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Effects of copper hydroxychloride on growth performance and abundance of genes involved in lipid metabolism of growing pigs

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Abstract

An experiment was conducted to test the hypothesis that copper (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 copper 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 ADG and G:F were greater ($P < 0.05$) for pigs fed the diet containing copper hydroxychloride compared with pigs fed the control diet. Pigs fed the diet supplemented with copper hydroxychloride also had increased ($P < 0.05$) abundance of cluster of differentiation 36 in the liver and increased ($P < 0.05$) abundance of fatty acid-binding protein 4 and lipoprotein lipase in subcutaneous adipose tissue. Inclusion of copper hydroxychloride also tended to increase ($P < 0.10$) the abundance of fatty acid-binding protein 1, peroxisome proliferator-activated receptor α, and carnitine palmitoyltransferase 1B in the liver, skeletal muscle, and subcutaneous adipose tissue, respectively. This indicates that dietary Cu may affect signaling pathways associated with lipid metabolism by improving the uptake, transport, and utilization of fatty acids. In conclusion, supplementation of copper hydroxychloride to the control diet improved growth performance and upregulated the abundance of some genes involved in postabsorptive metabolism of lipids.

Key words: copper, copper hydroxychloride, 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 vary 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 postweaning scouring and also improve ADG and 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 (**FATP**), fatty acid-binding protein (**FABP**), and carnitine palmitoyltransferase 1 (**CPT1**) in skeletal muscle (Lei et al., 2017), indicating that dietary Cu may influence postabsorptive metabolism of lipids. Supplementation of Cu to diets also increased lipogenesis and fatty acid uptake in fish by upregulating mRNA transcription of fatty acid synthase (**FAS**) and acetyl CoA carboxylase (**ACC**) in intestinal tissue (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 FABP. Hence, the effect of supplementing dietary Cu above the requirement on postabsorptive 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 postabsorptive 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 the initiation of the experiment. Pigs that are the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

Sixteen barrows and 16 gilts (15.05 ± 0.98 kg) that had been weaned for 28 d 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 (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 copper hydroxychloride (IntelliBond C^{II}; Micronutrients USA; Indianapolis, IN) to the control diet. Experimental diets were fed to pigs for 28 d (Tables 1 and 2).

Individual pig weights were recorded at the beginning of the experiment, on day 15, and at the conclusion of the experiment on day 28. Feed addition was recorded daily and weight of feed left in the feeder was recorded on days 15 and 28, respectively. At the conclusion of the experiment, data were summarized to calculate ADG, ADFI, and average G:F within each pen and treatment group. Data were summarized for the initial 15 d, the final 13 d, and the entire experiment. At the conclusion of the experiment, when pigs had an average BW of 35.14 kg, the pig with the greater BW in each pen was sacrificed via captive bolt stunning. Liver, skeletal muscle (longissimus dorsi), and adipose tissue from the 10th rib back fat were harvested and placed in 2 mL cryogenic tubes, snap-frozen in liquid N_2, and stored at –80 °C until used for gene expression analysis.

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.

qPCR 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) under the following

Table 1. Ingredient composition of experimental diets

Ingredient	Diet	
	Control	Control + Cu[1]
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]Diets were formulated to contain 3,315 kcal ME/kg, and the following quantities of standardized ileal digestible AA: Arg, 1.14%; His, 0.50%; Ile, 0.75%; Leu, 1.83%; Lys, 1.23%; Met, 0.36%; Met + Cys, 0.68%; Phe, 0.93%; Thr, 0.73%; Trp, 0.20%; Val, 0.86%.
[2]The diet containing added Cu was fortified with 150 mg/kg of Cu from copper hydroxychloride (IntelliBond C^{II}; Micronutrients USA; Indianapolis, IN). The phytase premix contained phytase (5,000 FTU/g of Quantum Blue 5G; AB Vista, Marlborough, United Kingdom) mixed with corn. The premix was formulated to provide 500 units of phytase per kilogram of complete diet if included by 1%.
[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-α 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 2. Analyzed composition of experimental diets

	Diet	
	Control	Control + Cu[1]
DM, %	87.39	87.15
Ash, %	4.83	4.88
GE, kcal/kg	3,995	4,009
CP, %	22.15	21.95
Acid hydrolyzed ether extract, %	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
Leu	1.91	1.85
Lys	1.40	1.40
Met	0.36	0.33
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 copper hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN).

