# COPPER FROM COPPER HYDROXYCHLORIDE IN DIETS FOR GROWING PIGS INCREASES FEED EFFICIENCY, IMPROVES ENERGY UTILIZATION AND CHANGES INTESTINAL MICROBIAL ACTIVITY

BY

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## ABSTRACT

Ten experiments were conducted to investigate effects of Cu from Cu hydroxychloride on growth performance, digestibility of nutrients, intestinal microbial protein, immune response, intestinal permeability, and lipid metabolism of pigs. The first 3 experiments were conducted to determine effects of Cu hydroxychloride on digestible energy (DE) and metabolizable energy (ME), apparent total tract digestibility (ATTD) of energy and acid hydrolyzed ether extract (AEE), diarrhea frequency, blood characteristics, and growth performance of nursery pigs (initial body weight: 6.80 ± 1.69 kg) fed a diet based on corn and soybean meal (SBM). Results indicated that supplementation of 150 mg Cu/kg from Cu hydroxychloride improved ($P < 0.05$) growth performance of pigs and reduced ($P < 0.05$) diarrhea frequency. However, no differences among treatments were observed for concentrations of tumor necrosis factor α (TNF-α), immunoglobulin A, blood urea N (BUN), total protein, or albumin, and the observed improvement in growth performance did not appear to be a result of increased digestibility of energy or AEE. In Exp. 4, the effects of Cu hydroxychloride on growth performance, diarrhea frequency, and blood characteristics of weanling pigs (initial body weight: 6.14 ± 0.90 kg) without or with exposure to heat stress were determined. Pigs were fed a control diet, the control diet plus 5% choice white grease (CWG), the control diet plus 100 mg/kg Cu from Cu hydroxychloride, or the control diet plus 5% CWG and 100 mg/kg Cu from Cu hydroxychloride. Results indicated that supplementation of Cu hydroxychloride to diets fed to weanling pigs without or with addition of CWG reduced ($P < 0.05$) diarrhea frequency and improved ($P < 0.05$) growth performance both if pigs were kept under normal temperature and if they were exposed to heat stress. There was also an increase ($P < 0.05$) in concentration of peptide YY and a reduction ($P < 0.05$) in TNF-α concentration on d 14 for pigs fed Cu hydroxychloride diets

compared with pigs fed diets without Cu hydroxychloride. It was, therefore, concluded that Cu hydroxychloride likely improves the immune system which may be the reason for the reduced diarrhea frequency of pigs fed Cu hydroxychloride. Experiments 5 and 6 were conducted to test the hypothesis that Cu from Cu hydroxychloride improves gain:feed (G:F) when fed to pigs (initial body weight: 15.40 ± 2.39 kg) by increasing ATTD of AEE. Results indicated that G:F linearly increased ($P < 0.05$) as CWG concentration increased in diets. Supplementation of Cu hydroxychloride to diets improved ($P < 0.05$) G:F of pigs, which resulted in a CWG equivalence of 2.8 to 3.8% for 150 mg Cu/kg from Cu hydroxychloride. Supplementation of Cu to diets improved ($P < 0.05$) ATTD of AEE by 20% due to reduced ($P < 0.10$) endogenous loss of fat (from 11.23 to 7.14 g/kg dry matter intake), but did not affect energy digestibility or true total tract digestibility of fat. This indicates that the increased G:F of pigs that was observed in Exp. 5 as a result of Cu supplementation to diets was not due to improved ATTD of gross energy or AEE, but may be a result of Cu influencing post-absorptive lipid metabolism. Experiment 7 was conducted to test the hypothesis that Cu hydroxychloride improves nutrient digestibility and alters the concentration of microbial protein in the small intestine or large intestine by pigs (initial body weight: 33.3 ± 3.40 kg) fed a corn-soybean meal diet or a diet based on corn, soybean meal, and distillers dried grains with solubles (DDGS). The apparent ileal digestibility (AID) and ATTD of crude protein were not affected by dietary Cu concentrations, but, the AID and ATTD of AEE were greater ($P < 0.05$) in diets supplemented with Cu hydroxychloride compared with diets without Cu hydroxychloride. There was also a reduction ($P < 0.05$) in the concentration of microbial protein and in the total concentration of volatile fatty acids in feces when diets were supplemented with Cu hydroxychloride indicating that Cu hydroxychloride reduced microbial activity in the intestinal tract, which likely was the reason for the increased

AID and ATTD of AEE. Experiment 8 was designed to test the hypothesis that Cu hydroxychloride improves growth performance by upregulating the mRNA transcription of genes involved in lipid metabolism of pigs (initial body weight: 15.05 ± 0.98 kg) fed a diet based on corn, SBM, and distillers dried grains with solubles (DDGS). Pigs were fed a corn-SBM-DDGS control diet that included Cu to meet the requirement. A second diet was formulated by adding 150 mg Cu/kg from Cu hydroxychloride to the control diet. Results indicated that overall average daily gain (ADG) and G:F were greater ($P < 0.05$) for pigs fed the diet containing Cu hydroxychloride compared with pigs fed the control diet. Pigs fed the diet supplemented with Cu hydroxychloride also had increased ($P < 0.05$) abundance of cluster of differentiation 36 in liver and increased ($P < 0.05$) abundance of fatty acid binding protein 4 and lipoprotein lipase in subcutaneous adipose tissue. Inclusion of Cu hydroxychloride also tended to increase ($P < 0.10$) abundance of fatty acid binding protein 1, peroxisome proliferator-activated receptor alpha, and carnitine palmitoyl transferase 1 B in liver, skeletal muscle, and subcutaneous adipose tissue, respectively. These results indicated that Cu from Cu hydroxychloride positively influences lipid metabolism, which may explain the increased G:F of pigs fed diets containing Cu hydroxychloride. Experiments 9 and 10 were designed to test the hypothesis that Cu hydroxychloride improves growth performance and blood characteristics, and reduces intestinal permeability of nursery pigs (2 wk post-weaning) fed diets without or with inclusion of cereal co-products. Overall ADG and G:F were greater ($P < 0.05$) for pigs fed diets with Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride; however, dietary Cu concentrations did not affect the lactulose:mannitol ratio in urine from pigs fed experimental diets. Supplementation of Cu hydroxychloride to diets also positively influenced ($P < 0.05$) BUN, albumin, and cytokine concentrations of nursery pigs indicating that Cu from Cu

hydroxychloride increases amino acid utilization for protein synthesis and improves the immune system of pigs. In conclusion, supplementation of Cu from Cu hydroxychloride to diets for pigs positively influences the immune system, changes intestinal microbial activity, and upregulates abundance of some genes involved in post-absorptive metabolism of lipids. Combined, these changes resulted in improved energy metabolism, improved G:F, and sometimes also improved ADG. These changes also resulted in improved intestinal health of pigs.

**Key words:** copper, digestibility, gene expression, growth performance, microbial protein, pigs

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# CHAPTER 1: INTRODUCTION

To maximize pork production, swine producers aim to achieve maximum pig growth performance while minimizing diseases and mortality. One of the main challenges in swine production is the restriction of antimicrobial use in food animals. The European Union introduced bans on the use of antibiotic growth promoters in food animal production, and other countries have followed this ban with the intention to reduce pools of resistance genes (Casewell, 2003). The main consequence of this ban is that pigs become more sensitive to infections and can develop diseases that may increase mortality and morbidity. As a consequence, alternatives to AGP have been introduced into the feed market. These alternatives include probiotics, acidifiers, prebiotics, phytobiotics, and dietary pharmacological levels of Zn and Cu (Liu et al., 2018).

Copper is a trace mineral needed for maintenance and growth by animals (Davis and Mertz, 1987; Ellingsen et al., 2007). Copper is required for cellular respiration, hemoglobin formation, proper cardiac function, keratinization, and connective tissue development (Turnlund, 1998; Gaetke and Chow, 2003). In commercial diets, 10 to 20 mg/kg of Cu is usually added, however, Cu may also be included at pharmacological concentrations (i.e., 100 to 250 mg/kg diet) for weanling and growing pigs (Cromwell et al., 1993; Cromwell et al., 1998; Hill, 2013; Ma et al., 2015) because high concentrations of dietary Cu fed to pigs improves growth performance (Cromwell et al., 1998; Hill et al., 2000; Perez et al., 2011).

Copper sulfate is the most common form of supplemental Cu used in animal feeding due to its availability and relatively low cost compared with other inorganic sources of Cu (Ma et al., 2006; 2007). However, using pharmacological concentrations of $CuSO_4$ in swine diets have resulted in environmental concerns due to high excretion of Cu in feces (Zhao et al., 2014).

Therefore, other forms of inorganic Cu have been introduced into the feed market, and one of these is Cu hydroxychloride ($Cu_2(OH)_3Cl$). It is believed that Cu hydroxychloride has high bioavailability (Liu et al., 2005) and, therefore, it may be possible to reduce the inclusion of Cu in diets without reducing animal performance if Cu hydroxychloride is fed. This may subsequently reduce the risk of soil pollution if less Cu will be excreted in the manure. There is, however, limited research about the use of Cu hydroxychloride in diets fed to pigs, and there is limited knowledge about the mechanisms or mode of action of dietary Cu. Copper may have a role in improving gut health and immune function of pigs (Højberg et al., 2005), but validation and information about mode of action are needed.

Therefore, the objectives of this dissertation were to determine effects of Cu hydroxychloride on growth performance, digestibility of nutrients, concentrations of volatile fatty acids (VFA) and intestinal microbial protein, and lipid metabolism in pigs. It was also the objective of this work to evaluate effects of Cu hydroxychloride on the immune response and intestinal permeability in weanling pigs upon exposure to environmental and nutritional challenges.

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# CHAPTER 2: NUTRITIONAL VALUE OF COPPER: LITERATURE REVIEW

## INTRODUCTION

Minerals are inorganic elements needed by pigs for maintenance, growth, and reproduction (Suttle, 2010). Discovery of the essentiality of minerals dates back to the late 18$^{th}$ century when it was recognized that deficiency of minerals caused certain diseases (Suttle, 2010). The first manifestation of nutritional significance of minerals originated in 1791 when Fordyce demonstrated that canaries require an adequate Ca supplementation for optimum health and egg production (McCollum, 1957). Thus, the early research with minerals was conducted to alleviate health problems (McCollum, 1957).

Minerals naturally occur in soil and composition and concentration of minerals in plants may vary with type of soil. The mineral profile of a particular type of soil may influence mineral content of forages and natural plant feed ingredients (McDowell, 1992). Other factors that may affect mineral concentrations of plants are the variety of the plants, type of soil on which plants grow, maturity stage, and climatic conditions during growth (Underwood and Suttle, 1999). Some of the minerals needed by animals are supplied by plant feed ingredients, and the amount of mineral supplementation to meet an animal's nutritional requirements is influenced by the mineral status of forage and plant feeds that the animal consumes (Kiatoko et al., 1982). Minerals may be recycled and returned to the soil via minerals in the litter of dead plants and animal excreta. The mineral net flow and status of soil varies in each cycle due to climatic conditions, plant maturity and type, and the amount of feces and urine the animal excretes (Underwood and Suttle, 1999).

Minerals have structural, physiological, catalytic, and regulatory functions in animals (Mateos et al., 2005). They act as components of body organs and tissues, are present in body fluids as electrolytes, catalysts or specific components in specific enzymes, and regulate cell replication and differentiation (Underwood, 1981). Minerals may also have metabolic interrelationships with other minerals that may affect growth performance and health status of the animal. Minerals are classified into 2 groups based on the amount that is required in the diet. Minerals needed by more than 100 mg/kg diet on a dry matter basis are called macrominerals, and this group includes Ca, Cl, K, Mg, Na, P, and S (Suttle, 2010). These minerals play a major role in acid-base balance, structural and regulatory functions in bones and teeth, and nerve transmission. Minerals that are required in quantities less than 100 mg/kg diet are called microminerals or trace minerals, and these include Cu, Fe, I, Mn, Mo, Co, Se, and Zn (McDowell, 1992). Microminerals primarily serve as components of enzymes, coenzymes, and hormones (Goff, 2015). Another group of minerals is the ultra-trace minerals, which are estimated to perhaps have dietary requirements of usually less than 50 ng/g (Nielsen, 1984). These minerals include B, Cd, F, Ni, Pb, and Si, and contribute mainly to mechanical stability of body organs such as bones and teeth. However, these elements are usually not supplemented in animal diets, because feed ingredients typically provide enough to meet the hypothesized requirements of animals.

## COPPER

Copper is an essential component of several metalloenzymes including ceruloplasmin (ferroxidase I), cytochrome C oxidase, lysyl oxidase, cytosolic Cu-Zn superoxide dismutase (**SOD 1**), extracellular Cu-Zn superoxide dismutase 3 (**SOD 3**), monoamine oxidase, and tyrosinase (Crapo et al., 1992; Manto, 2014). Copper, therefore, plays a role in oxidation-reduction reactions, transport of oxygen and electrons, and protection against oxidative stress (Hill, 2013; Manto, 2014). Copper is also involved in metabolic reactions including cellular respiration, tissue pigmentation, hemoglobin formation, and connective tissue development (Turnlund, 1998; Gaetke and Chow, 2003). Copper has been recognized as an essential mineral since 1928 when it was demonstrated that Cu plays an important role in red blood cell synthesis in rats. Rats suffering from anemia were fed animal or vegetable based diets supplemented with ash and were able to recuperate from the disease. It was subsequently discovered that ash contained Cu sulfide (Hart et al., 1928). This discovery led to further research to demonstrate that Cu is essential not only for preventing microcytic hypochromic anemia, but is also needed for maintenance and growth.

### *Copper Deficiency*

Animals deprived of Cu develop critical dysfunctions and hypocuprosis (Lorenzen and Smith, 1947; Gubler et al., 1952; Lahey et al., 1952; Gubler et al., 1957). Microcytic anemia is a sign of Cu deficiency due to its role in Fe metabolism, specifically in hemoglobin formation and development (Gubler et al., 1952; Suttle and Angus, 1978; Hart et al., 1987). Ceruloplasmin, which functions physiologically as a copper-dependent ferroxidase to promote transferrin formation, is essential for the catalysis of $Fe^{2+}$ to $Fe^{3+}$ (Roeser et al., 1970). Pigs may also suffer from bone abnormalities and unusual leg conditions with various degrees of crookedness if

dietary Cu is deficient because of deficiency in monoamine oxidase, which is needed for cartilage formation (Lorenzen and Smith, 1947; Lahey et al., 1952). Depigmentation, failure of hair keratinization, and cardiovascular disorders have also been demonstrated as signs of Cu deficiency (Savage et al., 1966; Carla, 1977). Pigs with hypocuprosis may also have impaired humoral response (Prohaska, 1983). Copper plays an important role in the development and function of T and B cells, neutrophils, and macrophages (Miller et al., 1979; Sorenson, 1989), and deficiency of Cu affects the immune system because of deficiency in cytochrome C oxidase and superoxide dismutase (Prohaska, 1983). Low concentrations of cytochrome C oxidase may result in impairment of the respiration burst in neutrophils, and subsequently a decrease in immunological function (Segal and Meshulam, 1979). Copper is also important for pigmentation in cattles, chickens, turkeys, and black-wool sheep by serving as a component of polyphenyl oxidase, which is the enzyme that catalyzes the conversion of tyrosine to the pigment melanin (Raper, 1928; Leeson, 2009).

The clinical signs and symptoms that are typically observed in pigs with Cu deficiency have always been associated with the role of Cu as a component of metalloenzymes needed for several metabolic reactions such as cellular respiration, hemoglobin formation, cartilage formation, and keratinization (Lahey et al., 1952). A reduction in growth performance and feed intake occurs when Cu is deficient in diets for all species, however, an unusual leg condition develops specifically in Cu-deficient pigs (Teague and Carpenter, 1951). Piglets fed diets that are deficient with Cu also manifest signs of cardiovascular and central nervous system disorders such as ataxia, posterior paresis, and horizontal nystagmus, and these observations may be attributed to a deficiency of cytochrome C oxidase needed for phospholipid synthesis (Miller et

al., 1979; Pletcher and Banting, 1983). Deficiency in Cu may also be related to the degree of saturation of the animals' lipid reserves and cholesterol profile because dietary Cu is believed to influence lipid metabolism in animals (Johnson and Engle, 2003; Kaya et al., 2006). Addition of increasing levels of dietary Cu as $CuSO_4$ reduced the concentration of serum polyunsaturated FA in pigs fed diets containing 5% fat as stabilized medium-chain triglycerides, whereas, the concentration of serum polyunsaturated FA increased in pigs fed diets without added fat (Dove, 1993). Copper supplementation may also affect the carcass FA composition of pigs where supplementation of Cu in diets resulted in an increased iodine value of backfat and increased proportion of unsaturated FA in the outer backfat, inner backfat, and perinephric backfat of pigs (Elliot and Bowland, 1968; Wu et al., 2018). Rats fed diets with high concentration of Cu had soft adipose tissues due to the role of Cu in increasing the activity of Δ-9-desaturase and Cu-hydroxylase enzymes for 16-C and 18-C fatty acids (Bureau et al., 1998). Deficiency of Cu causes hypercholesteremia and hypertriglyceridemia (Carr and Lei, 1989), and the reason for these conditions has been attributed to the role of Cu in increasing lipoprotein lipase and triolein hydrolase activities (Kaya et al., 2006). The effect of Cu deficiency on accumulation of long chain fatty acids has also been attributed to increased expression of fatty acid synthase with reduced concentration of ceruloplasmin in the serum (Burkhead and Lutsenko, 2013) because Cu is needed for ceruloplasmin to function. As Cu concentration increases, and is greater relative to the requirement, ceruloplasmin activity increases (Meagher and FitzGerald, 2000), which inhibits lipid peroxidation by inhibiting glutathione peroxidase and catalase (Abdel-Mageed and Oehme, 1990). In ruminants, dietary Cu supplementation may increase lipolytic rate and reduce animal backfat by increasing the concentration of epinephrine and norepinephrine in the body (Johnson and Engle, 2003). Copper is a component of dopamine β-hydroxylase, which is the

enzyme that catalyzes the conversion of dopamine to norepinephrine. Norepinephrine, a catecholamine, acts as a hormone and can cause physiological changes such as increased lipolytic activity in response to stress (Prohaska et al., 1990). Copper supplementation to diets was also been demonstrated to affect catecholamine metabolism in rats (Prohaska and Wells, 1974) and sheep (O'Dell et al., 1976).

***Requirement for Copper***

The requirement for Cu in pigs is influenced by dietary factors and age of the animal. Neonatal pigs usually require 5 to 10 mg of Cu/kg of diet for normal metabolism (Underwood, 1977; NRC, 2012; Hill et al., 1983) and as pigs get older, the requirement for Cu decreases if measured as milligram per kilogram of diet. A requirement of 4 mg of Cu per kg of diet has been suggested for growing pigs (ARC, 1981). Limited information is available about the Cu requirement for gestating and lactating sows, but including 60 mg of Cu/kg of diet for sows may improve reproductive performance compared with sows fed a diet containing 6 mg/kg of Cu (NRC, 2012). Sows fed diets containing 250 mg/kg of Cu from $CuSO_4$ had reduced culling rate, farrowed large litter of piglets, and had heavier piglets at birth and at weaning compared with sows fed diets without added Cu (Cromwell et al., 1993). Supplementation of Cu at 4 to 6 mg/d is necessary for sows during late pregnancy (Underwood and Suttle, 1999), but Close and Cole (2000) indicated that 10 mg of Cu per kg of diet is required by both primiparous and multiparous sows during gestation.

Dietary factors that may interfere with dietary Cu absorption, and therefore may influence the need of the animal for Cu, include dietary Zn, Fe, S, Mo, and phytate. Zinc is closely related to Cu, chemically and physiologically (Hill et al., 1984). Zinc is an essential component and activator of several metalloenzymes, and some of these metalloenzymes, such as

superoxide dismutase, have both Cu and Zn as one of its components (O'Dell, 2000). High concentrations of dietary Zn may increase the requirement for Cu (Underwood, 1977) by inducing high concentrations of intestinal metallothionein, which binds Cu, and decreases Cu absorption (Cousins, 1985). High Zn intake, therefore, will induce clinical signs of Cu deficiency (Bird, 1966; Hill et al., 1983; Esparza Gonzalez et al., 2005; Carlson et al., 2007). High dietary concentrations of Fe also decreases Cu absorption, which may lead to Cu deficiency (Hedges and Kornegay, 1973; Hansen et al., 2009). It is believed that Fe and Cu have antagonistic effects due to competition for absorption sites in intestinal mucosa (Hedges and Kornegay, 1973), and the interference of Fe in Cu absorption may involve formation of ferrous sulfide complexes (Collins et al., 2010). The sulfide part in the complex may form insoluble complexes with Cu (Suttle et al., 2009). The presence of phytate in the diet can also affect Cu absorption because phytic acid molecules bind dietary minerals such as Cu, rendering them unavailable for digestion and absorption (Martin and Evans, 1986). Phytase supplementation may, therefore, increase Cu absorption by releasing Cu from phytic acid (Adeola, 1995), but microbial phytase may also decrease Cu availability by releasing significant amounts of Zn bound to phytate (Aoyagi and Baker, 1995).

***Digestibility of Copper***

Mineral digestibility reflects the dissolution and absorption of minerals from the gastrointestinal lumen. Digestibility and absorption of minerals is difficult to accurately determine due to endogenous mineral secretions into the gastrointestinal tract from the pancreas, bile, and mucosal cells (Hambridge et al., 1986). Digestibility of Cu and Zn is also difficult to assess due to interference of homoeostatic regulation, which normally limits absorption of these minerals when animals are fed beyond the requirement (Lebel et al., 2014). The digestibility of

Cu for growing pigs range from 30 to 55% (Lebel et al., 2014; Liu et al., 2014), and the relatively low digestibility of Cu is due to antagonisms between Cu and other microminerals (Richards et al., 2010). Low pH in the stomach can reduce digestibility of Cu by causing dissociation of inorganic salts of dietary Cu (Underwood and Suttle, 1999). As pH increases in the small intestine, Zn and Cu may be trapped in insoluble hydroxide precipitates, rendering these minerals unavailable for absorption (Powell et al., 1999). The dietary source of Cu may also affect its digestibility in pigs (Lebel et al., 2014). Chelation of dietary trace minerals with proteinates (i.e., peptides, amino acids) improved apparent total tract digestibility and retention of Cu in pigs by preventing the formation of insoluble complexes along the gastrointestinal tract (Mullan and D'Souza, 2005; Liu et al., 2014).

***Copper Absorption and Metabolism***

Copper is mostly absorbed in the upper gastrointestinal tract, particularly in the duodenum, but some Cu is possibly absorbed in the stomach (van Campen and Mitchell, 1965). It is believed that Cu is not absorbed through simple diffusion because a saturable Cu transport system has been identified in rats, but it is also possible that Cu may be absorbed by both active transport and simple diffusion (Bronner and Yost, 1985). In non-ruminants, Cu is primarily absorbed through a transcellular saturable process (van den Berghe and Klomp, 2009). Copper can also be absorbed through solvent drag, which involves movement of Cu through the tight junction pores (Pappenheimer and Reiss, 1987).

Copper may exist in 2 forms of valency depending on its state of oxidation. Most dietary Cu is in the $Cu^{2+}$ form, but for Cu to be absorbed, it must be reduced to $Cu^{+}$, which is catalyzed by a Cu-reductase enzyme that is expressed by glands at the brush border (Georgatsou et al., 1997). Copper in grains may be associated with lectins and glycoproteins (Mills, 1956), and

proteolytic enzymes present in the small intestine may also mediate the release of ionized Cu. This metalloreductase belongs to Steap protein family, and is also a ferrireductase that stimulates cellular uptake of Fe and Cu (Ohgami et al., 2006). Following the reduction of dietary $Cu^{2+}$ into $Cu^{+}$, $Cu^{+}$ crosses the apical membrane and enters the enterocyte through Cu transport protein 1 (**CTR1**). Copper transport protein 1, which has a high affinity for Cu, is the main Cu transporter in enterocytes, and is found in most tissues with significant amounts found in the liver (Lee et al., 2002) because of the hepatic cells' significant need for Cu. The amount of CTR1 in the apical membrane decreases via degradation in endosomal compartments if Cu is in excess of the requirement (Hill and Link, 2009). Other Cu transporters involved in Cu uptake are the Cu transport protein 2 (**CTR2**) and divalent metal transporter (**DMT1**), but their affinity for Cu is less than that of CTR1 (Zhou and Gitschier, 1997). The DMT1 is located mainly on the brush border, and may be involved in transporting several metals such as Cu, Fe, Zn, and Mn across the apical membrane (Cater and Mercer, 2006). Thus, CTR1, CTR2, and DMT1 are the transport proteins specifically involved in increasing cellular Cu concentration if the body is in need of Cu.

Upon uptake of $Cu^{+}$ from the apical membrane, $Cu^{+}$ is transferred to chaperone proteins (Vonk et al., 2008) and chaperone proteins are also involved in maintaining the homeostatic Cu concentration in the body, and are associated with specific metalloenzymes and other Cu-containing proteins (Hill and Link, 2009). One of the chaperone proteins delivers $Cu^{+}$ to Cu/Zn-superoxide dismutase, which is an antioxidant enzyme. Another chaperone protein is the cytochrome C oxidase Cu chaperone (**COX17**), which transports $Cu^{+}$ in the mitochondria to cytochrome C oxidase, which is involved in energy transfer from NADH or $FADH_2$ to ATP (Cater and Mercer, 2006). Other chaperone proteins include antioxidant protein 1 (**Atox1**), which delivers Cu through the cytosol to the Golgi apparatus of intestinal cells (Lutsenko et al., 2007).

Copper is then transferred to the Cu transporting ATPase 1 protein (**ATP7A**), which can bind and translocate 6 $Cu^{+}$ ions into the basolateral membrane (Kim et al., 2009). This ATPase is also involved in sequestering excess Cu to avoid Cu toxicity (Axelsen and Palmgren, 1998). At the basolateral membrane, $Cu^{+}$ is then converted to $Cu^{2+}$ via a Cu oxidase for release into the interstitial space.

The homeostatic regulation of Cu absorption primarily involves the action of specific transporters and chaperone proteins (Peña et al., 1999). The rate of Cu absorption may be affected by the Cu status of the animal, and Cu digestibility may be increased if animals are Cu-deficient (Davis and Mertz, 1987). If animals are deficient in Cu, there is an increase in the synthesis of Cu transport proteins and a Cu-ATPase pump is used to move Cu across the basolateral membrane into the extracellular fluid (Davis and Mertz, 1987). If the Cu concentration of the animal is adequate, the amount of Cu transport proteins for uptake is low, and the liver can synthesize metalloenzymes and store Cu for future use. If dietary Cu is provided in excess of the requirement, enterocytes produce a sulfhydryl-rich protein called metallothionein, which binds to the freely ionized Cu. This results to a subsequent reduction in Cu absorption which can help prevent Cu toxicity (Cousins, 1985; Carlson et al., 1999). Metallothionein also binds other metals such as Zn and Cd (Carlson et al., 1999; Toriumi et al., 2005). Supplementing high concentrations of Cu may also increase gene and protein expression of Cu specific transporters and chaperone proteins (Huang et al., 2015) because high concentration of Cu triggers the ATP7A to become more active to release $Cu^{+}$ at a higher rate (Goff, 2018). However, research is needed to evaluate how pharmacological concentrations of Cu modulate expression of Cu transporters and chaperone proteins at the transcription level as well as at the level of translation.

In the hepatic portal vein, most of the absorbed $Cu^{2+}$ is bound to albumin and transcuprein (Linder, 1991) for transport to the liver, where it is taken up by hepatocytes as $Cu^{+}$ using Cu reductase. The CTR1 protein then moves $Cu^{+}$ across the hepatocyte cell membrane. For Cu to be transported from the liver to peripheral tissues, Atox1 delivers Cu to the transmembrane Golgi complex. Copper is then transferred to the Cu transporting ATPase 2 protein (**ATP7B**; Kim et al., 2009). The Cu bound to ATP7B can then be utilized to produce Cu-containing proteins for export from the liver. Most Cu in serum is contained in ceruloplasmin, which is the major protein carrier for export of Cu from liver to target organs (Roeser et al., 1970).

Ceruloplasmin is involved in Fe metabolism by having a ferroxidase activity which catalyzes the conversion of $Fe^{2+}$ to $Fe^{3+}$ (Osaki et al., 1966). The biological role of ceruloplasmin in pigs was demonstrated by Ragan et al. (1969) where effects of ceruloplasmin on plasma Fe in pigs fed diets deficient with Cu were determined. The deficiency of Cu in the diets resulted in reduced concentration of serum ceruloplasmin with a subsequent manifestation of anemia in pigs. Iron deficiency was only corrected by the administration of homologous ceruloplasmin or inorganic Cu to Cu-deficient pigs (Ragan et al., 1969). Porcine ceruloplasmin can be classified into 2 forms, ceruloplasmin I and ceruloplasmin II, which were purified by chromatography from pigs which ranged at an age of 2 d to 10 wk (Milne and Matrone, 1970). Ceruloplasmin I has greater copper content and specific enzymatic activity compared with ceruloplasmin II. Newly born piglets typically have high concentrations of liver Cu with ceruloplasmin II as the predominant form of ceruloplasmin. As pigs develop and grow older, ceruloplasmin I increases dramatically while the concentration of ceruloplasmin II remains constant (Milne and Matrone, 1970). Aside from Fe metabolism, ceruloplasmin is also believed to influence lipid metabolism in other species. Decreased concentration of ceruloplasmin in the serum alters lipid metabolism

in rats and cattle by increasing synthesis of fatty acids and cholesterol (Johnson and Engle, 2003).

***Toxicity of Copper***

Ruminants are more sensitive to Cu toxicity than monogastric animals (Goff, 2015). Sheep and lambs can only tolerate a Cu level of 10 mg/kg of DM and 70 mg/kg of DM, respectively (ARC, 1981; Lamand, 1981; Zervas et al., 2010). The differences in the tolerance level for Cu among species may be attributed to the capacity of the animal to excrete Cu in the bile, and in general, monogastric animals excrete more Cu compared with ruminants (Goff, 2015). Differences in efficiency of Cu absorption also exist among different breed, age, and species. In adult animals, not more than 5 to 10% of the Cu in the diet is absorbed, whereas young animals can absorb 15 to 30% of dietary Cu. Monogastric animals also absorb Cu more efficiently compared with ruminants (Suttle et al., 1975; Thompson, 2018). If the dietary level of Cu is in excess of the requirement, Cu accumulates in the liver and other vital organs. This may result in increased concentration of unbound free ionized Cu, which is a strong oxidant leading to hemolysis (NRC, 2012). Growth performance of broiler chickens fed diets containing 500 to 700 mg/kg of Cu declined due to a reduction in water and feed intake compared with broilers fed diets with 250 mg/kg Cu (Jensen and Maurice, 1979). Toxicity signs in birds also include proventriculitis, thick mucus secretion, black discoloration of the gizzard, and enlarged kidney, spleen, and bursa (Wideman et al., 1996). In pigs, Cu may be toxic if more than 250 mg/kg of diet is fed for an extended period, because this leads to hemolysis of red blood cells characterized by jaundice and necrosis (Jacela et al., 2010; NRC, 2012). Inclusion of 750 mg of Cu/kg of diet in growing pigs resulted in increased Cu and aspartate transaminase (**AST**) concentrations in the serum (Suttle and Mills, 2007), and the observed increase in serum AST

concentration indicates damage to tissues where AST is abundant (i.e., kidney, liver; Ellingsen et al., 2007). Suttle and Mills (2007) also observed signs of jaundice in pigs when fed diets with supplemental Cu at 750 mg/kg. However, addition of 500 mg/kg of Zn or Fe to diets with supplemental Cu at 750 mg/kg prevented clinical signs of copper toxicity and also resulted in normal concentrations of AST in the serum (Suttle and Mills, 2007).

***Assessment of Bioavailability of Copper***

One way to evaluate the efficacy of inorganic sources of Cu is to measure relative bioavailability or digestibility. Relative bioavailability of dietary Cu is defined as the proportion of the ingested dietary Cu that has been chemically absorbed and can be utilized by the animal for maintenance and growth (Baker and Ammerman, 1995). Bioavailability may also be defined as the proportion of an ingested nutrient that is absorbed, transported to its site of action, and be utilized to the physiologically active species (O'Dell, 1983). Estimates for relative bioavailability of different Cu sources is commonly obtained through slope-ratio assays (Littell et al., 1995). In this assay, diets with graded levels of Cu are formulated, and responses indicative of Cu status of the animals are evaluated (Aoyagi et al., 1993). Responses may include tissue concentrations of Cu, concentrations of metalloproteins, and enzymatic activity of animals fed the test diets, and the slope of the regression line is compared with that from animals fed a reference Cu source (L'Abbé and Fischer, 1984; Suttle, 2007).

Cupric sulfate pentahydrate is the most commonly used reference standard in estimating the bioavailability of Cu from various organic and inorganic sources (Lönnerdal et al., 1985). The relative bioavailability of Cu may also be evaluated *in vivo* using Cu radioisotopes and plethoric dietary supplementation (Baker and Ammerman, 1995). Another method to determine relative bioavailability of Cu is by conducting experiments, which involve diets with high

concentrations of Cu (Baker and Ammerman, 1995). Liver, bile, and gall bladder are usually harvested and Cu concentrations are measured to assess relative Cu bioavailability in pigs, broiler chickens, and rats (Lo et al., 1984; Cromwell et al., 1989; Aoyagi and Baker, 1995). Plasma Cu concentrations, metalloproteins, and metalloenzymatic activities (ceruloplasmin, cytochrome C oxidase, and Cu-superoxide dismutase) can also be analyzed to determine relative bioavailability of Cu (Kegley and Spears, 1994). Copper supplements such as Cu citrate and cupric carbonate have similar relative bioavailability, whereas Cu from Cu-Lys and Cu-Met are more available in poultry (Baker et al., 1991). In swine, Cu-Lys and Cu-Met are more bioavailable, whereas cupric carbonate and Cu citrate are less bioavailable (Table 2.1). In general, plant feed ingredients are variable in the bioavailability of Cu and have lower bioavailability of Cu than animal and inorganic sources of Cu (Baker and Ammerman, 1995), which may be due to the fact that the majority of Cu in plant feed ingredients is bound to phytate (Aoyagi et al., 1993). However, this may not always be the case. Copper from pork liver has low bioavailability compared with other sources, which may be due to the high Zn concentration in liver, which may inhibit Cu availability to body (Aoyagi et al., 1993). Copper oxide also has low bioavailability when fed to ruminants, poultry, and swine (Cromwell et al., 1989; Baker, 1999). This may be due to the inability of copper oxide to solubilize in acidic conditions with relatively high passage rate in the gastrointestinal tract (Kegley and Spears, 1994).

