and ADFI from feeding pharmacological levels of copper. For example, (Coble et al., 2017) in a study with growing-finishing pigs, found a linear improvements in live weight ADG, ADFI, and body weight at the end of the test period with increasing dietary copper levels compared to the unsupplemented control treatment. However, the results of the current study are in agreement with those of Carpenter et al. (2017) who found no improvement in growth performance from feeding supplemental copper to grow-finish swine. Additionally, Hastad et al. (2001) reported that supplementing diets in late finishing with copper did not improve growth performance. In general, the largest relative effect of feeding high levels of copper on growth performance has been observed in newly-weaned pigs (Braude, 1967; Cromwell et al., 1998; NRC, 2012) where higher levels (typically of 250 ppm) have generally been used.

In Study 1, there was no effect of adding 150 ppm of supplemental copper to the diet on carcass characteristics (Trt. 1 vs Trt. 4; Table 14). This is in contrast to the results of Coble et al. (2013), who suggested that increasing the level of supplementary copper in the diets of grow-finish swine linearly increased hot carcass weight, loin depth, and carcass lean content. However, these results are in contrast to results from other studies that have shown no effect of feeding high levels of copper on carcass measurements (Carpenter et al., 2017; Coble et al., 2017).

There was no effect ( $P > 0.05$ ) of copper supplementation on morbidity and mortality (Table 15). Most studies that have reported on the effects of feeding high levels of copper on

mortality levels have been carried out with newly-weaned pigs. It has been reported that high levels (250 ppm) of copper supplemented in the diet of weaned pigs can reduce the incidence of mortality by 6.5% (Stahly et al., 1980). There is evidence suggesting that copper can have antimicrobial properties when supplemented in pharmacological levels to weaned pig diets, improving conditions in the gut (Cromwell, 2002; Perez-Mendoza, 2008). Huang et al. (2015) suggested that feeding supplemental copper to weaned pigs reduces oxidative stress in the duodenum, which in turn can improve digestion and ultimately utilization of nutrients, however, research in this area with grow-finish swine is limited.

### *Conclusions*

These two studies that compared the effects of zinc oxide and zinc hydroxychloride on growth performance of growing-finishing pigs produced inconsistent results with one suggesting comparable or small improvements in performance for zinc hydroxychloride and the other study suggesting the opposite. The current research also suggests small improvements in growth performance from feeding high levels of copper to growing-finishing pigs. Further research is needed to clearly establish the advantage, if any, of replacing zinc oxide with zinc hydroxychloride and of including high levels of copper as tribasic copper chloride in diets for growing-finishing pigs.

## TABLES

**Table 2.** Study 1: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow-Finish Phase 1.

Item	Dietary Treatments <sup>1,2,3</sup>							
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
Zinc Source (assumed bioavailability)	Zinc Oxide (65%)		Zinc Hydroxychloride (65%)		Zinc Hydroxychloride (100%)		Zinc Oxide (65%)	
Supplemental Copper (150 ppm)			Tribasic Copper Chloride				No Supplemental Copper	
Ingredient, %								
Corn, fine	55.82		55.85		55.81		55.67	
DDGS	26.48		26.44		26.50		26.69	
Soybean Meal	14.55		14.55		14.54		14.51	
Limestone	1.37		1.37		1.37		1.37	
Water	0.50		0.50		0.50		0.50	
L-Lysine HCl (98%)	0.40		0.40		0.40		0.40	
Fat, Yellow Grease	0.35		0.35		0.35		0.35	
Salt	0.33		0.33		0.33		0.33	
Trace Mineral Premix <sup>4</sup>	0.08		0.08		0.08		0.08	
Vitamin Premix	0.03		0.03		0.03		0.03	
Tribasic Copper Chloride (58%)	0.03		0.03		0.03		-	
L-Threonine (98%)	0.02		0.02		0.02		0.02	
Zinc Hydroxychloride (55%)	-		0.02		0.02		-	
Zinc Oxide (72%)	0.02		-		-		0.02	
Growth Promotant <sup>5</sup>	0.02		0.02		0.02		0.02	
Phytase 2500	0.01		0.01		0.01		0.01	
HMTBa (88%)	0.01		0.01		0.01		0.01	
Ingredient Total:	100.00		100.00		100.00		100.00	
	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>
ME, Kcal/kg	3229.09	-	3229.09	-	3229.11	-	3229.11	-
SID Lys:ME, g/Mcal	2.86	-	2.86	-	2.86	-	2.86	-
Crude Protein, %	18.00	17.90	17.99	18.60	18.00	18.50	18.02	17.50
Moisture, %	13.52	12.40	13.52	14.17	13.52	13.69	13.51	13.75
Crude Fat, %	4.11	4.38	4.11	4.62	4.11	4.43	4.12	4.70
Crude Fiber, %	2.81	3.46	2.81	2.68	2.81	2.45	2.82	2.81
ADF, %	4.98	5.20	4.97	6.10	4.98	5.30	5.00	5.10

**Table 2 (Cont.)**

NDF, %	12.53	12.40	12.52	11.90	12.54	12.60	12.58	12.10
Ash, %	3.87	4.46	3.87	4.27	3.87	4.33	3.88	4.18
Phosphorus, %	0.49	0.50	0.49	0.52	0.49	0.52	0.50	0.51
Phosphorus, Available, %	0.26	-	0.26	-	0.26	-	0.25	-
Calcium, %	0.60	0.59	0.60	0.68	0.60	0.58	0.60	0.62
Sodium, %	0.20	0.20	0.20	0.22	0.20	0.22	0.20	0.22
Chloride, %	0.36	0.36	0.37	0.41	0.36	0.37	0.36	0.36
Magnesium, %	0.22	0.17	0.22	0.18	0.22	0.18	0.22	0.18
Potassium, %	0.80	0.81	0.80	0.88	0.80	0.84	0.80	0.81
Copper, ppm	169.93	178.00	169.93	145.00	169.93	194.00	19.14	29.60
Zinc, ppm	159.21	168.00	156.94	182.00	115.72	136.00	159.29	155.00
Iodine, ppm	0.22	-	0.22	-	0.22	-	0.22	-
Iron, ppm	231.50	283.00	231.49	246.00	231.51	276.00	231.57	281.00
Manganese, ppm	44.42	55.90	44.42	62.30	44.42	51.60	44.43	49.50
Lysine, Total, %	1.09	-	1.09	-	1.09	-	1.10	-
Lysine, SID, %	0.92	-	0.92	-	0.92	-	0.92	-
Isoleucine, Total, %	0.71	-	0.71	-	0.71	-	0.71	-
Isoleucine, SID, %	0.59	-	0.59	-	0.59	-	0.59	-
Leucine, Total, %	1.78	-	1.78	-	1.78	-	1.78	-
Leucine, SID, %	1.52	-	1.52	-	1.52	-	1.53	-
Met + Cys, Total, %	0.64	-	0.64	-	0.64	-	0.64	-
Met + Cys, SID, %	0.53	-	0.53	-	0.53	-	0.53	-
Threonine, Total, %	0.70	-	0.70	-	0.70	-	0.70	-
Threonine, SID, %	0.55	-	0.55	-	0.55	-	0.55	-
Tryptophan, Total, %	0.18	-	0.18	-	0.18	-	0.18	-
Tryptophan, SID, %	0.15	-	0.15	-	0.15	-	0.15	-
Valine, Total, %	0.85	-	0.85	-	0.85	-	0.85	-
Valine, SID, %	0.69	-	0.69	-	0.69	-	0.69	-

<sup>1</sup>Zinc oxide was used as the zinc source in Treatments 1 and 4 (average bioavailability of 65%).

<sup>2</sup>Treatments 2 and 3 contained zinc hydroxychloride used to provide 65% and 100% bioavailable zinc.

<sup>3</sup>Treatments 1-3 included 150 ppm of tribasic copper chloride (average bioavailability of 100%; Ammerman et al., 1995)

<sup>4</sup>The trace mineral premix was devoid of zinc, thus all zinc in the diet was provided by treatment zinc sources as well as other ingredients in the diet.

<sup>5</sup>A common industry growth promotant was added as a standard production practice of the participating production company.

<sup>6</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.



**Table 3.** Study1: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow-Finish Phase 2.

Item	Dietary Treatments <sup>1,2,3</sup>							
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Zinc Oxide (65%)		Zinc Hydroxychloride (65%)		Zinc Hydroxychloride (100%)		Zinc Oxide (65%)	
Zinc Source (assumed bioavailability)								
Supplemental Copper (150 ppm)			Tribasic Copper Chloride				No Supplemental Copper	
Ingredient Name								
Corn, fine	62.42		62.42		62.42		62.41	
DDGS	17.12		17.14		17.11		17.02	
Soybean Meal	11.78		11.78		11.78		11.77	
Corn germ meal	5.62		5.59		5.64		5.78	
Limestone	1.27		1.27		1.27		1.27	
Water	0.50		0.50		0.50		0.50	
Salt	0.38		0.38		0.38		0.38	
L-Lysine HCl (98%)	0.35		0.35		0.35		0.35	
Fat, Yellow Grease	0.35		0.35		0.35		0.35	
Trace Mineral Premix <sup>4</sup>	0.08		0.08		0.08		0.08	
L-Threonine (98%)	0.04		0.04		0.04		0.04	
Vitamin Premix	0.03		0.03		0.03		0.03	
Tribasic Copper chloride (58%)	0.03		0.03		0.03		-	
Zinc oxide (72%)	0.02		-		-		0.02	
Growth Promotant <sup>5</sup>	0.02		0.02		0.02		0.02	
Phytase 2500	0.02		0.02		0.02		0.02	
Zinc hydroxychloride (55%)	-		0.02		0.02		-	
	100.00		100.00		100.00		100.00	
	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>
ME, Kcal/kg	3226.99	-	3227.02	-	3226.99	-	3226.95	-
SID Lys:ME, g/Mcal	2.51	-	2.51	-	2.51	-	2.51	-
Crude Protein, %	16.03	14.90	16.03	16.80	16.03	14.60	16.03	15.70
Moisture, %	13.77	14.15	13.77	14.28	13.77	14.06	13.77	14.20
Crude Fat, %	3.70	4.16	3.70	4.09	3.70	4.96	3.69	4.22
Crude Fiber, %	2.64	1.20	2.64	2.20	2.64	2.83	2.65	2.10
ADF, %	4.56	4.30	4.55	4.90	4.56	4.70	4.56	4.20
NDF, %	12.10	11.10	12.09	12.00	12.10	13.60	12.12	12.70