Enzyme efficacy, digestibility, and *in vitro* bioavailability of Cu in feed ingredients have been studied (Kong et al., 2015). Results of an *in vitro* digestibility assay indicated that $CuSO_4$ and Cu hydroxychloride ($\mathbf{Cu_2(OH)_3Cl}$) were completely dissolved during the stomach digestion simulation, but the solubility of Cu from Cu hydroxychloride was more influenced by the pH of the digesta than Cu from $CuSO_4$ in poultry (Pang and Applegate, 2006). Pang and Applegate

(2007) demonstrated that Cu from $CuSO_4$ was completely dissolved at pH 6.8, 4.8, 3.0, and 2.0, however, Cu from Cu hydroxychloride was not soluble at pH 6.8, but solubility gradually increased as pH decreased. The concentration of Cu in diets can also affect its solubility in the stomach. Solubility of Cu during the simulated stomach digestion in pigs increased if 250 mg/kg of $CuSO_4$ or Cu hydoxychloride was included in a control diet that contained 15 mg/kg of Cu (Park and Kim, 2016). Due to the low concentration of Cu in the control diet, dissolution of Cu may be inhibited by other feed ingredients. Therefore, supplementation of $CuSO_4$ or Cu hydoxychloride to the control diet may have increased the proportion of Cu available for stomach dissolution in pigs (Park and Kim, 2016).

## GROWTH PROMOTING LEVELS OF COPPER

Supplementing Cu to diets fed to weanling pigs at 100 to 250 mg/kg may reduce postweaning diarrhea and improve average daily gain (**ADG**) and average daily feed intake (**ADFI**; Cromwell et al., 1998; Hill et al., 2000; Perez et al., 2011). Barber et al. (1955) also reported that high dietary concentrations of Cu increased growth rate and feed efficiency in growing pigs. The greater ADFI reported for pigs fed diets supplemented with Cu may be due to the role of Cu in upregulating the mRNA expression of neuropeptide Y (**NPY**; Li et al., 2008), a neuropeptide considered a feed intake inducer (Gehlert, 1999). Copper is also believed to stimulate growth hormone secretion (LaBella et al., 1973) and is involved in post-translational modification of regulatory peptides (Eipper and Mains, 1988). Addition of 60 to 250 mg of Cu/kg in sow diets during late gestation and lactation may also reduce preweaning mortality (Thacker, 1991) and increase pig weaning weights (Wallace et al., 1966), presumably because of increased milk production. Copper may also stimulate activities of enzymes involved in nutrient

digestion (Dove, 1995). Addition of high concentrations of Cu increased lipase and phospholipase A activities in the small intestine (Luo and Dove, 1996), which may result in increased absorption of fatty acids and improved growth performance. Inclusion of 45 mg of Cu/kg of diet improved body weight gain of rabbits by upregulating the mRNA transcription of fatty acid transport protein (**FATP**) and fatty acid-binding protein (**FABP**), and carnitine palmitoyl transferase 1 (**CPT1**; Lei et al., 2017). Supplementation of Cu to diets also increased lipogenesis and fatty acid uptake in fish (Chen et al., 2016). However, high concentrations of Cu in the diet can promote lipid peroxidation in cell membranes by inducing oxidative stress in diets as well as in the body (Bremner, 1998). Lipid peroxidation causes degradation of unsaturated fatty acids, which results in a reduction of energy in diets, and as a consequence, may negatively affect growth performance and health of pigs (Lykkesfeldt and Svendsen, 2007).

One method to determine the degree of peroxidation in the animal's body is through the use of malondialdehyde (**MDA;** Fry et al., 2012a). Malondialdehyde is commonly used as a biomarker of oxidative stress, and the thiobarbitoric acid (**TBA**) assay is a method frequently used to determine MDA in biological fluids and tissues (Khoubnasabjafari et al., 2015). The degree of oxidative stress varies and may be influenced by diet type and source of Cu. Dietary factors that act as antagonists for Cu absorption, such as high concentrations of Zn and phytate, may alleviate the prooxidant effects of excess Cu (Huang et al., 2015). The major sources of inorganic Cu fed to pigs include $CuSO_4$ and Cu hydroxychloride, and these sources vary greatly in their chemical characteristics (Miles et al., 1998). Pigs fed diets with 225 mg/kg Cu hydroxychloride had reduced duodenal MDA concentrations than pigs fed $CuSO_4$ at the same concentration, which resulted in less oxidative stress in the intestine (Huang et al., 2015).

### *Effect of Copper on the Gut Microbiome*

The growth-promoting effects of dietary Cu have been attributed to its bacteriostatic and bactericidal properties (Stahly et al., 1980) because Cu may alter the bacterial populations in the intestine, and thereby affect the growth and community structure of microorganisms in the cecum and colon (Hojberg et al., 2005). Copper may alter the 3-dimensional structure of bacterial proteins, which prevents bacteria from performing their normal functions (Thurman et al., 1989). Copper may also disrupt enzyme structures and functions of bacteria by binding to S or carboxylate-containing groups and amino groups of proteins (Sterritt and Lester, 1980). Shurson et al. (1990) demonstrated that high-Cu diet did not improve growth performance of germ-free pigs, however, high-Cu diet increased ADG and ADFI in conventionally reared pigs. Clostridium, salmonella, and coliform populations were reduced in the small intestine, as well as in the cecum and colon of pigs upon Cu supplementation (Ma et al., 2006; 2007; Song et al., 2013). Copper supplementation in weanling pig diets also reduced the counts of enterococci in the stomach and increased the lactobacilli population in the cecum of young pigs (Hojberg et al., 2005; Wang et al., 2012). Reduction in synthesis of lactate, short chain fatty acids (**SCFA)**, and biogenic amines (histamine, cadaverine, and putrescine), ammonia absorption, and urease activity in the gastrointestinal content of pigs were observed if 175 to 250 mg of $CuSO_4$/kg was supplemented to diets for weanling pigs compared with pigs fed diets containing 10 to 20 mg of $CuSO_4$/kg (Dierick et al., 1986; Yen and Nienaber, 1993; Hojberg et al., 2005; Song et al., 2013). The observed reduction in biogenic amines indicates a reduction of microbial degradation of AA in the small intestine, which may indicate that dietary Cu may increase the AA available for absorption (Dierick et al., 1986). The effect of Cu on altering the gut microbiome was also observed in poultry (Xia et al., 2004; Aydin et al., 2010; Kim et al., 2011). Supplementation of

Cu-Met chelate at 50 to 100 mg/kg to diets reduced the population of *E. coli* and increased the population of lactobacilli (Kim et al., 2011). However, in terms of the effect of Cu on the gastrointestinal populations of coliform bacteria, results were inconsistent and in most cases, no effect of Cu was observed (Jensen, 2016).

### *Effects of Copper on Intestinal Health and Gut Barrier Function*

Weanling pigs are susceptible to infections, diseases, and villous atrophy in the gut, which may result in physiological and pathological changes and altered intestinal tight junction barrier resulting in increased intestinal permeability (Al-Sadi et al., 2009; Wijtten et al., 2011). Tight junctions are made up of integral membrane proteins, mainly occludin and zonula occludens protein-1 (ZO-1), and the integrity of the tight junctions is one of the important components of the intestinal mucosal barrier function (Ballard et al., 1995). Intestinal permeability increases upon diarrhea, which may allow entry of toxins and pathogenic microorganism through the epithelial cells (Zhang and Guo, 2009). Inclusion of Cu at 100 to 200 mg/kg in diets fed to weanling pigs increases villus height and reduces crypt depth, thus improving intestinal health (Zhao et al., 2007). A reduction in the concentrations of plasma diamine oxidase (**DAO**) and D-lactate was also observed when diets were supplemented with Cu-exchanged montmorillonites (**Cu-MMT**) at 1,500 mg/kg (Song et al., 2013). Diamine oxidase is located exclusively in intestinal villus and its presence in blood plasma serves as a marker for mucosal injury (Hu et al., 2012). When pigs undergo stress and intestinal mucosal barrier is damaged, intestinal mucosal cells are being sloughed into the lumen, which leads to increased concentration of DAO (Song et al., 2013). Plasma D-lactate is a byproduct of intestinal bacteria, and excessive production of this metabolite may pass through the damaged mucosa (Hu et al., 2012). Therefore, the observed reduction in plasma DAO and D-lactate upon

supplementation of dietary of Cu to diets indicates reduction in intestinal permeability and improvement of intestinal health.

***Effects of Copper on Immune Status***

Copper plays an important role in improving the innate immune function of animals (Prohaska and Failla, 1993). Rats fed Cu-deficient diets had reduced relative percentage of T cells and antibody-forming cells, and acute and delayed inflammatory response and susceptibility to infection increased when rats were fed Cu-deficient diets (Prohaska and Failla, 1993). Exposure of pigs to pathogenic or nonpathogenic antigens results in activated immune system and subsequent release of cytokines such as tumor necrosis factor α (**TNF-α**), interleukin-1 (**IL-1**), and interleukin-6 (**IL-6**; Al-Sadi et al., 2009). Piglets fed diets containing 3,000 mg of Zn/kg and 250 mg of Cu/kg had reduced plasma cytokine circulation after a coliform lipopolysaccharide challenge, which may indicate that both Cu and Zn can reduce infection and alleviate stress responses induced by bacterial endotoxin (Namkung et al., 2006). In an experiment conducted by Gonzales-Eguia et al. (2009), diets were supplemented with 50 mg/kg of dietary Cu as nanoCu or $CuSO_4$. Pigs fed diets with nanoCu had greater ADG and feed efficiency and greater concentrations of γ-globulin, total globulin protein, and IgG compared with pigs fed the control diet, and supplementation of dietary Cu to diets resulted in increased activity of superoxide dismutase (**SOD**) activity in blood serum of weanling pigs (Gonzales-Eguia et al., 2009). This may indicate that the observed improvement in growth performance in pigs fed the Cu-supplemented diets was due to the improved humoral immune response which can prevent susceptibility of piglets to infections and diseases.

## SOURCES OF DIETARY COPPER

The Cu that is included in swine diets usually originates from plant or animal based feed ingredients or from inorganic sources. Cereal grains contain 4 to 10 mg/kg of Cu (Table 2.2), but the amount of Cu present within each plant feed ingredient may vary depending on the variety, type of soil on which plants grow, maturity stage, and climatic conditions during growth (Underwood and Suttle, 1999). Oilseed meals including soybean meal (**SBM**), cottonseed meal, and linseed meal have typically higher Cu concentration compared with cereal grains (O'Dell, 1962). Processing of plant feed ingredients such as fermentation improves digestibility and concentration of CP and ash because of the reduced concentration of carbohydrates, and therefore, may increase the concentration of Cu in these ingredients (Drew et al., 2007). Animal protein sources commonly used in swine diets include fish meal, poultry meal, and blood meal and these ingredients are generally comparable in Cu concentration with plant feed ingredients ranging from 8 to 36 mg/kg (NRC, 2012). The Cu in milk products such as skim milk powder, lactose, casein, and whey powder ranges from 0.10 to 6 mg/kg (NRC, 2012).

Supplemental Cu is provided by fortifying complete diets and premixes with inorganic Cu, which include $CuSO_4$, copper chloride (**CuCl**), chelated Cu, Cu amino acid complexes, and Cu hydroxychloride. Copper sulfate pentahydrate (**$CuSO_4 \cdot 5\ H_2O$**) is a blue crystalline Cu salt commonly used as a pesticide, fungicide, soil additive, and feed supplement (Milligan and Moyer, 1975). The production process of $CuSO_4$ involves 4 basic steps, which includes heap leaching, solvent extraction, crystallization, and recrystallization (Justel et al., 2015). Copper sulfate is soluble in water with a decreased solubility upon subjection to increased acid conditions (Justel et al., 2015). Copper sulfate is the most common form of supplemental Cu

used in animal feeding due to its availability, and is relatively low in cost compared with other inorganic sources of Cu (Shelton et al., 2009). Results of a number of experiments have documented the effects of $CuSO_4$ in enhancing growth performance and gut health in weanling pigs (Stahly et al., 1980; Ma et al., 2006; 2007; Perez et al., 2011). However, using pharmacological concentrations of $CuSO_4$ in swine diets have resulted in environmental concerns due to high excretion of Cu in feces (Zhao et al., 2014) and antagonisms with other dietary constituents are also of concern (Wang et al., 2007). Therefore, other forms of inorganic Cu, which are generally included in diets at a lower inclusion rate and are less reactive with other nutrients, have been introduced to the feed industry. Examples of other sources of Cu include chelated Cu and Cu hydroxychloride. Chelation involves binding of Cu to a ligand (i.e., EDTA, amino acids, or polysaccharides), and it is possible that Cu from these sources is absorbed more efficiently and have higher retentions compared with $CuSO_4$ (Fouad, 1976; Liu et al., 2014). Inclusion of chelated Cu in diets for weanling pigs is as effective as use of $CuSO_4$ in improving growth performance (Fouad, 1976; Stansbury et al., 1990; Cromwell et al., 1994). Addition of 100 to 200 mg of Cu/kg complexed with amino acids such as copper-lysine (**CuLys**) is also as effective, and in some cases more effective, than Cu from $CuSO_4$ in increasing ADFI and ADG in weanling pigs (Coffey et al., 1994; Windisch et al., 2001). In an experiment conducted by Ma et al. (2015), treatments included 2 supplemental levels of Cu (50 or 250 mg/kg) and 2 sources of Cu from either Cu(2-hydroxy-4-(methylthiobutanoic acid)$_2$ **(Cu(HMTBa)$_2$)** or $CuSO_4$. Results indicated that the linear slope for increasing Cu supplementation on G:F was higher for $Cu(HMTBa)_2$ than that of $CuSO_4$, which indicates that $Cu(HMTBa)_2$ is more effective than $CuSO_4$ in improving feed efficiency (Ma et al., 2015). Another source of inorganic Cu is Cu hydroxychloride, which also enhances growth rate and feed efficiency in pigs (Cromwell et al.,

1998), and the bioavailability of Cu from Cu hydroxychloride is greater for laying hens than from other sources of Cu (Liu et al., 2005). Copper hydroxychloride is insoluble in water due to covalent binding of Cu to hydroxyl groups, but highly soluble in acidic conditions, which makes it less reactive in vitamin-mineral premixes, less toxic, and has less prooxidant activity than $CuSO_4$ (Miles et al., 1998; Liu et al., 2005).

## TABLES

**Table 2.1**. Relative bioavailability of Cu sources for swine (Baker and Ammerman, 1995)

| Source | Relative bioavailability[1] |
|---|---|
| $CuSO_4$ | 100 |
| Cu-Met | 110 |
| Cu-Lys | 112 |
| Cupric carbonate | 85 |
| Cupric oxide | 0 |
| Cupric sulfide | 10 |
| Cu citrate | 84 |

[1]The relative bioavailability (as liver Cu) is expressed relative to the bioavailability of Cu in $CuSO_4$.

**Table 2.2**. Copper concentration in feed ingredients (O'Dell, 1962; NRC, 2012)

| Feed ingredients | Average Cu content (as-fed basis, mg/kg) |
|---|---|
| Corn, white | 4.4 |
| Corn, yellow | 4.7 |
| Rye | 5.9 |
| Oats | 6.8 |
| Barley | 7.2 |
| Oat groats | 7.6 |
| Wheat | 7.8 |
| Fish meal | 8.0 |
| Rice bran | 9.0 |
| Wheat germ | 9.0 |
| Millet, Japanese | 9.1 |
| Wheat middlings | 12.1 |
| Meat and bone meal | 11.0 |
| Blood meal | 13.1 |
| Flaxseed meal | 16.2 |
| Brewer's dried grain | 16.4 |
| Wheat bran | 16.4 |
| Wheat gluten | 17.2 |
| Cottonseed meal | 21.8 |
| Linseed oil meal | 21.8 |
| Soybeans | 22.7 |
| Meat meal | 23.1 |
| Corn gluten meal | 35.1 |
| Poultry meal | 35.7 |
| Distillers dried gains with solubles | 38.4 |

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# CHAPTER 3: COPPER HYDROXYCHLORIDE IMPROVES GROWTH PERFORMANCE AND REDUCES DIARRHEA FREQUENCY OF WEANLING PIGS FED A CORN-SOYBEAN MEAL DIET BUT DOES NOT CHANGE APPARENT TOTAL TRACT DIGESTIBILITY OF ENERGY AND ACID HYDROLYZED ETHER EXTRACT

## ABSTRACT

Three experiments were conducted to determine effects of Cu hydroxychloride on digestible energy (DE) and metabolizable energy (ME), apparent total tract digestibility (ATTD) of energy and acid hydrolyzed ether extract (AEE), and growth performance of pigs fed a diet based on corn and soybean meal (SBM). In Exp. 1, 80 weanling pigs (PIC, L359 × Camborough; 6.80 ± 1.69 kg) were allotted to 2 treatments with 4 pigs per pen and 10 pen replicates per diet. Pigs were fed a corn-SBM control diet that had Cu added to meet the requirement. A second diet was formulated by adding 150 mg of Cu/kg from Cu hydroxychloride to the control diet. Both diets were fed for 4 wk. Results indicated that average daily gain (ADG), gain:feed (G:F), and final body weight (BW) were greater ($P < 0.05$), but diarrhea scores were reduced ($P < 0.05$) for pigs fed the diet containing 150 mg Cu/kg as Cu hydroxychloride compared with pigs fed the control diet. In Exp. 2, 36 barrows (9.89 ± 1.21 kg) were randomly allotted to 3 dietary treatments and placed in metabolism crates. The control diet was based on corn and SBM and contained 20 mg of Cu/kg. Two additional diets were formulated by adding 100 or 200 mg of Cu/kg from Cu hydroxychloride to the control diet. Diets were fed for 28 d, with feces and urine being collected from d 9 to 14, d 16 to 21, and d 23 to 28. The DE and ME of diets and the ATTD of gross energy and AEE were not affected by dietary Cu concentrations, but increased (*P*

< 0.01) by collection period. In Exp. 3, 150 pigs (10.22 ± 1.25 kg) were fed the same 3 diets as in the second experiment, and diets were provided on an *ad libitum* basis for 4 wk. Fecal scores were recorded, and on the last day of the experiment, blood samples were collected and tumor necrosis factor-α (TNF-α), IgA, blood urea N, total protein, and albumin were measured. Phase 1 ADG and G:F and final body weight on d 28 were greater ($P < 0.05$) for pigs fed diets containing 100 or 200 mg of Cu/kg supplemented by Cu hydroxychloride compared with pigs fed the control diet. Pigs fed the diets supplemented with Cu hydroxychloride also had reduced ($P < 0.05$) overall diarrhea scores compared with pigs fed the control diet. However, no differences among treatments were observed for concentrations of TNF-α, IgA, blood urea N, total protein, or albumin. In conclusion, supplementation of Cu as Cu hydroxychloride to diets fed to weanling pig diets improved growth performance and reduced diarrhea frequency, but this did not appear to be a result of increased digestibility of energy or AEE.

**Key words:** copper, copper hydroxychloride, diarrhea, digestibility, growth performance, pigs

## INTRODUCTION

The requirement for Cu by weanling pigs is 5 to 6 mg/kg diet (NRC, 2012), and if this amount is included in diets, deficiency symptoms are not observed (Suttle, 2010). Copper is provided by most feed ingredients, including corn and soybean meal (SBM), but in practical diet formulations, the contribution of Cu from the plant feed ingredients is usually ignored, and 10 to 20 mg Cu/kg diet from an inorganic source of Cu are usually added to commercial diets (Cromwell et al., 1993). However, Cu may also be included at growth promoting concentrations (i.e., 100 to 200 mg/kg diet) in diets for weanling and growing pigs (Cromwell et al., 1998; Hill,

2013; Ma et al., 2015). Growth-promoting levels of Cu from copper sulfate usually improve average daily gain (**ADG**) and gain:feed (**G:F**), which may be a result of improved fat digestibility (Dove, 1995), although conclusive evidence of the effects of Cu on fat digestibility has not been reported.

Copper may be provided in sulfate, oxide, or chloride forms, as chelated Cu, or as Cu hydroxychloride. Copper hydroxychloride is as effective as $CuSO_4$ in enhancing growth rate and feed efficiency in pigs (Cromwell et al., 1998). Copper hydroxychloride is also less reactive in vitamin-mineral premixes, insoluble in water, and less toxic than $CuSO_4$ (Miles et al., 1998). There is, however, limited research about the use of Cu hydroxychloride when fed to pigs. Therefore, the objective of this work was to test the hypothesis that Cu hydroxychloride increases growth performance and the apparent total tract digestibility (**ATTD**) of gross energy (**GE**), ash, and acid hydrolyzed ether extract (**AEE**) and reduces the incidence of diarrhea in pigs fed a corn-SBM diet.

## MATERIALS AND METHODS

The protocols for these experiments were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. Pigs were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN). In all experiments, antibiotic growth promoters were not included in the diets, and pharmacological levels of Zn were also not used.

### *Experiment 1: Growth Performance and Diarrhea Frequency*

Eighty newly weaned pigs (6.80 ± 1.69 kg) were allotted to a 2 × 2 factorial design with 4 pigs per pen. Pens were 1.20 × 1.35 m and provided 0.40 $m^2$ per pig. There were 5 pens with

barrows and 5 pens with gilts for a total of 10 replicate pens per treatment. Pigs were fed a control diet based on corn and SBM or the control diet plus 150 mg Cu/kg from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN) for 4 wk (Table 3.1). The control diet contained 20 mg of Cu from $CuSO_4$ and $CuCl_2$, with 10 mg/kg being provided from each source. A 2-phase feeding program was used, with d 0 to 14 as phase 1 and d 14 to 28 as phase 2. Both diets in phases 1 and 2 were formulated to meet current estimates for nutrient requirements for 6 to 8 and 8 to 20 kg pigs, respectively (NRC, 2012). Individual pig weights were recorded at the beginning of the experiment and at the conclusion of each week. Diarrhea scores were assessed visually every other day by 2 independent observers using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Daily feed allotments were recorded, and at the conclusion of the experiment, data were summarized to calculate ADG, average daily feed intake (**ADFI**), and G:F for each pen and treatment group. Data were summarized for each phase and over the entire experiment. Diets were analyzed for dry matter (**DM**; method 930.15; AOAC Int., 2007), ash (method 942.05; AOAC Int., 2007), GE using bomb calorimetry (model 6300; Parr Instruments, Moline, IL), and crude protein (**CP**) using the combustion procedure (method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Amino acids (**AA**) were analyzed on a Hitachi AA Analyzer (model L8800; Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard, and Cu was analyzed by inductively coupled plasma optical emissions spectrometry using an internally validated method (method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (method 975.03 B[b]; AOAC Int., 2007). Analyses for AA and Cu were conducted at the Agricultural Experiment Station Chemical

Laboratories at the University of Missouri, Colombia, and all other analyses were conducted in the Monogastric Nutrition Laboratory at the University of Illinois at Urbana-Champaign, Urbana. Data were analyzed as a 2 × 2 factorial using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The model included sex, treatment, and sex × treatment as fixed effects and replicate as random effect. The $\chi 2$ test was used to analyze frequency of diarrhea between the 2 treatments, but sex was not included in this analysis. Statistical significance was considered at $P < 0.05$.

***Experiment 2: Digestibility of Gross Energy and Acid Hydrolyzed Ether Extract***

A corn-SBM basal diet was formulated to meet requirements for all nutrients for 11 to 25 kg pigs (NRC, 2012; Table 3.2). Vitamins and minerals were included to meet or exceed current requirement estimates (NRC, 2012). The basal diet contained 20 mg supplemental Cu/ kg, which was provided as $CuSO_4$ and $CuCl_2$ in equal amounts. Two additional diets were formulated by adding 100 or 200 mg Cu/kg from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN) to the basal diet. Thirty-six barrows (9.89 ± 1.21 kg) were allotted to the 3 diets with 12 replicate pigs per diet in a completely randomized design. Pigs were individually housed in metabolism crates (0.69 × 0.79 m) that were equipped with a self-feeder, a nipple waterer, a slatted floor, a screen floor, and a urine pan to allow for the total, but separate, collection of urine and fecal materials. An aluminum foil tray was placed on the screen under the feeder to minimize contamination of feces with feed. Urine was collected once daily, whereas, feces were collected both in the morning and in afternoon. All diets were fed in meal form at 3.2 times the energy requirement for maintenance (i.e., 197 kcal/kg × $BW^{0.60}$; NRC, 2012), which was provided each day in 2 equal meals at 0800 and 1600 h. Throughout the study, pigs had *ad libitum* access to water. Feed consumption was recorded daily, and pigs were fed the same diets

for 28 d. The initial 8 d were considered the adaptation period to diets, and urine and fecal materials were collected the following 5 d according to the marker-to-marker procedure (Adeola, 2001). Briefly, a marker (indigo carmine included at less than 10% of feed allowance) was included in the morning meal on d 9 and again on d 14. Fecal collections were initiated when the first marker appeared and ceased when the second marker appeared. Urine collection started in the afternoon of d 9 and ceased in the afternoon on d 14. Urine and feces were also collected from the feed fed from d 16 to 21 and from d 23 to 28, resulting in a total of 3 collection periods. Urine was collected in urine buckets over a preservative of 50 mL of HCl. Fecal samples and 20% of the collected urine were stored in plastic containers at −20°C immediately after collection. At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet, and a subsample was lyophilized before analysis. At the conclusion of the experiment, fecal samples were thawed and mixed within pig and diet and then dried to approximately 95% DM in a 50°C forced-air drying oven prior to analysis. Urine samples were filtered and then prepared for lyophilization as explained previously (Kim et al., 2009). Fecal, diet, and urine samples were analyzed for GE using bomb calorimetry as described for Exp. 1, and diets were analyzed for DM, ash, and Cu as described for Exp. 1. Dried fecal samples were also analyzed for DM and ash, and AEE was determined in diets and feces using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction using petroleum ether (method 2003.06; AOAC Int., 2007) on an Ankom fat analyzer (Ankom XT-15 Extractor, Ankom Technology). Following analysis, ATTD of ash, energy, and AEE were calculated for each diet and collection period, and the digestible energy (**DE**) and metabolizable energy (**ME**) in each diet within each collection period were calculated as well (Adeola, 2001). Data were analyzed as a completely randomized design with

the pig as the experimental unit. Results for all treatment groups were analyzed as repeated measures using the MIXED procedure of SAS (Littell et al., 1998; Stewart et al., 2010). Fixed effects included period, treatment, and the interaction between period and treatment. However, the final model included only period and treatment as fixed effects because no interactions between period and treatments were observed. Appropriate covariance structures were chosen on the basis of the Akaike information criterion (Littell et al., 1998). The PDIFF option was used to separate means for the main effects. Results were considered significant at $P \leq 0.05$.

***Experiment 3: Growth Performance and Blood Characteristics***

A total of 150 pigs (10.22 ± 1.25 kg) were randomly allotted to 3 treatment diets in a completely randomized design. There were 5 pigs per pen (3 gilts and 2 barrows) and 10 pen replicates per treatment. Pigs were fed 3 diets: 1) a control diet based on corn and SBM, 2) the control diet plus 100 mg Cu/kg from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN), or 3) the control diet plus 200 mg Cu/kg from Cu hydroxychloride. Diets used in this experiment were similar to those used in Exp. 2 (Table 2), and pigs were allowed *ad libitum* access to feed and water throughout the experiment. Individual pig weights were recorded at the beginning of the experiment, after 14 d, and at the conclusion of the 28-d experiment. Daily feed allotments were recorded as well, and the weight of the feed left in the feeders was recorded on d 14 and 28. Diarrhea scores were assessed visually every other day as explained for Exp. 1. Three blood samples were collected at the end of the experiment from 1 pig per pen via venipuncture. One of the 3 blood samples was collected in vacutainers without ethylenediaminetetraacetic acid (**EDTA**), and blood serum was obtained from this sample. The 2 remaining blood samples were collected in vacutainers with EDTA to harvest blood plasma. Serum samples were obtained by centrifuging blood samples without EDTA at 1,500 × *g* at 4°C

for 15 min. Serum samples were frozen at -20°C until analyzed for blood urea N (**BUN**), total protein, and albumin. Plasma was analyzed for IgA and tumor necrosis factor-α (**TNF-α**) as indicators for the humoral immune response. Tumor necrosis factor-α and IgA were measured using ELISA kits according to the recommendations from the manufacturer (R&D Systems Inc., Minneapolis, MN; and Bethyl Laboratories, Inc., Montgomery, TX, respectively). All samples were analyzed in duplicate. Serum samples were analyzed for BUN, albumin, and total protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter Inc., Brea, CA). At the conclusion of the experiment, data were summarized to calculate ADG, ADFI, and average G:F for each pen and treatment group. Data were summarized for the initial 14 d, the final 14 d, and the entire experiment. Diets were analyzed for DM, ash, GE, CP, AA, and Cu as explained for Exp. 1. Diets were also analyzed for Ca, P, and Fe using an ICP procedure as explained for Cu analysis. Data were analyzed using the MIXED procedure of SAS with the pen as the experimental unit. Treatment means were calculated using the LSMeans procedure. The PDIFF option was used to separate means. Frequency of diarrhea and diarrhea scores were analyzed as explained for Exp. 1. Statistical significance was considered at $P < 0.05$.

## RESULTS

### *Experiment 1*

Diet analyses indicated that the intended concentrations of Cu were present in both diets (Table 3.1). Greater ($P < 0.05$) BW was observed for pigs fed diets with 150 mg of Cu/kg as hydroxychloride compared with pigs fed the control diet on d 14 and on d 28 (Table 3.3). For ADFI, an interaction ($P < 0.05$) between sex and treatment (added Cu hydroxychloride) was

observed in phase 1, with barrows fed the Cu hydroxychloride diet having greater ($P < 0.05$) ADFI than barrows fed the control diet; whereas, no effect of Cu hydroxychloride on ADFI was observed for gilts. For the overall experimental period, pigs fed diets with 150 mg of Cu/kg as hydroxychloride had greater ($P < 0.05$) ADFI than pigs fed the control diets. During phase 1, pigs fed the Cu hydroxychloride diet also had greater ($P \leq 0.05$) ADG than pigs fed the control diet. In phase 2, an interaction ($P < 0.05$) between sex and Cu as hydroxychloride was observed, with barrows having increased ($P < 0.05$) ADG with the 150 mg of Cu/kg hydroxychloride diet, whereas no difference between the 2 diets was observed for gilts. Pigs fed the diet with 150 mg of Cu/kg as Cu hydroxychloride also had greater ($P < 0.05$) G:F in phase 1 compared with pigs fed the control diet.

In phase 2 and overall, a reduction ($P < 0.05$) in diarrhea scores was observed for pigs fed the diets with 150 mg of Cu/kg as hydroxychloride compared with pigs fed the control diet (Table 3.4). Reduction ($P < 0.05$) in diarrhea frequency was also observed for pigs fed diets with 150 mg of Cu/kg as hydroxychloride compared with pigs fed the control diet in both phases of the experiment and for the overall experimental period.

***Experiment 2***

The intended concentrations of Cu were analyzed in all diets (Table 3.2). The GE intake, fecal output, fecal GE loss, and DE and ME of diets increased ($P < 0.01$) as period increased but were not affected by dietary Cu concentrations (Table 3.5). Likewise, the ATTD of GE or ash, but not AEE, increased ($P < 0.05$) as period increased, but no differences were observed in the ATTD of ash, GE, or AEE among dietary treatments.

***Experiment 3***

The intended concentrations of Cu were present in all diets. Greater ($P \leq 0.01$) ADG and G:F from d 0 to 14 and greater ($P < 0.01$) body weight on d 14 were observed for pigs fed diets with 100 or 200 mg of Cu/kg as Cu hydroxychloride compared with pigs fed the control diet (Table 3.6). No differences were observed in the ADFI of pigs fed the dietary treatments from d 0 to 14, from d 14 to 28, or for the entire experimental period. Greater ($P < 0.01$) final body weight on d 28 was also observed for pigs fed diets containing 100 or 200 mg of Cu/kg as Cu hydroxychloride compared with pigs fed the control diet. No differences were observed between the diet supplemented with 100 mg of Cu/kg from Cu hydroxychloride and the diet supplemented with 200 mg of Cu/kg from Cu hydroxychloride. Reductions ($P < 0.05$) in diarrhea scores were observed for pigs fed the Cu hydroxychloride diets compared with pigs fed the control diet (Table 3.7). No differences among dietary treatments were observed in the concentrations of BUN, total protein, or albumin (Table 3.8). Concentrations of TNF-α and IgA were between 53.9 and 59.1 pg/mL and 0.4 and 0.6 mg/mL, respectively; these concentrations were not influenced by concentrations of Cu hydroxychloride in the diets.

## DISCUSSION

The greater ADG for pigs fed the Cu hydroxychloride diets in Exp. 1 from d 0 to 14 was due to a combination of greater ADFI and G:F. From d 14 to 28, the increased ADG observed for pigs fed the Cu hydroxychloride diet was a result of increased ADFI, which is the reason the G:F was not improved during this phase. The observation that ADG, G:F, and final BW were greater for pigs fed diets containing Cu hydroxychloride than for pigs fed the control diet is in agreement with results indicating that addition of 50 to 250 mg of Cu/kg in diets for weanling pigs

improved growth performance (Cromwell et al., 1998; Li et al., 2008; Ma et al., 2015). However, because the diets fed in Exp. 3 were similar to those fed in Exp. 2 and because no increase in ATTD of GE or AEE was observed in Exp. 2, it is unlikely that the increased ADG and G:F observed were due to increased digestibility of GE or AEE. Instead, it is possible that the improved ADG and G:F in pigs fed diets containing Cu hydroxychloride were a result of a positive effect of Cu on intestinal health of pigs, increased villus height, reduced crypt depth, or altered microbiota profile (Namkung et al., 2006; Zhao et al., 2007), but because we did not determine those parameters in this work, we were unable to identify the mechanism for the improved ADG and G:F. The reduced diarrhea score and reduced frequency of diarrhea observed in pigs fed diets containing Cu hydroxychloride in Exp. 1 and 3 are also in agreement with previous data (Rutkowska-Pejsak et al., 1998). Similar results were reported by Xia et al. (2004) and Song et al. (2013) and may be attributed to the bacteriostatic properties of dietary Cu, which is believed to affect the growth and community structure of microorganisms in the cecum and colon (Højberg et al., 2005). Concentrations of BUN, total protein, and albumin of pigs that were measured in this experiment were within the normal physiological ranges (Tumbleson and Kalish, 1972), and the lack of differences among treatments indicates that dietary Cu concentrations have no effect on serum protein components. The lack of differences in concentrations of TNF-α and IgA between pigs fed diets containing Cu hydroxychloride and pigs fed the control diet indicates that, under the conditions of this experiment, Cu hydroxychloride had no impact on the parameters of immune status of the pigs. It is possible that this is a result of the fact that pigs with high health status were used in this experiment. In the digestibility experiment, a 3-period design was used to test the hypothesis that Cu may improve the ATTD of energy and nutrients. The observation that the ATTD of GE and ash and DE and ME of diets

increased as period increased is in agreement with data indicating that ATTD of DM, GE, ADF, and NDF increases over time in growing-finishing pigs (Jaworski et al., 2016). This observation indicates that heavier or more mature pigs have a more developed digestive system and are better able to utilize nutrients in the feed (Graham et al., 1986; Lindberg, 2014). However, the lack of differences in the digestibility of AEE among diets containing different dietary Cu concentrations is in contrast with data indicating that addition of 5% fat and 250 mg of Cu/kg from $CuSO_4$ in weanling pig diets increased fat digestibility (Dove, 1995). Copper is involved in metabolic reactions as a component of several metalloenzymes (McDowell, 1992), which may stimulate enzyme activities involved in nutrient digestion. Addition of high concentrations of Cu may increase lipase and phospholipase A activities in the small intestine (Luo and Dove, 1996), which may result in increased absorption of fatty acids and improved growth performance. However, on the basis of the results of this experiment, it was concluded that the improved growth performance of pigs allowed *ad libitum* access to feed that was observed in the present experiments as a result of inclusion of Cu hydroxychloride in the diets is likely not a result of increased digestibility of energy or AEE. It is therefore likely that Cu influences other intestinal parameters such as the microbiome in the hindgut (Højberg et al., 2005; Holman and Chenier, 2015), but more research is needed to confirm this hypothesis. It is also possible that the improved growth performance is a result of increased synthesis of neuropeptide Y in the brain that was not determined in this study (Li et al., 2008).