**Table 3 (Cont.)**

Ash, %	3.42	3.49	3.42	3.74	3.42	3.89	3.42	3.74
Phosphorus, %	0.45	0.44	0.45	0.45	0.45	0.45	0.45	0.44
Phosphorus, Available, %	0.22	-	0.22	-	0.22	-	0.22	-
Calcium, %	0.55	0.58	0.55	0.56	0.55	0.69	0.55	0.57
Sodium, %	0.20	0.19	0.20	0.19	0.20	0.18	0.20	0.17
Chloride, %	0.36	0.35	0.37	0.36	0.37	0.33	0.36	0.32
Magnesium, %	0.20	0.15	0.20	0.15	0.20	0.15	0.20	0.14
Potassium, %	0.68	0.67	0.68	0.72	0.68	0.68	0.68	0.66
Copper, ppm	169.41	146.00	169.41	152.00	169.41	159.00	18.61	23.70
Zinc, ppm	158.58	169.00	156.31	172.00	115.09	142.00	158.66	120.00
Iodine, ppm	0.22	-	0.22	-	0.22	-	0.22	-
Iron, ppm	223.89	258.00	223.87	241.00	223.89	252.00	223.96	203.00
Manganese, ppm	42.82	56.00	42.82	49.20	42.82	50.10	42.82	40.70
Lysine, Total, %	0.96	-	0.96	-	0.96	-	0.96	-
Lysine, SID, %	0.81	-	0.81	-	0.81	-	0.81	-
Isoleucine, Total, %	0.62	-	0.62	-	0.62	-	0.62	-
Isoleucine, SID, %	0.52	-	0.52	-	0.52	-	0.52	-
Leucine, Total, %	1.56	-	1.56	-	1.56	-	1.56	-
Leucine, SID, %	1.33	-	1.33	-	1.33	-	1.33	-
Met + Cys, Total, %	0.28	-	0.28	-	0.28	-	0.28	-
Met + Cys, SID, %	0.46	-	0.46	-	0.46	-	0.46	-
Threonine, Total, %	0.63	-	0.63	-	0.63	-	0.63	-
Threonine, SID, %	0.50	-	0.50	-	0.50	-	0.50	-
Tryptophan, Total, %	0.16	-	0.16	-	0.16	-	0.16	-
Tryptophan, SID, %	0.13	-	0.13	-	0.13	-	0.13	-
Valine, Total, %	0.76	-	0.76	-	0.76	-	0.76	-
Valine, SID, %	0.62	-	0.62	-	0.62	-	0.62	-

<sup>1</sup>Zinc oxide was used as the zinc source in Treatments 1 and 4 (average bioavailability of 65%).

<sup>2</sup>Treatments 2 and 3 contained zinc hydroxychloride used to provide 65% and 100% bioavailable zinc.

<sup>3</sup>Treatments 1-3 included 150 ppm of tribasic copper chloride (average bioavailability of 100%; Ammerman et al., 1995).

<sup>4</sup>The trace mineral premix was devoid of zinc, thus all zinc in the diet was provided by treatment zinc sources as well as other ingredients in the diet.

<sup>5</sup>A common industry growth promotant was added as a standard production practice of the participating production company.

<sup>6</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 4.** Study 1: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow-Finish Phase 3.

Item	Dietary Treatments <sup>1,2,3</sup>							
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Zinc Source (assumed bioavailability)	Zinc Oxide (65%)	Zinc Hydroxychloride (65%)	Zinc Hydroxychloride (100%)	Zinc Hydroxychloride (100%)	Zinc Oxide (65%)	No Supplemental Copper	
Supplemental Copper (150 ppm)			Tribasic Copper Chloride				No Supplemental Copper	
Ingredient Name								
Corn, fine	68.75		68.76		68.74		68.69	
Corn germ meal	17.11		17.09		17.12		17.21	
Soybean Meal	10.98		10.99		10.98		10.96	
Limestone	1.02		1.02		1.02		1.02	
Fat, Yellow Grease	0.70		0.70		0.70		0.70	
Water	0.50		0.50		0.50		0.50	
Salt	0.46		0.46		0.46		0.46	
Lysine dry (98%)	0.25		0.25		0.25		0.25	
Trace Mineral Premix <sup>4</sup>	0.08		0.08		0.08		0.08	
Phytase 2500	0.04		0.04		0.04		0.04	
Vitamin premix	0.03		0.03		0.03		0.03	
Threonine (98%)	0.03		0.03		0.03		0.03	
Tribasic copper chloride (58%)	0.03		0.03		0.03		0.03	
Zinc oxide (72%)	0.02		-		-		0.02	
Growth Promotant <sup>5</sup>	0.02		0.02		0.02		0.02	
HMTBa (88%)	0.01		0.01		0.01		0.01	
Zinc hydroxychloride (55%)	-		0.02		0.02		-	
	100.00		100.00		100.00		100.00	
	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>
ME, Kcal/kg	3223.75	-	3223.75	-	3223.75	-	3223.73	-
SID Lys:ME, g/Mcal	2.17	-	2.17	-	2.17	-	2.17	-
Crude Protein, %	14.26	15.00	14.26	14.80	14.26	14.20	14.27	15.2
Moisture, %	14.12	14.11	14.12	13.80	14.12	13.99	14.12	13.64
Crude Fat, %	3.21	3.92	3.21	4.24	3.21	3.98	3.21	3.78
Crude Fiber, %	2.47	1.49	2.47	2.37	2.47	1.98	2.48	2.42
ADF, %	4.10	4.30	4.10	4.50	4.10	4.50	4.11	3.9
NDF, %	11.73	11.40	11.73	11.80	11.74	11.20	11.77	11.3

**Table 4 (Cont.)**

Ash, %	2.75	3.20	2.75	3.18	2.75	3.74	2.76	3.71
Phosphorus, %	0.39	0.39	0.39	0.41	0.39	0.41	0.39	0.42
Phosphorus, Available, %	0.18	-	0.18	-	0.18	-	0.18	-
Calcium, %	0.45	0.52	0.45	0.49	0.45	0.50	0.45	0.55
Sodium, %	0.20	0.20	0.20	0.17	0.20	0.18	0.20	0.17
Chloride, %	0.37	0.37	0.37	0.35	0.37	0.36	0.36	0.34
Magnesium, %	0.17	0.13	0.17	0.14	0.17	0.13	0.18	0.14
Potassium, %	0.54	0.57	0.54	0.59	0.54	0.58	0.54	0.63
Copper, ppm	169.18	186.00	169.18	179.00	169.18	184.00	18.39	71.7
Zinc, ppm	159.96	170.00	157.70	180.00	116.47	161.00	160.04	182.00
Iodine, ppm	0.22	-	0.22	-	0.22	-	0.22	-
Iron, ppm	215.59	289.00	215.58	232.00	215.60	260.00	215.66	276
Manganese, ppm	41.21	51.70	41.21	45.00	41.21	49.20	41.22	51.1
Lysine, Total, %	0.82	-	0.82	-	0.82	-	0.82	-
Lysine, Dig, %	0.70	-	0.70	-	0.70	-	0.70	-
Isoleucine, Total, %	0.55	-	0.55	-	0.55	-	0.55	-
Isoleucine, SID, %	0.46	-	0.46	-	0.46	-	0.46	-
Leucine, Total, %	1.29	-	1.29	-	1.29	-	1.29	-
Leucine, SID, %	1.10	-	1.10	-	1.10	-	1.10	-
Met + Cys, Total, %	0.49	-	0.49	-	0.49	-	0.49	-
Met + Cys, SID, %	0.40	-	0.40	-	0.40	-	0.40	-
Threonine, Total, %	0.15	-	0.15	-	0.15	-	0.15	-
Threonine, SID, %	0.44	-	0.44	-	0.44	-	0.44	-
Tryptophan, Total, %	0.15	-	0.15	-	0.15	-	0.15	-
Tryptophan, SID, %	0.13	-	0.13	-	0.13	-	0.13	-
Valine, Total, %	0.69	-	0.69	-	0.69	-	0.69	-
Valine, SID, %	0.56	-	0.56	-	0.56	-	0.56	-

<sup>1</sup>Zinc oxide was used as the zinc source in Treatments 1 and 4 (average bioavailability of 65%).

<sup>2</sup>Treatments 2 and 3 contained zinc hydroxychloride used to provide 65% and 100% bioavailable zinc.

<sup>3</sup>Treatments 1-3 included 150 ppm of tribasic copper chloride (average bioavailability of 100%; Ammerman et al., 1995).

<sup>4</sup>The trace mineral premix was devoid of zinc, thus all zinc in the diet was provided by treatment zinc sources as well as other ingredients in the diet.

<sup>5</sup>A common industry growth promotant was added as a standard production practice of the participating production company.

<sup>6</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 5.** Study1: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow-Finish Phase 4.