## CONCLUSION

In conclusion, supplementation of Cu as hydroxychloride to diets fed to weanling pigs improved final BW and reduced the incidence of diarrhea. However, the observed improvements

in final BW and diarrhea frequency were not a result of improved energy and AEE digestibility, and there were no measurable changes in concentrations of blood proteins, TNF-α, or IgA. Therefore, further research is needed to determine the mode of action of Cu hydroxychloride in diets fed to weanling pigs.

## TABLES

**Table 3.1.** Composition of experimental diets used in Exp. 1

| | Phase 1 diets | | Phase 2 diets | |
|---|---|---|---|---|
| Item | Control | Cu[1] | Control | Cu |
| Ingredient, % | | | | |
| Corn | 47.95 | 47.95 | 49.69 | 49.69 |
| Soybean meal | 26.50 | 26.50 | 33.50 | 33.50 |
| Whey, dried | 15.00 | 15.00 | 10.00 | 10.00 |
| Fish meal | 6.00 | 6.00 | 3.50 | 3.50 |
| Soybean oil | 2.10 | 2.10 | 1.27 | 1.27 |
| Limestone | 1.05 | 1.05 | 0.63 | 0.63 |
| Cornstarch | 0.10 | 0.074 | 0.10 | 0.074 |
| Copper hydroxychloride, 58% Cu | - | 0.026 | - | 0.026 |
| L-Lys, HCl, | 0.35 | 0.35 | 0.37 | 0.37 |
| DL-Met | 0.13 | 0.13 | 0.13 | 0.13 |
| L-Thr | 0.10 | 0.10 | 0.09 | 0.09 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[2] | 0.20 | 0.20 | 0.20 | 0.20 |
| Phytase premix[3] | 0.02 | 0.02 | 0.02 | 0.02 |
| Analyzed values | | | | |
| Dry matter, % | 88.34 | 88.41 | 88.16 | 88.34 |
| Ash, % | 6.50 | 6.42 | 6.08 | 5.85 |
| Gross energy, kcal/kg | 3,995 | 3,998 | 4,034 | 4,074 |

**Table 3.1. (cont.)**

| | | | | |
|---|---|---|---|---|
| Crude protein, % | 22.44 | 22.71 | 19.97 | 22.42 |
| Cu, mg/kg | 20.50 | 171.00 | 14.30 | 154.00 |
| Amino acids % | | | | |
| Arg | 1.35 | 1.38 | 1.45 | 1.48 |
| His | 0.51 | 0.52 | 0.62 | 0.62 |
| Ile | 0.99 | 1.01 | 1.03 | 1.02 |
| Leu | 1.83 | 1.87 | 1.89 | 1.88 |
| Lys | 1.64 | 1.58 | 1.60 | 1.61 |
| Met | 0.44 | 0.46 | 0.45 | 0.45 |
| Phe | 1.03 | 1.05 | 1.12 | 1.12 |
| Thr | 0.93 | 0.97 | 0.95 | 0.94 |
| Trp | 0.28 | 0.28 | 0.27 | 0.29 |
| Val | 1.08 | 1.10 | 1.11 | 1.10 |

[1]The diet containing added Cu was fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin $D_3$ as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin $B_{12}$, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine

**Table 3.1. (cont.)**

dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]Provided 400 units of phytase (Optiphos 2000, Huvepharma, Sofia, Bulgaria) per kilogram of complete diet.

**Table 3.2.** Composition of experimental diets used in Exp. 2 and Exp. 3

| Item | Diets | | |
|---|---|---|---|
| | Control | $Cu^2$-100 mg/kg | Cu- 200 mg/kg |
| Ingredient, % | | | |
| Corn | 58.80 | 58.80 | 58.80 |
| Soybean meal | 32.75 | 32.75 | 32.75 |
| Soybean oil | 5.00 | 5.00 | 5.00 |
| Limestone | 1.16 | 1.16 | 1.16 |
| Dicalcium phosphate | 0.82 | 0.82 | 0.82 |
| Cornstarch | 0.10 | 0.0827 | 0.0653 |
| Copper hydroxychloride, 58% Cu | - | 0.0173 | 0.0347 |
| L-Lys, HCl | 0.41 | 0.41 | 0.41 |
| DL-Met | 0.12 | 0.12 | 0.12 |
| L-Thr | 0.12 | 0.12 | 0.12 |
| Salt | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[2] | 0.20 | 0.20 | 0.20 |
| Phytase premix[3] | 0.02 | 0.02 | 0.02 |
| Analyzed values | | | |
| Dry matter, % | 87.74 | 87.83 | 88.37 |
| Ash, % | 5.01 | 4.99 | 5.04 |
| Gross energy, kcal/kg | 4,125 | 4,142 | 4,137 |
| Acid hydrolyzed ether extract, % | 7.13 | 6.91 | 7.02 |
| Crude protein, % | 20.70 | 21.11 | 21.32 |

**Table 3.2. (cont.)**

| | | | |
|---|---|---|---|
| Ca, % | 0.78 | 0.75 | 0.77 |
| P, % | 0.54 | 0.53 | 0.51 |
| Fe, mg/kg | 160.00 | 144.00 | 143.00 |
| Cu, mg/kg | 13.10 | 120.00 | 205.00 |
| Amino acids, % | | | |
| Arg | 1.37 | 1.33 | 1.37 |
| His | 0.53 | 0.57 | 0.54 |
| Ile | 0.92 | 0.92 | 0.93 |
| Leu | 1.74 | 1.74 | 1.77 |
| Lys | 1.52 | 1.54 | 1.56 |
| Met | 0.39 | 0.37 | 0.37 |
| Phe | 1.03 | 1.02 | 1.05 |
| Thr | 0.88 | 0.90 | 0.89 |
| Trp | 0.26 | 0.25 | 0.27 |
| Val | 1.01 | 1.01 | 1.02 |

[1]Cu from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN).

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin $D_3$ as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin $B_{12}$, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as

**Table 3.2. (cont.)**

copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]Provided 400 units of phytase (Optiphos 2000, Huvepharma, Sofia, Bulgaria) per kilogram of complete diet.

**Table 3.3.** Growth performance of pigs fed diets without or with 150 mg of Cu/kg from Cu hydroxychloride (Exp. 1)[1]

| Item | Barrow | | Gilt | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Cu[2] | Control | Cu | | Sex × Diet | Diet | Sex |
| d 0 to 7 | | | | | | | | |
| Initial BW[3], kg | 7.096 | 7.128 | 6.414 | 6.559 | 1.21 | 0.821 | 0.479 | 0.002 |
| ADFI[3], kg | 0.051 | 0.103 | 0.092 | 0.096 | 0.02 | 0.193 | 0.073 | 0.275 |
| ADG[3], kg | -0.040 | 0.012 | 0.014 | 0.042 | 0.02 | 0.527 | 0.016 | 0.010 |
| G:F[3] | -7.552 | 0.078 | 0.138 | 0.435 | 2.80 | 0.234 | 0.195 | 0.190 |
| Final BW, kg | 6.786 | 7.237 | 6.536 | 6.828 | 1.35 | 0.769 | 0.021 | 0.050 |
| d 7 to 14 | | | | | | | | |
| ADFI, kg | 0.184 | 0.345 | 0.325 | 0.314 | 0.03 | 0.042 | 0.017 | 0.069 |
| ADG, kg | 0.117 | 0.197 | 0.210 | 0.241 | 0.03 | 0.485 | 0.041 | 0.025 |
| G:F | 0.657 | 0.614 | 0.649 | 0.700 | 0.07 | 0.523 | 0.959 | 0.594 |
| Final BW, kg | 7.614 | 8.549 | 7.993 | 8.580 | 2.01 | 0.718 | 0.015 | 0.462 |
| d 0 to 14 | | | | | | | | |
| ADFI, kg | 0.114 | 0.226 | 0.212 | 0.203 | 0.02 | 0.037 | 0.012 | 0.056 |
| ADG, kg | 0.037 | 0.104 | 0.112 | 0.142 | 0.02 | 0.408 | 0.010 | 0.007 |
| G:F | 0.238 | 0.425 | 0.510 | 0.702 | 0.10 | 0.983 | 0.033 | 0.009 |
| d 14 to 21 | | | | | | | | |
| ADFI, kg | 0.441 | 0.555 | 0.596 | 0.555 | 0.05 | 0.199 | 0.362 | 0.095 |
| ADG, kg | 0.292 | 0.415 | 0.410 | 0.450 | 0.03 | 0.260 | 0.006 | 0.012 |

**Table 3.3. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| G:F | 0.655 | 0.755 | 0.701 | 0.834 | 0.05 | 0.763 | 0.035 | 0.216 |
| Final BW, kg | 9.653 | 11.418 | 10.872 | 11.768 | 2.56 | 0.454 | 0.002 | 0.037 |
| d 21 to 28 | | | | | | | | |
| ADFI, kg | 0.743 | 0.952 | 0.748 | 0.908 | 0.07 | 0.734 | 0.032 | 0.786 |
| ADG, kg | 0.521 | 0.620 | 0.575 | 0.597 | 0.04 | 0.388 | 0.156 | 0.702 |
| G:F | 0.711 | 0.652 | 0.789 | 0.663 | 0.05 | 0.552 | 0.123 | 0.427 |
| Final BW, kg | 12.836 | 16.118 | 15.357 | 15.590 | 3.46 | 0.095 | 0.004 | 0.063 |
| d 14 to 28 | | | | | | | | |
| ADFI, kg | 0.753 | 0.845 | 0.774 | 0.817 | 0.05 | 0.658 | 0.135 | 0.937 |
| ADG, kg | 0.382 | 0.534 | 0.517 | 0.507 | 0.03 | 0.039 | 0.006 | 0.032 |
| G:F | 0.530 | 0.620 | 0.655 | 0.633 | 0.03 | 0.090 | 0.280 | 0.046 |
| d 0 to 28 | | | | | | | | |
| ADFI, kg | 0.432 | 0.536 | 0.495 | 0.510 | 0.03 | 0.251 | 0.048 | 0.508 |
| ADG, kg | 0.211 | 0.318 | 0.314 | 0.326 | 0.02 | 0.090 | 0.005 | 0.011 |
| G:F | 0.502 | 0.592 | 0.628 | 0.636 | 0.03 | 0.177 | 0.089 | 0.013 |

[1]Data are least squares means of 10 observations for all treatments.

[2]The diet containing added Cu was fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN).

[3]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

**Table 3.4.** Diarrhea score and frequency of diarrhea of pigs fed diets without or with 150 mg of Cu/kg from Cu hydroxychloride (Exp. 1)[1]

| Item | Treatment | | SEM | *P*-value |
|---|---|---|---|---|
| | Control | Cu[2] | | |
| Diarrhea score[3] | | | | |
| d 0 to 14 | 2.314 | 2.014 | 0.106 | 0.076 |
| d 14 to 28 | 2.643 | 2.157 | 0.102 | 0.008 |
| d 0 to 28 | 2.479 | 2.086 | 0.087 | 0.011 |
| Frequency of diarrhea | | | | |
| d 0 to 14 | | | | |
| Pen days[4] | 70 | 70 | | |
| Frequency[5] | 52.86 | 32.86 | - | 0.017 |
| d 14 to 28 | | | | |
| Pen days | 70 | 70 | | |
| Frequency | 55.71 | 27.14 | - | <0.001 |
| Overall, d 0 to 28 | | | | |
| Pen days | 140 | 140 | | |
| Frequency | 54.29 | 30.00 | - | <0.001 |

[1]Data are least squares means of 10 observations for all treatments.

[2]The diet containing added Cu was fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN).

[3]Diarrhea score = 1, normal feces, 2, moist feces, 3, mild diarrhea, 4, severe diarrhea, 5, watery diarrhea.

**Table 3.4. (cont.)**

[4]Pen days = number of pens × the number of days assessing diarrhea scores.

[5]Frequency = (number of pen days with diarrhea scores ≥ 3/pen days)*100.

**Table 3.5.** Effect of feeding period and Cu hydroxychloride on the apparent total tract digestibility (ATTD) of gross energy (GE) and nutrients and on the concentration of digestible energy and metabolizable energy in corn-soybean meal diets (Exp. 2)[1]

| | Period effect | | | | | Treatment effect | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | 1 | 2 | 3 | SEM | *P*-value | Control | Cu[2]-100 mg/kg | Cu-200 mg/kg | SEM | *P*-value[3] |
| GE intake, kcal/d | 2,707[c] | 3,594[b] | 4,253[a] | 77.532 | <0.001 | 3,458 | 3,553 | 3,542 | 119.750 | 0.829 |
| Fecal output, g/d | 65[c] | 81[b] | 90[a] | 2.810 | <0.001 | 78 | 80 | 78 | 4.097 | 0.879 |
| Fecal GE loss, kcal/d | 312[c] | 395[b] | 437[a] | 13.754 | <0.001 | 375 | 397 | 428 | 20.216 | 0.909 |
| DE of diet, kcal/kg | 3,685[b] | 3,701[b] | 3,734[a] | 11.056 | <0.001 | 3,688 | 3,713 | 3,720 | 14.624 | 0.270 |
| ME of diet, kcal/kg | 3,504[b] | 3,538[a] | 3,544[a] | 12.679 | 0.011 | 3506 | 3543 | 3538 | 16.742 | 0.245 |
| ATTD, GE, % | 88.61[b] | 89.00[b] | 89.79[a] | 0.266 | <0.001 | 88.99 | 89.13 | 89.28 | 0.352 | 0.850 |
| ATTD, Ash, % | 77.82[b] | 79.71[a] | 80.48[a] | 0.697 | 0.021 | 78.90 | 79.30 | 79.83 | 0.723 | 0.713 |
| ATTD, AEE[4], % | 80.04 | 79.54 | 80.32 | 0.429 | 0.394 | 79.68 | 80.31 | 79.91 | 0.495 | 0.658 |

[a-c]Means within a row that do not have a common superscript differ, $P < 0.05$.

[1]Data are least squares means of 12 observations for all treatments.

[2]Cu from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN).

[3]Period × treatment effects were not significant, therefore, only main effects were indicated.

**Table 3.5. (cont.)**

[4]AEE = acid hydrolyzed ether extract.

**Table 3.6.** Growth performance of pigs fed diets with 0, 100, or 200 mg/kg of Cu from Cu hydroxychloride (Exp. 3)[1]

| | Treatment | | | | |
|---|---|---|---|---|---|
| Item | Control | Cu[2]- 100 mg/kg | Cu- 200 mg/kg | SEM | *P*-value |
| d 0 to 14 | | | | | |
| Initial BW[3], kg | 10.214 | 10.230 | 10.221 | 0.409 | 0.844 |
| ADG[3], kg | 0.359[b] | 0.461[a] | 0.464[a] | 0.025 | 0.002 |
| ADFI[3], kg | 0.872 | 0.873 | 0.883 | 0.066 | 0.991 |
| G:F[3] | 0.414[b] | 0.530[a] | 0.531[a] | 0.025 | 0.005 |
| Final BW, kg | 15.251[b] | 16.732[a] | 16.710[a] | 0.565 | 0.001 |
| d 14 to 28 | | | | | |
| ADG, kg | 0.589 | 0.580 | 0.616 | 0.038 | 0.713 |
| ADFI, kg | 0.950 | 1.000 | 1.056 | 0.041 | 0.163 |
| G:F | 0.612 | 0.583 | 0.586 | 0.032 | 0.698 |
| Final BW, kg | 23.780[b] | 25.199[a] | 25.886[a] | 0.827 | 0.006 |
| d 0 to 28 | | | | | |
| ADG, kg | 0.485 | 0.525 | 0.538 | 0.027 | 0.174 |
| ADFI, kg | 0.903 | 0.945 | 0.966 | 0.041 | 0.174 |
| G:F | 0.542 | 0.550 | 0.561 | 0.025 | 0.614 |

[a,b]Means within a row that do not have a common superscript differ, $P < 0.05$.

[1]Data are least squares means of 10 observations for all treatments.

[2]Cu from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN).

**Table 3.6. (cont.)**

[3]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

**Table 3.7.** Diarrhea score and frequency of diarrhea of pigs fed diets with 0, 100, or 200 mg/kg of Cu from Cu hydroxychloride (Exp. 3)[1]

| | Treatment | | | | |
|---|---|---|---|---|---|
| Item | Control | Cu[2]- 100 mg/kg | Cu- 200 mg/kg | SEM | *P*-value |
| Diarrhea score[3] | | | | | |
| d 0 to 14 | 2.286 | 2.129 | 2.157 | 0.072 | 0.201 |
| d 14 to 28 | 2.171 | 2.057 | 2.071 | 0.041 | 0.129 |
| d 0 to 28 | 2.229[a] | 2.093[b] | 2.114[b] | 0.078 | 0.037 |
| Frequency of diarrhea | | | | | |
| d 0 to 14 | | | | | |
| Pen days[4] | 70 | 70 | 70 | | |
| Frequency[5] | 25.71 | 12.86 | 21.43 | - | 0.153 |
| d 14 to 28 | | | | | |
| Pen days | 70 | 70 | 70 | | |
| Frequency | 11.43 | 2.86 | 7.14 | - | 0.144 |
| Overall, d 0 to 28 | | | | | |
| Pen days | 140 | 140 | 140 | | |
| Frequency | 18.57 | 7.86 | 14.29 | - | 0.031 |

[a,b]Means within a row that do not have a common superscript differ, $P < 0.05$.

[1]Data are least squares means of 10 observations for all treatments.

[2]Cu from Cu hydroxychloride (IntelliBond C; Micronutrients USA; Indianapolis, IN)

**Table 3.7. (cont.)**

[3]Diarrhea score = 1, normal feces, 2, moist feces, 3, mild diarrhea, 4, severe diarrhea, 5, watery diarrhea.

[4]Pen days = number of pens × the number of days assessing diarrhea scores.

[5]Frequency = (number of pen days with diarrhea scores ≥ 3/pen days)*100.

**Table 3.8.** Blood urea nitrogen (BUN), total protein, and albumin in serum and tumor necrosis factor-α (TNF-α) and IgA in plasma of pigs fed diets with 0, 100, or 200 mg of Cu/kg from Cu hydroxychloride (Exp. 3)[1]

| | Treatment | | | | |
|---|---|---|---|---|---|
| Item | Control | $Cu^2$- 100 mg/kg | Cu- 200 mg/kg | SEM | *P*-value |
| BUN, mg/dL | 11.400 | 12.600 | 11.700 | 0.686 | 0.452 |
| Total protein, g/dL | 5.680 | 5.660 | 5.520 | 0.107 | 0.459 |
| Albumin, g/dL | 3.370 | 3.540 | 3.460 | 0.072 | 0.271 |
| TNF-α, pg/mL | 59.121 | 53.924 | 58.248 | 7.516 | 0.846 |
| IgA, mg/mL | 0.524 | 0.444 | 0.562 | 0.057 | 0.255 |

[1]Data are least squares means of 10 observations for all treatments.

[2]Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

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# CHAPTER 4: EFFECTS OF COPPER HYDROXYCHLORIDE AND CHOICE WHITE GREASE ON GROWTH PERFORMANCE AND BLOOD CHARACTERISTICS OF WEANLING PIGS KEPT AT NORMAL AMBIENT TEMPERATURE OR UNDER HEAT STRESS CONDITIONS

## ABSTRACT

An experiment was conducted to test the hypothesis that inclusion of copper (Cu) hydroxychloride to diets without or with addition of choice white grease (CWG) improves growth performance and blood characteristics, and reduces diarrhea incidence in weanling pigs without or with exposure to heat stress. One hundred sixty pigs (6.14 ± 0.90 kg) were allotted to a 2 × 2 factorial arrangement with 2 levels of CWG (0 or 5%) and 2 levels of added Cu from Cu hydroxychloride (0 or 100 mg/kg). There were 5 pigs per pen and 8 pen replicates per diet. Diarrhea scores were visually assessed using a score from 1 to 5 (1 = normal feces to 5 = watery diarrhea). From d 40 to 44, ambient temperature was increased from 24°C to 32°C to create a mild heat stress. On d 14, d 28, d 40, and on d 44, blood samples were collected from 1 pig per pen and tumor necrosis factor-α (TNF-α), peptide YY, immunoglobulin G, blood urea N, total protein, and albumin were analyzed. Results indicated that there were no interactions between CWG and Cu hydroxychloride for overall growth performance. Greater ($P < 0.05$) gain:feed was observed from d 28 to 40 and from d 40 to 44 for pigs fed diets with 5% CWG compared with pigs fed diets without supplemental fat. Average daily gain was greater ($P < 0.05$) from d 14 to 28 and also during exposure to heat stress, and fecal scores were reduced over the entire period ($P < 0.05$) for pigs fed diets with Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride. There was also an increase ($P < 0.05$) in concentration of peptide YY and a

reduction ($P < 0.05$) in TNF-α concentration on d 14 for pigs fed Cu hydroxychloride diets compared with pigs fed diets without Cu hydroxychloride. This may be attributed to the effect of Cu in enhancing the expression of hypothalamic appetite regulators and its bacteriostatic property in reducing inflammation caused by pathogens. In conclusion, supplementation of Cu hydroxychloride to diets fed to weanling pigs without or with addition of CWG reduces diarrhea incidence and improves growth performance and some blood parameters.

**Key words:** copper, copper hydroxychloride, growth performance, blood characteristics, heat stress, pigs

## INTRODUCTION

Heat stress is one of the contributing causes of reduction in growth performance and occurrence of diseases in pigs (Le Dividich, 1981; Hicks et al., 1998). Heat stress may affect behavior, endocrine responses, and immune responses of pigs, and may increase concentrations of serum cortisol and tumor necrosis factor-α (**TNF-α**) in pigs upon exposure (Hicks et al., 1998; Carroll et al., 2012). Heat stress also induces increased gut permeability and inflammation, which may affect growth performance and intestinal function of pigs (Lee et al., 2016).

Copper is a trace mineral that has bacteriostatic and bactericidal properties (Stahly et al., 1980). Dietary Cu may result in reduced or altered bacterial populations in the intestine and affect growth and community structure of microorganisms in the cecum and colon, and also contribute to improved intestinal health (Hojberg et al., 2005). Copper hydroxychloride (IntelliBond $C^{II}$; Micronutrients, USA LLC, Indianapolis, IN 46241) is one of the sources of Cu

that may be used in diets for pigs, and Cu hydroxychloride has low water solubility, but is highly soluble under acidic conditions (Spears et al., 2004). Copper hydroxychloride also improves growth rate and feed efficiency in pigs (Cromwell et al., 1998; Fry et al., 2012; Espinosa et al., 2017). It is, therefore, possible that dietary Cu hydroxychloride may result in an improved immune response of pigs and contribute to prevention of diseases, and thus, improve growth performance of pigs exposed to heat stress. Pigs under heat stress often respond by reducing average daily feed intake (**ADFI**), but addition of fat to the diet may help pigs maintain energy intake (Kellner et al., 2016). However, there is no information about the effects of adding both Cu and fat to diets during heat stress. Therefore, an experiment was conducted to test the hypothesis that inclusion of 100 mg/kg of supplemental Cu from Cu hydroxychloride in diets fed to pigs without or with addition of choice white grease (**CWG**) will improve growth performance and blood concentrations of indicators for protein utilization, inflammatory responses, and hormonal effects if pigs are kept under normal temperature or if they are exposed to heat stress.

## MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used. No antibiotic growth promoters were included in the diets, and pharmacological levels of Zn were also not used.

A total of 160 weanling pigs (6.14 ± 0.90 kg) were allotted to a 2 × 2 factorial design with 2 levels of CWG (0 or 5%) and 2 concentrations of Cu from added Cu hydroxychloride (0

or 100 mg/kg). Weanling pigs were allotted by initial body weight in a randomized complete block design. Pens were 1.20 × 1.35 m and provided 0.40 $m^2$ per pig, and each pen was equipped with a feeder and a nipple drinker. There were 5 pigs per pen (3 gilts and 2 barrows) and 8 replicate pens per treatment. A 3-phase feeding program was used with d 0 to 14 as phase 1, d 14 to 28 as phase 2, and d 28 to 44 as phase 3. All diets from d 0 to 14, d 14 to 28, and d 28 to 44 were formulated to meet current estimates for nutrient requirements for 6 to 8, 8 to 12, and 12 to 25 kg pigs, respectively (NRC, 2012). Diets were formulated based on corn and soybean meal (Tables 1 and 2). Within each period, pigs were fed a control diet, the control diet plus 5% CWG, the control diet plus 100 mg/kg Cu from Cu hydroxychloride, or the control diet plus 5% CWG and 100 mg/kg Cu from Cu hydroxychloride. The control diet contained 20 mg of Cu from CuCl that was provided by the vitamin-mineral premix.

Individual pig weights were recorded at the beginning of the experiment, on d 14, d 28, d 40, and on d 44. Feed addition was recorded daily and the weight of feed left in the feeder was recorded on d 14, 28, 40, and 44. Diarrhea scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Diarrhea frequency was obtained by totaling the number of pen days with diarrhea scores ≥ 3 divided by the total number of pen days multiplied by 100. At the conclusion of the experiment, data were summarized to calculate ADFI, average daily gain (**ADG**), and gain:feed (**G:F**) within each pen and treatment group. Data were summarized for d 0 to 14, d 14 to 28, d 28 to 40, d 40 to 44, d 0 to 40, and for the entire experiment.

On d 14, d 28, d 40, and on d 44, 2 blood samples were collected from 1 pig per pen via vena puncture. The same pigs were bled at each bleeding. These samples were collected in vacutainers that contained either heparin or ethylenediaminetetraacetic acid (**EDTA)**.

Heparinized samples were frozen at -20ºC and were analyzed for blood urea N, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA). Tumor necrosis factor-α, immunoglobulin G (**IgG**), and peptide YY were measured in plasma samples collected in the vacutainer with EDTA using ELISA kits according to the recommendations from the manufacturer (R&D Systems, Inc., Minneapolis, MN; Bethyl Laboratories, Inc., Montgomery, TX; and Phoenix Pharmaceuticals, Inc., Burlingame, CA, respectively).

The temperature from d 1 to 40 followed normal farm practices (i.e., approximately 28°C from d 1 to 7, 26°C from d 7 to 14, and 24°C from d 18 to 40). However, from d 40 to 44, the temperature in the barn was increased from 24°C to 32°C to create a mild heat stress for the pigs. Pigs were allowed *ad libitum* access to feed and water during the entire experiment.

All diet samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. Diets were analyzed for dry matter (Method 930.15; AOAC Int., 2007), ash (Method 942.05; AOAC Int., 2007), gross energy using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL), acid hydrolyzed ether extract by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY), and crude protein using the combustion procedure (Method 990.03; AOAC Int., 2007). Amino acids were analyzed on a Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard (Method 982.30 E (a, b, c); (AOAC Int., 2007). Minerals were analyzed by inductively coupled plasma optical emission spectrometry using an internally validated method (Method 985.01 A, B, and C; AOAC, 2007) after wet ash sample preparation (Method 975.03

B[b]; AOAC, 2007). Analyses for AA and minerals were conducted at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Colombia, and all other analyses were conducted in the Monogastric Nutrition Laboratory at the University of Illinois at Urbana-Champaign, Urbana.

Data and diarrhea scores were analyzed following a 2 × 2 factorial arrangement using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pen as the experimental unit. The model included CWG, Cu, and CWG × Cu as fixed effects, and replicate as a random effect. Least square means were calculated for each independent variable and means were separated using the PDIFF option. The chi-squared test was used to analyze the frequency of diarrhea among treatments. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

## RESULTS

Diet analyses indicate that the intended concentrations of acid hydrolyzed ether extract and Cu were present in all diets and concentrations of other nutrients were not affected by dietary treatment. Likewise, no differences in amino acid composition among diets within each phase were observed (Tables 4.1 and 4.2).

An interaction between CWG and Cu hydroxychloride was observed for ADFI of pigs from d 0 to 14 (Table 4.3). Addition of 5% CWG to the control diet resulted in increased ADFI of pigs, whereas addition of 5% CWG to the diet supplemented with Cu hydroxychloride did not increase ADFI of pigs. Greater ($P < 0.05$) ADFI was observed by pigs fed diets with 100 mg/kg Cu from Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride from d 14 to 28, d 0 to 40, and during the overall experimental period. Greater ($P < 0.05$) ADG and final

bodyweight were observed from d 14 to 28 for pigs fed the Cu hydroxychloride diets compared with pigs fed diets without Cu hydroxychloride. However, pigs fed the Cu hydroxychloride tended to have reduced ($P < 0.10$) G:F compared with pigs fed diets without supplemental Cu hydroxychloride from d 28 to 40. Pigs fed diets with 5% CWG tended to have less ($P < 0.10$) ADFI from d 0 to 40 and over the entire experimental period, but had greater G:F ($P < 0.05$) compared with pigs fed diets without supplemental fat. When heat stress was induced, no interactions between CWG and Cu hydroxychloride were observed. However, pigs fed diets with Cu hydroxychloride had greater ($P < 0.05$) ADG and G:F and tended ($P < 0.10$) to have greater final body weight compared with pigs fed diets without Cu hydroxychloride. An improvement ($P < 0.05$) in G:F was also observed during the heat stress period when pigs were fed diets with 5% CWG compared with pigs fed diets without CWG.

Pigs fed diets containing Cu hydroxychloride had reduced ($P < 0.05$) diarrhea scores compared with pigs fed diets without Cu hydroxychloride from d 0 to 14, d 14 to 28, d 0 to 40, and over the entire experimental period (Table 4.4). Pigs fed diets with 5% CWG had reduced ($P < 0.05$) diarrhea scores compared with pigs fed diets without supplemental fat from d 28 to 40. A reduction ($P < 0.05$) in diarrhea frequency was also observed for pigs fed the Cu hydroxychloride diets compared with pigs fed diets without supplemental Cu from d 14 to 28, d 0 to 40, and for the overall experimental period.

On d 14, pigs fed diets with 100 mg/kg of Cu from Cu hydroxychloride had increased ($P < 0.05$) albumin concentration and reduced ($P < 0.05$) concentration of TNF-α in comparison with pigs fed diets without Cu hydroxychloride (Table 4.5). Likewise, supplementation of Cu hydroxychloride to diets tended to increase ($P < 0.10$) peptide YY concentration on d 14 and albumin concentration on d 40. Pigs fed diets with 5% CWG had greater ($P < 0.05$)

concentration of peptide YY compared with pigs fed diets without CWG on d 14. An interaction between CWG and Cu was observed for the concentration of albumin on d 44 because addition of 5% CWG to the control diet resulted in increased albumin concentration in plasma of pigs, whereas no difference was observed when 5% CWG was added to the diet supplemented with Cu hydroxychloride.

## DISCUSSION

Pigs undergo heat stress when environmental temperature is greater than their thermoneutral zone, and in this condition, pigs reduce their metabolic function by altering behavioral and physiological activity (Rauw et al., 2017). Under heat stress, pigs suppress feed intake as a mechanism to reduce heat production, and as a result, ADG of heat-stressed pigs is reduced compared with pigs kept under normal temperature (Kellner et al., 2016). Within 2 to 6 h of exposure to heat stress, a reduction in feed intake with a subsequent reduction in intestinal function and gut integrity may be observed in pigs (Pearce et al., 2013; Gabler et al., 2018). Pigs also become hyperglycemic due to an altered digestive capacity and changed post-absorptive metabolism within 24 h of exposure to heat stress (Pearce et al., 2012). To ameliorate these negative effects, ingredients with low heat increment such as crystalline amino acids and dietary fat are often used during periods where pigs may observe heat stress. However, it is not known if supplementation of Cu as Cu hydroxychloride without or with addition of CWG to diets can have beneficial effects in pigs exposed to heat stress.

Dietary fat increases energy concentration of diets (Kerr et al., 2015), and the tendency for a reduction in ADFI that was observed in pigs fed diets with supplemental fat over the entire experimental period is likely due to the greater energy density in these diets. This observation is

in agreement with data indicating that ADFI of pigs linearly decreased as the concentration of CWG increased in the diets (De la Llata et al., 2001). The observed improvement in G:F of pigs fed diets containing 5% CWG without or with heat stress is in agreement with results indicating that addition of 4% soybean oil improved G:F of pigs kept at 20°C or 30°C (Hsia and Lu, 2004). The reason no change in G:F was observed from d 0 to 14 and from d 14 to 28 may be that pigs do not utilize added dietary animal fat efficiently before they have a body weight close to 15 kg (Cera et al., 1990; Reinhart et al., 1992; Adeola et al., 2013).

The observation that ADG and ADFI were greater for pigs fed diets containing Cu hydroxychloride than for pigs fed the control diet under normal temperature is in agreement with previous data (Cromwell et al., 1998; Espinosa et al., 2017). The reduced diarrhea score and reduced frequency of diarrhea observed in pigs fed diets with supplemental Cu are also in agreement with previous data (Rutkowska-Pejsak et al., 1998; Espinosa et al., 2017). It is possible that the observed increase in ADG and ADFI may be attributed to the effect of Cu in stimulating the secretion of growth-promoting regulatory peptides such as growth hormone-releasing hormone (LaBella et al., 1973; Zhou et al., 1994) and its involvement in post-translational modification of regulatory peptides (Eipper and Mains, 1988).