Item	Dietary Treatments <sup>1,2,3</sup>							
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Zinc Source (assumed bioavailability)	Zinc Oxide (65%)	Zinc Hydroxychloride (65%)	Zinc Hydroxychloride (100%)	Zinc Hydroxychloride (100%)	Zinc Oxide (65%)	Supplemental Copper (150 ppm)	
			Tribasic Copper Chloride			No Supplemental Copper		
Ingredient, %								
Corn, fine	74.51		74.51		74.52		74.54	
Corn germ meal	11.84		11.84		11.83		11.83	
Soybean Meal	10.35		10.35		10.35		10.36	
Limestone	1.00		1.00		1.00		1.00	
Fat, Yellow Grease	0.70		0.70		0.70		0.70	
Water	0.50		0.50		0.50		0.50	
Salt	0.50		0.50		0.50		0.50	
L-Lysine HCl (98%)	0.23		0.23		0.23		0.23	
Phytogenic Feed Additive <sup>4</sup>	0.10		0.10		0.10		0.10	
Monocalcium Phosphate (21%)	0.07		0.07		0.07		0.07	
Trace Mineral Premix <sup>5</sup>	0.06		0.06		0.06		0.06	
Phytase 2500	0.04		0.04		0.04		0.04	
L-Threonine (98%)	0.03		0.03		0.03		0.03	
Tribasic Copper Chloride (58%)	0.03		0.03		0.03		-	
Vitamin Premix	0.02		0.02		0.02		0.02	
Zinc oxide (72%)	0.02		-		-		0.02	
Growth Promotant <sup>6</sup>	0.02		0.02		0.02		0.02	
Zinc hydroxychloride (55%)	-		0.02		0.01		-	
	100.00		100.00		100.00		100.00	
	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>
ME, Kcal/kg	3266.28	-	3266.08	-	3266.37	-	3267.21	-
SID Lys:ME, g/Mcal	1.96	-	1.96	-	1.96	-	1.96	-
Crude Protein, %	13.13	13.6	13.13	13.3	13.13	13.1	13.14	13.6
Moisture, %	14.22	14.57	14.22	14.13	14.22	13.86	14.22	13.85
Crude Fat, %	3.23	3.77	3.23	3.62	3.23	3.70	3.24	3.69
Crude Fiber, %	2.11	1.55	2.11	1.53	2.11	2.14	2.11	1.78
ADF, %	3.51	4.10	3.51	3.50	3.51	3.70	3.51	4.00

**Table 5 (Cont.)**

NDF, %	10.09	10.70	10.09	10.90	10.09	10.70	10.09	10.40
Ash, %	2.69	3.60	2.69	3.71	2.69	3.53	2.69	3.37
Phosphorus, %	0.37	0.40	0.37	0.38	0.37	0.39	0.37	0.38
Phosphorus, Available, %	0.18	-	0.18	-	0.18	-	0.18	-
Calcium, %	0.45	0.49	0.45	0.53	0.45	0.52	0.45	0.51
Sodium, %	0.22	0.21	0.22	0.20	0.22	0.19	0.22	0.20
Chloride, %	0.40	0.39	0.41	0.38	0.41	0.37	0.40	0.42
Magnesium, %	0.17	0.13	0.17	0.12	0.17	0.12	0.17	0.12
Potassium, %	0.52	0.57	0.52	0.55	0.52	0.55	0.52	0.54
Copper, %	166.77	176.00	166.77	181.00	166.77	189.00	15.97	29.60
Zinc, %	137.49	145.00	139.49	162.00	98.24	118.00	137.49	145.00
Iodine, %	0.19	-	0.19	-	0.19	-	0.19	-
Iron, %	187.74	233.00	187.74	225.00	187.74	230.00	187.75	233.00
Manganese, %	35.51	51.60	35.51	48.60	35.51	49.00	35.51	43.10
Lysine, Total, %	0.75	-	0.75	-	0.75	-	0.75	-
Lysine, SID, %	0.64	-	0.64	-	0.64	-	0.64	-
Isoleucine, Total, %	0.51	-	0.51	-	0.51	-	0.51	-
Isoleucine, SID, %	0.42	-	0.42	-	0.42	-	0.42	-
Leucine, Total, %	1.22	-	1.22	-	1.22	-	1.22	-
Leucine, SID, %	1.05	-	1.05	-	1.05	-	1.05	-
Met + Cys, Total, %	0.45	-	0.45	-	0.45	-	0.45	-
Met + Cys, SID, %	0.37	-	0.37	-	0.37	-	0.37	-
Threonine, Total, %	0.51	-	0.51	-	0.51	-	0.52	-
Threonine, SID, %	0.41	-	0.41	-	0.41	-	0.41	-
Tryptophan, Total, %	0.14	-	0.14	-	0.14	-	0.14	-
Tryptophan, SID, %	0.12	-	0.12	-	0.12	-	0.12	-
Valine, Total, %	0.63	-	0.63	-	0.63	-	0.63	-
Valine, SID, %	0.52	-	0.52	-	0.52	-	0.52	-

<sup>1</sup>Zinc oxide was used as the zinc source in Treatments 1 and 4 (average bioavailability of 65%).

<sup>2</sup>Treatments 2 and 3 contained zinc hydroxychloride used to provide 65% and 100% bioavailable zinc.

<sup>3</sup>Treatments 1-3 included 150 ppm of tribasic copper chloride (average bioavailability of 100%; Ammerman et al., 1995).

<sup>4</sup>A phytogetic feed additive was added to the diet in Grow-Finish phase 4 as a standard production practice of the participating production company.

<sup>5</sup>The trace mineral premix was devoid of zinc, thus all zinc in the diet was provided by treatment zinc sources as well as other ingredients in the diet.

<sup>6</sup>A common industry growth promotant was added as a standard production practice of the participating production company.

<sup>7</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 6.** Study 1: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow-Finish Phase 5.

Item	Dietary Treatments <sup>1,2,3</sup>							
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Zinc Oxide (65%)		Zinc Hydroxychloride (65%)		Zinc Hydroxychloride (100%)		Zinc Oxide (65%)	
Zinc Source (assumed bioavailability)								
Supplemental Copper (150 ppm)			Tribasic Copper Chloride				No Supplemental Copper	
Ingredient, %								
Corn, fine	66.48		66.48		66.49		66.50	
Soybean Meal	19.42		19.42		19.42		19.43	
Corn germ meal	10.00		10.00		10.00		10.00	
Fat, Yellow Grease	1.20		1.20		1.20		1.20	
Limestone	1.02		1.02		1.02		1.03	
Water	0.50		0.50		0.50		0.50	
Salt	0.50		0.50		0.50		0.50	
L-Lysine HCl (98%)	0.30		0.30		0.30		0.30	
Phytogenic Feed Additive <sup>4</sup>	0.10		0.10		0.10		0.10	
Monocalcium phospahte (21%)	0.10		0.10		0.10		0.10	
L-Threonine (98%)	0.09		0.09		0.09		0.09	
HMTBa (88%)	0.08		0.08		0.08		0.08	
Trace Mineral Premix <sup>5</sup>	0.06		0.06		0.06		0.06	
Phytase 2500	0.04		0.04		0.04		0.04	
Ractopamine Hydrochloride (19.8 g/kg) <sup>6</sup>	0.04		0.04		0.04		0.04	
Tribasic Copper Chloride (58%)	0.03		0.03		0.03		-	
Zinc oxide (72%)	0.02		-		-		0.02	
Zinc hydroxychloride (55%)	-		0.03		0.02		-	
Vitamin Premix	0.02		0.02		0.02		0.02	
	100.00		100.00		100.00		100.00	
Composition	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>
ME, Kcal/kg	3291.99	-	3291.72	-	3292.07	-	3292.87	-
SID Lys:ME, g/Mcal	2.77	-	2.77	-	2.77	-	2.77	-
Crude Protein, %	16.54	16.30	16.53	16.00	16.54	16.60	16.54	16.80
Moisture, %	14.05	13.63	14.05	13.36	14.05	13.42	14.05	14.16
Crude Fat, %	3.58	4.06	3.58	4.59	3.58	4.10	3.58	3.90
Crude Fiber, %	2.10	2.28	2.10	1.06	2.10	2.17	2.10	1.35
ADF, %	3.71	3.60	3.71	3.80	3.71	3.60	3.71	3.40

**Table 6 (Cont.)**

NDF, %	9.65	9.60	9.65	9.90	9.65	9.70	9.65	9.70
Ash, %	3.14	4.11	3.14	4.00	3.14	4.20	3.15	3.79
Phosphorus, %	0.41	0.42	0.41	0.40	0.41	0.40	0.41	0.41
Phosphorus, Available, %	0.20	-	0.20	-	0.20	-	0.20	-
Calcium, %	0.50	0.53	0.50	0.60	0.50	0.50	0.50	0.53
Sodium, %	0.22	0.22	0.22	0.19	0.22	0.22	0.22	0.20
Chloride, %	0.40	0.43	0.41	0.40	0.41	0.37	0.40	0.40
Magnesium, %	0.18	0.14	0.18	0.14	0.18	0.14	0.18	0.14
Potassium, %	0.68	0.71	0.68	0.66	0.68	0.67	0.68	0.71
Copper, ppm	168.17	163.00	168.17	165.00	168.17	175.00	17.37	20.20
Zinc, ppm	193.39	189.00	196.39	212.00	141.39	179.00	193.40	50.10
Iodine, ppm	0.19	-	0.19	-	0.19	-	0.19	-
Iron, ppm	200.98	243.00	200.97	229.00	200.98	240.00	201.00	138.00
Manganese, ppm	38.23	43.40	38.23	44.80	38.23	45.30	38.24	30.10
Lysine, Total, %	1.04	-	1.04	-	1.04	-	1.04	-
Lysine, SID, %	0.91	-	0.91	-	0.91	-	0.91	-
Isoleucine, Total, %	0.66	-	0.66	-	0.66	-	0.66	-
Isoleucine, SID, %	0.57	-	0.57	-	0.57	-	0.57	-
Leucine, Total, %	1.45	-	1.45	-	1.45	-	1.45	-
Leucine, SID, %	1.25	-	1.25	-	1.25	-	1.25	-
Met + Cys, Total, %	0.61	-	0.61	-	0.61	-	0.61	-
Met + Cys, SID, %	0.52	-	0.52	-	0.52	-	0.52	-
Threonine, Total, %	0.70	-	0.70	-	0.70	-	0.70	-
Threonine, SID, %	0.58	-	0.58	-	0.58	-	0.58	-
Tryptophan, Total, %	0.19	-	0.19	-	0.19	-	0.19	-
Tryptophan, SID, %	0.16	-	0.16	-	0.16	-	0.16	-
Valine, Total, %	0.78	-	0.78	-	0.78	-	0.78	-
Valine, SID, %	0.65	-	0.65	-	0.65	-	0.65	-

<sup>1</sup>Zinc oxide was used as the zinc source in Treatments 1 and 4: average bioavailability of 65% (50 to 80%, NRC, 2012).