The beneficial effect of adding high concentrations of Cu to diets is well established; however, to our knowledge, there are no published data indicating the effect of supplementing Cu hydroxychloride to diets fed to pigs exposed to heat stress. The observation that ADG and G:F were greater for pigs fed diets containing Cu hydroxychloride than for pigs fed diets without supplemental Cu during heat stress is possibly a result of the beneficial effects of Cu on gastrointestinal health and immune function (Zhao et al., 2007). Dietary Cu is also believed to have bacteriostatic properties because it may reduce clostridium, salmonella, and coliform

populations in the small intestine, as well as in the cecum and colon of pigs (Ma et al., 2006; Ma et al., 2007). During heat stress, a decrease in intestinal integrity and immune competence can be observed, which may result in disease and sudden reduction in nutrient absorption (Pearce et al., 2015). Therefore, addition of Cu hydroxychloride to diets may have prevented these negative effects, which subsequently resulted in improved growth performance.

Concentrations of blood urea N, total protein, and albumin were within the normal physiological ranges (Tumbleson and Kalish, 1972) and were in agreement with previous data (Casas and Stein, 2016). The lack of differences in blood urea N and total protein concentration among treatments indicate that dietary Cu concentrations and fat supplementation have no effect on the efficiency of N utilization of pigs. However, the increase in the concentration of albumin that was observed on d 14 and 40 in pigs fed the Cu hydroxychloride diets may be due to an increased absorption of Cu. Albumin constitutes approximately 60% of the total plasma protein and is involved in the binding and transport of fatty acids, amino acids, and trace element ions such as Zn and Cu (Quinlan et al., 2005; Francis, 2010). Therefore, the increased absorption of Cu and increased ADFI in pigs fed diets containing Cu hydroxychloride may have increased the need for albumin to transport nutrients to the liver and from the liver to extrahepatic tissues (Ramos et al., 2016).

The reduction in the concentration of TNF-α on d 14 that was observed when diets were supplemented with Cu hydroxychloride may be a result of the impact of Cu hydroxychloride on improving the immune status of pigs. It is possible that this effect also is due to the bacteriostatic property of Cu in reducing inflammation caused by pathogens. The observed increase in the concentration of PYY on d 14 by pigs fed the Cu hydroxychloride diets may be a consequence of Cu enhancing the expression of hypothalamic appetite regulators (Zhu et al., 2011). Peptide YY

is a gastrointestinal hormone, which belongs to the pancreatic polypeptide family together with neuropeptide Y (Batterham and Bloom, 2003). Peptide YY is the most potent stimulator of food intake; therefore, the increase in peptide YY that was observed on d 14 may have contributed to the observed increase in ADFI of pigs fed the Cu hydroxychloride diets. The increased concentration of peptide YY in pigs fed diets with CWG on d 14 may also be a result of the increased energy intake of these pigs (Batterham et al., 2006) because the concentration of peptide YY depends on the type of diet ingested by the animal, with fat being the most potent nutrient in terms of stimulating the release of peptide YY (Batterham and Bloom, 2003).

## CONCLUSION

In conclusion, supplementation of diets for weanling pigs with CWG resulted in improved G:F of pigs under both normal and heat-stressed conditions, which is likely due to the high energy density and low heat increment of dietary fat. Supplementation of Cu hydroxychloride without or with addition of CWG also improved growth performance both if pigs were kept under normal temperature and if they were exposed to heat stress, and there were positive changes in concentrations of TNF-α, albumin, and peptide YY on d 14 in pigs fed diets containing copper hydroxychloride. These measurable changes may be a result of the bacteriostatic property of Cu hydroxychloride and its effect on enhancing the secretion and expression of growth-promoting regulatory peptides and hypothalamic appetite regulators, but further research is needed to validate these hypotheses.

## TABLES

**Table 4.1.** Composition of experimental diets (d 0 to 14 and d 14 to 28) without or with choice white grease (CWG) and without or with copper from copper hydroxychloride

| | d 0 to 14 Diets | | | | d 14 to 28 Diets | | | |
|---|---|---|---|---|---|---|---|---|
| | No Cu[1] | | 100 mg of Cu/kg | | No Cu | | 100 mg of Cu/kg | |
| Item | -CWG | + CWG | -CWG | + CWG | -CWG | + CWG | -CWG | + CWG |
| Ingredient, % | | | | | | | | |
| Ground corn | 50.13 | 45.09 | 50.13 | 45.09 | 44.71 | 39.67 | 44.71 | 39.67 |
| Soybean meal | 26.50 | 26.50 | 26.50 | 26.50 | 27.00 | 27.00 | 27.00 | 27.00 |
| Whey, dried | 15.00 | 15.00 | 15.00 | 15.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Corn DDGS[1] | - | - | - | - | 15.00 | 15.00 | 15.00 | 15.00 |
| Fish meal | 6.00 | 6.00 | 6.00 | 6.00 | - | - | - | - |
| Choice white grease | - | 5.00 | - | 5.00 | - | 5.00 | - | 5.00 |
| Limestone | 1.05 | 1.05 | 1.05 | 1.05 | 1.44 | 1.44 | 1.44 | 1.44 |
| Dicalcium phosphate | - | - | - | - | 0.38 | 0.38 | 0.38 | 0.38 |
| Cornstarch | 0.10 | 0.10 | 0.0815 | 0.0815 | 0.10 | 0.10 | 0.0815 | 0.0815 |
| Copper hydroxychloride, 54% Cu | - | - | 0.0185 | 0.0185 | - | - | 0.0185 | 0.0185 |

**Table 4.1. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| L-Lys HCl | 0.35 | 0.36 | 0.35 | 0.36 | 0.51 | 0.52 | 0.51 | 0.52 |
| DL-Met | 0.12 | 0.14 | 0.12 | 0.14 | 0.10 | 0.12 | 0.10 | 0.12 |
| L-Thr | 0.09 | 0.10 | 0.09 | 0.10 | 0.10 | 0.11 | 0.10 | 0.11 |
| Phytase premix[2] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Analyzed composition | | | | | | | | |
| Dry matter, % | 85.94 | 87.02 | 86.18 | 87.87 | 86.10 | 87.29 | 86.33 | 87.09 |
| Ash, % | 6.36 | 6.17 | 5.40 | 6.28 | 6.37 | 5.76 | 5.85 | 5.96 |
| Gross energy, kcal/kg | 3,828 | 4,127 | 3,831 | 4,136 | 3,869 | 4,183 | 3,862 | 4,147 |
| Crude protein, % | 17.15 | 16.86 | 18.70 | 17.53 | 17.27 | 18.71 | 18.94 | 17.60 |
| AEE[4], % | 3.40 | 8.20 | 2.98 | 7.53 | 3.49 | 8.01 | 3.43 | 8.42 |
| Ca, % | 0.88 | 0.89 | 0.89 | 0.80 | 0.82 | 0.77 | 0.72 | 0.82 |
| P, % | 0.58 | 0.59 | 0.60 | 0.57 | 0.58 | 0.57 | 0.57 | 0.59 |
| Mn, mg/kg | 84.80 | 73.60 | 65.00 | 67.50 | 72.60 | 53.60 | 74.80 | 66.90 |

**Table 4.1. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fe, mg/kg | 335.00 | 197.00 | 195.00 | 296.00 | 198.00 | 163.00 | 303.00 | 177.00 |
| Zn, mg/kg | 175.00 | 168.00 | 156.00 | 153.00 | 171.00 | 125.00 | 148.00 | 152.00 |
| Cu, mg/kg | 30.40 | 26.40 | 121.00 | 121.00 | 26.90 | 19.00 | 108.00 | 125.00 |
| Amino acids, % | | | | | | | | |
| Arg | 1.23 | 1.28 | 1.26 | 1.25 | 1.19 | 1.33 | 1.26 | 1.26 |
| His | 0.52 | 0.53 | 0.52 | 0.51 | 0.53 | 0.57 | 0.55 | 0.55 |
| Ile | 0.94 | 0.97 | 0.97 | 0.94 | 0.91 | 0.98 | 0.98 | 0.97 |
| Leu | 1.70 | 1.71 | 1.73 | 1.68 | 1.83 | 1.92 | 1.90 | 1.88 |
| Lys | 1.51 | 1.58 | 1.58 | 1.54 | 1.42 | 1.56 | 1.52 | 1.51 |
| Met | 0.44 | 0.46 | 0.46 | 0.44 | 0.39 | 0.46 | 0.40 | 0.39 |
| Met + Cys | 0.74 | 0.76 | 0.76 | 0.74 | 0.69 | 0.76 | 0.70 | 0.69 |
| Phe | 0.96 | 0.99 | 0.98 | 0.96 | 1.00 | 1.08 | 1.05 | 1.04 |
| Thr | 0.88 | 0.94 | 0.92 | 0.91 | 0.90 | 0.96 | 0.90 | 0.94 |
| Trp | 0.25 | 0.22 | 0.25 | 0.25 | 0.23 | 0.24 | 0.24 | 0.25 |
| Val | 1.02 | 1.05 | 1.05 | 1.02 | 1.01 | 1.08 | 1.08 | 1.07 |

**Table 4.1. (cont.)**

[1]The diet containing added Cu was fortified with 100 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN); DDGS = distillers dried grains with solubles.

[2]Quantum Blue 5G (AB Vista, Marlborough, UK) contains 5,000 phytase units per gram. At 0.01% inclusion, the premix was calculated to provide 500 units of phytase per kilogram in the complete diet.

[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin $D_3$ as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin $B_{12}$, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[4]AEE = acid hydrolyzed ether extract.

**Table 4.2.** Composition of experimental diets (d 28 to 44) without or with choice white grease (CWG) and without or with copper from copper hydroxychloride

| | No Cu | | 100 mg/kg Cu[1] | |
|---|---|---|---|---|
| Item | -CWG | + CWG | -CWG | + CWG |
| Ingredient | | | | |
| Ground corn | 44.97 | 39.92 | 44.97 | 39.92 |
| Soybean meal | 22.00 | 22.00 | 22.00 | 22.00 |
| Corn DDGS[1] | 30.00 | 30.00 | 30.00 | 30.00 |
| Choice white grease | - | 5.00 | - | 5.00 |
| Limestone | 1.55 | 1.53 | 1.55 | 1.53 |
| Dicalcium phosphate | 0.06 | 0.10 | 0.06 | 0.10 |
| Cornstarch | 0.10 | 0.10 | 0.0815 | 0.0815 |
| Copper hydroxychloride, 54% Cu | - | - | 0.0185 | 0.0185 |
| L-Lys HCl, | 0.55 | 0.56 | 0.55 | 0.56 |
| DL-Met | 0.03 | 0.04 | 0.03 | 0.04 |
| L-Thr | 0.08 | 0.09 | 0.08 | 0.09 |
| Phytase premix[2] | 0.01 | 0.01 | 0.01 | 0.01 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 |
| Analyzed values | | | | |
| Dry matter, % | 85.38 | 86.20 | 85.76 | 86.44 |
| Ash, % | 4.85 | 5.24 | 5.48 | 4.47 |
| Gross energy, kcal/kg | 3,997 | 4,312 | 3,983 | 4,319 |

**Table 4.2. (cont.)**

| | | | | |
|---|---|---|---|---|
| Crude protein, % | 19.76 | 17.27 | 19.19 | 17.77 |
| AEE[4], % | 4.89 | 10.65 | 4.50 | 9.68 |
| Ca, % | 0.65 | 0.67 | 0.68 | 0.64 |
| P, % | 0.56 | 0.54 | 0.55 | 0.55 |
| Mn, mg/kg | 66.40 | 68.60 | 60.80 | 76.20 |
| Fe, mg/kg | 243.00 | 171.00 | 290.00 | 166.00 |
| Zn, mg/kg | 190.00 | 178.00 | 152.00 | 193.00 |
| Cu, mg/kg | 25.10 | 25.60 | 102.00 | 126.00 |
| Amino acids, % | | | | |
| Arg | 1.23 | 1.17 | 1.24 | 1.22 |
| His | 0.55 | 0.54 | 0.56 | 0.55 |
| Ile | 0.90 | 0.90 | 0.91 | 0.90 |
| Leu | 1.93 | 1.94 | 1.93 | 1.92 |
| Lys | 1.33 | 1.49 | 1.42 | 1.41 |
| Met | 0.35 | 0.39 | 0.35 | 0.35 |
| Met + Cys | 0.65 | 0.69 | 0.65 | 0.65 |
| Phe | 1.04 | 1.04 | 1.06 | 1.03 |
| Thr | 0.87 | 0.85 | 0.85 | 0.86 |
| Trp | 0.21 | 0.21 | 0.21 | 0.22 |
| Val | 1.06 | 1.05 | 1.07 | 1.05 |

**Table 4.2. (cont.)**

[1]The diet containing added Cu was fortified with 100 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN); DDGS = distillers dried grains with solubles.

[2]Quantum Blue 5G (AB Vista, Marlborough, UK) contains 5,000 phytase units per gram. At 0.01% inclusion, the premix was calculated to provide 500 units of phytase per kilogram in the complete diet.

[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin $D_3$ as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin $B_{12}$, 0.03 mg; $_{D\text{-}}$pantothenic acid as $_{D\text{-}}$calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[4]AEE = acid hydrolyzed ether extract.

**Table 4.3.** Growth performance for pigs fed diets containing 0 or 5% choice white grease (CWG) and 0 or 100 mg/kg Cu from Cu hydroxychloride[1]

| Item | No Cu | | 100 mg/kg Cu[2] | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | -CWG | + CWG | -CWG | + CWG | | CWG × Cu | CWG | Cu |
| d 0 to 14 | | | | | | | | |
| Initial body weight, kg | 6.12 | 6.14 | 6.14 | 6.14 | 0.056 | 0.868 | 0.810 | 0.801 |
| ADG[3], kg | 0.101 | 0.136 | 0.155 | 0.138 | 0.017 | 0.137 | 0.627 | 0.112 |
| ADFI[3], kg | 0.179 | 0.206 | 0.239 | 0.212 | 0.017 | 0.039 | 0.997 | 0.013 |
| G:F[3] | 0.562 | 0.664 | 0.646 | 0.647 | 0.095 | 0.335 | 0.323 | 0.468 |
| Final body weight, kg | 7.54 | 8.04 | 8.31 | 8.07 | 0.342 | 0.139 | 0.607 | 0.111 |
| d 14 to 28 | | | | | | | | |
| ADG, kg | 0.412 | 0.402 | 0.455 | 0.450 | 0.023 | 0.756 | 0.901 | 0.048 |
| ADFI, kg | 0.714 | 0.679 | 0.821 | 0.769 | 0.035 | 0.980 | 0.168 | 0.001 |
| G:F | 0.580 | 0.594 | 0.551 | 0.589 | 0.050 | 0.359 | 0.212 | 0.264 |
| Final body weight, kg | 13.30 | 13.67 | 14.68 | 14.37 | 0.686 | 0.472 | 0.422 | 0.014 |
| d 28 to 40 | | | | | | | | |

**Table 4.3. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ADG, kg | 0.639 | 0.641 | 0.629 | 0.634 | 0.024 | 0.929 | 0.820 | 0.616 |
| ADFI, kg | 1.073 | 0.999 | 1.085 | 1.053 | 0.053 | 0.597 | 0.184 | 0.400 |
| G:F | 0.599 | 0.643 | 0.582 | 0.608 | 0.019 | 0.519 | 0.020 | 0.071 |
| Final body weight, kg | 20.97 | 21.36 | 22.23 | 21.98 | 0.536 | 0.882 | 0.739 | 0.213 |
| d 40 to 44[4] | | | | | | | | |
| ADG, kg | 0.538 | 0.601 | 0.637 | 0.685 | 0.054 | 0.859 | 0.206 | 0.041 |
| ADFI, kg | 1.289 | 1.235 | 1.309 | 1.222 | 0.062 | 0.715 | 0.128 | 0.936 |
| G:F | 0.420 | 0.487 | 0.489 | 0.564 | 0.034 | 0.901 | 0.048 | 0.042 |
| Final body weight, kg | 23.12 | 23.76 | 24.78 | 24.72 | 0.841 | 0.846 | 0.496 | 0.077 |
| d 0 to 40 | | | | | | | | |
| ADG, kg | 0.371 | 0.380 | 0.400 | 0.396 | 0.018 | 0.309 | 0.905 | 0.204 |
| ADFI, kg | 0.617 | 0.607 | 0.680 | 0.633 | 0.023 | 0.269 | 0.093 | 0.013 |
| G:F | 0.599 | 0.622 | 0.588 | 0.621 | 0.032 | 0.693 | 0.314 | 0.818 |
| d 0 to 44 | | | | | | | | |
| ADG, kg | 0.386 | 0.401 | 0.424 | 0.422 | 0.017 | 0.264 | 0.874 | 0.081 |

**Table 4.3. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ADFI, kg | 0.756 | 0.743 | 0.817 | 0.764 | 0.023 | 0.249 | 0.064 | 0.023 |
| G:F | 0.515 | 0.542 | 0.522 | 0.553 | 0.027 | 0.709 | 0.126 | 0.716 |

[1]Data are least squares means of 8 observations (pen as the experimental unit; 5 pigs per pen) for all treatments.

[2]The diet containing added Cu was fortified with 100 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[3]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

[4]D 40 to 44 = heat stress period.

**Table 4.4.** Diarrhea score and frequency of diarrhea for pigs fed diets containing 0 or 5% choice white grease (CWG) and 0 or 100 mg/kg Cu from Cu hydroxychloride[1]

| | No Cu | | 100 mg of Cu[2]/kg | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | -CWG | + CWG | -CWG | + CWG | SEM | CWG × Cu | CWG | Cu |
| Diarrhea score[3] | | | | | | | | |
| d 0 to 14 | 2.00 | 2.18 | 1.84 | 1.73 | 0.088 | 0.117 | 0.689 | 0.002 |
| d 14 to 28 | 2.29 | 2.25 | 1.84 | 1.79 | 0.112 | 0.937 | 0.694 | <0.001 |
| d 28 to 40 | 1.69 | 1.56 | 1.60 | 1.35 | 0.086 | 0.473 | 0.038 | 0.101 |
| d 40 to 44 | 1.25 | 1.25 | 1.25 | 1.13 | 0.192 | 0.667 | 0.667 | 0.667 |
| d 0 to 40 | 1.99 | 2.00 | 1.76 | 1.62 | 0.067 | 0.289 | 0.327 | <0.001 |
| d 0 to 44 | 1.81 | 1.81 | 1.63 | 1.50 | 0.062 | 0.235 | 0.266 | <0.001 |
| Frequency of diarrhea | | | | | | | | |
| d 0 to 14 | | | | | | | | |
| Pen days[4] | 56 | 56 | 56 | 56 | | | | |
| Frequency[5] | 28.57 | 33.93 | 23.21 | 17.86 | - | | 0.242 | |
| d 14 to 28 | | | | | | | | |

**Table 4.4. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Pen days | 56 | 56 | 56 | 56 | | |
| Frequency | 25.00 | 32.14 | 8.93 | 14.29 | - | 0.009 |
| d 28 to 40 | | | | | | |
| Pen days | 48 | 48 | 48 | 48 | | |
| Frequency | 2.08 | 4.17 | 2.08 | 0.00 | - | 0.564 |
| d 40 to 44 | | | | | | |
| Pen days | 16 | 16 | 16 | 16 | - | |
| Frequency | 0.00 | 0.00 | 0.00 | 0.00 | | <0.999 |
| d 0 to 40 | | | | | | |
| Pen days | 160 | 160 | 160 | 160 | - | |
| Frequency | 19.38 | 24.38 | 11.88 | 11.25 | | 0.003 |
| d 0 to 44 | | | | | | |
| Pen days | 176 | 176 | 176 | 176 | - | |
| Frequency | 17.61 | 22.16 | 10.80 | 10.23 | | 0.004 |

[1]Data are least squares means of 8 observations for all treatments.

**Table 4.4. (cont.)**

[2]The diet containing added Cu was fortified with 100 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[3]Diarrhea score = 1, normal feces; 2, moist feces; 3, mild diarrhea; 4, severe diarrhea; 5, watery diarrhea.

[4]Pen days = number of pens × the number of days assessing diarrhea scores.

[5]Frequency = (number of pen days with diarrhea scores ≥ 3/pen days)*100.

**Table 4.5.** Blood characteristics for pigs fed diets containing 0 or 5% choice white grease (CWG) and 0 or 100 mg/kg Cu from Cu hydroxychloride[1]

| | No Cu | | 100 mg of Cu[2]/kg | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | - CWG | + CWG | - CWG | + CWG | SEM | CWG | Cu | CWG × Cu |
| d 14 | | | | | | | | |
| BUN[3], mg/dL | 10.50 | 7.86 | 8.63 | 8.50 | 1.66 | 0.243 | 0.485 | 0.275 |
| Total protein, g/dL | 5.00 | 4.77 | 4.97 | 5.09 | 0.22 | 0.763 | 0.424 | 0.312 |
| Albumin, g/dL | 2.48 | 2.60 | 2.71 | 2.69 | 0.11 | 0.460 | 0.048 | 0.371 |
| TNF-α[4], pg/mL | 181.44 | 168.54 | 129.06 | 144.13 | 18.17 | 0.952 | 0.039 | 0.436 |
| IgG, mg/mL | 20.75 | 21.10 | 17.99 | 18.67 | 3.44 | 0.882 | 0.457 | 0.963 |
| Peptide YY, ng/mL | 1.33 | 1.63 | 1.57 | 1.99 | 0.20 | 0.038 | 0.082 | 0.732 |
| d 28 | | | | | | | | |
| BUN, mg/dL | 8.00 | 9.63 | 7.25 | 7.25 | 0.95 | 0.398 | 0.111 | 0.398 |
| Total protein, g/dL | 4.88 | 4.80 | 4.76 | 4.79 | 0.13 | 0.850 | 0.636 | 0.705 |
| Albumin, g/dL | 2.63 | 2.69 | 2.75 | 2.70 | 0.09 | 0.931 | 0.345 | 0.439 |
| TNF-α, pg/mL | 216.91 | 202.44 | 188.41 | 190.62 | 19.35 | 0.770 | 0.341 | 0.691 |

**Table 4.5. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| IgG, mg/mL | 18.96 | 18.88 | 16.04 | 18.70 | 5.29 | 0.773 | 0.809 | 0.798 |
| Peptide YY, ng/mL | 1.33 | 1.13 | 1.32 | 1.35 | 0.15 | 0.513 | 0.416 | 0.337 |
| d 40 | | | | | | | | |
| BUN, mg/dL | 10.92 | 12.04 | 11.67 | 10.79 | 0.83 | 0.845 | 0.696 | 0.126 |
| Total protein, g/dL | 5.17 | 5.08 | 5.07 | 5.02 | 0.16 | 0.619 | 0.557 | 0.892 |
| Albumin, g/dL | 2.86 | 2.99 | 3.08 | 3.00 | 0.09 | 0.647 | 0.090 | 0.127 |
| TNF-α, pg/mL | 175.88 | 156.89 | 168.36 | 171.75 | 8.70 | 0.386 | 0.681 | 0.217 |
| IgG, mg/mL | 16.73 | 12.94 | 16.32 | 14.74 | 2.95 | 0.322 | 0.795 | 0.682 |
| Peptide YY, ng/mL | 1.41 | 1.39 | 1.28 | 1.26 | 0.11 | 0.832 | 0.115 | 0.975 |
| d 44 | | | | | | | | |
| BUN, mg/dL | 12.13 | 11.50 | 11.63 | 10.88 | 0.70 | 0.201 | 0.293 | 0.906 |
| Total protein, g/dL | 5.23 | 5.13 | 5.16 | 4.98 | 0.15 | 0.331 | 0.471 | 0.768 |
| Albumin, g/dL | 2.84 | 3.14 | 3.02 | 2.94 | 0.07 | 0.856 | 0.110 | 0.010 |
| TNF-α, pg/mL | 114.09 | 117.40 | 110.63 | 114.34 | 7.99 | 0.670 | 0.692 | 0.981 |
| IgG, mg/mL | 6.39 | 4.87 | 4.98 | 5.24 | 0.66 | 0.354 | 0.449 | 0.193 |

**Table 4.5. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Peptide YY, ng/mL | 1.19 | 0.98 | 0.95 | 0.96 | 0.13 | 0.360 | 0.266 | 0.333 |

[1]Data are least squares means of 8 observations for all treatments.

[2]The diet containing added Cu was fortified with 100 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[3]BUN = blood urea N.

[4]TNF-α = tumor necrosis factor-α.

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# CHAPTER 5: EFFECTS OF COPPER HYDROXYCHLORIDE AND INCREASING CONCENTRATIONS OF DIETARY FAT ON GROWTH PERFORMANCE, TOTAL TRACT ENDOGENOUS LOSS OF FAT, AND APPARENT TOTAL TRACT DIGESTIBILITY OF FAT BY GROWING PIGS


## ABSTRACT

Two experiments were conducted to test the hypothesis that Cu from Cu hydroxychloride improves gain:feed (G:F) when fed to pigs by increasing apparent total tract digestibility (ATTD) of fat. In experiment 1, 144 pigs (15.40 ± 2.39 kg) were allotted to 6 treatments with 2 pigs per pen and 12 replicate pens per diet. Pigs were fed diets with increasing concentrations of extracted fat by adding 2.0, 4.0, or 6.0% choice white grease (CWG) to a diet based on corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS) which contained no CWG. Two additional diets were formulated by adding 150 mg/kg of Cu from Cu hydroxychloride to the diet without added CWG and to the diet with 2% added CWG. Diets were fed for 4 wk. Results indicated that supplementation of diets with either CWG or Cu hydroxychloride improved ($P \leq 0.05$) G:F of pigs, and the improvement obtained by Cu hydroxychloride supplementation was similar to the improvement in G:F obtained by adding 2.8 to 3.8% CWG to the diets. In experiment 2, 80 pigs (18.24 ± 1.81 kg) were housed individually in metabolism crates and randomly allotted to 10 diets with 8 replicate pigs per diet. Two basal diets based on corn, SBM, corn bran, cornstarch, and casein were formulated. The only difference between the 2 diets was that one diet contained no Cu hydroxychloride; whereas, the other diet contained 150 mg/kg of Cu from Cu hydroxychloride. Six additional diets were formulated by adding 15, 30, or 45% DDGS to both basal diets, and the last 2 diets were

formulated by adding 2% CWG to diets containing 15% DDGS without or with Cu hydroxychloride. Feces were collected using the marker to marker approach with 5-d adaptation and 4-d collection periods. Supplementation of Cu hydroxychloride to diets improved ($P \leq 0.05$) the ATTD of acid hydrolyzed ether extract (AEE), but did not affect ATTD of DM or GE. Supplementation of Cu hydroxychloride to diets also reduced ($P \leq 0.05$) total tract endogenous loss of fat, but did not affect true total tract digestibility of AEE. This indicates that the increased G:F of pigs that was observed in Exp. 1 as a result of Cu supplementation to diets was not due to improved ATTD of GE or AEE, but may be a result of Cu influencing post-absorptive lipid metabolism. In conclusion, supplementation of Cu from Cu hydroxychloride to diets improved G:F in pigs, which may be due to effects of Cu on post-absorptive metabolism of energy and fat.
**Key words:** copper, copper hydroxychloride, digestibility, fat, growth performance, pigs

## INTRODUCTION

Dietary lipids are commonly included in diets for pigs to increase energy density, reduce dust, and improve palatability of the diets (Kerr et al., 2015). Pigs easily digest dietary lipids, and inclusion of dietary lipids to diets usually improves gain:feed (**G:F**) of pigs due to the high ME in lipids (Adeola et al., 2013). However, digestibility of fat may vary due to differences in the age of the animal, degree of saturation of fatty acids, carbon chain length, free fatty acid concentration, form of dietary fat (i.e., extracted or intact), and dietary inclusion rate of fat in the diet (Cera et al., 1988; Kellner and Patience, 2017). Most published values for the digestibility of fat are based on apparent total tract digestibility (**ATTD**), but to determine the true total tract

digestibility (**TTTD**) of fat, values for apparent digestibility need to be corrected for the endogenous loss of fat (Kil et al., 2010).

Supplementing Cu to diets fed to weanling pigs at 100 to 250 mg/kg may reduce post-weaning diarrhea and also improve average daily gain (**ADG**) and average daily feed intake (**ADFI**; Cromwell et al., 1998; Perez et al., 2011; Espinosa et al., 2017). Addition of Cu at 250 mg/kg in diets for weanling pigs containing 5% animal fat improved growth performance, and it was speculated that this was due to the ability of Cu to improve animal fat utilization and enzymatic activity (Dove and Haydon, 1992; Dove, 1993). Dietary Cu is also believed to have bacteriostatic and bactericidal properties (Stahly et al., 1980) because Cu may reduce bacterial populations in the intestine, which may affect growth and community structure of microorganisms in the cecum and colon (Højberg et al., 2005). It is, therefore, possible that inclusion of Cu in diets alters microbial activity and synthesis of endogenous microbial fat in the hindgut, but to our knowledge, effects of Cu on the endogenous loss of fat have not been reported. Therefore, the objective of this work was to test the hypothesis that inclusion of 150 mg/kg Cu from Cu hydroxychloride to diets based on corn, soybean meal (**SBM**), and distillers dried grains with solubles (**DDGS**) improves G:F of pigs by increasing the ATTD of fat due to reduced endogenous loss of fat.

## MATERIALS AND METHODS

Protocols for 2 experiments were submitted to the Institutional Animal Care and Use Committee at the University of Illinois, and both protocols were approved prior to initiation of the experiments. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used. In both experiments, antibiotic

growth promoters were not included in the diets, and pharmacological levels of Zn were also not used.

***Experiment 1: Effects of Cu on Growth Performance***

A total of 144 growing pigs (15.40 ± 2.39 kg) were used in a 4-wk experiment. Pigs were randomly allotted to 6 dietary treatments with 2 pigs per pen for a total of 12 replicate pens per treatment. Diets were formulated based on corn, SBM, and DDGS to meet current estimates for nutrient requirements for 11 to 25 kg pigs (Table 5.1; NRC, 2012). A basal diet was formulated based on corn, SBM, and DDGS which contained no choice white grease (**CWG**). Three diets with increasing concentrations of extracted fat were then formulated by adding 2.0, 4.0, or 6.0% CWG to the basal corn-SBM-DDGS diet. Two additional diets were formulated by adding 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN) to the diet without added CWG and to the diet with 2% added CWG.

Individual pig weights were recorded at the beginning of the experiment, after 2 wk, and at the conclusion of the experiment. Feed addition was recorded daily and the weight of feed left in the feeder was recorded after 2 wk and at the conclusion of the experiment. At the end of the 28-d experiment, data were summarized to calculate ADG, ADFI, and G:F within each pen and treatment group. Data were summarized for the initial 14 d, the final 14 d, and for the entire experiment.

All diet samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. Diets were analyzed for dry matter (**DM**; Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007), and gross energy (**GE**) was determined using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL). Acid hydrolyzed ether extract (**AEE**) was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl

Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY), and crude protein was determined using the combustion procedure (Method 990.03; AOAC Int., 2007). Amino acids were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard (Method 982.30 E (a, b, c); AOAC Int., 2007). Minerals were analyzed by inductively coupled plasma optical emissions spectrometry using an internally validated method (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B[b]; AOAC Int., 2007). Analyses for AA and minerals were conducted at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Colombia, and all other analyses were conducted in the Monogastric Nutrition Laboratory at the University of Illinois at Urbana-Champaign, Urbana.

Data were analyzed using the Mixed Procedure of SAS with the pen as the experimental unit. Homogeneity of the variances was confirmed using the UNIVARIATE procedure in SAS (SAS Institute Inc., Cary, NC). Treatment means were calculated using LSMeans in SAS. Linear and quadratic effects of increasing concentration of CWG were determined using orthogonal CONTRAST statements. Regression equations to estimate the improvement in G:F that was observed for each unit of CWG in the diets were developed using the REG procedure in SAS. Single degree of freedom contrasts were used to determine effects of Cu supplementation on growth performance by comparing the diet without added CWG and no Cu hydroxychloride with the diet containing Cu hydroxychloride without added CWG. Single degree of freedom contrasts were also used between the diet containing 2% CWG without Cu hydroxychoride and the diet containing 2% CWG with Cu hydroxychloride. The CWG equivalency of Cu from Cu

hydroxychloride was estimated using the prediction equation derived from the regression analysis. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

***Experiment 2: Digestibility of Gross Energy and Acid Hydrolyzed Ether Extract***

The same batches of corn, SBM, DDGS, and corn bran were used in the manufacturing of 10 diets (Table 5.2). Two basal diets based on corn, SBM, corn bran, cornstarch, and casein were formulated to meet requirements for all nutrients for 11 to 25 kg pigs (NRC, 2012). No extracted fat was added to these diets, but the intrinsic fat from corn, SBM, and corn bran resulted in these diets containing approximately 2% AEE (Tables 5.3 and 5.4). The only difference between the 2 diets was that one diet contained no Cu hydroxychloride, whereas the other diet contained 150 mg/kg of Cu from Cu hydroxychloride. Vitamins and minerals were included in both diets to meet or exceed current requirement estimates (NRC, 2012). Six additional diets were formulated by adding 15, 30, or 45% DDGS to both basal diets. Because of the intrinsic fat in DDGS, the diets with 15, 30, and 45% DDGS contained approximately 3, 4, and 5% AEE, respectively. The concentration of corn bran was reduced as DDGS was increased to maintain a constant concentration of NDF among diets (Kim et al., 2013). Likewise, inclusion of crystalline AA was adjusted to maintain constant concentrations of standardized ileal digestible AA among diets. Two additional diets were formulated by adding 2% CWG to diets containing 15% DDGS without or with Cu hydroxychloride. Thus, there were 5 diets without Cu hydroxychloride and 5 diets with Cu hydroxychloride. A sample of each diet was collected at the time of diet mixing and this sample was used for analysis.

A total of 80 growing pigs (18.24 ± 1.81 kg) were randomly allotted to the 10 diets with 8 replicate pigs per diet. Eight pigs were assigned to each treatment group in a randomized

complete block design with 2 blocks of 40 pigs and 4 replicate pigs per diet within each block. Pigs were placed in individual metabolism crates that were equipped with a self-feeder, a nipple waterer, a slatted floor, and a urine pan to allow for the total, but separate, collection of urine and fecal materials. All diets were fed in meal form. Pigs were limit fed at 3.2 times the energy requirement for maintenance (i.e., 197 kcal/kg × $BW^{0.60}$; NRC, 2012), which was provided each day in 2 equal meals at 0800 and 1600 h. Pigs had *ad libitum* access to water during the experiment. Feed consumption was recorded daily. The initial 5 d was considered the adaptation period to the diet, whereas fecal materials were collected during the following 4 days according to standard procedures using the marker to marker approach (indigo carmine included at less than 10% of feed allowance; Adeola, 2001). Fecal samples were stored at -20°C immediately after collection.