<sup>2</sup>Treatments 2 and 3 contained zinc hydroxychloride used to provide 65% and 100% bioavailable zinc.

<sup>3</sup>Treatments 1-3 included 150 ppm of tribasic copper chloride (average bioavailability of 100%)

<sup>4</sup>A phytogetic feed additive was added to the diet in Grow-Finish phase 4 as a standard production practice of the participating production company.

<sup>5</sup>The trace mineral premix was devoid of zinc, thus all zinc in the diet was provided by treatment zinc sources as well as other ingredients in the diet.

<sup>6</sup>Ractopamine Hydrochloride was added to all treatments in Grow-Finish Phase 5 for 28 days.

<sup>7</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.



**Table 7.** Study 2: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow-Finish Phase 1.

	Dietary Treatments <sup>1</sup>											
	Treatment 1 A <sup>2</sup>		Treatment 2 A <sup>3</sup>		Treatment 1 B <sup>2</sup>		Treatment 2 B <sup>3</sup>		Treatment 1 C <sup>2</sup>		Treatment 2 C <sup>3</sup>	
Growth Promotant <sup>4</sup>	Growth Promotant		Growth Promotant		Growth Promotant		Growth Promotant		Growth Promotant		Growth Promotant	
Zinc Source (assumed bioavailability)	Zinc Oxide (65%)		Zinc Hydroxy-chloride (100%)		Zinc Oxide (65%)		Zinc Hydroxy-chloride (100%)		Zinc Oxide (65%)		Zinc Hydroxy-chloride -100%	
Phytogenic Additive <sup>5</sup>	None		None		Additive		Additive		None		None	
Item												
Ingredient, %												
Corn, Fine	62.37		62.31		62.30		62.30		62.37		62.31	
Bakery Energy Blend	14.00		14.00		14.00		14.00		14.00		14.00	
Soybean Meal,	12.79		12.76		12.75		12.76		12.79		12.76	
DDGS	7.50		7.50		7.50		7.50		7.50		7.50	
Limestone	1.12		1.12		1.12		1.12		1.12		1.12	
Water	0.50		0.50		0.50		0.50		0.50		0.50	
Salt	0.40		0.50		0.50		0.50		0.40		0.50	
Fat, Yellow Grease	0.35		0.35		0.35		0.35		0.35		0.35	
L-Lysine HCl (98%)	0.35		0.35		0.35		0.35		0.35		0.35	
Monocalcium Phosphate (21%)	0.30		0.30		0.30		0.30		0.30		0.30	
Trace Mineral Premix (ZnO)	0.08		-		0.08		-		0.08		-	
Trace Mineral Premix (IBZ)	-		0.08		-		0.08		-		0.08	
L-Threonine (98%)	0.07		0.07		0.07		0.07		0.07		0.07	
Mycotoxin Binder	0.05		0.05		0.05		0.05		0.05		0.05	
HMTBa (88%)	0.04		0.04		0.04		0.04		0.04		0.04	
Tribasic Copper Chloride (54%)	0.03		0.03		0.03		0.03		0.03		0.03	
Vitamin Premix	0.03		0.03		0.03		0.03		0.03		0.03	
Phytase 2500	0.01		0.01		0.01		0.01		0.01		0.01	
Growth Promotant	0.02		0.02		0.02		0.02		0.02		0.02	
Phytogenic Feed Additive	-		-		0.02		0.02		-		-	
Ingredient Total:	100.00		100.00		100.00		100.00		100.00		100.00	
Composition	Calculated	Analyzed <sup>5</sup>	Calculated	Analyzed <sup>5</sup>	Calculated	Analyzed <sup>5</sup>	Calculated	Analyzed <sup>5</sup>	Calculated	Analyzed <sup>5</sup>	Calculated	Analyzed <sup>5</sup>
ME, Kcal/kg	3262.86	-	3259.16	-	3258.45	-	3258.59	-	3262.86	-	3259.16	-
SID Lys:ME, g/Mcal	2.51	-	2.51	-	2.51	-	2.51	-	2.51	-	2.51	-

**Table 7 (Cont.)**

Crude Protein, %	15.48	16.10	15.46	16.10	16.60	16.60	15.46	16.80	15.48	16.10	15.46	16.10
Moisture, %	13.72	13.76	13.71	13.38	13.70	13.66	13.71	13.23	13.72	13.76	13.71	13.38
Crude Fat, %	3.49	4.72	3.49	4.49	3.50	4.46	3.50	4.08	3.49	4.72	3.49	4.49
Crude Fiber, %	2.76	3.50	2.76	3.64	2.76	3.57	2.76	2.84	2.76	3.50	2.76	3.64
ADF, %	2.67	4.40	2.67	4.90	2.67	4.10	2.67	4.10	2.67	4.40	2.67	4.90
NDF, %	10.84	10.40	10.83	9.90	10.83	10.50	10.83	10.60	10.84	10.40	10.83	9.90
Ash, %	3.65	3.80	3.65	4.02	3.65	3.96	3.64	4.14	3.65	3.80	3.65	4.02
Phosphorus, %	0.46	0.52	0.46	0.52	0.46	0.50	0.46	0.50	0.46	0.52	0.46	0.52
Phosphorus, Available, %	0.23	-	0.23	-	0.23	-	0.23	-	0.23	-	0.23	-
Calcium, %	0.55	0.58	0.55	0.66	0.55	0.58	0.55	0.62	0.55	0.58	0.55	0.66
Sodium, %	0.23	0.27	0.27	0.26	0.27	0.26	0.27	0.27	0.23	0.27	0.27	0.26
Chloride, %	0.42	0.44	0.48	0.42	0.48	0.41	0.48	0.44	0.42	0.44	0.48	0.42
Magnesium, %	0.16	0.17	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.17
Potassium, %	0.69	0.72	0.69	0.75	0.69	0.70	0.69	0.72	0.69	0.72	0.69	0.75
Copper, ppm	170.30	205.00	170.05	184.00	170.29	190.00	170.04	212.00	170.30	205.00	170.05	184.00
Zinc, ppm	150.53	177.00	106.09	162.00	150.49	159.00	106.08	133.00	150.53	177.00	106.09	162.00
Iodine, ppm	0.22	-	0.23	-	0.22	-	0.23	-	0.22	-	0.23	-
Iron, ppm	221.14	281.00	212.16	305.00	221.10	275.00	212.15	284.00	221.14	281.00	212.16	305.00
Manganese, ppm	48.94	73.00	48.21	59.00	48.92	67.00	48.20	72.00	48.94	73.00	48.21	59.00
Lysine, Total, %	0.95	-	0.95	-	0.95	-	0.95	-	0.95	-	0.95	-
Lysine, SID, %	0.82	-	0.82	-	0.82	-	0.82	-	0.82	-	0.82	-
Isoleucine, Total, %	0.62	-	0.62	-	0.62	-	0.62	-	0.62	-	0.62	-
Isoleucine, SID, %	0.52	-	0.52	-	0.52	-	0.52	-	0.52	-	0.52	-
Leucine, Total, %	1.46	-	1.46	-	1.46	-	1.46	-	1.46	-	1.46	-
Leucine, SID, %	1.25	-	1.25	-	1.25	-	1.25	-	1.25	-	1.25	-
Met + Cys, Total, %	0.56	-	0.56	-	0.56	-	0.56	-	0.56	-	0.56	-
Met + Cys, SID, %	0.47	-	0.47	-	0.47	-	0.47	-	0.47	-	0.47	-
Threonine, Total, %	0.63	-	0.63	-	0.63	-	0.63	-	0.63	-	0.63	-
Threonine, SID, %	0.51	-	0.51	-	0.51	-	0.51	-	0.51	-	0.51	-
Tryptophan, Total, %	0.16	-	0.16	-	0.16	-	0.16	-	0.16	-	0.16	-
Tryptophan, SID, %	0.13	-	0.13	-	0.13	-	0.13	-	0.13	-	0.13	-
Valine, Total, %	0.74	-	0.74	-	0.74	-	0.74	-	0.74	-	0.74	-
Valine, SID, %	0.61	-	0.61	-	0.61	-	0.61	-	0.61	-	0.61	-

<sup>1</sup>There were 3 main plot Treatments (A, B, and C) with the subplot being 2 zinc source treatments (Trt. 1: zinc oxide; Trt. 2: zinc hydroxychloride). This resulted in 6 different diets, 3 for each zinc source treatment.

<sup>2</sup>Zinc oxide was used as the zinc source in Treatments 1 A, B, and C (average bioavailability of 65%).

<sup>3</sup>Treatments 2 A, B, and C contained zinc hydroxychloride used to provide (assumed 100% bioavailable).

<sup>4</sup>A common industry growth promotant was added to the diet in Grow-Finish phase 1 as a treatment of the main plot which was independent of the current study.

<sup>5</sup>A phytogetic feed additive was added to the diet in Grow-Finish phase 1 as a treatment of the main plot which was independent of the zinc study.

<sup>6</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 8.** Study 2: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow-Finish Phase 2.

Item	Dietary Treatments <sup>1</sup>											
	Treatment 1 A <sup>2</sup>		Treatment 2 A <sup>3</sup>		Treatment 1 B <sup>2</sup>		Treatment 2 B <sup>3</sup>		Treatment 1 C <sup>2</sup>		Treatment 2 C <sup>3</sup>	
Growth Promotant <sup>4</sup>	Growth Promotant		Growth Promotant		None		None		None		None	
Zinc Source (assumed bioavailability)	Zinc Oxide (65%)		Zinc Hydroxy-chloride (100%)		Zinc Oxide (65%)		Zinc Hydroxy-chloride (100%)		Zinc Oxide (65%)		Zinc Hydroxy-chloride (100%)	
Phytogenic Additive <sup>5</sup>	Additive 1		Additive 1		Additive 2		Additive 2		Additive 1		Additive 1	
Ingredient, %												
Corn, fine	66.11		66.12		66.22		66.22		66.13		66.13	
DDGS	10.76		10.76		10.73		10.73		10.76		10.76	
Soybean Meal	10.14		10.14		10.16		10.16		10.14		10.14	
Bakery Energy Blend	10.00		10.00		10.00		10.00		10.00		10.00	
Limestone	1.03		1.03		1.03		1.03		1.03		1.03	
Fat, Yellow Grease	0.60		0.60		0.60		0.60		0.60		0.60	
Water	0.50		0.50		0.50		0.50		0.50		0.50	
Salt	0.33		0.33		0.33		0.33		0.33		0.33	
L-Lysine HCl (98%)	0.22		0.22		0.22		0.22		0.22		0.22	
Phytogenic Feed Additive 1	0.10		0.10		-		-		0.10		0.10	
Trace Mineral Premix (ZnO)	0.06		-		0.06		-		0.06		-	
Trace Mineral Premix (ZnHyd)	-		0.06		-		0.06		-		0.06	
Mycotoxin Binder	0.05		0.05		0.05		0.05		0.05		0.05	
Tribasic Copper Chloride (54%)	0.03		0.03		0.03		0.03		0.03		0.03	
Vitamin Premix	0.02		0.02		0.02		0.02		0.02		0.02	
Phytase 2500	0.02		0.02		0.02		0.02		0.02		0.02	
Growth Promotant	0.02		0.02		-		-		-		-	
L-Threonine (98%)	0.01		0.01		0.01		0.01		0.01		0.01	
Phytogenic Feed Additive 2	-		-		0.02		0.02		-		-	
Ingredient Total:	100.00		100.00		100.00		100.00		100.00		100.00	
	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>	Calculated	Analyzed <sup>6</sup>
ME, Kcal/kg	3282.18	-	3282.29	-	3285.64	-	3285.75	-	3282.7	-	3282.81	-
SID Lys:ME, g/Mcal	1.96	-	1.96	-	1.96	-	1.96	-	1.96	-	1.96	-
Crude Protein, %	13.98	14.50	13.98	14.70	13.98	16.00	13.98	16.10	13.98	15.10	13.98	15.70
Moisture, %	13.84	13.54	13.84	13.23	13.85	13.63	13.85	13.17	13.84	14.18	13.84	13.46
Crude Fat, %	4.05	4.60	4.05	4.26	4.07	4.27	4.07	4.07	4.05	4.49	4.05	3.41

**Table 8 (Cont.)**

Crude Fiber, %	2.58	2.92	2.58	3.71	2.58	3.33	2.58	3.20	2.58	3.50	2.58	2.87
ADF, %	2.97	4.40	2.97	4.00	2.97	4.70	2.97	3.50	2.97	3.60	2.97	3.10
NDF, %	9.75	10.40	9.75	11.50	9.75	9.40	9.75	9.10	9.75	10.10	9.75	8.80
Ash, %	3.22	3.54	3.22	3.48	3.22	3.56	3.22	3.74	3.22	3.19	3.22	3.46
Phosphorus, %	0.38	0.42	0.38	0.41	0.38	0.43	0.38	0.43	0.38	0.44	0.38	0.44
Phosphorus, Available, %	0.18	-	0.18	-	0.18	-	0.18	-	0.18	-	0.18	-
Calcium, %	0.46	0.48	0.46	0.46	0.46	0.56	0.46	0.44	0.46	0.56	0.46	0.55
Sodium, %	0.22	0.25	0.22	0.25	0.22	0.27	0.22	0.25	0.22	0.25	0.22	0.27
Chloride, %	0.38	0.40	0.38	0.38	0.38	0.40	0.38	0.38	0.38	0.39	0.38	0.41
Magnesium, %	0.16	0.14	0.16	0.14	0.16	0.15	0.16	0.15	0.16	0.15	0.16	0.15
Potassium, %	0.60	0.63	0.60	0.62	0.60	0.69	0.60	0.70	0.60	0.66	0.60	0.70
Copper, ppm	166.67	172.00	166.46	156.00	166.68	195.00	166.47	191.00	166.67	202.00	166.46	182.00
Zinc, ppm	126.97	127.00	89.96	99.80	126.98	137.00	89.97	112.00	126.97	147.00	89.96	115.00
Iodine, ppm	0.19	-	0.19	-	0.19	-	0.19	-	0.19	-	0.19	-
Iron, ppm	183.92	235.00	176.46	200.00	183.97	253.00	176.51	217.00	183.92	250.00	176.46	243.00
Manganese, ppm	35.39	55.30	34.80	55.10	35.40	54.90	34.81	52.20	35.40	55.20	34.80	60.10
Lysine, Total, %	0.77	-	0.77	-	0.77	-	0.77	-	0.77	-	0.77	-
Lysine, SID, %	0.64	-	0.64	-	0.64	-	0.64	-	0.64	-	0.64	-
Isoleucine, Total, %	0.57	-	0.57	-	0.57	-	0.57	-	0.57	-	0.57	-
Isoleucine, SID, %	0.48	-	0.48	-	0.48	-	0.48	-	0.48	-	0.48	-
Leucine, Total, %	1.42	-	1.42	-	1.42	-	1.42	-	1.42	-	1.42	-
Leucine, SID, %	1.22	-	1.22	-	1.22	-	1.22	-	1.22	-	1.22	-
Met + Cys, Total, %	0.5	-	0.5	-	0.5	-	0.5	-	0.5	-	0.5	-
Met + Cys, SID, %	0.41	-	0.41	-	0.42	-	0.42	-	0.41	-	0.41	-
Threonine, Total, %	0.53	-	0.53	-	0.53	-	0.53	-	0.53	-	0.53	-
Threonine, SID, %	0.41	-	0.41	-	0.41	-	0.41	-	0.41	-	0.41	-
Tryptophan, Total, %	0.14	-	0.14	-	0.14	-	0.14	-	0.14	-	0.14	-
Tryptophan, SID, %	0.12	-	0.12	-	0.12	-	0.12	-	0.12	-	0.12	-
Valine, Total, %	0.68	-	0.68	-	0.68	-	0.68	-	0.68	-	0.68	-
Valine, SID, %	0.56	-	0.56	-	0.56	-	0.56	-	0.56	-	0.56	-

<sup>1</sup>There were 3 main plot Treatments (A, B, and C) with the subplot being 2 zinc source treatments (Trt. 1: zinc oxide; Trt. 2: zinc hydroxychloride). This resulted in 6 different diets, 3 for each zinc source treatment.

<sup>2</sup>Zinc oxide was used as the zinc source in Treatments 1 A, B, and C (average bioavailability of 65%).

<sup>3</sup>Treatments 2 A, B, and C contained zinc hydroxychloride used to provide (assumed 100% bioavailable).

<sup>4</sup>A common industry growth promotant was added to the diet in Grow-Finish phase 1 as a treatment of the main plot which was independent of the current study.

<sup>5</sup>Two different phytogetic feed additives were added to the diet in Grow-Finish phase 2 as treatments of the main plot which was independent of the zinc study.

<sup>6</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 9.** Study 2: Diet formulation and calculated and analyzed nutrient content for each dietary treatment for Grow Finish Phase 3.

	Dietary Treatments <sup>1,6</sup>											
	Treatment 1 A <sup>2</sup>		Treatment 2 A <sup>3</sup>		Treatment 1 B <sup>2</sup>		Treatment 2 B <sup>3</sup>		Treatment 1 C <sup>2</sup>		Treatment 2 C <sup>3</sup>	
Growth Promotant <sup>4</sup>	Growth Promotant		Growth Promotant		Growth Promotant		Growth Promotant		Growth Promotant		Growth Promotant	
Zinc Source (assumed bioavailability)	Zinc Oxide (65%)		Zinc Hydroxychloride (100%)		Zinc Oxide (65%)		Zinc Hydroxychloride (100%)		Zinc Oxide (65%)		Zinc Hydroxychloride (100%)	
Phytogenic Additive <sup>5</sup>	Additive 1		Additive 1		Additive 2		Additive 2		Additive 1		Additive 1	
Item												
Ingredient, %												
Corn, fine	45.98		45.99		46.17		46.18		45.98		45.99	
Soybean Meal	23.53		23.53		23.52		23.52		23.53		23.53	
DDGS	11.88		11.88		11.84		11.84		11.88		11.88	
Bakery Energy Blend	10.00		10.00		10.00		10.00		10.00		10.00	
Fat, Yellow Grease (Pelleter)	5.10		5.09		5.03		5.03		5.10		5.09	
Fat, Yellow Grease	1.20		1.20		1.20		1.20		1.20		1.20	
Limestone	0.91		0.91		0.91		0.91		0.91		0.91	
Water	0.50		0.50		0.50		0.50		0.50		0.50	
L-Lysine HCl (98%)	0.25		0.25		0.25		0.25		0.25		0.25	
Salt	0.25		0.25		0.25		0.25		0.25		0.25	
Phytogenic Feed Additive	0.10		0.10		-		-		0.10		0.10	
Trace Mineral Premix (ZnO)	0.06		-		0.06		-		0.06		-	
Trace Mineral Premix (IBZ)	-		0.06		-		0.06		-		0.06	
Mycotoxin Binder	0.05		0.05		0.05		0.05		0.05		0.05	
L-Threonine (98%)	0.05		0.05		0.05		0.05		0.05		0.05	
Ractopamine Hydrochloride (19.8g/kg)	0.04		0.04		0.04		0.04		0.04		0.04	
Alimet (88%)	0.04		0.04		0.04		0.04		0.04		0.04	
Copper Chloride (54%)	0.03		0.03		0.03		0.03		0.03		0.03	
Vitamin Premix	0.02		0.02		0.02		0.02		0.02		0.02	
Phytase 2500	0.01		0.01		0.01		0.01		0.01		0.01	
Phytogenic Feed Additive	-		-		0.02		0.02		-		-	
Zinc Oxide (72%)	0.01		-		0.01		-		0.01		-	
Zinc Hydroxychloride (55%)	-		0.01		-		0.01		-		0.01	
Ingredient Total:	100.00		100.00		100.00		100		100		100	
	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>	Calculated	Analyzed <sup>7</sup>