Fecal samples were thawed and mixed within pig and diet, and then dried at 50°C in a forced air drying oven prior to analysis. All ingredient, diet, and fecal samples were ground through a 1-mm screen in a Wiley mill before analysis. Fecal, ingredient, and diet samples were analyzed for DM, GE, and AEE as described for Exp. 1. Diets and ingredients were also analyzed for ash, crude protein, amino acids, and minerals as described for Exp. 1. Ingredient samples were also analyzed for NDF and ADF using Ankom Technology method 12 (Ankom$^{2000}$ fiber analyzer, Ankom Technology, Macedon, NY) and diets were analyzed for NDF as well. Following analysis, data were summarized and the ATTD of DM, AEE, and GE was calculated for each diet (Adeola, 2001).

Data were analyzed as a randomized complete block design with the pig as the experimental unit. Homogeneity of the variances was confirmed, and linear and quadratic effects of increasing concentration of DDGS without or with Cu supplementation were determined as

described for Exp. 1. Single degree of freedom contrasts were used to determine effects of Cu supplementation on ATTD of DM, GE, and AEE by comparing the diet containing 15% DDGS, 2% CWG, and no Cu hydroxychloride with the diet containing 15% DDGS and 2% CWG supplemented with Cu hydroxychloride. Single degree of freedom contrasts were also used between diets containing 0, 15, 30, or 45% DDGS without Cu hydroxychloride and diets containing 0, 15, 30, or 45% DDGS with Cu hydroxychloride, respectively. The REG procedure of SAS was used to estimate the Y-intercept for determining endogenous loss of AEE, and the slope was used to determine TTTD of AEE (Kil et al., 2010). Intercepts and slopes were compared between diets without and with Cu supplementation using confidence intervals derived from the SE of the respective regression coefficients (Dilger and Adeola, 2006). Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

## RESULTS

### *Experiment 1: Effects of Cu on Growth Performance*

Diet analyses indicated that the intended concentrations of AEE and Cu were present in all diets and concentrations of other nutrients were not affected by dietary treatment (Table 5.1). Average daily feed intake of pigs linearly decreased ($P < 0.05$) as the concentration of CWG increased in the diets from d 14 to 28 and in the overall experimental period (Table 5.5). A tendency for a quadratic decrease ($P < 0.10$) in ADFI was also observed from d 0 to 14 and from d 14 to 28, and a quadratic decrease ($P < 0.05$) in ADFI for the overall phase as CWG inclusion increased in the diets was observed. There was a tendency for a linear increase ($P < 0.10$) in G:F of pigs from d 0 to 14, and a linear increase ($P < 0.05$) in G:F was observed from d 14 to 28 and

for the overall experiment as the concentration of CWG increased in the diets. There was no effect of increasing concentrations of CWG on ADG of pigs.

Supplementation of 150 mg/kg of Cu from Cu hydroxychloride to the diet without added CWG improved ($P < 0.05$) final body weight, ADG, and G:F of pigs from d 14 to 28 and for the entire experimental period (Table 5.5). Supplementation of 150 mg/kg of Cu from Cu hydroxychloride to the diet with 2% added CWG reduced ($P < 0.05$) ADFI, but improved ($P < 0.05$) G:F of pigs from d 14 to 28 and for the overall phase. For the overall experimental period, prediction equations for G:F in diets containing CWG were derived from regression analysis (Table 5.6), which indicated that the intercept and slope were 0.526 and 0.022, respectively. Therefore, G:F increases by 0.022 for each percentage unit change of CWG inclusion in the diet. From these prediction equations, it was calculated that the improvement obtained by Cu hydroxychloride supplementation was similar to the improvement in G:F obtained by adding 2.8 to 3.8% CWG to the diets (Table 5.7).

***Experiment 2: Digestibility of Gross Energy and Acid Hydrolyzed Ether Extract***

The intended concentrations of AEE, NDF, and Cu were present in all diets (Table 5.4). All pigs consumed their diets and remained healthy throughout the experiment. Values for ATTD of DM, GE, and AEE linearly increased ($P < 0.05$) as the concentration of DDGS increased in diets without or with 150 mg/kg Cu as Cu hydroxychloride (Table 5.8). A tendency ($P < 0.10$) for a quadratic increase in the ATTD of GE as the concentration of DDGS increased was also observed in diets without Cu hydroxychloride. The ATTD of DM and GE in diets containing Cu hydroxychloride was not different from values for diets without supplemental Cu hydroxychloride. However, supplementation of Cu hydroxychloride to the control diet and to the diet with 15% DDGS improved ($P < 0.05$) the ATTD of AEE. Likewise, the ATTD of AEE in

the diet containing 30% DDGS with supplemental Cu tended to be greater ($P < 0.10$) compared with the diet containing 30% DDGS and no Cu. Addition of Cu hydroxychloride to the diet containing 15% DDGS with 2% CWG did not affect ATTD of DM and GE. Likewise, no difference in the ATTD of AEE was observed between the diet with 2% CWG and no Cu hydroxychloride and the diet with 2% CWG and Cu hydroxychloride.

The estimated endogenous loss of fat for diets with supplemental Cu as Cu hydroxychloride was 7.14 g/kg dry matter intake (**DMI**), and this value tended to be less ($P < 0.10$) compared with the estimated endogenous loss of fat for diets without Cu hydroxychloride (11.23 g/kg DMI; Table 5.9). However, values for the TTTD of AEE in diets without or with Cu hydroxychloride were not different.

## DISCUSSION

The digestibility of lipids from ingredients of animal origin increases with age, and is less compared with the digestibility of lipids from plant feed ingredients (Frobish et al., 1970). Pigs weaned at 21 d of age also have difficulty digesting and absorbing dietary fat efficiently due to insufficient production of bile salt and pancreatic lipase (Cera et al., 1990; Adeola et al., 2013; Kellner and Patience, 2017). However, during the initial 2 to 4 wk post-weaning, pigs gradually develop the ability to digest dietary lipids (Stahly, 1984; Jung et al., 2003). The linear increase in G:F of pigs fed diets with increasing concentration of CWG is likely a result of the increased energy density of diets that is due to increased inclusion of CWG in the diets. The reduced ADFI that was observed in Exp. 1 as CWG was increased in the diet without a change in ADG is the reason for the increase in G:F that was observed as CWG was added to the diet. This observation

is in agreement with previous data (De la Llata et al., 2001). Choice white grease is efficiently utilized by pigs because of its high digestibility and low heat increment compared with protein and carbohydrates (Forbes and Swift, 1944; Noblet et al., 1994). The reduced heat increment that is a result of adding CWG to diets may result in a greater percentage of the dietary energy being available for maintenance and growth (Swift and Black, 1949; Coffey et al., 1982).

The greater ADG and G:F of pigs fed diets with 0 or 2% CWG and 150 mg/kg of Cu from Cu hydroxychloride compared with pigs fed diets with the same quantities of CWG and no Cu hydroxychloride may be due to the bacteriostatic properties of Cu. The antibacterial properties of Cu may improve intestinal health and growth performance of pigs (Højberg et al., 2005). The observed improvement in ADG and G:F upon Cu supplementation may also be due to the effect of Cu on post-absorptive lipid metabolism (Lei et al., 2017). Supplementation of 250 mg/kg of Cu to diets improved ADG of weanling pigs, and it was speculated that this was due to increased digestibility or absorption of fatty acids caused by the Cu in the diet (Dove and Haydon, 1992). Addition of 45 mg/kg of Cu to diets also improved body mass gain in rabbits by upregulating mRNA transcription of fatty acid binding protein and fatty acid transport proteins (Lei et al., 2017), indicating an increase in cellular uptake of fatty acids (Chen et al., 2016). Therefore, supplementation of Cu hydroxychloride to diets may have improved CWG utilization in pigs by improving lipid uptake, transport, and oxidation, but further research is needed to verify this speculation.

In Exp. 2, corn bran was included in all diets to equalize the concentration of NDF among diets. Natural fiber in feed ingredients is more effective in stimulating microbial fat synthesis compared with purified fiber (Sambrook, 2007; Kil et al., 2010). Although the concentration of NDF was equalized among diets, the linear increase that was observed in the

ATTD of DM and GE as DDGS concentration increased in the diets is due to the greater digestibility of dietary fiber in DDGS compared with corn bran (Gutierrez et al., 2014). The observed linear increase in ATTD of AEE as dietary fat concentration increased is in agreement with previous data (Jørgensen et al., 1993; Kil et al., 2010). This response is a result of the fact that the endogenous AEE contributes a larger proportion to fecal AEE output in diets that have low AEE concentration compared with diets with greater AEE concentration (Kil et al., 2010; Kim et al., 2013).

The linear regression procedure was used to estimate endogenous loss and TTTD of AEE in diets due to the linear relationship between apparent total tract digested AEE and dietary AEE intake (Fan et al., 2001). The estimated TTTD of intact fat in diets used in this experiment (82.8%) was in agreement with TTTD values for fat from corn germ (84.1%; Kil et al., 2010), but greater than TTTD values reported by Kim et al. (2013) and Adams and Jensen (1984) for DDGS (51.9%) and corn (77.6%), respectively. Improvements in technological treatment and further processing of corn co-products may have increased nutrient digestibility, and therefore may explain the greater TTTD of fat observed in this experiment compared with previous data. Moreover, diets used in this experiments contained fat that originated from 4 feed ingredients and not only from DDGS, which may have also influenced digestibility. Differences in digestibility may be caused by dietary fat source, diet composition, and daily DM intake (Jørgensen et al., 1992).

To our knowledge, no data demonstrating effects of Cu hydroxychloride on total endogenous loss of fat by pigs have been reported. The origin of endogenous fat includes excreted bile and desquamated cells from the mucosa (Jørgensen et al., 1993), but the majority of endogenous fat is synthesized in the hindgut and consists of microbial fat (Just et al., 1980; Kim

et al., 2013). Therefore, the observation that pigs had reduced endogenous loss of fat if diets were supplemented with Cu hydroxychloride likely is a result of the effect of Cu on changing the microbial population in the gastrointestinal tract of pigs (Højberg et al., 2005; Bikker et al., 2016). Dietary Cu reduces a number of bacterial species in the small intestine and in the cecum and colon of pigs (Ma et al., 2006; Jensen, 2016), and this may have reduced fermentation of fiber in the hindgut with a subsequent reduction in endogenous loss of fat due to reduced microbial synthesis (Eggum et al., 1982). Dietary intact fat from corn germ results in increased endogenous loss of fat compared with extracted fat because of a greater concentration of fiber in diets with corn germ, which promotes microbial growth in the hindgut (Bach Knudsen and Hansen, 2007; Kil et al., 2010). Supplementing diets with neomycin sulfate and bacitracin resulted in a reduction in fecal fat excretion, and therefore, improved ATTD of fat in diets based on potato starch, which is likely due to the modulating effects of antibiotics on intestinal microbes (Mason and Just, 1975). It is, therefore, likely that the ability of Cu hydroxychloride to modulate the microbial population in the hindgut is the reason for the reduced endogenous loss of fat observed in this experiment. The observation that there was no difference in TTTD of AEE between diets without and with Cu further indicates that the effects of Cu on ATTD of AEE was caused by a reduction in endogenous loss of AEE. The lack of a difference in the ATTD of AEE between the diet with 2% CWG and no Cu hydroxychloride and the diet with 2% CWG and Cu hydroxychloride indicates that Cu hydroxychloride had less impact on diets containing extracted fat and low amounts of fiber compared with diets containing more fiber.

The observation from Exp. 1 that Cu hydroxychloride improves G:F combined with the observation from Exp. 2 that TTTD of AEE is not increased by Cu hydroxychloride indicates that the increased G:F observed in Exp. 1 is a result of improved post-absorptive utilization of

AEE. Thus, it appears that Cu hydroxychloride in addition to modulating microbial populations in the hindgut of pigs also impacts certain aspects of energy metabolism. This may be a result of Cu influencing mRNA expression of proteins involved in fatty acid uptake and utilization (Coble et al., 2018), but it is also possible that the reduced microbial activity in the hindgut of pigs fed diets containing Cu hydroxychloride results in reduced heat increment and therefore more energy is used for gain. However, additional research is needed to confirm these hypotheses.

## CONCLUSION

In conclusion, supplementation of 150 mg/kg Cu as Cu hydroxychloride to diets improved G:F of 15 to 35 kg pigs. The improvement obtained by adding Cu hydroxychloride to the diets was similar to the improvement in G:F obtained by adding 2.8 to 3.8% CWG to the diets. However, the observed improvements were not a result of improved energy or AEE digestibility, because the TTTD of AEE was not improved by Cu. Instead, the increased G:F may be a result of the effect of Cu on post-absorptive metabolism of energy and fat. The improved ATTD of AEE that was observed as Cu was added to diets was likely caused by reduced microbial synthesis of fat in the hindgut, and therefore reduced endogenous loss of fat.

## TABLES

**Table 5.1.** Composition of experimental diets, Exp. 1

| | Choice white grease, % | | | | Cu[1], 150 mg/kg | |
|---|---|---|---|---|---|---|
| Item | 0.0 | 2.0 | 4.0 | 6.0 | 0% CWG | 2% CWG |
| Ingredient | | | | | | |
| Ground corn | 38.55 | 38.55 | 38.55 | 38.55 | 38.522 | 38.522 |
| Soybean meal | 22.50 | 22.50 | 22.50 | 22.50 | 22.50 | 22.50 |
| Corn DDGS[1] | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 |
| Cornstarch | 6.00 | 4.00 | 2.00 | - | 6.00 | 4.00 |
| Choice white grease | - | 2.00 | 4.00 | 6.00 | - | 2.00 |
| Limestone | 1.53 | 1.53 | 1.53 | 1.53 | 1.53 | 1.53 |
| Dicalcium phosphate | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Copper hydroxychloride, 54% Cu | - | - | - | - | 0.028 | 0.028 |
| L-Lys HCl, 78% Lys | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| DL-Met | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| L-Thr | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |

**Table 5.1. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Phytase[2] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Analyzed values | | | | | | |
| Dry matter, % | 87.18 | 87.28 | 86.75 | 87.31 | 86.90 | 86.62 |
| Ash, % | 4.81 | 5.14 | 4.84 | 4.72 | 4.96 | 4.80 |
| Gross energy, kcal/kg | 4,011 | 4,104 | 4,250 | 4,359 | 4,001 | 4,090 |
| Crude protein, % | 22.16 | 21.07 | 21.48 | 21.48 | 22.25 | 21.76 |
| Acid hydrolyzed ether extract, % | 4.23 | 6.20 | 8.03 | 9.97 | 4.22 | 6.20 |
| Ca, % | 0.67 | 0.68 | 0.73 | 0.68 | 0.76 | 0.67 |
| P, % | 0.57 | 0.54 | 0.55 | 0.55 | 0.56 | 0.57 |
| Mn, mg/kg | 57.40 | 59.90 | 65.50 | 54.10 | 70.20 | 70.30 |
| Fe, mg/kg | 198.00 | 189.00 | 190.00 | 153.00 | 187.00 | 200.00 |
| Zn, mg/kg | 147.00 | 154.00 | 150.00 | 130.00 | 156.00 | 146.00 |
| Cu, mg/kg | 22.10 | 27.10 | 20.80 | 31.80 | 165.00 | 150.00 |

**Table 5.1. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Amino acids % | | | | | | |
| Arg | 1.21 | 1.18 | 1.24 | 1.23 | 1.26 | 1.24 |
| His | 0.59 | 0.58 | 0.60 | 0.60 | 0.61 | 0.60 |
| Ile | 0.91 | 0.89 | 0.93 | 0.92 | 0.95 | 0.93 |
| Leu | 1.99 | 1.95 | 1.99 | 2.03 | 2.04 | 2.01 |
| Lys | 1.42 | 1.45 | 1.42 | 1.40 | 1.48 | 1.46 |
| Met | 0.36 | 0.36 | 0.37 | 0.35 | 0.37 | 0.37 |
| Met + Cys | 0.37 | 0.35 | 0.37 | 0.36 | 0.39 | 0.38 |
| Phe | 1.04 | 1.02 | 1.06 | 1.07 | 1.09 | 1.07 |
| Thr | 0.85 | 1.22 | 0.85 | 0.85 | 0.89 | 0.88 |
| Trp | 0.24 | 0.23 | 0.24 | 0.20 | 0.25 | 0.25 |
| Val | 1.05 | 1.03 | 1.08 | 1.07 | 1.09 | 1.07 |

[1]DDGS = distillers dried grains with solubles; Cu as Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[2]Quantum Blue 5G; AB Vista, Marlborough, United Kingdom (5,000 units of phytase per gram).

**Table 5.1. (cont.)**

[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin $D_3$ as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin $B_{12}$, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

**Table 5.2.** Chemical composition of corn, soybean meal (SBM), distillers dried grains with solubles (DDGS), and corn bran used in Exp. 2

| Item | Corn | SBM | DDGS | Corn bran |
|---|---|---|---|---|
| Dry matter, % | 87.33 | 89.73 | 87.55 | 91.36 |
| Ash, % | 1.04 | 5.65 | 5.67 | 0.50 |
| Gross energy, kcal/kg | 3,873 | 4,192 | 4,507 | 4,098 |
| Crude protein, % | 7.68 | 44.66 | 27.27 | 5.84 |
| AEE[1], % | 4.18 | 1.51 | 10.74 | 1.54 |
| NDF[2], % | 7.01 | 8.14 | 36.26 | 65.94 |
| ADF[2], % | 1.99 | 5.11 | 13.73 | 15.32 |
| Ca, % | 0.01 | 0.31 | 0.02 | 0.01 |
| P, % | 0.24 | 0.60 | 0.92 | 0.07 |
| Cu (mg/kg) | 1.35 | 20.80 | 5.82 | 2.32 |
| Fe (mg/kg) | 36.00 | 184.00 | 59.50 | 13.40 |
| Mn (mg/kg) | 4.54 | 33.20 | 11.50 | 10.40 |
| Zn (mg/kg) | 46.60 | 66.50 | 57.80 | 10.90 |
| Amino acids, % | | | | |
| Arg | 0.30 | 3.10 | 1.26 | 0.19 |
| His | 0.20 | 1.13 | 0.70 | 0.12 |
| Ile | 0.26 | 2.07 | 1.08 | 0.17 |
| Leu | 0.80 | 3.36 | 2.85 | 0.46 |
| Lys | 0.25 | 2.74 | 0.74 | 0.30 |

**Table 5.2. (cont.)**

| | | | | |
|---|---|---|---|---|
| Met | 0.15 | 0.57 | 0.44 | 0.08 |
| Met + Cys | 0.32 | 1.18 | 0.92 | 0.18 |
| Phe | 0.34 | 2.25 | 1.28 | 0.23 |
| Thr | 0.25 | 1.66 | 0.97 | 0.32 |
| Trp | 0.06 | 0.59 | 0.20 | 0.04 |
| Val | 0.33 | 2.14 | 1.36 | 0.24 |

[1]AEE = acid hydrolyzed ether extract.

[2]NDF = neutral detergent fiber; ADF = acid detergent fiber.

**Table 5.3.** Ingredient composition of experimental diets, Exp. 2

| Item | Control | 15% DDGS[1] | 30% DDGS | 45% DDGS | 15% DDGS + 2% CWG[1] | Control+ Cu[1] | 15% DDGS + Cu | 30% DDGS + Cu | 45% DDGS + Cu | 15% DDGS + 2% CWG + Cu |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingredient | | | | | | | | | | |
| Ground corn | 30.58 | 30.58 | 30.58 | 30.58 | 30.58 | 30.552 | 30.552 | 30.552 | 30.552 | 30.552 |
| Soybean meal | 21.70 | 21.70 | 21.70 | 21.70 | 21.70 | 21.70 | 21.70 | 21.70 | 21.70 | 21.70 |
| Corn DDGS | - | 15.00 | 30.00 | 45.00 | 15.00 | - | 15.00 | 30.00 | 45.00 | 15.00 |
| Corn bran | 27.30 | 18.20 | 9.20 | - | 18.20 | 27.30 | 18.20 | 9.20 | - | 18.20 |
| Choice white grease | - | - | - | - | 2.00 | - | - | - | - | 2.00 |
| Cornstarch | 12.28 | 8.25 | 4.05 | - | 6.25 | 12.28 | 8.25 | 4.05 | - | 6.25 |
| Casein | 5.63 | 3.74 | 1.90 | - | 3.74 | 5.63 | 3.74 | 1.90 | - | 3.74 |
| Limestone | 0.88 | 1.20 | 1.49 | 1.56 | 1.20 | 0.88 | 1.20 | 1.49 | 1.56 | 1.20 |
| Dicalcium phosphate | 0.65 | 0.30 | - | - | 0.30 | 0.65 | 0.30 | - | - | 0.30 |
| Copper hydroxychloride, 54% Cu | - | - | - | - | - | 0.028 | 0.028 | 0.028 | 0.028 | 0.028 |
| L-Lys HCl, 78% Lys | 0.20 | 0.30 | 0.40 | 0.50 | 0.30 | 0.20 | 0.30 | 0.40 | 0.50 | 0.30 |
| DL-Met | 0.07 | 0.03 | - | - | 0.03 | 0.07 | 0.03 | - | - | 0.03 |
| L-Thr | 0.05 | 0.04 | 0.02 | - | 0.04 | 0.05 | 0.04 | 0.02 | - | 0.04 |

**Table 5.3. (cont.)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Phytase[2] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |

[1]DDGS = distillers dried grains with solubles; CWG = choice white grease; Cu = 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[2]Quantum Blue 5G; AB Vista, Marlborough, United Kingdom. (5,000 units of phytase per gram).

[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin $D_3$ as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin $B_{12}$, 0.03 mg; $_{D\text{-}}$pantothenic acid as $_{D\text{-}}$calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

**Table 5.4.** Analyzed composition of experimental diets, Exp. 2

| Item | Control | 15% DDGS[1] | 30% DDGS | 45% DDGS | 15% DDGS + 2% CWG[1] | Control + Cu[1] | 15% DDGS + Cu | 30% DDGS + Cu | 45% DDGS + Cu | 15% DDGS + 2% CWG + Cu |
|---|---|---|---|---|---|---|---|---|---|---|
| Dry matter, % | 87.34 | 88.49 | 87.96 | 87.23 | 88.52 | 88.55 | 88.38 | 87.85 | 87.06 | 88.48 |
| Ash, % | 3.23 | 4.15 | 4.50 | 5.95 | 4.16 | 3.33 | 4.38 | 4.98 | 5.81 | 4.43 |
| Gross energy, kcal/kg | 3,975 | 4,070 | 4,104 | 4,123 | 4,141 | 3,971 | 4,018 | 4,061 | 4,120 | 4,122 |
| Crude protein, % | 17.75 | 20.90 | 22.72 | 24.11 | 19.93 | 17.20 | 20.09 | 22.77 | 23.52 | 18.85 |
| Acid hydrolyzed ether extract, % | 1.84 | 2.51 | 3.61 | 4.91 | 4.60 | 2.02 | 2.67 | 4.02 | 4.75 | 4.87 |
| Neutral detergent fiber, % | 20.96 | 20.97 | 19.78 | 20.07 | 20.23 | 21.49 | 19.34 | 19.56 | 18.88 | 20.22 |
| Ca, % | 0.57 | 0.61 | 0.63 | 0.79 | 0.66 | 0.50 | 0.68 | 0.60 | 0.72 | 0.57 |
| P, % | 0.41 | 0.47 | 0.52 | 0.66 | 0.46 | 0.39 | 0.46 | 0.53 | 0.66 | 0.47 |
| Mn, mg/kg | 70.00 | 67.00 | 65.00 | 68.00 | 59.00 | 73.00 | 70.00 | 73.00 | 65.00 | 63.00 |
| Fe, mg/kg | 233.00 | 197.00 | 168.00 | 186.00 | 193.00 | 201.00 | 203.00 | 174.00 | 197.00 | 194.00 |
| Zn, mg/kg | 157.00 | 153.00 | 160.00 | 161.00 | 168.00 | 156.00 | 164.00 | 168.00 | 169.00 | 166.00 |
| Cu, mg/kg | 24.00 | 23.00 | 21.00 | 23.00 | 27.00 | 151.00 | 156.00 | 148.00 | 170.00 | 163.00 |
| Amino acids % | | | | | | | | | | |
| Arg | 0.99 | 1.10 | 1.21 | 1.31 | 1.09 | 0.94 | 1.07 | 1.19 | 1.35 | 1.17 |
| His | 0.52 | 0.57 | 0.62 | 0.66 | 0.56 | 0.52 | 0.58 | 0.61 | 0.67 | 0.60 |

**Table 5.4. (cont.)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ile | 0.85 | 0.90 | 0.98 | 1.01 | 0.89 | 0.86 | 0.94 | 0.97 | 1.03 | 0.96 |
| Leu | 1.60 | 1.84 | 2.08 | 2.26 | 1.82 | 1.61 | 1.87 | 2.05 | 2.32 | 1.92 |
| Lys | 1.33 | 1.38 | 1.49 | 1.48 | 1.38 | 1.33 | 1.47 | 1.43 | 1.45 | 1.39 |
| Met | 0.41 | 0.38 | 0.37 | 0.39 | 0.37 | 0.39 | 0.41 | 0.38 | 0.39 | 0.40 |
| Met + Cys | 0.68 | 0.67 | 0.73 | 0.81 | 0.66 | 0.62 | 0.72 | 0.75 | 0.81 | 0.71 |
| Phe | 0.25 | 0.29 | 0.36 | 0.42 | 0.29 | 0.23 | 0.31 | 0.37 | 0.42 | 0.31 |
| Thr | 0.92 | 1.01 | 1.10 | 1.16 | 0.99 | 0.92 | 1.03 | 1.09 | 1.19 | 1.06 |
| Trp | 0.81 | 0.84 | 0.87 | 0.89 | 0.81 | 0.79 | 0.85 | 0.86 | 0.92 | 0.87 |
| Val | 0.24 | 0.25 | 0.26 | 0.26 | 0.26 | 0.23 | 0.24 | 0.26 | 0.24 | 0.26 |

[1]DDGS = distillers dried grains with solubles; CWG = choice white grease; Cu = 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN).

**Table 5.5.** Growth performance for pigs fed diets containing 0, 2, 4, or 6% choice white grease (CWG) and 150 mg/kg Cu from Cu hydroxychloride without or with 2% CWG, Exp. 1[1]

| | CWG, % | | | | | *P*-value | | Cu[2], 150 mg/kg | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | 2.0 | 4.0 | 6.0 | SEM | Linear | Quadratic | - CWG | SEM[3] | *P*-value[3] | 2% CWG | SEM[4] | *P*-value[4] |
| D 0 to 14 | | | | | | | | | | | | | |
| Initial BW[5], kg | 15.274 | 15.494 | 15.416 | 15.520 | 0.356 | 0.109 | 0.279 | 15.338 | 0.360 | 0.765 | 15.341 | 0.356 | 0.465 |
| ADG[5], kg | 0.571 | 0.632 | 0.601 | 0.602 | 0.027 | 0.557 | 0.214 | 0.621 | 0.027 | 0.172 | 0.645 | 0.026 | 0.706 |
| ADFI[5], kg | 1.049 | 1.130 | 1.045 | 1.012 | 0.031 | 0.150 | 0.063 | 1.092 | 0.033 | 0.304 | 1.083 | 0.032 | 0.260 |
| G:F[5] | 0.548 | 0.561 | 0.574 | 0.593 | 0.020 | 0.074 | 0.868 | 0.568 | 0.019 | 0.431 | 0.597 | 0.019 | 0.152 |
| Final BW, kg | 23.267 | 24.342 | 23.833 | 23.950 | 0.566 | 0.312 | 0.163 | 24.058 | 0.579 | 0.199 | 24.375 | 0.563 | 0.955 |
| D 14 to 28 | | | | | | | | | | | | | |
| ADG, kg | 0.784 | 0.836 | 0.838 | 0.831 | 0.026 | 0.186 | 0.217 | 0.865 | 0.028 | 0.028 | 0.799 | 0.026 | 0.294 |
| ADFI, kg | 1.483 | 1.512 | 1.344 | 1.170 | 0.055 | <0.001 | 0.054 | 1.359 | 0.061 | 0.116 | 1.231 | 0.058 | <0.001 |
| G:F | 0.530 | 0.555 | 0.637 | 0.733 | 0.030 | <0.001 | 0.250 | 0.645 | 0.032 | 0.009 | 0.666 | 0.031 | 0.010 |
| Final BW, kg | 34.242 | 36.050 | 35.567 | 35.583 | 0.757 | 0.156 | 0.110 | 36.136 | 0.768 | 0.032 | 35.562 | 0.744 | 0.564 |
| D 0 to 28 | | | | | | | | | | | | | |
| ADG, kg | 0.677 | 0.734 | 0.720 | 0.717 | 0.020 | 0.248 | 0.136 | 0.744 | 0.020 | 0.020 | 0.722 | 0.019 | 0.661 |

**Table 5.5. (cont.)**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ADFI, kg | 1.266 | 1.321 | 1.195 | 1.091 | 0.039 | <0.001 | 0.032 | 1.225 | 0.043 | 0.450 | 1.157 | 0.041 | 0.003 |
| G:F | 0.536 | 0.557 | 0.608 | 0.666 | 0.018 | <0.001 | 0.320 | 0.609 | 0.018 | 0.003 | 0.632 | 0.017 | 0.003 |

[1]Data are least squares means of 12 observations for all treatments.

[2]Cu as Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN).

[3]SEM and *P*-values for contrast between diets without CWG and 0 or 150 mg/kg Cu from Cu hydroxychloride.

[4]SEM and *P*-values for contrast between diets with 2% CWG and 0 or 150 mg/kg Cu from Cu hydroxychloride.

[5]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

**Table 5.6.** Regression coefficients used for estimating the gain:feed (G:F) response to including choice white grease (CWG) in diets, Exp.1[1]

| | | SE | | *P*-value | | | |
|---|---|---|---|---|---|---|---|
| Dependent variable | Prediction equation | Intercept | Slope | Intercept | Slope | $R^2$ | RMSE[2] |
| G:F, d 0 to 14 | 0.546 + 0.0075 × (CWG, %) | 0.017 | 0.004 | <0.001 | 0.099 | 0.058 | 0.069 |
| G:F, d 14 to 28 | 0.510 + 0.0347 × (CWG, %) | 0.025 | 0.007 | <0.001 | <0.001 | 0.366 | 0.104 |
| G:F, d 0 to 28 | 0.526 + 0.0220 × (CWG, %) | 0.015 | 0.004 | <0.001 | <0.001 | 0.402 | 0.061 |

[1]Data were subjected to linear regression analysis with the percent inclusion of CWG as the independent variable and the G:F as the dependent variable. The regression coefficients indicate the change in G:F for each percentage point change of CWG included in the diet.

[2]RMSE = root mean square error.

**Table 5.7.** Choice white grease (CWG) equivalence of Cu from Cu hydroxychloride supplemented at 150 mg/kg to diets based on corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS) without or with 2% added CWG, Exp. 1[1]

| | Cu[2], 150 mg/kg | |
|---|---|---|
| Items | - CWG | 2% CWG |
| D 0 to 14 | | |
| Gain:feed | 0.568 | 0.597 |
| CWG equivalency, %[3] | 2.933 | 4.800 |
| D 14 to 28 | | |
| Gain:feed | 0.645 | 0.666 |
| CWG equivalency, % | 3.890 | 2.478 |
| D 0 to 28 | | |
| Gain:feed | 0.609 | 0.632 |
| CWG equivalency, % | 3.773 | 2.818 |

[1]Data are least squares means of 12 observations for all treatments.

[2]Cu as Cu hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN).

[3]Derived from the prediction equation used for estimating gain:feed in CWG.

**Table 5.8.** Apparent total tract digestibility (% ATTD) of dry matter, gross energy, and acid hydrolyzed ether extract (AEE) of pigs fed diets with increasing concentrations of distillers dried grains with solubles (DDGS) without or with 150 mg/kg Cu from Cu hydroxychloride, Exp. 2[1]

| Item | Dry matter[2,3] | Gross energy[2,3] | AEE[2,4,5,6] |
|---|---|---|---|
| DDGS without Cu | | | |
| Control | 81.9 | 81.7 | 41.4 |
| 15% DDGS | 81.8 | 81.2 | 38.5 |
| 30% DDGS | 83.8 | 82.1 | 53.6 |
| 45% DDGS | 84.6 | 83.7 | 63.9 |
| SEM | 0.553 | 0.641 | 5.051 |
| *P*-value | | | |
| Linear | <0.001 | 0.014 | 0.001 |
| Quadratic | 0.143 | 0.069 | 0.157 |
| DDGS with Cu | | | |
| Control | 81.2 | 80.9 | 52.7 |
| 15% DDGS | 81.8 | 81.0 | 55.0 |

**Table 5.8. (cont.)**

| | | | |
|---|---|---|---|
| 30% DDGS | 82.9 | 81.7 | 63.7 |
| 45% DDGS | 84.1 | 82.7 | 69.9 |
| SEM | 0.539 | 0.592 | 3.294 |
| *P*-value | | | |
| Linear | <0.001 | 0.004 | <0.001 |
| Quadratic | 0.454 | 0.301 | 0.531 |

[1]Data are least squares means of 8 observations for all treatments.

[2]15% DDGS + 2% CWG without Cu hydroxychloride vs. 15% DDGS + 2% CWG with Cu hydroxychloride: $P > 0.10$.

[3]Control, 15% DDGS, 30% DDGS, or 45% DDGS without Cu hydroxychloride vs. Control, 15% DDGS, 30% DDGS, or 45% DDGS with Cu hydroxychloride: $P > 0.10$.

[4]Control without Cu hydroxychloride vs. Control with Cu hydroxychloride: $P < 0.05$.

[5]15% DDGS without Cu hydroxychloride vs. 15% DDGS with Cu hydroxychloride: $P < 0.05$.

[6]30% DDGS without Cu hydroxychloride vs. 30% DDGS with Cu hydroxychloride: $P < 0.10$.

**Table 5.9.** Regression coefficients of apparent total tract digested acid hydrolyzed ether extract (AEE; g/kg dry matter intake) on dietary AEE intake (g/kg dry matter) of pigs fed diets with increasing concentrations of distillers dried grains with solubles (DDGS) without or with 150 mg/kg Cu from Cu hydroxychloride, Exp. 2[1]

| Item | Regression equation | Slope | | Intercept | | $R^2$ | Estimated TTTD[2] of AEE,% | Estimated EFL[2], g/ kg DMI[2] |
|---|---|---|---|---|---|---|---|---|
| | | SE | *P*-value | SE | *P*-value | | | |
| DDGS | y = 0.8282*x* – 11.23 | 0.0660 | <0.001 | 2.6737 | <0.001 | 0.85 | 82.8 | 11.23[y] |
| DDGS + Cu[3] | y = 0.8185*x* – 7.14 | 0.0404 | <0.001 | 1.6305 | <0.001 | 0.94 | 81.9 | 7.14[x] |

[x,y]Means within a row that do not have a common superscript tended to differ ($P < 0.10$).

[1]Regression analyses of apparent total tract digested AEE on dietary AEE intake was linear ($P < 0.01$).

[2]TTTD = true total tract digestibility; EFL = endogenous loss of fat; DMI = dry matter intake.