**Table 9** (Cont.)

ME, Kcal/kg	3527.40	-	3527.40	-	3527.40	-	3527.40	-	3527.40	-	3527.40	-
SID Lys:ME, g/Mcal	2.77	-	2.77	-	2.77	-	2.77	-	2.77	-	2.77	-
Crude Protein, %	19.18	21.10	19.18	19.10	19.17	19.80	19.17	19.90	19.18	21.10	19.18	19.10
Moisture, %	12.76	13.01	12.76	12.32	12.78	11.94	12.78	12.42	12.76	13.01	12.76	12.32
Crude Fat, %	9.04	7.44	9.04	9.62	9.00	9.44	8.99	7.50	9.04	7.44	9.04	9.62
Crude Fiber, %	2.81	2.68	2.81	2.93	2.81	2.18	2.81	3.46	2.81	2.68	2.81	2.93
ADF, %	3.40	3.40	3.40	4.00	3.40	3.60	3.40	3.50	3.40	3.40	3.40	4.00
NDF, %	9.75	10.00	9.75	9.40	9.75	9.60	9.75	9.50	9.75	10.00	9.75	9.40
Ash, %	3.76	3.66	3.75	3.66	3.76	3.69	3.75	3.89	3.76	3.66	3.75	3.66
Phosphorus, %	0.43	0.47	0.43	0.44	0.43	0.46	0.43	0.46	0.43	0.47	0.43	0.44
Phosphorus, Available, %	0.20		0.20		0.20		0.20		0.20		0.20	
Calcium, %	0.45	0.49	0.45	0.52	0.45	0.52	0.45	0.50	0.45	0.49	0.45	0.52
Sodium, %	0.19	0.19	0.19	0.19	0.19	0.21	0.19	0.21	0.19	0.19	0.19	0.19
Chloride, %	0.34	0.31	0.34	0.31	0.34	0.34	0.34	0.36	0.34	0.31	0.34	0.31
Magnesium, %	0.18	0.18	0.18	0.16	0.18	0.16	0.18	0.16	0.18	0.18	0.18	0.16
Potassium, %	0.83	0.90	0.83	0.83	0.83	0.88	0.83	0.85	0.83	0.90	0.83	0.83
Copper, ppm	168.73	207.00	168.52	180.00	168.73	180.00	168.52	191.00	168.73	207.00	168.52	180.00
Zinc, ppm	185.66	237.00	129.03	192.00	185.67	218.00	129.03	158.00	185.66	237.00	129.03	192.00
Iodine, ppm	0.19	-	0.19	-	0.19	-	0.19	-	0.19	-	0.19	-
Iron, ppm	199.86	249.00	192.40	301.00	199.86	264.00	192.40	252.00	199.86	249.00	192.40	301.00
Manganese, ppm	39.28	62.00	38.68	66.80	39.28	50.10	38.69	65.70	39.28	62.00	38.68	66.80
Lysine, Total, %	1.13	-	1.13	-	1.13	-	1.13	-	1.13	-	1.13	-
Lysine, SID, %	0.98	-	0.98	-	0.98	-	0.98	-	0.98	-	0.98	-
Isoleucine, Total, %	0.82	-	0.82	-	0.82	-	0.82	-	0.82	-	0.82	-
Isoleucine, SID, %	0.71	-	0.71	-	0.71	-	0.71	-	0.71	-	0.71	-
Leucine, Total, %	1.75	-	1.75	-	1.75	-	1.75	-	1.75	-	1.75	-
Leucine, SID, %	1.52	-	1.52	-	1.52	-	1.52	-	1.52	-	1.52	-
Met + Cys, Total, %	0.65	-	0.65	-	0.65	-	0.65	-	0.65	-	0.65	-
Met + Cys, SID, %	0.56	-	0.56	-	0.56	-	0.56	-	0.56	-	0.56	-
Threonine, Total, %	0.76	-	0.76	-	0.76	-	0.76	-	0.76	-	0.76	-
Threonine, SID, %	0.63	-	0.63	-	0.63	-	0.63	-	0.63	-	0.63	-
Tryptophan, Total, %	0.21	-	0.21	-	0.21	-	0.21	-	0.21	-	0.21	-
Tryptophan, SID, %	0.18	-	0.18	-	0.18	-	0.18	-	0.18	-	0.18	-
Valine, Total, %	0.92	-	0.92	-	0.92	-	0.92	-	0.92	-	0.92	-
Valine, SID, %	0.77	-	0.77	-	0.77	-	0.77	-	0.77	-	0.77	-

<sup>1</sup>There were 3 main plot Treatments (A, B, and C) with the subplot being 2 zinc source treatments (Trt. 1: zinc oxide; Trt. 2: zinc hydroxychloride). This resulted in 6 different diets, 3 for each zinc source treatment.

<sup>2</sup>Zinc oxide was used as the zinc source in Treatments 1 A, B, and C (average bioavailability of 65%).

<sup>3</sup>Treatments 2 A, B, and C contained zinc hydroxychloride used to provide (assumed 100% bioavailable).

<sup>4</sup>A common industry growth promotant was added to the diet in Grow-Finish phase 1 as a treatment of the main plot which was independent of the current study.

<sup>5</sup>Two different phytogenic feed additives were added to the diet in Grow-Finish phase 2 as treatments of the main plot which was independent of the zinc study.

<sup>6</sup>Ractopamine Hydrochloride was added to all diets (7.4 ppm) during Grow-Finish phase 3 for 28 days.

<sup>7</sup>Chemical composition analysis and mineral analyses were conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 10.** Study 1: Inclusion levels and levels of added and bioavailable zinc and copper by treatment and dietary phase.<sup>1</sup>

Zinc												
Source (Bioavailability)	Treatment 1			Treatment 2			Treatment 3			Treatment 4		
	Zinc Oxide 65% Bioavailability			Zinc Hydroxychloride 65% Bioavailability			Zinc Hydroxychloride 100% Bioavailability			Zinc Oxide 65% Bioavailability		
	Inclusion Level, kg/1,000 kg	Total Added Zinc, ppm	Bioavailable Zinc, ppm <sup>2</sup>	Inclusion Level, kg/1,000 kg	Total Added Zinc, ppm	Bioavailable Zinc, ppm <sup>2</sup>	Inclusion Level, kg/1,000 kg	Total Added Zinc, ppm	Bioavailable Zinc, ppm <sup>2</sup>	Inclusion Level, kg/1,000 kg	Total Added Zinc, ppm	Bioavailable Zinc, ppm <sup>2</sup>
Dietary Phase												
Grow-Finish Phase 1	0.18	126	82	0.23	123	80	0.15	83	83	0.18	126	82
Grow-Finish Phase 2	0.18	126	82	0.23	123	80	0.15	83	83	0.18	126	82
Grow-Finish Phase 3	0.18	126	82	0.23	123	80	0.15	83	83	0.18	126	82
Grow-Finish Phase 4	0.15	109	71	0.20	111	72	0.13	59	69	0.15	109	71
Grow-Finish Phase 5 <sup>4</sup>	0.23	163	106	0.30	165	107	0.20	100	100	0.23	163	106

  

Copper												
Source (Bioavailability)	Treatment 1			Treatment 2			Treatment 3			Treatment 4		
	Tribasic Copper Chloride (100%) <sup>3</sup>			Tribasic Copper Chloride (100%) <sup>3</sup>			Tribasic Copper Chloride (100%) <sup>3</sup>			Tribasic Copper Chloride (100%) <sup>3</sup>		
	Inclusion Level, kg/1,000 kg	Total Added Copper, ppm	Bioavailable Copper, ppm	Inclusion Level, kg/1,000 kg	Total Added Copper, ppm	Bioavailable Copper, ppm	Inclusion Level, kg/1,000 kg	Total Added Copper, ppm	Bioavailable Copper, ppm	Inclusion Level, kg/1,000 kg	Total Added Copper, ppm	Bioavailable Copper, ppm
Dietary Phase												
Grow-Finish Phase 1	0.26	150	150	0.26	150	150	0.26	150	150	0	0	0
Grow-Finish Phase 2	0.26	150	150	0.26	150	150	0.26	150	150	0	0	0
Grow-Finish Phase 3	0.26	150	150	0.26	150	150	0.26	150	150	0	0	0
Grow-Finish Phase 4	0.26	150	150	0.26	150	150	0.26	150	150	0	0	0
Grow-Finish Phase 5 <sup>4</sup>	0.26	150	150	0.26	150	150	0.26	150	150	0	0	0

<sup>1</sup>The trace mineral premix was devoid of zinc, therefore, all zinc additions for treatments were made by hand at the mixer with zinc oxide or zinc hydroxychloride (100% bioavailable).