[3]Cu as Cu hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN.

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# CHAPTER 6: EFFECTS OF COPPER HYDROXYCHLORIDE AND DISTILLERS DRIED GRAINS WITH SOLUBLES ON INTESTINAL MICROBIAL PROTEIN CONCENTRATION AND DIGESTIBILITY OF ENERGY, CRUDE PROTEIN, AMINO ACIDS, AND ACID HYDROLYZED ETHER EXTRACT BY GROWING PIGS


## ABSTRACT

An experiment was conducted to test the hypothesis that Cu hydroxychloride improves nutrient digestibility and alters the concentration of microbial protein in the small intestine or large intestine by pigs fed a corn-soybean meal diet or a diet based on corn, soybean meal, and distillers dried grains with solubles (DDGS). Twenty four barrows (33.3 ± 3.4 kg) that had a T-cannula installed in the distal ileum, were allotted to a 2 × 2 factorial design with 2 levels of DDGS (0 or 45%) and 2 levels of Cu from Cu hydroxychloride (0 or 150 mg/kg). Pigs were allotted to a 2-period switch back design with 4 diets and 6 replicate pigs per diet in each period resulting in 12 replicate pigs per diet for the 2 periods. The initial 9 d of each period was considered an adaptation period to the experimental diets. For each period, feces were collected on d 10, 11, and 12, and ileal digesta were collected for 8 h on d 13 and 14. Results indicated that inclusion of 45% DDGS to diets reduced ($P < 0.05$) the apparent ileal digestibility (AID) and the apparent total tract digestibility (ATTD) of dry matter, gross energy, crude protein, and amino acids. Inclusion of DDGS to diets increased ($P < 0.05$) the concentration of microbial protein in the hindgut. However, concentrations of VFA from carbohydrate fermentation in ileal digesta and in feces from pigs fed the DDGS diets were not different from concentrations in pigs fed diets without DDGS. The AID and ATTD of crude protein were not affected by dietary Cu

concentrations, but, the AID and ATTD of acid hydrolyzed ether extract were greater ($P < 0.05$) in diets supplemented with Cu hydroxychloride compared with diets without Cu hydroxychloride. There was also a reduction ($P < 0.05$) in the concentration of microbial protein and concentration of acetate in feces when diets were supplemented with Cu hydroxychloride. In conclusion, supplementation of Cu hydroxychloride to diets improves AID and ATTD of acid hydrolyzed ether extract and reduces concentration of microbial protein in the large intestine.

**Keywords:** copper, copper hydroxychloride, digestibility, microbial protein, pigs

## INTRODUCTION

Copper is involved in several metabolic reactions including cellular respiration, tissue pigmentation, and connective tissue development (Turnlund, 1998; Gaetke and Chow, 2003), and is an essential component of several metalloenzymes such as cytochrome C oxidase and lysyl oxidase. The requirement for Cu for normal metabolism by weanling pigs is usually 5 to 6 mg/kg (NRC, 2012), but it is common practice to include additional Cu in diets for pigs to enhance growth performance (Ma et al., 2015). Several modes of action for the improved growth performance have been proposed, and one proposed mode of action is the ability of Cu to alter microbial activity (Højberg et al., 2005) and subsequently decrease deamination and decarboxylation of amino acids (Dierick et al., 1986). Copper may, therefore, reduce microbial degradation of amino acids, which may increase amino acid absorption (Dierick et al., 1986). The effect of dietary Cu has also been attributed to its bacteriostatic and bactericidal properties (Stahly et al., 1980), and clostridium, salmonella, total anaerobe bacteria, and coliform populations were reduced in the small intestine, as well as in the cecum and colon, upon Cu

supplementation (Ma et al., 2006; 2007; Song et al., 2013). Apparent total tract digestibility (**ATTD**) of acid hydrolyzed ether extract (**AEE**) was also improved if Cu hydroxychloride was supplemented to high fiber diets (Espinosa et al., 2019). It is, therefore, possible that pigs consuming diets with Cu hydroxychloride have reduced fermentation in the small intestine, which may increase amino acid and fat absorption. Therefore, an experiment was conducted to test the hypothesis that inclusion of 150 mg/kg of Cu from Cu hydroxychloride to diets fed to growing pigs improves apparent ileal digestibility (**AID**) and ATTD of AEE, and the AID of crude protein and amino acids. The second objective was to test the hypothesis that supplementing diets with Cu hydroxychloride can reduce the concentration of microbial protein in the small intestine or in the large intestine by pigs fed a corn-soybean meal diet or a diet based on corn, soybean meal, and distillers dried grains with solubles (**DDGS**).

## MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

Twenty four barrows (33.3 ± 3.4 kg) were surgically fitted with a T-cannula in the distal ileum (Stein et al., 1998). After surgery, pigs were housed individually in pens (1.2 × 1.5 m) that had a fully slatted tribar floor, a feeder, and a nipple drinker. Pigs were allotted to a 2-period switch back design with 4 diets and 6 replicate pigs per diet in each period resulting in 12 replicate pigs per diet for the 2 periods. Feed and water were available at all times. Pigs were randomly allotted to a 2 × 2 factorial design with 2 levels of DDGS (0 or 45%) and 2 levels of

Cu from Cu hydroxychloride (0 or 150 mg/kg). For diets without DDGS, two diets based on corn and soybean meal were formulated to meet the nutrient requirements for 25 to 50 kg pigs (Table 6.1; NRC, 2012). The only difference between the 2 diets was that one diet contained no Cu hydroxychloride whereas the other diet contained 150 mg/kg of Cu from Cu hydroxychloride. Two additional diets were formulated based on corn, soybean meal, and DDGS without and with Cu hydroxychloride. All diets contained 0.40% titanium dioxide as an indigestible marker.

The initial 9 d of each period was considered an adaptation period to the experimental diets. In each period, feces were collected by grab-sampling on d 10, 11, and 12. Ileal digesta samples were collected for 8 h on d 13 and 14 by attaching a 225-mL plastic bag to the cannula barrel using a cable tie. Bags were removed every 30 min, or whenever full, and replaced with a new bag. On the completion of the first experimental period, animals were deprived of feed overnight, and the following morning, a new experimental diet was offered. Ileal digesta and fecal samples were stored at – 20°C immediately after collection.

For the analysis of concentrations of volatile fatty acids (**VFA**), approximately 5 g of feces and ileal digesta were collected on d 10 and d 13, respectively. After collection, ileal digesta and fecal samples were placed in small wide-mouth plastic jars and samples were stabilized in 2*N* HCl and stored at – 20°C until analyzed for concentrations of VFA. Concentrations of VFA (acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate) were analyzed using a Hewlett-Packard (Hewlett Packard, Avondale, PA) Model 5890A gas chromatograph equipped with a flame ionization detector on a column (1.8 m × 4 mm i.d.) packed with GP 10% SP-1200/1% H3P04 on 80/100 chromosorb W/AW (Chromosorb® W/AW-DMCS, Supelco, Bellefonte, PA). The carrier gas was N that was used with a flow rate of 45 mL/min. The oven, injection port, and detector port temperatures were 125, 175, and

180°C, respectively.

To analyze for intestinal concentrations of microbial protein, approximately 10 g of feces and 10 g of ileal digesta were collected on d 10 and d 13, respectively. Ileal digesta and fecal samples were immediately stored at – 20°C. Samples were fractionated using differential centrifugation following the procedure of Metges et al., 1999). Samples were centrifuged first at 250 relative centrifugal force for 15 min at 4°C, which resulted in fractions that were expected to contain undigested feed particles and porcine cells in the precipitate and supernatant, respectively (Miner-Williams et al., 2009). The supernatant from the 250 relative centrifugal force was then centrifuged at 14,500 relative centrifugal force for 30 min at 4°C to give a precipitate that was expected to contain microbial cells (Miner-Williams et al., 2009). Microbial cells from the 14,500 relative centrifugal force precipitate were subjected in a lysis buffer which contained 100 mM of tris(hydroxymethyl)aminomethane at pH 7.2, 0.5% sodium dodecyl sulfate, and 0.5% sodium deoxycholate. The protein concentration of the lysed microbial cells was then analyzed using Pierce™ Bicinchoninic Acid Assay Kit (ThermoFisher Scientific, Waltham, MA).

At the conclusion of the experiment, fecal samples collected on d 10, 11, and 12, and ileal digesta samples collected on d 13 and 14 in each period were thawed, mixed within animal and diet, and a sub-sample was collected for other chemical analysis. Digesta samples were lyophilized and fecal samples were dried in a 50°C forced air drying oven prior to analysis. Dried ileal digesta and fecal samples were finely ground through a 1-mm screen in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ). Diets, ileal digesta, and fecal samples were analyzed for titanium following the procedure of Myers et al. (2004). All diets, ileal digesta, and fecal samples were analyzed for gross energy using bomb calorimetry (Model 6400; Parr

Instruments, Moline, IL), dry matter (Method 930.15; AOAC Int., 2007), crude protein (Method 990.03; AOAC Int., 2007) using the combustion procedure on a Leco FP628 protein apparatus (Leco Corp., St Joseph, MI), and AEE using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction using petroleum ether (Method 2003.06, AOAC Int., 2007) on an Ankom fat analyzer (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). All diets and ileal digesta samples were also analyzed for amino acids (Method 982.30 E (a, b, c); AOAC Int., 2007) on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Diets were analyzed for ash (Method 942.05; AOAC Int., 2007) and were also analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 (AOAC Int., 2007) using the Ankom$^{TDF}$ Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Total dietary fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber. Analyses for amino acids and minerals were conducted at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Colombia, and all other analyses were conducted in the Monogastric Nutrition Laboratory at the University of Illinois at Urbana-Champaign, Urbana. Following analysis, the ATTD of dry matter, AEE, gross energy, and crude protein, and AID of dry matter, AEE, gross energy, crude protein, and amino acids were calculated for all dietary treatments as previously described (Stein et al., 2007).

Data were analyzed as a randomized complete block design in a 2 × 2 factorial arrangement using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. Fixed effects included Cu, DDGS, and the interaction between Cu and DDGS. Random effects included pig and period. Least square means were calculated for each

independent variable and means were separated using the PDIFF option. Results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

## RESULTS

Diet analyses indicated that the intended concentrations of AEE, total dietary fiber, amino acids, and Cu were present in all diets (Table 6.2). Interactions between DDGS and Cu were observed for AID and ATTD of dry matter and gross energy (Table 6.3). Inclusion of 45% DDGS to the diet without added Cu resulted in a greater ($P < 0.05$) reduction of AID and ATTD of dry matter and gross energy compared with the diet containing 45% DDGS and 150 mg/kg of Cu from Cu hydroxychloride. Greater ($P < 0.01$) AID and ATTD of AEE was observed in the DDGS diets compared with diets without DDGS, but a reduction ($P < 0.01$) in AID and ATTD of crude protein was observed in diets containing DDGS compared with diets without DDGS. No difference was observed in AID and ATTD of crude protein between diets without and with supplemental Cu, but supplementation of Cu hydroxychloride to diets improved ($P < 0.01$) the AID and ATTD of AEE.

The AID of all amino acids was less ($P < 0.01$) in diets containing DDGS compared with diets without DDGS (Table 6.4). Supplementation of Cu to diets did not affect AID of amino acids except for a reduction ($P < 0.05$) in the AID of Cys.

Inclusion of DDGS or Cu hydroxychloride in diets did not affect the concentration of microbial protein in ileal digesta (Table 6.5). However, fecal microbial protein concentration increased ($P < 0.01$) if diets contained DDGS, whereas Cu supplementation reduced ($P < 0.05$) the concentration of microbial protein in feces. The proportion of butyrate produced in ileal digesta was less ($P < 0.05$) in the diets containing DDGS compared with the diets without

DDGS, whereas the proportion of isobutyrate and valerate were greater ($P < 0.05$) in ileal digesta from pigs fed DDGS-containing diets. However, the total concentration of VFA in ileal digesta was not affected by inclusion of DDGS in the diets. Dietary Cu concentrations did not affect the total concentration and proportions of VFA produced in ileal digesta.

The proportion of acetate produced from the fermentation process in the hindgut of pigs was greater ($P < 0.01$) from pigs fed DDGS diets compared from pigs fed no DDGS. However, reduced ($P < 0.01$) proportions of propionate and isobutyrate were observed in feces from pigs fed diets containing DDGS compared with diets without DDGS. Likewise, the proportion of valerate in feces tended to be less ($P < 0.10$) if pigs were fed DDGS diets than diets without DDGS. The proportion of acetate produced tended to be less ($P < 0.10$) in feces from pigs fed diets with Cu from Cu hydroxychloride compared with diets without Cu hydroxychloride. This resulted in a tendency for a reduction ($P < 0.10$) in the total concentration of VFA in feces when Cu hydroxychloride was added to diets.

## DISCUSSION

Distillers dried grains with solubles is a co-product from ethanol production, which contains 30 to 35% total dietary fiber (NRC, 2012; Espinosa and Stein, 2018). Therefore, inclusion of 45% DDGS in diets resulted in a greater concentration of total dietary fiber compared with diets without DDGS. Dietary fiber includes non-starch polysaccharides, resistant starch, and oligosaccharides that are resistant to enzymatic hydrolysis in the small intestine (Bindelle et al., 2008), and therefore, can affect a wide range of physiological processes in pigs. The observation that the AID and ATTD of dry matter and gross energy decreased as the concentration of total dietary fiber increased in the diets is likely due to the insoluble portion of

dietary fiber in DDGS that cannot be fermented and utilized by pigs (Lindberg, 2014). Fermentation of dietary fiber results in production of methane and microbial biomass, and this may reduce the efficiency of energy utilization from dietary fiber (Noblet and Le Goff, 2001; Bindelle et al., 2008). The observation that the AID and ATTD of crude protein decreased as total dietary fiber concentration increased in the diets is in agreement with previous data (Zhang et al., 2013). This is likely a result of increased endogenous secretions, or faster gastric emptying, which subsequently reduces the time that feed proteins are exposed to proteolytic enzymes (Eggum, 1992) due to the insoluble portion of dietary fiber in DDGS.

The origin of endogenous protein includes salivary and gastric secretions, pancreatic and bile secretions, sloughed epithelial cells, and mucus (Jansman et al., 2002), but some of the endogenous N is also synthesized in the gastrointestinal tract as microbial protein (Jansman et al., 2002; Miner-Williams et al., 2009). High concentration of dietary fiber in diets results in an increased growth of microbial mass (Noblet and Le Goff, 2001; Jarrett and Ashworth, 2018), and this may increase endogenous protein secretions through increased fecal N of microbial origin.

The observation that inclusion of DDGS resulted in an improved AID and ATTD of AEE is due to the greater concentration of AEE in diets containing DDGS. This response is in agreement with previous data (Jørgensen et al., 1993) indicating that the ATTD of fat increased upon inclusion of graded concentrations of soybean oil to a diet with low concentration of fat.

Increased concentration of TDF in diets results in increased fermentation by microbes in the gastrointestinal tract. Therefore, the observation that the concentration of fecal microbial protein increased when DDGS was included in the diets is likely due to an increased growth of bacteria in the hindgut (Bach Knudsen and Hansen, 2007; Bindelle et al., 2008). The undigested portion of dietary fiber serves as a substrate for intestinal microbes to hydrolyze and metabolize

through a series of anaerobic energy-yielding reactions (Jha and Berrocoso, 2015). Substrate-level phosphorylation reactions lead to synthesis of ATP needed by microbes for maintenance and growth (Macfarlane and Macfarlane, 2007), and increased microbial growth may, therefore, result in increased synthesis of VFA because of increased fermentation. Volatile fatty acids are the major end products of microbial metabolism, and they can be absorbed via passive diffusion and utilized by pigs as a source of energy (Dierick et al., 1989; Macfarlane and Macfarlane, 2007). However, in the current experiment, DDGS inclusion reduced the proportions of some VFA produced in the small intestine and in the hindgut of pigs, which is contrary to our hypothesis. It is possible that the reason for this observation is that pigs fed high-fiber diets have greater fecal output compared with pigs fed low-fiber diets (Jaworski et al., 2017). Fecal dry matter excretion increases by 0.75 g per d for each g increase of wheat fiber inclusion in the diet (de Vries et al., 2015). As a consequence, even if the proportions of some VFA in the feces is reduced, it is possible that the daily synthesis is increased, and the total quantity of VFA synthesized from pigs fed the DDGS diets may, therefore, have been greater than the total VFA produced from pigs fed diets without DDGS. However, because a balance study was not conducted to determine the total fecal output from pigs, further investigation is needed to address this possibility. Similar results were reported by Jaworski et al. (2017) who observed that the concentration of VFA in rectum contents from pigs fed low-fiber diets was greater compared with the VFA concentration from pigs fed high-fiber diets.

Inclusion of pharmacological concentrations of dietary Cu improves growth performance of pigs (Cromwell et al., 1998; Hill et al., 2000; Ma et al., 2015), but the effect of dietary Cu on nutrient digestibility remains inconsistent. The lack of differences in the AID and ATTD of gross energy among diets containing 0 or 150 mg Cu/kg from Cu hydroxychloride is in agreement with

data indicating that supplementation of Cu to diets for pigs did not improve energy digestibility (Castell and Bowland, 1968; Espinosa et al., 2017). However, this is in contrast with results by Gonzales-Eguia et al. (2009) and Coble et al. (2018), who demonstrated that supplementation of 50 to 150 mg Cu/kg from Cu hydroxychloride or $CuSO_4$ resulted in an improved ATTD of gross energy.

The observation that the AID of Cys decreased when diets contained additional Cu is in agreement with data indicating that the AID of Cys was reduced in broilers fed diets with Cu supplementation (Rochell et al., 2016). Cysteine is a sulfur-containing amino acid and may directly react with Cu by forming a chelate in the lumen that is poorly absorbed by the animal (Maurice and Jensen, 1979; Baker and Czarnecki-Maulden, 1987). Two mols of Cys are involved and are bound to one mole of Cu, with both the sulfhydryl moiety and amino group of Cys being involved in the binding (Baker and Czarnecki-Maulden, 1987). The observation that supplementation of Cu hydroxychloride to diets did not affect AID of most amino acids is in contrast with results by Rochell et al. (2016), who demonstrated that inclusion of 116 mg Cu/kg to diets for broilers with low amino acid density improved AID of total amino acids.

The improvement in AID and ATTD of AEE that was observed when diets were supplemented with Cu concurs with previous data (Espinosa et al., 2019). Dietary fiber present from corn, SBM, and DDGS facilitates microbial growth in the intestinal tract, which may result in increased endogenous loss of fat (Kil et al., 2010). Therefore, the observed response is possibly due to the effect of Cu in reducing the endogenous loss of fat by pigs (Espinosa et al., 2019) and subsequently improve AID and ATTD of AEE.

To our knowledge, no data demonstrating the effects of Cu hydroxychloride on intestinal microbial protein concentrations of pigs have been reported. The observation that the concentration of microbial protein in feces was reduced when diets were supplemented with Cu hydroxychloride is likely a result of the bacteriostatic properties of dietary Cu. Dietary Cu may alter the microbial populations in the intestine, and thereby affect the growth and community structure of microorganisms in the cecum and colon (Stahly et al., 1980; Højberg et al., 2005). Copper may also disrupt enzyme structures and functions of bacteria by binding to S or carboxylate-containing groups and amino groups of proteins (Sterritt and Lester, 1980). Therefore, it is possible that Cu hydroxychloride inhibited the growth of microbes and subsequently reduced the concentration of microbial protein in the hindgut. The observed response in the concentration of fecal microbial protein also supports the observed improvement in the ATTD of AEE upon supplementation of Cu hydroxychloride to diets. The ability of Cu hydroxychloride to alter microbial growth and activity, and therefore, prevent microbes from performing normal functions (Thurman et al., 1989) resulted in reduced endogenous loss of fat with a subsequent improvement in AID and ATTD of AEE. The observed reduction in the total concentration of VFA in feces from pigs fed diets with Cu hydroxychloride is in contrast with data indicating that the concentrations of VFA were greater in cecal contents from pigs fed diets supplemented with 250 mg Cu/kg from $CuSO_4$ (Mei et al., 2010). However, the observed response for VFA is in agreement with the observed reduction of microbial protein concentration in the hindgut. Copper may reduce the fermentation of fiber in the hindgut by altering the growth, activity, and metabolism of microbes, and therefore, may have reduced the concentration of VFA from carbohydrate fermentation.

# CONCLUSION

In conclusion, inclusion of 45% DDGS in diets resulted in a reduced AID and ATTD of dry matter, gross energy, crude protein, and amino acids, which is likely due to increased endogenous losses of nutrients and reduced utilization of energy. Supplementation of Cu hydroxychloride to diets reduced the concentrations of total VFA from carbohydrate fermentation and microbial protein in feces, indicating reduced microbial activity in the hindgut of pigs fed diets containing Cu hydroxychloride. The improved AID and ATTD of AEE that was, therefore, likely caused by reduced endogenous loss of fat due to reduced synthesis of microbial protein.

## TABLES

**Table 6.1.** Ingredient composition of experimental diets

| | No added Cu[1] | | 150 mg/kg Cu[1] | |
|---|---|---|---|---|
| | No DDGS | 45% DDGS | No DDGS | 45% DDGS |
| Ingredient | | | | |
| Ground corn | 68.13 | 31.74 | 68.102 | 31.712 |
| Soybean meal, 48% CP | 28.00 | 19.50 | 28.00 | 19.50 |
| Distillers dried grains with solubles | - | 45.00 | - | 45.00 |
| Limestone | 1.22 | 1.46 | 1.22 | 1.46 |
| Dicalcium phosphate | 0.45 | - | 0.45 | - |
| Copper hydroxychloride, 54% Cu | - | - | 0.028 | 0.028 |
| L-Lys, HCl | 0.15 | 0.25 | 0.15 | 0.25 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 |
| Phytase premix[2] | 1.00 | 1.00 | 1.00 | 1.00 |
| Titanium dioxide | 0.40 | 0.40 | 0.40 | 0.40 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 |

[1]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[2]The phytase premix contained phytase (5,000 FTU/g of Quantum Blue 5G; AB Vista, Marlborough, United Kingdom) mixed with corn. The mixture was formulated to provide 500 units of phytase per kilogram of complete diet.

[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU;

**Table 6.1. (cont.)**

vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

**Table 6.2.** Analyzed composition of experimental diets

| | No added Cu[1] | | 150 mg/kg Cu[1] | |
|---|---|---|---|---|
| Item | No DDGS | 45% DDGS | No DDGS | 45% DDGS |
| Dry matter, % | 87.98 | 87.75 | 88.20 | 87.29 |
| Ash, % | 5.75 | 5.85 | 5.79 | 5.91 |
| Gross energy, kcal/kg | 3,829 | 4,091 | 3,837 | 4,086 |
| Crude protein, % | 17.90 | 23.33 | 18.18 | 23.47 |
| Acid hydrolyzed ether extract, % | 2.36 | 4.16 | 2.57 | 4.43 |
| Insoluble dietary fiber, % | 12.10 | 20.80 | 13.10 | 20.60 |
| Soluble dietary fiber, % | 1.40 | 2.40 | 1.10 | 2.65 |
| Total dietary fiber, % | 13.50 | 23.12 | 14.20 | 23.25 |
| Minerals | | | | |
| Ca, % | 0.65 | 0.72 | 0.63 | 0.73 |
| P, % | 0.41 | 0.61 | 0.42 | 0.62 |
| Na, % | 0.19 | 0.30 | 0.18 | 0.31 |
| Mg, % | 0.13 | 0.21 | 0.13 | 0.20 |
| K, % | 0.78 | 1.09 | 0.78 | 1.07 |
| S, % | 0.19 | 0.27 | 0.19 | 0.27 |
| Mn, mg/kg | 75.10 | 76.20 | 71.90 | 80.70 |
| Fe, mg/kg | 234.00 | 203.00 | 232.00 | 205.00 |
| Zn, mg/kg | 152.00 | 179.00 | 143.00 | 179.00 |
| Cu, mg/kg | 28.60 | 33.80 | 189.00 | 220.00 |

**Table 6.2. (cont.)**

| | | | | |
|---|---|---|---|---|
| Indispensable amino acids, % | | | | |
| Arg | 1.14 | 1.22 | 1.07 | 1.25 |
| His | 0.47 | 0.59 | 0.44 | 0.60 |
| Ile | 0.79 | 0.96 | 0.75 | 0.97 |
| Leu | 1.51 | 2.18 | 1.42 | 2.19 |
| Lys | 1.08 | 1.15 | 1.05 | 1.13 |
| Met | 0.28 | 0.37 | 0.25 | 0.36 |
| Met + Cys | 0.56 | 0.75 | 0.50 | 0.73 |
| Phe | 0.90 | 1.13 | 0.85 | 1.14 |
| Thr | 0.69 | 0.87 | 0.63 | 0.86 |
| Trp | 0.22 | 0.22 | 0.21 | 0.23 |
| Val | 0.86 | 1.14 | 0.82 | 1.14 |
| Dispensable amino acids, % | | | | |
| Ala | 0.88 | 1.30 | 0.84 | 1.32 |
| Asp | 1.69 | 1.85 | 1.62 | 1.85 |
| Cys | 0.28 | 0.38 | 0.25 | 0.37 |
| Glu | 3.13 | 3.48 | 2.90 | 3.49 |
| Gly | 0.75 | 0.97 | 0.71 | 0.97 |
| Ser | 0.78 | 0.95 | 0.72 | 0.95 |
| Tyr | 0.63 | 0.83 | 0.60 | 0.83 |
| All amino acids, % | 17.59 | 21.40 | 16.54 | 21.55 |

**Table 6.2. (cont.)**

[1]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

**Table 6.3.** Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of energy and nutrients in experimental diets[1]

| | No added Cu[2] | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | - DDGS[3] | 45% DDGS | - DDGS | 45% DDGS | SEM | DDGS | Cu | DDGS × Cu |
| AID, % | | | | | | | | |
| Dry matter | 72.6 | 52.3 | 69.3 | 54.3 | 1.5 | <0.001 | 0.628 | 0.039 |
| Gross energy | 70.3 | 53.7 | 67.7 | 55.8 | 1.2 | <0.001 | 0.818 | 0.054 |
| AEE[4] | 37.6 | 44.0 | 39.1 | 53.0 | 2.2 | 0.004 | 0.025 | 0.108 |
| Crude protein | 77.8 | 65.6 | 78.0 | 66.2 | 1.3 | <0.001 | 0.674 | 0.820 |
| ATTD, % | | | | | | | | |
| Dry matter | 82.4 | 66.9 | 79.8 | 68.6 | 1.1 | <0.001 | 0.592 | 0.019 |
| Gross energy | 81.0 | 66.7 | 78.0 | 69.1 | 1.1 | <0.001 | 0.763 | 0.004 |
| AEE | 8.2 | 19.4 | 13.8 | 34.3 | 4.1 | <0.001 | 0.036 | 0.114 |
| Crude protein | 78.3 | 69.2 | 76.8 | 70.4 | 1.4 | <0.001 | 0.893 | 0.193 |

[1]Data are least squares means of 12 observations for treatments without DDGS and 11 observations for treatments with DDGS.

[2]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN).

**Table 6.3. (cont.)**

[3]DDGS = distillers dried grains with solubles.

[4]AEE = acid hydrolyzed ether extract.

**Table 6.4.** Apparent ileal digestibility (AID) of amino acids (AA) in experimental diets[1]

| | No added Cu[2] | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | - DDGS[3] | 45% DDGS | - DDGS | 45% DDGS | SEM | DDGS | Cu | DDGS × Cu |
| Crude protein, % | 77.8 | 65.6 | 78.0 | 66.2 | 1.3 | <0.001 | 0.674 | 0.820 |
| Indispensable AA, % | | | | | | | | |
| Arg | 88.5 | 80.7 | 88.3 | 81.1 | 0.6 | <0.001 | 0.869 | 0.583 |
| His | 84.6 | 70.5 | 83.6 | 69.3 | 1.1 | <0.001 | 0.210 | 0.905 |
| Ile | 81.1 | 70.6 | 79.6 | 70.9 | 0.9 | <0.001 | 0.524 | 0.305 |
| Leu | 81.5 | 75.0 | 79.6 | 74.6 | 1.0 | <0.001 | 0.183 | 0.347 |
| Lys | 85.4 | 71.3 | 84.8 | 71.5 | 1.1 | <0.001 | 0.857 | 0.659 |
| Met | 83.4 | 74.7 | 81.2 | 74.4 | 1.0 | <0.001 | 0.149 | 0.282 |
| Phe | 82.2 | 73.3 | 80.8 | 73.7 | 0.9 | <0.001 | 0.520 | 0.233 |
| Thr | 74.6 | 60.6 | 71.8 | 61.1 | 1.3 | <0.001 | 0.266 | 0.127 |
| Trp | 81.4 | 69.6 | 83.1 | 68.9 | 0.9 | <0.001 | 0.576 | 0.194 |
| Val | 78.3 | 66.7 | 77.2 | 67.0 | 1.2 | <0.001 | 0.640 | 0.441 |
| Total | 82.4 | 72.0 | 81.2 | 72.1 | 1.0 | <0.001 | 0.488 | 0.421 |

**Table 6.4. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dispensable AA, % | | | | | | | | |
| Ala | 74.6 | 66.8 | 75.1 | 67.5 | 1.4 | <0.001 | 0.567 | 0.914 |
| Asp | 78.4 | 64.1 | 78.6 | 63.6 | 1.2 | <0.001 | 0.854 | 0.734 |
| Cys | 64.6 | 47.9 | 61.6 | 39.5 | 2.2 | <0.001 | 0.010 | 0.213 |
| Glu | 83.5 | 72.2 | 82.8 | 71.6 | 1.4 | <0.001 | 0.619 | 0.940 |
| Gly | 64.7 | 50.3 | 64.7 | 48.5 | 2.1 | <0.001 | 0.645 | 0.625 |
| Ser | 80.0 | 69.8 | 80.0 | 70.6 | 1.2 | <0.001 | 0.754 | 0.532 |
| Tyr | 81.6 | 75.6 | 81.0 | 75.7 | 0.8 | <0.001 | 0.745 | 0.607 |
| Total | 78.7 | 67.0 | 78.4 | 66.4 | 1.4 | <0.001 | 0.685 | 0.922 |
| All AA | 80.6 | 69.5 | 79.8 | 69.3 | 1.2 | <0.001 | 0.762 | 0.762 |

[1]Data are least squares means of 12 observations for treatments without DDGS and 11 observations for treatments with DDGS.

[2]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[3]DDGS = distillers dried grains with solubles.

**Table 6.5.** Intestinal microbial protein concentrations (mg/g, DM basis) and rate of fermentation of volatile fatty acids in ileal digesta and feces of pigs fed experimental diets[1]

| | No added Cu[2] | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | - DDGS[3] | 45% DDGS | - DDGS | 45% DDGS | SEM | DDGS | Cu | DDGS × Cu |
| Ileal digesta | | | | | | | | |
| Microbial protein | 23.38 | 19.91 | 19.09 | 19.37 | 2.36 | 0.501 | 0.312 | 0.430 |
| Volatile fatty acids (µmol/100 µmol) | | | | | | | | |
| Acetate | 93.13 | 93.49 | 91.22 | 94.91 | 1.86 | 0.200 | 0.868 | 0.276 |
| Propionate | 2.54 | 3.66 | 3.88 | 2.88 | 1.08 | 0.954 | 0.797 | 0.329 |
| Butyrate | 3.63 | 1.59 | 3.93 | 1.08 | 0.69 | <0.001 | 0.847 | 0.453 |
| Isobutyrate | 0.53 | 0.78 | 0.68 | 0.74 | 0.07 | 0.019 | 0.434 | 0.139 |
| Isovalerate | 0.16 | 0.37 | 0.28 | 0.27 | 0.07 | 0.170 | 0.874 | 0.123 |
| Valerate | 0.01 | 0.11 | 0.01 | 0.12 | 0.07 | 0.010 | 0.885 | 0.897 |
| Total [VFA[4]], µmol/g, DM basis | 7.88 | 9.05 | 7.88 | 7.00 | 1.10 | 0.866 | 0.229 | 0.230 |
| Feces | | | | | | | | |

**Table 6.5. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Microbial protein | 169.32 | 195.15 | 142.08 | 182.07 | 9.63 | 0.002 | 0.046 | 0.469 |
| Volatile fatty acids (µmol/100 µmol) | | | | | | | | |
| Acetate | 69.66 | 75.70 | 61.75 | 75.10 | 1.73 | 0.006 | 0.096 | 0.277 |
| Propionate | 20.27 | 16.17 | 25.08 | 16.69 | 2.52 | 0.005 | 0.215 | 0.317 |
| Butyrate | 1.15 | 1.09 | 1.25 | 1.12 | 0.10 | 0.384 | 0.523 | 0.718 |
| Isobutyrate | 5.56 | 4.15 | 7.48 | 4.12 | 0.72 | 0.002 | 0.195 | 0.180 |
| Isovalerate | 1.34 | 1.18 | 1.60 | 1.27 | 0.18 | 0.168 | 0.342 | 0.644 |
| Valerate | 2.02 | 1.71 | 2.84 | 1.70 | 0.42 | 0.067 | 0.300 | 0.280 |
| Total [VFA], µmol/g, DM basis | 12.36 | 10.68 | 10.06 | 10.42 | 0.67 | 0.333 | 0.065 | 0.140 |

[1]Data are least squares means of 12 observations for treatments without DDGS and 11 observations for treatments with DDGS.

[2]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[3]DDGS = distillers dried grains with solubles.

[4]Volatile fatty acids.

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# CHAPTER 7: EFFECTS OF COPPER HYDROXYCHLORIDE ON GROWTH PERFORMANCE AND ABUNDANCE OF GENES INVOLVED IN LIPID METABOLISM OF GROWING PIGS


## ABSTRACT

An experiment was conducted to test the hypothesis that Cu hydroxychloride improves growth performance by upregulating the mRNA transcription of genes involved in lipid metabolism of pigs fed a diet based on corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS). Thirty two pigs (15.05 ± 0.98 kg) were allotted to 2 dietary treatments with 2 pigs per pen for a total of 8 replicate pens per treatment. Pigs were fed a corn-SBM-DDGS control diet that included Cu to meet the requirement. A second diet was formulated by adding 150 mg Cu/kg from Cu hydroxychloride to the control diet. On the last day of the experiment, one pig per pen was sacrificed and samples from liver, skeletal muscle, and subcutaneous adipose tissue were collected to analyze relative mRNA abundance of genes involved in lipid metabolism. Results indicated that overall average daily gain (ADG) and gain:feed (G:F) were greater ($P < 0.05$) for pigs fed the diet containing Cu hydroxychloride compared with pigs fed the control diet. Pigs fed the diet supplemented with Cu hydroxychloride also had increased ($P < 0.05$) abundance of cluster of differentiation 36 in liver and increased ($P < 0.05$) abundance of fatty acid binding protein 4 and lipoprotein lipase in subcutaneous adipose tissue. Inclusion of Cu hydroxychloride also tended to increase ($P < 0.10$) abundance of fatty acid binding protein 1, peroxisome proliferator-activated receptor alpha, and carnitine palmitoyl transferase 1 B in liver, skeletal muscle, and subcutaneous adipose tissue, respectively. This

indicates that dietary Cu affects signaling pathways associated with lipid metabolism by improving the uptake, transport, and utilization of fatty acids. In conclusion, supplementation of Cu hydroxychloride to the control diet improved growth performance and upregulated abundance of some genes involved in post-absorptive metabolism of lipids.

**Keywords:** copper, copper hydroxychloride, gene expression, lipid metabolism, pigs

## INTRODUCTION

Dietary lipids are commonly included in swine diets to increase energy density, reduce dust, and improve palatability (Cera et al., 1990; Kerr et al., 2015). Differences in fat digestibility and utilization by pigs may be related to differences in age of the animal, form of dietary fat (i.e., extracted or intact fat), degree of saturation, and dietary inclusion rate of fat (Kerr et al., 2015). Most lipids in feed ingredients are present in the form of triglycerides, but concentration and fatty acid composition varies among ingredients (Stahly, 1984). The apparent total tract digestibility of dietary lipids in different feed ingredients also varies and ranges from 25 to 77%, which is less compared with values for extracted fat (Kil et al., 2010; Kim et al., 2013).

Supplementing Cu to diets fed to weanling pigs at 100 to 250 mg/kg may reduce post-weaning scouring and also improve average daily gain (**ADG**) and average daily feed intake (**ADFI**; Cromwell et al., 1998; Perez et al., 2011). Addition of Cu at 250 mg/kg in diets for weanling pigs containing 5% animal fat improved growth performance, and it was speculated that this is due to the ability of Cu to improve animal fat utilization and enzymatic activity (Dove and Haydon, 1992; Dove, 1993). Inclusion of 45 mg/kg of dietary Cu in diets for rabbits

improved body mass gain by upregulating mRNA transcription of fatty acid transport protein, fatty acid binding protein, and carnitine palmitoyl transferase 1 (Lei et al., 2017), indicating that dietary Cu may influence post-absorptive metabolism of lipids. Supplementation of Cu to diets also increased lipogenesis and fatty acid uptake in fish (Chen et al., 2016). However, Coble et al., 2018) reported that adding 150 mg Cu/kg to corn-soybean meal (**SBM**) diets for finishing pigs decreased mRNA expression of intestinal fatty acid binding protein. Hence, the effect of supplementing dietary Cu on post-absorptive lipid metabolism in pigs remains inconclusive. It was, therefore, the objective of this experiment to test the hypothesis that addition of 150 mg Cu/kg to a diet based on corn, SBM, and distillers dried grains with solubles (**DDGS**) improves growth performance of pigs, and that dietary Cu influences mRNA abundance of genes involved in post-absorptive metabolism of lipids in pigs.

## MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved prior to initiation of the experiment. Pigs that are the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

A total of 32 pigs (15.05 ± 0.98 kg) were used in the experiment. Pigs were randomly allotted to 2 dietary treatments with 2 pigs per pen for a total of 8 replicate pens per treatment. A control diet was formulated based on corn, SBM, and DDGS to meet the current estimates for nutrient requirements for 11 to 25 kg pigs (NRC, 2012). The control diet contained 20 mg Cu from CuCl that was included in the vitamin-mineral premix. An additional diet was formulated

by adding 150 mg Cu/kg as Cu hydroxychloride to the control diet. Experimental diets were fed to pigs for 28 d (Tables 7.1 and 7.2).

Individual pig weights were recorded at the beginning of the experiment, on d 15, and at the conclusion of the experiment on d 28. Feed addition was recorded daily and weight of feed left in the feeder was recorded on d 15 and on d 28. At the conclusion of the experiment, data were summarized to calculate ADG, ADFI, and average gain:feed (**G:F**) within each pen and treatment group. Data were summarized for the initial 15 d, the final 13 d, and for the entire experiment. At the conclusion of the experiment, one pig per pen was sacrificed. Liver, skeletal muscle (longissimus dorsi), and adipose tissue were harvested and placed in 2 mL cryogenic tubes, snap frozen in liquid $N_2$, and stored at -80$^{o}$C until used for mRNA and protein determination.

Total RNA was extracted from 40 ± 0.2 mg of frozen liver, 50 ± 0.2 mg of skeletal muscle, and 200 ± 0.2 mg of subcutaneous adipose tissue using QIAzol® Lysis Reagent according to the miRNeasy® Mini Kit (QIAGEN, Germantown, MD) manufacturer's instructions. Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The RNA quality was determined using a Fragment Analyzer™ Automated CE System (Method DNF-471-33-SS Total RNA 15nt; Advanced Analytical, Ankeny, IA) and RNA samples with an RNA quality number greater than 7 were used for cDNA synthesis. A portion of the RNA was diluted to 100 ng/µL with DNase/RNase-free water for cDNA synthesis as described by Vailati-Riboni et al. (2015) using 2 µl of diluted RNA from each tissue. The cDNA was then diluted 1:4 for liver and skeletal muscle and 1:3 for subcutaneous adipose tissue with DNase/RNase-free water, prior to quantitative polymerase chain reaction (**qPCR**) analysis.

Quantitative PCR was performed using 4 μL of diluted cDNA combined with 6 μL of a mixture composed of 5 μL of SYBR Green master mix (PerfeCTa SYBR Green FastMix, ROX™; Quanta BioSciences, Beverly, MA), 0.4 μL each of 10 μM forward and reverse primers, and 0.2 μL DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA). All samples were run in triplicate using a 7-point standard curve that was developed with samples and which was run in triplicate. The reactions were performed in a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA) using the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. Data were analyzed using the QuantStudio™ Real-Time PCR Software (version 1.3; Applied Biosystems, Foster City, CA).

Two internal control genes, β-actin (Fry et al., 2012) and glyceraldehyde 3-phosphate dehydrogenase (Vigors et al., 2014), were used to normalize the abundance of tested genes. The geometric mean of the internal control genes was determined for the target normalization and relative abundance of specified genes. Tested genes included fatty acid synthase, acetyl coA carboxylase, fatty acid translocase or cluster of differentiation 36, and peroxisome proliferator-activated receptor α in liver, skeletal muscle, and subcutaneous adipose tissue. Genes expressed in specific tissues were also tested. Carnitine palmitoyl transferase 1 A and fatty acid binding protein 1 were analyzed in liver tissue, whereas carnitine palmitoyl transferase 1 B and fatty acid transport protein 1 were tested for both skeletal muscle and adipose tissues. Fatty acid binding protein 3 was also analyzed in skeletal muscle. Fatty acid binding protein 4, peroxisome proliferator-activated receptor ɤ, lipoprotein lipase, and hormone sensitive lipase were also

analyzed in subcutaneous adipose tissue. All genes are important for storage, uptake, utilization, or oxidation of fatty acids. Primers are listed in Table 7.3 and were commercially synthesized by Integrated DNA Technologies (Skokie, IL).

All diet samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. Diets were analyzed for dry matter (Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007), and gross energy was determined using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL). Acid hydrolyzed ether extract was quantified by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY), and crude protein was analyzed using the combustion procedure (Method 990.03; AOAC Int., 2007) on a Leco FP628 protein apparatus (Leco Corporation, St. Joseph, MI). Amino acids were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard, and minerals were analyzed by inductively coupled plasma optical emissions spectrometry using an internally validated method (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B[b]; AOAC Int., 2007). Analyses for amino acids and minerals were conducted at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Colombia, and all other analyses were conducted in the Monogastric Nutrition Laboratory at the University of Illinois at Urbana-Champaign, Urbana.

Data were analyzed using the Mixed Procedure of SAS with the pen as the experimental unit. Homogeneity of the variances was confirmed using the UNIVARIATE procedure in SAS (SAS Institute Inc., Cary, NC). Diet was the fixed effect and replicate was the random effect.

Least squares means were calculated using the LSMeans procedure. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

## RESULTS

Diet analyses indicated that the intended concentrations of acid hydrolyzed ether extract and Cu were present in both diets and concentrations of other nutrients were not affected by dietary treatment. A tendency for greater ($P < 0.10$) ADG and G:F was observed for pigs fed the diet containing Cu hydroxychloride compared with pigs fed the control diet from d 0 to 15 (Table 7.4). Addition of Cu hydroxychloride to the control diet resulted in a tendency for an improved ($P < 0.05$) final body weight on d 28. For the overall experimental period, pigs fed the diet with Cu hydroxychloride had greater ($P < 0.05$) ADG, which resulted in a greater ($P < 0.05$) G:F compared with pigs fed the control diet.

Inclusion of Cu hydroxychloride in the diet increased ($P < 0.05$) the abundance of cluster of differentiation 36 and tended to increase ($P < 0.10$) the abundance of fatty acid binding protein 1 in liver (Table 7.5). Pigs fed the Cu hydroxychloride diet also had a tendency to have greater ($P < 0.10$) expression of peroxisome proliferator-activated receptor α in skeletal muscle compared with pigs fed the control diet. Expression of fatty acid binding protein 4 and lipoprotein lipase also increased ($P < 0.05$) and expression of carnitine palmitoyl transferase 1 B tended to increase ($P < 0.10$) in subcutaneous adipose tissue if Cu hydroxychloride was added to the diet.

## DISCUSSION

The observed improvement in final body weight, ADG, and G:F of pigs fed the Cu hydroxychloride diet is in agreement with previous data (Cromwell et al., 1989; Cromwell et al., 1998; Hill et al., 2000; Ma et al., 2015; Espinosa et al., 2017), but there is limited research about the mechanism or mode of action of dietary Cu. One hypothesized mode of action for Cu is that Cu affects the bacteriostatic properties in the intestinal tract with a subsequent improvement in gastrointestinal health and immune function of pigs (Namkung et al., 2006). Addition of 175 mg Cu/kg reduced the number of coliforms in the large intestine, and may have suppressed pathogen adhesion and invasion in the intestinal mucosa of pigs (Højberg et al., 2005), and pigs fed diets containing added dietary Cu at 200 mg/kg had less crypt depth and greater villous length than pigs fed a control diet containing 20 mg/kg of Cu (Zhao et al., 2007). Dietary Cu may also affect the cholesterol profile and the degree of saturation of the animals' lipid reserves (Elliot and Bowland, 1968; Kaya et al., 2006). Addition of increasing levels of dietary Cu reduced the concentration of serum polyunsaturated fatty acids, increased iodine value of backfat, and increased proportion of unsaturated fatty acids in the outer backfat, inner backfat, and perinephric backfat of pigs (Elliot and Bowland, 1968; Dove, 1993; Zhao et al., 2007; Wu et al., 2018). Based on these results, it was hypothesized that dietary Cu improves growth performance by influencing expression of specific genes involved in lipid metabolism.

To determine if dietary Cu influences post-absorptive metabolism of lipids in pigs, the abundance of genes involved in the uptake, transport, synthesis, and oxidation of fatty acids were analyzed. Tested genes involved in lipogenesis include acetyl coA carboxylase and fatty acid synthase. Acetyl CoA carboxylase catalyzes the irreversible carboxylation of acetyl CoA to malonyl CoA (Coutanson et al., 2004), whereas fatty acid synthase is a multifunctional enzyme

complex that catalyzes the synthesis of saturated fatty acids using acetyl CoA and malonyl CoA as substrates (Chirala and Wakil, 2004). Tested genes also include lipoprotein lipase which hydrolyzes chylomicrons and very low-density lipoproteins (**VLDL**) to monoglycerides and free fatty acids (Eckel, 1989). Tested genes involved in the catabolism and oxidation of fatty acids include carnitine palmitoyl transferase 1, hormone sensitive lipase, and peroxisome proliferator-activated receptor alpha. Carnitine palmitoyl transferase 1 is the rate-limiting enzyme in the ß-oxidation of fatty acids and facilitate the transfer of fatty acids into the mitochondria through formation of acyl carnitines (Brown et al., 1997), whereas hormone sensitive lipase is an intracellular lipase that catalyzes the hydrolysis of stored triacylglycerols, diacyglycerols, and cholesteryl esters to free fatty acids for energy provision (Kraemer and Shen, 2002). Peroxisome proliferator-activated receptor α is a ligand-activated transcription factor, which regulates genes involved in the uptake, transport, and mitochondrial oxidation of fatty acids (Xu et al., 2002). The nuclear receptor family includes peroxisome proliferator-activated receptor α and peroxisome proliferator-activated receptor ɤ. Peroxisome proliferator-activated receptor ɤ is predominantly expressed in adipose tissues and promotes *de novo* adipogenesis (Chawla et al., 2001). Genes involved in the uptake and transport of fatty acids include fatty acid binding protein, fatty acid transport protein, and cluster of differentiation 36. Fatty acid transport protein and fatty acid binding protein are intracellular chaperone proteins that bind long-chain fatty acids and actively facilitate the transport of lipids to specific compartments in the cell (Pohl et al., 2004; Furuhashi and Hotamisligil, 2008). Cluster of differentiation 36, also known as fatty acid translocase, is also a multifunctional protein present at the plasma membrane that enhances cellular uptake and transport of fatty acids (Febbraio et al., 2001).

Fat is the largest reserve of energy in pigs, and the tissues that are mainly involved in fatty acid metabolism include adipose tissue, liver, and skeletal muscle (Frayn et al., 2006). These 3 main organs hydrolyze triacylglycerol in a regulated way, and lipids present in these tissues can be stored or exported depending on the nutritional state of the tissues (Lei et al., 2017). The observation that the mRNA expression of lipoprotein lipase in subcutaneous adipose tissue increased in pigs upon supplementation of Cu to the control diet is possibly due to the role of Cu as a cofactor of the activator complex of lipoprotein lipase. Lipoprotein lipase is synthesized in the parenchymal cells of adipose tissue, cardiac muscle, and skeletal muscle (Wang and Eckel, 2009). After synthesis, lipoprotein lipase is transported and is attached at the luminal surface of capillary endothelial cells by heparin sulfated proteoglycans (Braun and Severson, 1992), and the activation of lipoprotein lipase is influenced by apolipoprotein C-II and divalent cations (Srinivasan et al., 1975). Therefore, addition of Cu hydroxychloride to the diet may have promoted lipoprotein lipase activation in a similar manner to divalent cations. This is may be the case because Lau and Klevay (1982) demonstrated that lipoprotein lipase activity was reduced by 40% when rats were fed diets deficient in Cu. The increased mRNA abundance of lipoprotein lipase that was observed for pigs fed Cu hydroxychloride, therefore, indicates an increased uptake and utilization of fatty acids from the hydrolysis of the triacylglycerol component of chylomicrons. The fatty acids derived from lipoprotein lipase may be used for uptake by other tissues such as heart, liver, and muscle. The increased abundance of fatty acid binding protein 4 in pigs fed the Cu hydroxychloride diet is likely a result of increased concentration of fatty acids released by lipoprotein lipase. Fatty acid binding protein 4 is specifically induced by fatty acids (Amri et al., 1991), and therefore, may facilitate transport of fatty acids across the cell membrane (Distel et al., 1992). The observation that mRNA abundance

of carnitine palmitoyl transferase 1 B tended to increase when pigs were fed the Cu hydroxychloride diet is in agreement with data indicating that dietary addition of Cu at 45 mg/kg increased the abundance of carnitine palmitoyl transferase 1 in the adipose tissue of rabbits (Lei et al., 2017). The observed increase in carnitine palmitoyl transferase 1 B abundance indicates that dietary Cu enhances oxidation of fatty acids and subsequently increases the synthesis of ATP (Houten and Wanders, 2010), which may contribute to the increase in G:F that was observed for the pigs fed the Cu hydroxychloride diet compared with pigs fed the control diet.

The increase in mRNA abundance of fatty acid binding protein 1 and cluster of differentiation 36 in liver tissue that was observed in pigs fed the Cu hydroxychloride diet indicates an increased uptake of fatty acids by hepatocytes. Lipids in the liver may originate from non-esterified fatty acids and lipoprotein remnants formed from the action of lipoprotein lipase (Nguyen et al., 2008). Therefore, the increase in mRNA abundance of lipoprotein lipase in the adipose tissue that was observed upon Cu supplementation may have resulted in an increased concentration of non-esterified fatty acids and remnant particles in the plasma membrane. This increased flux of fatty acids may have activated and increased abundance of fatty acid binding protein 1 and cluster of differentiation 36 in the liver because these genes have high affinities for fatty acids (Pepino et al., 2014). Fatty acids taken up by hepatocytes can be activated and esterified. Esterified fatty acids in the liver can be stored intracellularly, be used as a substrate for the formation of VLDL, or can be oxidized completely for ATP synthesis (Mashek et al., 2002).

Fatty acids are major sources of energy in skeletal muscle. Skeletal muscle accounts for a significant portion of the total energy expenditure of the body, and approximately 70% of this energy is derived from fatty acids (Phua et al., 2018). The fatty acids present in skeletal muscle are derived primarily from the adipose tissue and from lipoprotein lipase-mediated hydrolysis of

chylomicrons and VLDL (Watt and Hoy, 2012). The observation that mRNA expression of peroxisome proliferator-activated receptor α tended to increase in pigs fed the diet with Cu hydroxychloride indicates an increased uptake of fatty acids in skeletal muscle by pigs fed the diet containing Cu hydroxychloride. Peroxisome proliferator-activated receptor α is a transcription factor present in tissues with high metabolic rates such as liver, heart, intestine, kidney, and skeletal muscle (Burri et al., 2010). This transcription factor can be activated by specific ligands, which include hypolipidemic fibrates, xenobiotics, and saturated and/or unsaturated fatty acids (Forman et al., 1997; Kliewer et al., 1997). Activation of peroxisome proliferator-activated receptor α favors upregulation of genes involved in the uptake and oxidation of fatty acids (Dreyer et al., 1992). It is, therefore, possible that supplementation of Cu hydroxychloride to the diet improved fatty acid oxidation and subsequently increased ATP synthesis by increasing mRNA expression of peroxisome proliferator-activated receptor α in skeletal muscle. As a consequence, skeletal muscle cells may have obtained more energy from fatty acids, which possibly improved protein synthesis for skeletal muscle growth.

## CONCLUSION

In conclusion, supplementation of Cu from Cu hydroxychloride to the control diet improved ADG and G:F of pigs and increased mRNA abundance of genes involved in uptake, transport, and oxidation of fatty acids. This improvement may be a result of Cu hydroxychloride increasing cellular absorption of fatty acids by increasing the concentration of albumin, but because we did not measure these parameters in this experiment, this warrants further research. The observed increase in mRNA abundance of genes involved in lipid metabolism upon dietary

inclusion of Cu hydroxychloride resulted in an improved efficiency of fatty acid utilization, and therefore, more energy was generated for maintenance and growth of pigs. This may partially explain the increased ADG and G:F of pigs fed the Cu-supplemented diet compared with pigs fed the control diet.

## TABLES

**Table 7.1.** Ingredient composition of experimental diets

| | Diet | |
|---|---|---|
| | Control | Control + Cu[1] |
| Ingredient | | |
| Ground corn | 37.56 | 37.532 |
| Soybean meal | 22.50 | 22.50 |
| Distillers dried grains with solubles | 30.00 | 30.00 |
| Cornstarch | 6.00 | 6.00 |
| Limestone | 1.53 | 1.53 |
| Dicalcium phosphate | 0.10 | 0.10 |
| Copper hydroxychloride, 54% Cu | - | 0.028 |
| L-Lys, HCl | 0.55 | 0.55 |
| DL-Met | 0.03 | 0.03 |
| L-Thr | 0.08 | 0.08 |
| Salt | 0.50 | 0.50 |
| Phytase premix[2] | 1.00 | 1.00 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 |

[1]The diet containing added Cu was fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[2]The phytase premix contained phytase (5,000 FTU/g of Quantum Blue 5G; AB Vista, Marlborough, United Kingdom) mixed with corn. The mixture was formulated to provide 500 units of phytase per kilogram of complete diet.

**Table 7.1. (cont.)**

[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

**Table 7.2.** Analyzed composition of experimental diets

| | Diet | |
|---|---|---|
| | Control | Control + Cu[1] |
| Dry matter, % | 87.39 | 87.15 |
| Ash, % | 4.83 | 4.88 |
| Gross energy, kcal/kg | 3,995 | 4,009 |
| Crude protein, % | 22.15 | 21.95 |
| AEE[2], % | 3.98 | 4.00 |
| Minerals | | |
| Ca, % | 0.69 | 0.61 |
| P, % | 0.51 | 0.52 |
| Na, % | 0.27 | 0.25 |
| Mg, % | 0.17 | 0.17 |
| K, % | 0.95 | 0.96 |
| S, % | 0.23 | 0.23 |
| Mn, mg/kg | 58.10 | 58.10 |
| Fe, mg/kg | 179.00 | 142.00 |
| Zn, mg/kg | 145.00 | 139.00 |
| Cu, mg/kg | 25.90 | 166.00 |
| Indispensable AA, % | | |
| Arg | 1.20 | 1.15 |
| His | 0.54 | 0.52 |
| Ile | 0.89 | 0.86 |

**Table 7.2. (cont.)**

| | | |
|---|---|---|
| Leu | 1.91 | 1.85 |
| Lys | 1.40 | 1.40 |
| Met | 0.36 | 0.33 |
| Met + Cys | 0.72 | 0.68 |
| Phe | 1.04 | 1.00 |
| Thr | 0.86 | 0.84 |
| Trp | 0.22 | 0.20 |
| Val | 1.02 | 1.00 |
| Dispensable AA, % | | |
| Ala | 1.14 | 1.10 |
| Asp | 1.82 | 1.73 |
| Cys | 0.36 | 0.35 |
| Glu | 3.33 | 3.20 |
| Gly | 0.88 | 0.84 |
| Ser | 0.86 | 0.82 |
| Tyr | 0.74 | 0.70 |

[1]The diet containing added Cu was fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

[2]AEE = acid hydrolyzed ether extract.

**Table 7.3.** Forward and reverse primer sequences used for quantitative reverse transcription-PCR

| Gene[1] | Direction[2] | Primer sequence | Reference |
|---|---|---|---|
| Internal control gene | | | |
| *ß-ACTIN* | F | 5′-CAC GCC ATC CTG CGT CTG GA-3′ | Fry et al., 2012 |
| | R | 5′-AGC ACC GTG TTG GCG TAG AG-3′ | |
| *GAPDH* | F | 5′-CAG CAA TGC CTC CTG TAC CA-3′ | Vigors et al., 2014 |
| | R | 5′-ACG ATG CCG AAG TTG TCA TG-3′ | |
| Target gene | | | |
| *FAS* | F | 5′-CAC AAC TCC AAA GAC ACG-3′ | Kellner et al., 2017 |
| | R | 5′-AGG AAC TCG GAC ATA GCG-3′ | |
| *CD36* | F | 5′-CTG GTG CTG TCA TTG GAG CAG T-3′ | Li et al., 2017 |
| | R | 5′-CTG TCT GTA AAC TTC CGT GCC TGT T-3′ | |
| *ACC* | F | 5′-ATG GAT GAA CCG TCT CCC-3′ | Kellner et al., 2017 |
| | R | 5′-TGT AAG GCC AAG CCA TCC-3′ | |
| *PPAR-α* | F | 5′-GCC GAA GTC ATC CAA GAA GG-3′ | Kellner et al., 2017 |
| | R | 5′-TGA CCT CAC AGG ACA CTC CAA G-3′ | |

**Table 7.3. (cont.)**

| | | | |
|---|---|---|---|
| *CPT1A* | F | 5′-GCA TTT GTC CCA TCT TTC GT-3′ | Varady et al., 2012 |
| | R | 5′-GCA CTG GTC CTT CTG GGA TA -3′ | |
| *FABP1* | F | 5′-ACA TCA AGG GGA CAT CGG-3′ | Kellner et al., 2017 |
| | R | 5′-GTC TCC ATC TCA CAC TCC-3′ | |
| *CPT1B* | F | 5′-GGA CGA GGA GTC TCA CCA CTA TGA C-3′ | Li et al., 2017 |
| | R | 5′-TCT TGA ACG CGA TGA GGG TGA-3′ | |
| *FATP1* | F | 5′-CCC TCT GCG TCG CTT TGA TG-3′ | Li et al., 2017 |
| | R | 5′-GCT GCG GTC CCG GAA ATA CA-3′ | |
| *FABP3* | F | 5′-CCA ACA TGA CCA AGC CTA CCA CA-3′ | Li et al., 2017 |
| | R | 5′-ACA AGT TTG CCT CCA TCC AGT G-3′ | |
| *FABP4* | F | 5′-CAG GAA AGT CAA GAG CAC CA-3′ | Zhao et al., 2010 |
| | R | 5′-TCG GGA CAA TAC ATC CAA CA-3′ | |
| *PPAR-ɤ* | F | 5′-GTG GAG ACC GCC CAG GTT TG-3′ | Li et al., 2017 |
| | R | 5′-GGG AGG ACT CTG GGT GGT TCA-3′ | |
| *LPL* | F | 5′-CCC TAT ACA AGA GGG AAC CGG AT-3′ | Li et al., 2017 |

**Table 7.3. (cont.)**

| | | | |
|---|---|---|---|
| | R | 5′-CCG CCA TCC AGT CGA TAA ACG T-3′ | |
| *HSL* | F | 5′-AAC GCA ATG AAA CAG GCC-3′ | Kellner et al., 2017 |
| | R | 5′-TGT ATG ATC CGC TCA ACT CG-3′ | |

[1]*GAPDH* = glyceraldehyde 3-phosphate dehydrogenase; *FAS* = fatty acid synthase; *CD36* = cluster of differentiation 36/fatty acid translocase; *ACC* = acetyl CoA carboxylase; *PPAR-α* = peroxisome proliferator-activated receptor alpha; *CPT1A* = carnitine palmitoyl transferase 1 A; *FABP1* = fatty acid binding protein 1; *CPT1B* = carnitine palmitoyl transferase 1 B; *FATP1* = fatty acid transport protein 1; *FABP3* = fatty acid binding protein 3; *FABP4* = fatty acid binding protein 4; *PPAR-ɤ* = peroxisome proliferator-activated receptor gamma; *LPL* = lipoprotein lipase; *HSL* = hormone sensitive lipase.

[2]Direction of primer (F = forward; R = reverse).

**Table 7.4.** Growth performance for pigs fed diets containing 0 or 150 mg Cu/kg from Cu hydroxychloride[1]

| Item | Diet | | SEM | *P*-value |
|---|---|---|---|---|
| | Control | Control + Cu[2] | | |
| d 0 to 15 | | | | |
| Initial bodyweight, kg | 15.150 | 14.949 | 0.455 | 0.132 |
| ADG[3], kg | 0.578 | 0.654 | 0.031 | 0.070 |
| ADFI[3], kg | 1.018 | 1.009 | 0.038 | 0.876 |
| G:F[3] | 0.570 | 0.648 | 0.027 | 0.080 |
| Final bodyweight, kg | 23.825 | 24.757 | 0.510 | 0.150 |
| d 15 to 28 | | | | |
| ADG, kg | 0.827 | 0.843 | 0.033 | 0.747 |
| ADFI, kg | 1.570 | 1.448 | 0.048 | 0.113 |
| G:F | 0.530 | 0.586 | 0.028 | 0.157 |
| Final bodyweight, kg | 34.575 | 35.713 | 0.492 | 0.076 |
| d 0 to 28 | | | | |
| ADG, kg | 0.694 | 0.742 | 0.021 | 0.037 |
| ADFI, kg | 1.275 | 1.214 | 0.033 | 0.119 |
| G:F | 0.546 | 0.612 | 0.018 | 0.024 |

[1]Data are least squares means of 8 observations for all treatments.

[2]The diet containing added Cu was fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN).

[3]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

**Table 7.5.** Least squares means ($log_2$-backtransformed) for expression of genes in the liver, skeletal muscle, and subcutaneous adipose tissue of pigs fed diets containing 0 or 150 mg Cu/kg from Cu hydroxychloride[1]

| | Diet | | | |
|---|---|---|---|---|
| Item[2] | Control | Control + Cu[3] | SEM | *P*-value |
| Liver | | | | |
| *FAS* | 0.681 | 0.926 | 0.192 | 0.329 |
| *CD36* | 0.839 | 1.094 | 0.064 | 0.017 |
| *ACC* | 0.823 | 0.886 | 0.136 | 0.746 |
| *PPAR-α* | 0.857 | 0.923 | 0.089 | 0.451 |
| *CPT1A* | 1.031 | 1.000 | 0.078 | 0.678 |
| *FABP1* | 0.774 | 1.137 | 0.098 | 0.067 |
| Skeletal muscle | | | | |
| *FAS* | 0.678 | 1.314 | 0.315 | 0.742 |
| *CD36* | 1.031 | 1.391 | 0.219 | 0.215 |
| *ACC* | 0.564 | 0.809 | 0.245 | 0.418 |
| *PPAR-α* | 0.732 | 0.877 | 0.046 | 0.082 |
| *CPT1B* | 0.877 | 0.835 | 0.077 | 0.682 |
| *FABP3* | 0.889 | 0.797 | 0.062 | 0.308 |
| *FATP1* | 0.826 | 0.687 | 0.069 | 0.158 |
| Subcutaneous adipose tissue | | | | |
| *FAS* | 0.559 | 0.951 | 0.334 | 0.312 |
| *CD36* | 1.031 | 1.391 | 0.219 | 0.215 |

**Table 7.5. (cont.)**

| | | | | |
|---|---|---|---|---|
| *ACC* | 0.564 | 0.809 | 0.245 | 0.418 |
| *PPAR-α* | 0.784 | 0.966 | 0.119 | 0.188 |
| *CPT1B* | 0.960 | 1.395 | 0.126 | 0.075 |
| *FABP4* | 0.998 | 1.320 | 0.149 | 0.035 |
| *FATP1* | 1.081 | 1.159 | 0.107 | 0.651 |
| *PPAR-ɤ* | 1.049 | 1.396 | 0.182 | 0.160 |
| *LPL* | 0.985 | 1.462 | 0.143 | 0.004 |
| *HSL* | 0.802 | 1.135 | 0.184 | 0.126 |

[1]Data are least squares means of 7 or 8 observations for all treatments.

[2]*FAS* = fatty acid synthase; *CD36* = cluster of differentiation 36/fatty acid translocase; *ACC* = acetyl CoA carboxylase; *PPAR-α* = peroxisome proliferator-activated receptor alpha; *CPT1A* = carnitine palmitoyl transferase 1 A; *FABP1* = fatty acid binding protein 1; *CPT1B* = carnitine palmitoyl transferase 1 B; *FATP1* = fatty acid transport protein 1; *FABP3* = fatty acid binding protein 3; *FABP4* = fatty acid binding protein 4; *PPAR-ɤ* = peroxisome proliferator-activated receptor gamma; *LPL* = lipoprotein lipase; *HSL* = hormone sensitive lipase.

[3]The diet containing added Cu was fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

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# CHAPTER 8: EFFECTS OF COPPER HYDROXYCHLORIDE AND DIETARY FIBER ON INTESTINAL PERMEABILITY, GROWTH PERFORMANCE, AND BLOOD CHARACTERISTICS OF NURSERY PIGS


## ABSTRACT

Two experiments were conducted to test the hypothesis that copper (Cu) hydroxychloride improves growth performance and blood characteristics, and reduces intestinal permeability of nursery pigs fed diets without or with inclusion of cereal co-products. In experiment 1, 32 pigs (13.53 ± 1.27 kg) were allotted to a 2 × 2 factorial arrangement with 2 types of diets (low-fiber or high-fiber) and 2 levels of Cu from Cu hydroxychloride (0 or 150 mg/kg). Pigs were adapted to diets for 5 d, followed by the oral administration of lactulose and mannitol on d 6. After administration, urine was collected during 2 6-h periods. Results indicated that pigs fed high-fiber diets tended to increase ($P < 0.10$) urinary lactulose:mannitol during the first 6-h period; whereas, dietary Cu concentrations did not affect lactulose:mannitol of pigs. In experiment 2, 128 pigs (8.33 ± 1.32 kg) were allotted to the same dietary treatments as in experiment 1. There were 4 pigs per pen and 8 replicate pens per diet. On d 7, d 14, and on d 21, blood samples were collected from 1 pig per pen and tumor necrosis factor-α (TNF-α), immunoglobulin G, interleukin-1 ß (IL-1ß), interleukin-10 (IL-10), blood urea N (BUN), total protein, and albumin were analyzed. Results indicated that there were no interactions between diet type and Cu hydroxychloride for overall growth performance and blood characteristics of pigs. Overall average daily gain and gain:feed were greater ($P < 0.05$) for pigs fed diets with Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride. The level of dietary

fiber did not affect overall growth performance of pigs. However, BUN concentration tended to increase ($P < 0.10$), and a reduction ($P < 0.05$) in albumin and IL-10 concentrations on d 14 was observed for pigs fed high-fiber diets compared with pigs fed low-fiber diets. Supplementation of Cu hydroxychloride to diets positively influenced ($P < 0.05$) BUN, albumin, and cytokine concentrations of nursery pigs. These changes may be a result of Cu hydroxychloride modulating bacterial populations in the intestinal tract, which further results in improved intestinal health and growth performance.

**Key words:** copper, copper hydroxychloride, dietary fiber, intestinal permeability, pigs

## INTRODUCTION

Post weaning diarrhea is one of the contributing causes of reduction in growth performance and mortality in weanling pigs (Pluske et al., 1997). Weanling pigs are susceptible to infections, diseases, and villous atrophy in the gut, which indicate that the intestinal barrier function is disturbed after weaning (Wijtten et al., 2011). Intestinal permeability increases if pigs have diarrhea, and this may allow entry of toxins and pathogenic microorganisms through the epithelial cells (Zhang and Guo, 2009). Exposure of pigs to pathogenic or nonpathogenic antigens results in activated immune system and subsequent release of cytokines such as tumor necrosis factor α (**TNF-α**), interleukin-1 (**IL-1**), and interleukin-6 (**IL-6**). Cytokines exert physiological and pathological effects on intestinal tight junction barrier proteins, which results in further increasing intestinal permeability (Al-Sadi et al., 2009).

Most diets for weanling pigs contain highly digestible plant and animal proteins, but, there is a growing trend to include more fibrous co-products due to reduced diet costs (Li et al., 2018). However, feeding diets to weanling pigs with high concentrations of dietary fiber may

reduce nutrient digestibility, induce intestinal inflammation, and subsequently depress growth performance in weanling pigs (Tsai et al., 2017).