<sup>2</sup>Parts per million of bioavailable zinc assuming zinc oxide at 65% (50 to 80%, NRC, 2012), zinc hydroxychloride at 65% and zinc hydroxychloride at 100%.

<sup>3</sup>Tribasic copper chloride was considered to have a bioavailable value of 100% (Ammerman et al., 1995).

<sup>4</sup>Additional zinc from the respective zinc source for each treatment was added in the presence of Ractopamine Hydrochloride in Grow-Finish Phase 5

**Table 11.** Study 2: Inclusion levels and levels of added and bioavailable zinc by treatment and dietary phase.<sup>1</sup>

Zinc	Treatments <sup>2</sup>					
	Treatment 1A,B,C <sup>3</sup>			Treatment 2A,B,C <sup>4</sup>		
Source (Bioavailability)	Zinc Oxide 65% Bioavailability <sup>2</sup>			Zinc Hydroxychloride 100 % Bioavailability <sup>2</sup>		
Dietary phase	GF-1	GF-2	GF-3	GF-1	GF-2	GF-3 <sup>5</sup>
Trace Mineral Premix Inclusion Level, kg/1,000 kg						
Added Zinc Oxide (65% Bioavailability)	0.68	0.57	0.57	-	-	-
Added Zinc Hydroxychloride (100% Bioavailability)	-	-	-	0.68	0.57	0.63
Total Added Zinc Concentration: Premix, ppm <sup>1</sup>						
Added Zinc Oxide (65% Bioavailability)	123	103	103	-	-	-
Added Zinc Hydroxychloride (100% Bioavailability)	-	-	-	81	68	68
Total Added Bioavailable Zn Concentration, ppm <sup>2</sup>						
Added Zinc Oxide (65% Bioavailability)	80	67	67	-	-	-
Added Zinc Hydroxychloride (100% Bioavailability)	-	-	-	81	68	104
Added via hand during GF-3 <sup>5</sup>			35			36
Total Bioavailable Zn, ppm	80	67	102	81	68	104

<sup>1</sup>Zinc oxide and zinc hydroxychloride were included in the respective diets via specially manufactured mineral premixes.

<sup>2</sup>There were 3 main plot treatments (A, B, and C) with the subplot being 2 zinc source treatments (Trt. 1: zinc oxide; Trt. 2: zinc hydroxychloride). This resulted in 6 different diets; 3 for each zinc source treatment.

<sup>3</sup>Treatment 1 A, B, and C used zinc oxide as the source of zinc (average bioavailability of 65%).

<sup>4</sup>Treatments 2 A, B, and C used zinc hydroxychloride as the zinc source (assumes 100% bioavailable).

<sup>5</sup>In grow-finish phase 3, zinc oxide or zinc hydroxychloride was added via hand at the mixer in the presence of Ractopamine Hydrochloride.



**Table 12.** Study 1: Formulated and analyzed total zinc and copper levels by treatment and dietary phase.<sup>1</sup>

Zinc	Treatments <sup>2</sup>							
	Treatment 1 <sup>3</sup>		Treatment 2 <sup>4</sup>		Treatment 3 <sup>4</sup>		Treatment 4 <sup>3</sup>	
Source (Bioavailability)	Zinc Oxide 65% Bioavailability		Zinc Hydroxychloride 65% Bioavailability		Zinc Hydroxychloride 100% Bioavailability		Zinc Oxide 65% Bioavailability	
	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>
Dietary Phase	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Grow-Finish Phase 1	159	168	157	182	116	136	159	155
Grow-Finish Phase 2	159	169	156	172	115	142	159	120
Grow-Finish Phase 3	160	170	158	180	116	161	160	182
Grow-Finish Phase 4	137	145	139	162	98	118	137	145
Grow-Finish Phase 5 <sup>6</sup>	193	189	196	212	141	179	193	50

  

Copper	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	Tribasic Copper Chloride (100%) <sup>5</sup>		Tribasic Copper Chloride (100%) <sup>5</sup>		Tribasic Copper Chloride (100%) <sup>5</sup>		No Supplemental Copper	
Source (Bioavailability)	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Grow-Finish Phase 1	170	178	170	145	170	194	19	27
Grow-Finish Phase 2	169	146	169	152	169	159	19	24
Grow-Finish Phase 3	169	186	169	179	169	184	18	72
Grow-Finish Phase 4	167	176	167	181	167	189	16	30
Grow-Finish Phase 5 <sup>6</sup>	168	163	168	165	168	175	17	20

<sup>1</sup>Diets were formulated on a total zinc basis; values represent zinc supplemented to the diet as well as other ingredients in the diet for total zinc content.

<sup>2</sup>The trace mineral premix was devoid of zinc, therefore, all zinc additions for treatments were made by hand at the mixer with zinc oxide or zinc hydroxychloride.

<sup>3</sup>Treatments 1 and 4 used zinc oxide as the source of zinc (average bioavailability 65%)

<sup>4</sup>Treatments 2 and 3 used zinc hydroxychloride as the source of zinc, 65% and 100% assumed bioavailability.

<sup>5</sup>Tribasic copper chloride was considered to have a bioavailable value of 100% (Ammerman et al., 1995).

<sup>6</sup>Additional zinc from the respective zinc source for each treatment was added to all diets in the presence of Ractopamine Hydrochloride in Grow-Finish Phase 5.

<sup>7</sup>Mineral analysis was conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 13.** Study 2: Formulated and analyzed total zinc levels by treatment and dietary phase.<sup>1</sup>

Source (Bioavailability)	Treatments <sup>2,3</sup>											
	Treatment 1A <sup>4</sup>		Treatment 2A <sup>5</sup>		Treatment 1B <sup>4</sup>		Treatment 2B <sup>5</sup>		Treatment 1C <sup>4</sup>		Treatment 2C <sup>5</sup>	
	Zinc Oxide 65% Bioavailability		Zinc Hydroxychloride 100% Bioavailability		Zinc Oxide 65% Bioavailability		Zinc Hydroxychloride 100% Bioavailability		Zinc Oxide 65% Bioavailability		Zinc Hydroxychloride 100% Bioavailability	
	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>	Formulated	Analyzed <sup>7</sup>
Dietary Phase	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Grow-Finish Phase 1	150	177	106	162	150	159	106	133	150	177	106	162
Grow-Finish Phase 2	127	127	90	99	127	137	90	112	127	147	90	115
Grow-Finish Phase 3 <sup>6</sup>	185	237	129	192	185	218	129	158	185	237	129	192

<sup>1</sup>Diets were formulated on a total zinc basis; values represent zinc supplemented to the diet as well as zinc from other ingredients in the diet for total zinc content.

<sup>2</sup>There were 3 main plot treatments (A, B, and C) with the subplot being 2 zinc source treatments (Trt. 1: zinc oxide; Trt. 2: zinc hydroxychloride). This resulted in 6 different diets, 3 for each zinc source treatment.

<sup>3</sup>Zinc oxide and zinc hydroxychloride were included in the respective diets via specially manufactured mineral premixes.

<sup>4</sup>Treatments 1 A, B, and C used zinc oxide as the source of zinc (average bioavailability 65%).

<sup>5</sup>Diets 2 A, B, and C used zinc hydroxychloride (assumed 100% bioavailable).

<sup>6</sup>In grow-finish phase 3, zinc oxide or zinc hydroxychloride was added via hand at the mixer in the presence of Ractopamine Hydrochloride, not from the mineral premixes.

<sup>7</sup>Mineral analysis was conducted based on AOAC 985.01 methodology at Midwest Labs, Omaha, NE.

**Table 14.** Study 1: Least-squares means for the effects of zinc source and supplemental copper in growing-finishing pig diets on growth performance, carcass characteristics, and morbidity and mortality.