Copper hydroxychloride is one of the inorganic sources of Cu that may be used in diets for pigs. It has low water solubility, but is highly soluble under acidic conditions (Spears et al., 2004). Addition of 100 to 200 mg Cu/kg from Cu hydroxychloride to diets also improves feed efficiency and reduces post weaning diarrhea in pigs (Cromwell et al., 1998; Fry et al., 2012; Espinosa et al., 2017). It is, therefore, possible that inclusion of Cu hydroxychloride may result in an improved intestinal barrier integrity and immune response, and thus, improved growth performance of pigs. However, there are at this point no data to demonstrate the effect of Cu hydroxychloride on intestinal barrier integrity of pigs fed low-fiber or high-fiber diets. Therefore, the objective of this work was to test the hypothesis that inclusion of 150 mg Cu/kg from Cu hydroxychloride reduces intestinal permeability and subsequently improves growth performance of pigs fed diets without or with high concentration of dietary fiber.

## MATERIALS AND METHODS

Protocols for 2 experiments were submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved prior to initiation of the experiments. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used in both experiments.

### *Experiment 1: Intestinal Permeability*

A total of 32 pigs (13.53 ± 1.27 kg) were used. Pigs were randomly allotted to a 2 × 2 factorial arrangement with 2 types of diets (low-fiber or high-fiber) and 2 levels of Cu from Cu hydroxychloride (0 or 150 mg/kg). For the low-fiber diets, two diets based on corn and soybean

meal (**SBM**) were formulated to meet the nutrient requirements for 11 to 25 kg pigs (Table 8.1; NRC, 2012). The only difference between the 2 diets was that one diet contained no Cu hydroxychloride, whereas the other diet contained 150 mg Cu/kg from Cu hydroxychloride. Two additional diets were formulated based on corn, SBM, wheat middlings, and distillers dried grains with solubles (**DDGS**) without and with Cu hydroxychloride. No antibiotic growth promoters or vaccines were administered to the pigs during the experimental period, and Zn was not included at pharmacological levels. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). A sample of each diet was collected and sub-sampled.

Pigs were placed in individual metabolism crates that were equipped with a self-feeder, a nipple waterer, a slatted floor, and a urine pan to allow for the total, but separate, collection of urine. Pigs were limit fed at 3 times the energy requirement for maintenance (i.e., 197 kcal/kg × $BW^{0.60}$; NRC, 2012), which was provided each day in 2 equal meals at 0800 and 1600 h. Throughout the study, pigs had *ad libitum* access to water. Pigs had a 5 d adaptation period to the crates. On d 6, individual weights were recorded and intestinal permeability was assessed through the oral administration of lactulose and mannitol using a syringe with an extension tube to reach the back of the mouth. Pigs were overnight fasted before a solution of lactulose (500 mg/kg body weight; Sigma, St. Louis, MO, USA) and mannitol (50 mg/kg body weight; Sigma, St. Louis, MO, USA) were mixed with 10 ml distilled water. After administration, urine was collected in urine buckets during 2 6-h periods. A preservative of 50 mL of 6*N* HCl was added to the buckets, and 20% of the collected urine was stored at -20ºC immediately after collection.

Diets were analyzed for dry matter (Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007). Crude protein was analyzed using the combustion procedure (Method

990.03; AOAC Int., 2007) on a Leco FP628 protein apparatus (Leco Corporation, St. Joseph, MI), and gross energy was determined using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). Acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY), and amino acids were analyzed (Method 982.30 E (a, b, c); AOAC Int., 2007) on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Minerals were analyzed by inductively coupled plasma optical emissions spectrometry using an internally validated method (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B[b]; AOAC Int., 2007). Analyses for amino acids and minerals were conducted at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Colombia, and all other analyses were conducted in the Monogastric Nutrition Laboratory at the University of Illinois at Urbana-Champaign, Urbana. Urinary lactulose and mannitol concentrations were determined by high-performance liquid chromatography (**HPLC**) using ion-exchange chromatography with pulsed amperometric detection (Generoso et al., 2003). The lactulose:mannitol was calculated based on the percent recovery basis of lactulose and mannitol, and this was considered as an index of intestinal permeability (Wijtten et al., 2011).

***Experiment 2: Growth Performance and Blood Characteristics***

A total of 128 pigs (8.33 ± 1.32 kg) were randomly allotted to a 2 × 2 factorial arrangement using the same 4 diets as those used in Exp. 1. Diets for Exp. 1 and 2 were mixed in one batch. There were 4 pigs per pen with 8 replicate pens per treatment. No antibiotic growth promoters or vaccines were administered to the pigs during the experimental period.

Individual pig weights were recorded at the beginning of the experiment, on d 7, on d 14, and at the conclusion of the experiment on d 21. Feed addition was recorded daily and the weight of feed left in the feeders was recorded on d 7, d 14, and d 21. Data were summarized to calculate average daily feed intake (**ADFI**), average daily gain (**ADG**), and average gain:feed ratio (**G:F**) within each pen and treatment group. Data were summarized for d 0 to 7, d 7 to 14, d 14 to 21, and for the entire experiment from d 0 to 21.

On d 7, d 14, and on d 21, 2 blood samples were collected from 1 pig per pen via vena puncture. The same pigs were bled at each bleeding. These samples were collected in vacutainers that contained either heparin or ethylenediaminetetraacetic acid (**EDTA)**. Heparinized samples were frozen at -20ºC and were analyzed for blood urea N (**BUN**), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA). Heparinized samples were also analyzed for interleukin-1 beta (**IL-1ß**; R&D Systems, Inc., Minneapolis, MN). Tumor necrosis factor-α and interleukin 10 (**IL-10**) were measured in plasma samples collected in the vacutainer with EDTA using ELISA kits according to the recommendations from the manufacturer (R&D Systems, Inc., Minneapolis, MN). Samples collected in the EDTA-containing tubes were also analyzed for immunoglobulin G (**IgG**; Bethyl Laboratories, Inc., Montgomery, TX).

***Statistical Analyses***

Data from both experiments were analyzed following a 2 × 2 factorial arrangement using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pig or pen as the experimental unit. Fixed effects included Cu, diet type, and the interaction between Cu and diet type. Random effects included replicate. Least square means were calculated for each independent variable and

means were separated using the PDIFF option. Results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

## RESULTS

Diet analyses indicated that the intended concentrations of total dietary fiber and Cu were present in all diets and concentrations of other nutrients were not affected by dietary treatment (Table 8.2). For Exp. 1, there were no diet type × Cu interactions observed for urinary lactulose:mannitol of pigs (Table 8.3). Inclusion of DDGS and wheat middlings in diets tended to increase ($P < 0.10$) the lactulose:mannitol ratio during the initial 6-h period. However, no difference was observed in the lactulose:mannitol ratio between pigs fed low-fiber diets and pigs fed high-fiber diets in the second 6-h period. The level of dietary Cu did not affect urinary lactulose:mannitol ratios of pigs.

For Exp. 2, no interactions between diet type and Cu were observed for the overall growth performance of pigs (Table 8.4). Greater ($P < 0.05$) ADG and G:F were observed from d 0 to 7 in pigs fed diets containing 150 mg/kg Cu from Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride. Pigs fed the Cu hydroxychloride diets also had greater ($P < 0.05$) G:F from d 7 to 14 compared with pigs fed diets without added Cu. For the overall experimental period, pigs fed diets containing Cu hydroxychloride had greater ($P < 0.05$) ADG and G:F compared with pigs fed diets without Cu hydroxychloride. The level of dietary fiber did not affect growth performance of pigs.

No interactions between diet type and Cu were observed for the overall blood No interactions between diet type and Cu were observed for the overall blood characteristics of pigs (Table 8.5). Inclusion of Cu hydroxychloride to diets reduced ($P < 0.05$) the concentrations of

BUN and TNF-α on d 7 and d 21, respectively. On d 14, pigs fed diets with 150 mg Cu/kg from Cu hydroxychloride had greater ($P < 0.05$) concentration of IL-10, and tended to have greater ($P < 0.10$) concentration of albumin compared with pigs fed diets without Cu hydroxychloride. Likewise, pigs fed the Cu hydroxychloride diets had increased ($P < 0.05$) albumin concentration compared with pigs fed diets without added Cu on d 21. Inclusion of DDGS and wheat middlings to diets resulted in a tendency for a reduction ($P < 0.10$) in the concentration of albumin on d 7. On d 14, pigs fed high-fiber diets had a tendency to have greater ($P < 0.10$) BUN concentration, and pigs fed high-fiber diets had reduced ($P < 0.05$) concentrations of albumin and IL-10 compared with pigs fed the low-fiber diets. Pigs fed high-fiber diets also had reduced ($P < 0.05$) albumin concentration compared with pigs fed low-fiber diets on d 21. However, on d 21, concentrations of total protein, IgG, and IL-1ß were not affected by dietary Cu concentrations or inclusion of fibrous co-products in the diets.

## DISCUSSION

Distillers dried grains with solubles and wheat middlings are cereal co-products that are commonly included in diets for growing-finishing pigs. These co-products are usually less expensive than corn and soybean meal due to the high concentration of dietary fiber, which are resistant to enzymatic hydrolysis in the small intestine, and therefore, cannot be digested by pigs (Anguita et al., 2006; Bindelle et al., 2008). Growing-finishing pigs may obtain energy from dietary fiber via synthesis of volatile fatty acids as a result of microbial fermentation in the hindgut (Dierick et al., 1989; Macfarlane and Macfarlane, 2007). The observation that inclusion of fibrous co-products to diets did not affect overall growth performance of nursery pigs

indicates that under the conditions of this experiment, weanling pigs from approximately 8 to 18 kg tolerated diets containing 20% DDGS and 10% wheat middlings without apparent negative effects on growth performance. However, all diets were formulated to contain similar concentrations of ME and standardized ileal digestible amino acids, which likely contributed to the lack of differences in growth performance (Gutierrez et al., 2013; Jaworski et al., 2014). However, these results are in contrast with some previous data indicating that inclusion of 30% DDGS to diets resulted in a reduction in nutrient digestibility and reduced ADG of nursery pigs (Tsai et al., 2017). However, Jones et al. (2010) reported that inclusion of high concentration of DDGS to diets did not influence overall ADG, ADFI, or G:F of nursery pigs. Thus, pigs appear to react differently to inclusion of DDGS and wheat middlings in diets for pigs, which possibly is an effect of differences in the nutritional quality of the sources of DDGS and wheat middlings that are used.

The observation that supplementation of Cu hydroxychloride to diets resulted in an improvement in ADG and G:F of pigs were in agreement with previous data (Cromwell et al., 1989; Cromwell et al., 1998; Espinosa et al., 2017). Inclusion of 125 mg Cu/kg from $CuSO_4$ increased the mRNA abundance of growth hormone-releasing hormone and also suppressed the mRNA abundance of somatostatin in the hypothalamus of pigs (Zhou et al., 1994). Therefore, it is possible that the observed improvement in growth performance of pigs upon supplementation with Cu hydroxychloride to the diets is due to the effect of Cu on stimulating growth-promoting regulatory peptides such as growth hormone-releasing hormone (LaBella et al., 1973; Zhou et al., 1994). It is also possible that Cu stimulates post-translational modification of regulatory peptides, which may also contribute to improved growth performance (Eipper and Mains, 1988). Improved ADG and G:F in pigs fed diets containing Cu hydroxychloride may also be a result of

the beneficial effect of Cu on intestinal health of pigs, increased villus height, or changed microbiota profile in the gastointestinal tract of pigs (Namkung et al., 2006; Zhao et al., 2007). Dietary Cu may also improve growth performance of pigs by upregulating mRNA abundance of proteins involved in the uptake and utilization of fatty acids. Addition of 45 mg/kg of Cu to diets for rabbits improved body mass gain by upregulating mRNA transcription of fatty acid binding proteins and fatty acid transport proteins (Lei et al., 2017), indicating an increase in cellular uptake of fatty acids (Chen et al., 2016).

The oral administration of lactulose and mannitol as marker probes to assess intestinal permeability in pigs is considered to be reliable and more advantageous than Ussing chambers because multiple measurements can be obtained from the same animal over time (Wijtten et al., 2011). Lactulose is a disacharride that transverses the intestinal epithelium through paracellular route if gut barrier function is compromised; whereas, mannitol is a monosaccharide that is absorbed by either paracellular or transcellular routes (Wijtten et al., 2011). These sugars are not metabolized in the body and the majority of the absorbed sugars is, therefore, excreted in the urine. The ratio between lactulose and mannitol recovered in the urine serves as an index for intestinal barrier function (Bjarnason et al., 1995). The increase in the lactulose:mannitol ratio during the initial 6-hr period after administration of sugars of pigs fed high-fiber diets indicates that the intestinal barrier function of these pigs was disturbed compared with pigs fed the low-fiber diets. Pigs experience a variety of stresses after weaning such as physiological, nutritional, environmental, and social challenges (Campbell et al., 2013). Inclusion of fibrous co-products in diets, which are less digestible and palatable than some low-fiber ingredients, may induce stress (Yang et al., 2016), and result in compromised intestinal health and subsequently increase intestinal permeability. The lack of differences in the lactulose:mannitol ratio of pigs fed diets

containing 0 or 150 mg Cu/kg from Cu hydroxychloride indicates that high concentration of dietary Cu did not impact intestinal permeability of pigs. This is in contrast with data indicating that addition of 1,500 mg/kg of Cu-exchanged montmorillonite to diets reduced intestinal permeability of pigs, with plasma diamine oxidase and D-lactate used as marker probes for intestinal barrier integrity (Song et al., 2013).

Concentrations of blood urea N, total protein, and albumin that were observed in Exp. 2 were within the normal physiological ranges (Tumbleson and Kalish, 1972) and were in agreement with previous data (Casas and Stein, 2016; Espinosa et al., 2017). The observed reduction in the concentration of albumin on d 7, 14, and 21 in pigs fed the high-fiber diets is possibly due to a reduced absorption of nutrients from the small intestine caused by an increased concentration of dietary fiber. Inclusion of fibrous co-products results in a reduction in the apparent ileal digestibility and apparent total tract digestibility of organic matter (Graham et al., 1986; Bach Knudsen and Hansen, 2007), and energy from fiber is absorbed following fermentation in the large intestine rather than absorption of glucose from the small intestine. Albumin constitutes approximately 60% of total plasma protein and one of its main functions is to bind and transport nutrients including fatty acids, glucose, amino acids, and metal ions such as Zn and Cu (Quinlan et al., 2005; Francis, 2010). Therefore, if inclusion of fibrous co-products results in reduced glucose absorption, pigs fed high-fiber diets may have reduced need for albumin to transport nutrients from the liver to extrahepatic tissues (Ramos et al., 2016).

Blood urea N is often used as an index to predict efficiency of amino acid utilization and N excretion in pigs (Coma et al., 1995; Russek-Cohen et al., 2005). The observed tendency for an increase in the concentration of BUN on d 14 in pigs fed high-fiber diets indicates that high concentrations of dietary fiber reduces the efficiency of N utilization of pigs. Inclusion of high

concentration of dietary fiber in diets often results in a reduction in digestibility of crude protein (Zhang et al., 2013), and this may result in a reduction in the efficiency of amino acid utilization with a subsequent increase in the concentration of BUN.

Interleukin-10 is an anti-inflammatory cytokine, which inhibits activation and effector function of T cells, monocytes, and macrophages (Moore et al., 2001). The reduction in the concentration of IL-10 on d 14 in pigs fed the high-fiber diets is in contrast with data indicating that inclusion of 7.5% DDGS in diets for weanling pigs increased mRNA abundance of IL-10 in ileal tissue of pigs (Weber et al., 2008). However, the observed reduction for IL-10 is in agreement with the observed increase in the intestinal permeability of pigs. Anti-inflammatory agents, which include IL-10, contribute to protecting the intestinal barrier integrity from effects of interferon-ɤ and TNF-α in inducing barrier disruption (Al-Sadi et al., 2009). Effects of dietary fiber on cytokine concentrations of pigs may vary and may depend on the age of the animal, as well as fermentability and viscosity of the fiber, and inclusion level of cereal co-products in the diet (Ferrandis Vila et al., 2018).

The increase in the concentration of albumin that was observed on d 14 and 21 in pigs fed the Cu hydroxychloride diets may be due to an increased absorption of Cu. Most of the absorbed $Cu^{2+}$ in the hepatic portal vein needs to be bound to albumin (Linder, 1991) for transport to the liver, where it is taken up by hepatocytes as $Cu^{+}$ using Cu reductase. The increased albumin concentration in pigs fed Cu hydroxychloride may also result in an increased efficiency of transporting other nutrients to the liver and from the liver to peripheral tissues (Ramos et al., 2016). The reduction in the concentration of BUN that was observed on d 7 in pigs fed diets containing Cu hydroxychloride indicates that Cu hydroxychloride improves the efficiency of

amino acid utilization by pigs (Whang and Easter, 2000), and this may also result in an increased availability of amino acids for protein synthesis and skeletal muscle growth.

The reduction in the concentration of TNF-α on d 21 that was observed when diets were supplemented with Cu hydroxychloride is in agreement with previous data (Song et al., 2013). Tumor necrosis factor-α is a cytokine produced by macrophages, lymphocyte cell lines, and monocytes in response to diseases and infections caused by parasites and pathogens (Pauli, 1995). Therefore, the observed reduction in the concentration of TNF-α upon Cu supplementation may be a result of the impact of dietary Cu on improving the intestinal health and immune response of pigs. Dietary Cu may reduce bacterial populations in the intestinal tract of pigs (Højberg et al., 2005), and this may result in a reduced inflammation caused by pathogens. The observed increase in IL-10 concentration of pigs fed diets containing Cu hydroxychloride may contribute to an increased protection and an improved ability to combat diseases and infection, and thereby improve growth performance of pigs.

## CONCLUSION

In conclusion, inclusion of 20% DDGS and 10% wheat middlings in diets for nursery pigs did not affect overall growth performance. However, negative changes were observed in intestinal permeability and BUN concentration, as well as in concentrations of albumin and IL-10, in pigs fed diets with DDGS and wheat middlings. Supplementation of Cu hydroxychloride to low-fiber or high-fiber diets improved growth performance of pigs and Cu hydroxychloride positively influenced BUN, albumin, and cytokine concentrations in pigs.

## TABLES

**Table 8.1.** Ingredient composition of experimental diets used in Exp. 1 and 2

| | No added Cu | | 150 mg/kg Cu[1] | |
|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber |
| Ingredient | | | | |
| Ground corn | 52.99 | 38.71 | 52.962 | 38.682 |
| Soybean meal, 48% CP | 32.00 | 25.00 | 32.00 | 25.00 |
| Distillers dried grains with solubles | - | 20.00 | - | 20.00 |
| Wheat middlings | - | 10.00 | - | 10.00 |
| Dried whey | 10.00 | - | 10.00 | - |
| Soybean oil | 1.50 | 2.50 | 1.50 | 2.50 |
| Limestone | 1.30 | 1.58 | 1.30 | 1.58 |
| Dicalcium phosphate | 0.18 | - | 0.18 | - |
| Copper hydroxychloride, 54% Cu | - | - | 0.028 | 0.028 |
| L-Lys, HCl | 0.26 | 0.45 | 0.26 | 0.45 |
| DL-Met | 0.07 | 0.04 | 0.07 | 0.04 |
| Threonine | 0.05 | 0.07 | 0.05 | 0.07 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 |
| Phytase premix[2] | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 |
| Calculated ME[4], kcal/kg | 3,354 | 3,361 | 3,354 | 3,364 |

[1]Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN).

**Table 8.1. (cont.)**

[2]The phytase premix contained phytase (5,000 FTU/g of Quantum Blue 5G; AB Vista, Marlborough, United Kingdom) mixed with corn. The mixture was formulated to provide 500 units of phytase per kilogram of complete diet.

[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[4]ME = metabolizable energy.

**Table 8.2.** Analyzed composition of experimental diets used in Exp. 1 and 2

| | No added Cu | | 150 mg/kg Cu[1] | |
|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber |
| Dry matter, % | 88.39 | 88.22 | 88.35 | 87.89 |
| Ash, % | 5.69 | 5.63 | 5.62 | 5.53 |
| Gross energy, kcal/kg | 3,964 | 4,175 | 3,954 | 4,138 |
| Crude protein, % | 20.71 | 22.94 | 21.94 | 22.54 |
| Acid hydrolyzed ether extract, % | 2.43 | 3.60 | 2.25 | 3.68 |
| Insoluble dietary fiber, % | 9.70 | 16.80 | 9.80 | 17.40 |
| Soluble dietary fiber, % | 0.70 | 1.20 | 0.50 | 0.90 |
| Total dietary fiber, % | 10.40 | 18.00 | 10.30 | 18.30 |
| Minerals | | | | |
| Ca, % | 0.91 | 0.77 | 0.81 | 0.78 |
| P, % | 0.46 | 0.57 | 0.47 | 0.58 |
| Na, % | 0.31 | 0.26 | 0.27 | 0.28 |
| Mg, % | 0.16 | 0.22 | 0.16 | 0.22 |
| K, % | 1.12 | 1.06 | 1.14 | 1.08 |
| S, % | 0.21 | 0.23 | 0.21 | 0.23 |
| Mn, mg/kg | 76.20 | 85.20 | 76.50 | 81.10 |
| Fe, mg/kg | 210.00 | 181.00 | 241.00 | 207.00 |
| Zn, mg/kg | 141.00 | 164.00 | 154.00 | 163.00 |
| Cu, mg/kg | 30.90 | 26.30 | 189.00 | 200.00 |
| Indispensable AA, % | | | | |

**Table 8.2. (cont.)**

| | | | | |
|---|---|---|---|---|
| Arg | 1.31 | 1.32 | 1.29 | 1.31 |
| His | 0.53 | 0.56 | 0.53 | 0.57 |
| Ile | 0.97 | 0.96 | 0.98 | 0.96 |
| Leu | 1.81 | 1.94 | 1.82 | 1.93 |
| Lys | 1.40 | 1.42 | 1.38 | 1.48 |
| Met | 0.36 | 0.35 | 0.32 | 0.37 |
| Met + Cys | 0.68 | 0.73 | 0.65 | 0.74 |
| Phe | 1.06 | 1.10 | 1.05 | 1.10 |
| Thr | 0.83 | 0.85 | 0.92 | 1.00 |
| Trp | 0.25 | 0.23 | 0.24 | 0.25 |
| Val | 1.02 | 1.06 | 1.03 | 1.06 |
| Dispensable AA, % | | | | |
| Ala | 1.02 | 1.15 | 1.03 | 1.16 |
| Asp | 2.12 | 1.99 | 2.10 | 1.99 |
| Cys | 0.32 | 0.38 | 0.33 | 0.37 |
| Glu | 3.72 | 3.72 | 3.75 | 3.76 |
| Gly | 0.86 | 0.95 | 0.86 | 0.96 |
| Ser | 0.81 | 0.86 | 0.81 | 0.89 |
| Tyr | 0.73 | 0.75 | 0.70 | 0.74 |

[1]Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN).

**Table 8.3.** Urinary lactulose:mannitol (percent recovery basis) of pigs fed low fiber or high fiber diets containing 0 or 150 mg/kg Cu from Cu hydroxychloride[1]

| | No added Cu | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber | SEM | Fiber | Cu | Fiber × Cu |
| 0 to 6 h | | | | | | | | |
| Lactulose:mannitol | 0.105 | 0.148 | 0.077 | 0.141 | 0.03 | 0.062 | 0.530 | 0.993 |
| 6 to 12 h | | | | | | | | |
| Lactulose:mannitol | 0.232 | 0.375 | 0.217 | 0.300 | 0.08 | 0.175 | 0.580 | 0.711 |

[1]Data are least squares means of 8 observations (pen as the experimental unit; 4 pigs per pen) for all treatments.

[2]Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN).

**Table 8.4.** Growth performance for pigs fed low fiber or high fiber diets containing 0 or 150 mg/kg Cu from Cu hydroxychloride[1]

| | No added Cu | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber | SEM | Fiber | Cu | Fiber × Cu |
| d 0 to 7 | | | | | | | | |
| Initial body weight, kg | 8.314 | 8.318 | 8.334 | 8.353 | 0.475 | 0.981 | 0.955 | 0.988 |
| ADG, kg | 0.324 | 0.308 | 0.417 | 0.377 | 0.024 | 0.252 | 0.002 | 0.616 |
| ADFI, kg | 0.593 | 0.543 | 0.626 | 0.594 | 0.041 | 0.329 | 0.316 | 0.829 |
| G:F | 0.549 | 0.570 | 0.670 | 0.634 | 0.028 | 0.946 | 0.003 | 0.307 |
| Final body weight, kg | 10.583 | 10.476 | 11.250 | 10.990 | 0.592 | 0.759 | 0.327 | 0.898 |
| d 7 to 14 | | | | | | | | |
| ADG, kg | 0.520 | 0.476 | 0.558 | 0.515 | 0.031 | 0.196 | 0.185 | 0.950 |
| ADFI, kg | 0.875 | 0.790 | 0.830 | 0.724 | 0.064 | 0.145 | 0.392 | 0.872 |
| G:F | 0.589 | 0.605 | 0.675 | 0.711 | 0.051 | 0.443 | 0.032 | 0.887 |
| Final body weight, kg | 14.240 | 13.806 | 15.156 | 14.598 | 0.745 | 0.511 | 0.261 | 0.934 |
| d 14 to 21 | | | | | | | | |
| ADG, kg | 0.558 | 0.560 | 0.609 | 0.577 | 0.033 | 0.639 | 0.307 | 0.601 |
| ADFI, kg | 0.988 | 0.911 | 0.975 | 0.967 | 0.046 | 0.366 | 0.642 | 0.464 |

**Table 8.4. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| G:F | 0.571 | 0.620 | 0.629 | 0.594 | 0.031 | 0.785 | 0.645 | 0.178 |
| Final body weight, kg | 18.148 | 17.726 | 19.421 | 18.635 | 0.878 | 0.497 | 0.224 | 0.837 |
| d 0 to 21 | | | | | | | | |
| ADG, kg | 0.468 | 0.448 | 0.528 | 0.490 | 0.022 | 0.196 | 0.028 | 0.677 |
| ADFI, kg | 0.825 | 0.748 | 0.810 | 0.762 | 0.045 | 0.178 | 0.993 | 0.760 |
| G:F | 0.570 | 0.601 | 0.656 | 0.646 | 0.022 | 0.511 | 0.010 | 0.267 |

[1]Data are least squares means of 8 observations (pen as the experimental unit; 4 pigs per pen) for all treatments.

[2]Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients USA; Indianapolis, IN).

**Table 8.5.** Blood characteristics for pigs fed with low fiber or high fiber diets containing 0 or 150 mg/kg Cu from Cu hydroxychloride[1]

| | No Cu | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber | SEM | Fiber | Cu | Fiber × Cu |
| d 7 | | | | | | | | |
| BUN[3], mg/dL | 12.13 | 13.71 | 10.71 | 11.38 | 0.85 | 0.199 | 0.037 | 0.591 |
| Total protein, g/dL | 4.58 | 4.56 | 4.63 | 4.44 | 0.12 | 0.396 | 0.749 | 0.457 |
| Albumin, g/dL | 2.55 | 2.39 | 2.65 | 2.43 | 0.10 | 0.073 | 0.514 | 0.766 |
| TNF-α[3], pg/mL | 167.21 | 152.37 | 151.72 | 156.85 | 11.04 | 0.663 | 0.622 | 0.374 |
| IgG[3], mg/mL | 18.71 | 19.24 | 19.29 | 20.00 | 1.35 | 0.649 | 0.622 | 0.947 |
| Interleukin-1 beta, pg/mL | 34.27 | 34.48 | 34.58 | 34.89 | 0.70 | 0.687 | 0.584 | 0.942 |
| Interleukin-10, pg/mL | 20.69 | 19.74 | 22.20 | 20.92 | 1.29 | 0.398 | 0.307 | 0.901 |
| d 14 | | | | | | | | |
| BUN, mg/dL | 10.50 | 12.75 | 10.43 | 11.13 | 0.82 | 0.083 | 0.310 | 0.352 |
| Total protein, g/dL | 4.74 | 4.68 | 5.00 | 4.68 | 0.13 | 0.138 | 0.310 | 0.310 |
| Albumin, g/dL | 2.76 | 2.50 | 2.99 | 2.71 | 0.12 | 0.035 | 0.081 | 0.959 |
| TNF-α, pg/mL | 116.5 | 131.04 | 117.17 | 101.59 | 12.21 | 0.963 | 0.205 | 0.185 |

**Table 8.5. (cont.)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| IgG, mg/mL | 20.03 | 21.09 | 21.46 | 20.13 | 0.72 | 0.856 | 0.749 | 0.110 |
| Interleukin-1 beta, mg/mL | 18.58 | 17.92 | 18.39 | 18.30 | 0.24 | 0.119 | 0.675 | 0.222 |
| Interleukin-10, mg/mL | 16.19 | 14.34 | 18.96 | 15.25 | 1.15 | 0.003 | 0.039 | 0.280 |
| d 21 | | | | | | | | |
| BUN, mg/dL | 13.37 | 13.12 | 13.00 | 12.00 | 0.92 | 0.504 | 0.423 | 0.688 |
| Total protein, g/dL | 5.23 | 5.04 | 5.18 | 5.20 | 0.14 | 0.558 | 0.685 | 0.445 |
| Albumin, g/dL | 3.10 | 2.70 | 3.15 | 3.11 | 0.11 | 0.061 | 0.048 | 0.117 |
| TNF-α, pg/mL | 113.22 | 121.61 | 92.75 | 85.90 | 13.27 | 0.954 | 0.044 | 0.570 |
| IgG, mg/mL | 15.61 | 18.51 | 16.21 | 15.84 | 1.47 | 0.245 | 0.342 | 0.136 |
| Interleukin-1 beta, mg/mL | 15.16 | 15.58 | 15.71 | 15.50 | 0.38 | 0.780 | 0.538 | 0.419 |
| Interleukin-10, mg/mL | 10.97 | 10.33 | 11.41 | 11.15 | 0.89 | 0.623 | 0.490 | 0.837 |

[1]Data are least squares means of 8 observations (pen as the experimental unit; 4 pigs per pen) for all treatments.

[2]Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN).

[3]BUN = blood urea N; TNF-α = tumor necrosis factor-α; IgG = immunoglobulin G.

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# CHAPTER 9: OVERALL CONCLUSIONS

One of the main challenges in swine production is the restriction of antimicrobial use in food animals. The European Union introduced bans on the use of antibiotic growth promoters in food animal production, and other countries have followed this ban with the intention to reduce pools of resistance genes. One of the alternatives for antibiotic growth promoters is dietary pharmacological levels of Cu. Copper sulfate is the most commonly used inorganic source of supplemental Cu in animal feeding due to its availability, however, pharmacological concentrations of $CuSO_4$ in diets have resulted in environmental concerns due to high excretion of Cu in feces. Therefore, other forms of inorganic Cu have been introduced into the feed market, and one of these is Cu hydroxychloride ($Cu_2(OH)_3Cl$). The overall focus of this dissertation was to determine mechanisms and modes of action of dietary Cu as Cu hydroxychloride in improving growth performance of pigs. It was also the intent of this dissertation to investigate effects of Cu hydroxychloride on growth performance and immune response of pigs upon exposure to environmental and nutritional challenges.

The use of Cu by human civilization dates back between the $5^{th}$ and $6^{th}$ millennia B. C. where Cu was used as treatment for burns, intestinal worms, and infections. The effects of supplementing Cu hydroxychloride to diets for nursery pigs on growth performance, nutrient digestibility, blood characteristics, concentrations of volatile fatty acids and intestinal microbial protein, mRNA abundance of genes involved in post-absorptive metabolism of lipids, and intestinal permeability were investigated. Results demonstrated that supplementation of Cu as Cu hydroxychloride consistently improved feed efficiency and reduced incidence of diarrhea. It was also demonstrated that Cu hydroxychloride positively influences immune response of pigs. The observed improvement in growth performance upon supplementation of high concentration of

dietary Cu was in agreement with results of research with $CuSO_4$ from the early 1950's. However, the mechanism by which dietary Cu exert these positive effects on pig performance has never been fully elucidated.

One hypothesized mode of action for Cu is that Cu affects the bacteriostatic properties in the intestinal tract with a subsequent improvement in gastrointestinal health and immune function of pigs. This is partly due to innate or natural bactericidal properties of Cu because Cu may induce oxidative damage to most biological molecules including bacterial membranes. Therefore, an experiment was designed to test the hypothesis that Cu from Cu hydroxychloride changes concentration of microbial protein in the small intestine or in the large intestine by pigs. Results indicated that supplementation of Cu hydroxychloride to diets reduced the concentrations of total volatile fatty acids and microbial protein in feces. Therefore, these observations indicate that the improved feed efficiency that was observed in pigs upon Cu supplementation is likely due to the effect of Cu in reducing microbial populations in the intestinal tract. This may have reduced the number of toxins and pathogenic microorganisms that could have negatively affected intestinal health, which may have reduced incidence of diarrhea and positively influenced immune response in pigs.

In addition to the impact on intestinal health of pigs fed dietary Cu, because of reduced microbial population, the mode of action of Cu also was hypothesized to be related to systemic effects. Administration of high concentration of Cu via intravenous injection improved growth performance in previous experiments. This response was hypothesized to be attributed to the effect of Cu on increasing the mRNA expressions of growth hormone releasing hormone and neuropeptide Y in the hypothalamus of pigs. In rabbits and fish, the effect of Cu on improving body mass gain is attributed to its role in increasing mRNA expression of fatty acid binding

proteins and fatty acid transport proteins. To test the hypothesis that similar results can be obtained in pigs, another experiment was designed to investigate effects of Cu hydroxychloride on feed efficiency and mRNA abundance of genes involved in lipid metabolism of pigs. It was demonstrated that supplementation of Cu hydroxychloride to diets increased mRNA abundance of lipoprotein lipase, fatty acid binding proteins, peroxisome proliferator-activated receptor alpha, and carnitine palmitoyl transferase 1 B. Therefore, it was concluded that it is possible that the improved growth performance of pigs fed the Cu-supplemented diets is a result of improved lipid metabolism, which may have improved energy utilization.

In summary, results of experiments included in this dissertation confirmed that inclusion of Cu from Cu hydroxychloride improves growth performance and reduces diarrhea from pigs fed diets containing no antibiotic growth promoters. The consistent improvement in feed efficiency upon Cu supplementation to diets is likely a result of the ability of dietary Cu to modulate intestinal microbial populations and its effect on increasing mRNA abundance of some genes involved in post-absorptive metabolism of lipids.

Sources of inorganic Cu used in animal feeding have different physical and chemical characteristics and different sources of Cu may have different effects on pig growth performance, intestinal health, and post-absorptive energy metabolism. Results from this dissertation that were obtained using Cu hydroxychloride can, therefore, not be extrapolated to other sources of Cu without prior confirmation that similar effects can be obtained using other sources. It is also necessary to determine effects of Cu supplementation on the net energy of diets because Cu may reduce heat increment by reducing microbial activity in the hindgut of pigs. Effects of dietary Cu on growth performance, carcass characteristics, intestinal microbial protein concentrations, and

mRNA abundance of genes involved in post-absorptive metabolism of lipids of growing-finishing pigs also need to be investigated.