	Treatments <sup>1</sup>				SEM	P-values				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4		Trt	Trt 1	Trt 1	Trt 1	Trt 2
	Zinc Oxide 65% Bioavailability	Zinc Hydroxychloride 65% Bioavailability	Zinc Hydroxychloride 100% Bioavailability	No Supplemental Copper			vs. 2	vs. 3	vs. 4	vs. 3
Number of pens	22	27	27	27	-	-	-	-	-	-
Growth performance										
Live weight, kg										
Start of test	46.9	47.0	47.0	47.0	1.00	0.99	0.88	0.85	0.80	0.96
Week 4	76.4 <sup>a</sup>	75.8 <sup>ab</sup>	76.2 <sup>a</sup>	75.0 <sup>b</sup>	1.17	0.03	0.23	0.72	0.01	0.36
Week 6	91.7 <sup>a</sup>	91.1 <sup>a</sup>	91.4 <sup>a</sup>	89.5 <sup>b</sup>	1.23	0.004	0.40	0.75	0.002	0.58
Start of final dietary phase <sup>2</sup>	112.7	114.2	113.9	112.9	0.72	0.27	0.11	0.21	0.85	0.69
End of test	126.4	128.0	127.3	126.5	0.79	0.30	0.11	0.35	0.91	0.45
Days on test	79.9	80.7	79.9	81.5	1.21	0.23	0.43	0.78	0.13	0.25
Average daily gain, kg										
Start – Week 4	1.052 <sup>a</sup>	1.043 <sup>a</sup>	1.057 <sup>a</sup>	1.007 <sup>b</sup>	0.0159	0.002	0.45	0.85	0.002	0.32
Week 4 – Week 6	1.048 <sup>ab</sup>	1.057 <sup>a</sup>	1.057 <sup>a</sup>	1.011 <sup>b</sup>	0.0181	0.04	0.63	0.60	0.07	0.96
Week 6 – Start of final dietary phase <sup>2</sup>	0.866	0.916	0.921	0.916	0.0299	0.12	0.04	0.04	0.05	0.95
Start of final dietary phase <sup>2</sup> – End of test	0.875	0.884	0.894	0.880	0.0299	0.98	0.83	0.68	0.86	0.83
Overall (live weight)	0.966 <sup>bc</sup>	0.975 <sup>ab</sup>	0.989 <sup>a</sup>	0.953 <sup>c</sup>	0.0141	0.004	0.39	0.04	0.17	0.22
Overall (carcass) <sup>3</sup>	0.739 <sup>ab</sup>	0.744 <sup>a</sup>	0.753 <sup>a</sup>	0.723 <sup>b</sup>	0.0104	0.01	0.68	0.19	0.07	0.33
Average daily feed intake, kg										
Start – Week 4	2.44 <sup>a</sup>	2.44 <sup>a</sup>	2.43 <sup>a</sup>	2.35 <sup>b</sup>	0.035	0.01	0.80	0.60	0.004	0.77
Week 4 – Week 6	2.91	2.93	2.93	2.83	0.061	0.10	0.75	0.74	0.09	0.99
Week 6 – Start of final dietary phase <sup>2</sup>	2.82	2.93	2.93	2.89	0.063	0.14	0.04	0.04	0.17	0.99
Start of final dietary phase <sup>2</sup> – End of test	2.69	2.71	2.69	2.76	0.053	0.58	0.67	0.90	0.22	0.74
Overall	2.68	2.70	2.70	2.66	0.038	0.11	0.23	0.22	0.48	0.99
Gain:feed, kg:kg										
Start – Week 4	0.433	0.430	0.436	0.428	0.0037	0.10	0.57	0.30	0.22	0.09
Week 4 – Week 6	0.363	0.363	0.364	0.359	0.0047	0.77	0.97	0.90	0.44	0.86
Week 6 – Start of final dietary phase <sup>2</sup>	0.310	0.313	0.313	0.315	0.0059	0.74	0.53	0.49	0.27	0.94
Start of final dietary phase <sup>2</sup> – End of test	0.326	0.324	0.332	0.320	0.0091	0.78	0.91	0.62	0.64	0.52
Overall (live weight)	0.362 <sup>ab</sup>	0.361 <sup>b</sup>	0.366 <sup>a</sup>	0.359 <sup>b</sup>	0.0024	0.03	0.67	0.16	0.16	0.05
Overall (carcass) <sup>4</sup>	0.277 <sup>a</sup>	0.275 <sup>ab</sup>	0.279 <sup>a</sup>	0.272 <sup>b</sup>	0.0019	0.01	0.38	0.39	0.03	0.07
Carcass characteristics <sup>5</sup>										
Harvest live weight, kg	126.5	128.0	127.3	126.6	0.79	0.30	0.11	0.35	0.91	0.45
Hot carcass weight, kg <sup>5</sup>	96.1	97.4	96.8	96.1	0.65	0.23	0.09	0.31	0.93	0.45
Carcass yield, % <sup>6</sup>	76.3	76.1	76.1	75.9	0.20	0.48	0.46	0.41	0.12	0.93
Longissimus muscle depth, cm <sup>5</sup>	6.91	6.93	6.93	6.93	0.038	0.99	0.89	0.84	0.81	0.94
Backfat depth, cm <sup>5</sup>	2.06	2.03	2.03	2.01	0.048	0.72	0.51	0.62	0.26	0.86
Morbidity and mortality, %	9.30	7.98	6.65	8.99	-	0.41	0.46	0.13	0.87	0.41

<sup>a,b</sup> Means within a row with different superscripts are different ( $P \leq 0.05$ ).

<sup>1</sup>Trt 1: Control – (zinc oxide; assumed bioavailability 65%) + tribasic copper chloride; Trt 2: zinc hydroxychloride – assumed bioavailability 65% + tribasic copper chloride; Trt 3: zinc hydroxychloride – assumed bioavailability 100% + tribasic copper chloride; No supplemental copper – As Trt. 1 without supplemental copper.

<sup>2</sup> Feeding of the final dietary phase (containing ractopamine) initiated at 113.4 kg body weight and was fed to entire pens for 14 ± 1 day and the lightest half of the pigs in the pen for 28 ± 1 day.

<sup>3</sup>Carcass average daily gain = overall average daily gain × carcass yield.

<sup>4</sup>Carcass gain:feed = Carcass average daily gain / overall average daily feed intake.

<sup>5</sup>Measurements taken using the Fat-O-Meater on the slaughter line at approximately the 10<sup>th</sup> rib.

**Table 15.** Study 2: Least-squares means for the effect of zinc source in growing-finishing pig diets on pig growth performance, carcass

	Treatments <sup>1</sup>		SEM	P-values
	Treatment 1	Treatment 2		
	Zinc Oxide 65% Bioavailability	Zinc Hydroxychloride 100% Bioavailability		
Pens	132	132	-	-
Number of pigs	1,444	1,444	-	-
Growth performance				
Live weight, kg				
Start of test	42.0	42.0	0.34	0.91
Week 3	64.5 <sup>a</sup>	63.6 <sup>b</sup>	0.30	<0.001
Week 6	79.5 <sup>a</sup>	78.8 <sup>b</sup>	0.82	0.02
Start of final dietary phase <sup>2</sup>	111.6	111.1	0.50	0.23
Start of study – Start of final dietary phase <sup>2</sup>	111.3	111.5	0.51	0.68
Removal of first harvest group	126.6 <sup>a</sup>	125.2 <sup>b</sup>	0.44	0.002
End of test <sup>3</sup>	132.7	132.0	0.65	0.32
Days on test	81.3	81.7	0.62	0.14
Average daily gain, kg				
Start – Week 3	1.030 <sup>a</sup>	0.980 <sup>b</sup>	0.0091	<0.001
Week 3 – Week 6	0.948	0.957	0.0290	0.51
Week 6 – Start of final dietary phase <sup>2</sup>	1.030	1.034	0.0145	0.81
Start of study – Start of final dietary phase <sup>2</sup>	1.007	1.007	0.0073	0.92
Removal of first harvest group	1.066 <sup>a</sup>	1.002 <sup>b</sup>	0.0313	0.01
End of test	0.966 <sup>b</sup>	1.043 <sup>a</sup>	0.0354	0.003
Start of final dietary phase <sup>2</sup> - End of test <sup>4</sup>	0.404	0.399	0.0122	0.70
Overall (live weight)	1.021 <sup>a</sup>	1.002 <sup>b</sup>	0.0082	0.01
Overall (carcass) <sup>5</sup>	0.821 <sup>a</sup>	0.780 <sup>b</sup>	0.0227	0.02
Average daily feed intake, kg				
Start – Week 3	2.25 <sup>a</sup>	2.18 <sup>b</sup>	0.020	<0.001
Week 3 – Week 6	2.61	2.59	0.045	0.37
Week 6 – Start of final dietary phase <sup>2</sup>	2.86	2.88	0.033	0.46
Start of study – Start of final dietary phase <sup>2</sup>	2.59	2.60	0.022	0.50
Removal of first harvest group	2.69 <sup>a</sup>	2.60 <sup>b</sup>	0.059	0.005
End of test	2.65	2.69	0.056	0.52
Start of final dietary phase <sup>2</sup> - End of test <sup>4</sup>	1.05	1.03	0.030	0.41
Overall	2.62 <sup>a</sup>	2.59 <sup>b</sup>	0.029	0.001
Gain:Feed, kg:kg				
Start – Week 3	0.458	0.452	0.0045	0.06
Week 3 – Week 6	0.363	0.370	0.0079	0.16
Week 6 – Start of final dietary phase <sup>2</sup>	0.361	0.360	0.0047	0.77
Start of study – Start of final dietary phase <sup>2</sup>	0.390	0.388	0.0024	0.43
Removal of first harvest group	0.396	0.385	0.0082	0.10
End of test	0.363 <sup>b</sup>	0.388 <sup>a</sup>	0.0092	0.001
Start of final dietary phase <sup>2</sup> - End of test	0.386	0.389	0.0069	0.67
Overall (live weight)	0.389	0.388	0.0023	0.79
Overall (carcass) <sup>6</sup>	0.307	0.302	0.0059	0.37
Carcass characteristics <sup>7</sup>				
Harvest live weight, kg	133.4 <sup>a</sup>	132.4 <sup>b</sup>	0.52	0.03
Hot carcass weight, kg	101.5	100.7	0.40	0.08
Carcass yield, %	76.1	76.1	0.13	0.83
10 <sup>th</sup> rib <i>Longissimus</i> muscle depth, in	6.83	6.81	0.051	0.50
10th rib backfat depth, in	1.78	1.78	0.043	0.88
Morbidity and Mortality, %	6.30	6.79	-	0.60

<sup>a,b</sup> Means within a row with different superscripts are different ( $P \leq 0.05$ ).

<sup>1</sup> Trt. 1: Control – zinc oxide; (assumed bioavailability 65%); Trt. 2: zinc hydroxychloride (assumed bioavailability 100%)

<sup>2</sup> Feeding of the final dietary phase, which contained ractopamine, was initiated at 111.1 kg body weight and was fed to the entire pen for 14 ±

<sup>3</sup> End of test was considered the last weight collected at the removal of the second harvest group.

<sup>4</sup> Start of final dietary phase - End of test was considered from the start of ractopamine to the marketing of the second harvest group (~Week

<sup>5</sup> Carcass average daily gain = Overall average daily gain × carcass yield.

<sup>6</sup> Carcass gain:feed = Carcass average daily gain / overall average daily feed intake.

<sup>7</sup> Measurements taken using the Fat-O-Meater on the slaughter line at approximately the 10th rib.

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