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).

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 *FAS*, *ACC*, fatty acid translocase or cluster of differentiation 36 (***CD36***), and peroxisome proliferator-activated receptor-α (***PPAR-α***) in the liver, skeletal muscle, and subcutaneous adipose tissue. Genes expressed in specific tissues were also tested. Carnitine palmitoyltransferase 1A (***CPT1A***) and fatty acid-binding protein-1 (***FABP1***) were analyzed in liver tissue, whereas carnitine palmitoyltransferase 1B (***CPT1B***) and fatty acid transport protein-1 (***FATP1***) were tested for both skeletal muscle and adipose tissues. Fatty acid-binding protein 3 (***FABP3***) was also analyzed in skeletal muscle. Fatty acid-binding protein 4 (***FABP4***), peroxisome proliferator-activated receptor γ (***PPAR-γ***), lipoprotein lipase (***LPL***), and hormone-sensitive lipase (***HSL***) 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 3 and were commercially synthesized by Integrated DNA Technologies (Skokie, IL).

Methyl esters of fatty acids were extracted from liver, skeletal, and adipose tissues (Method Ce-266; AOCS, 2017), and the concentrations of total fatty acids and free fatty acids (% of total fat) were measured using capillary gas–liquid chromatography (Method 996.06; AOAC Int., 2007). 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 DM (Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007), and GE 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), and CP was analyzed using the combustion procedure (Method 990.03; AOAC Int., 2007) on a Leco FP628 protein apparatus (Leco Corporation, St. Joseph, MI). AAs were analyzed on a Hitachi AA 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).

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 (pen) 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 (Table 2). A tendency for greater ($P < 0.10$) ADG and G:F was observed for pigs fed the diet containing copper hydroxychloride compared with pigs fed the control diet from days 0 to 15 (Table 4). Inclusion of copper hydroxychloride to the control diet resulted in a tendency for improved ($P < 0.10$) final BW on day 28. For the overall experimental period, pigs fed the diet with copper hydroxychloride had greater ($P < 0.05$) ADG, which resulted in greater ($P < 0.05$) G:F compared with pigs fed the control diet.

Inclusion of copper hydroxychloride in the diet increased ($P < 0.05$) the abundance of *CD36* and tended to increase ($P < 0.10$) the abundance of *FABP1* in the liver (Table 5). Pigs fed the copper hydroxychloride diet also had a tendency to have greater ($P < 0.10$) expression of *PPAR-α* in skeletal muscle compared with pigs fed the control diet. Expression of *FABP4* and *LPL* also increased ($P < 0.05$) and expression of *CPT1B* tended to increase ($P < 0.10$) in subcutaneous adipose tissue if copper hydroxychloride was added to the diet. However, no differences were observed among treatments in the

Table 3. Forward and reverse primer sequences used for quantitative reverse transcription PCR

Gene[1]	Direction[2]	Primer sequence	Reference
Internal control gene			
β-*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′	
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-γ	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)
	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-ϒ*, peroxisome proliferator-activated receptor gamma; *LPL*, lipoprotein lipase; *HSL*, hormone sensitive lipase.
[2]Direction of primer (F, forward; R, reverse).

concentrations of total fatty acid and free fatty acids in the liver, skeletal muscle, and subcutaneous adipose tissue of pigs (Table 6).

Discussion

The observed improvement in final BW, ADG, and G:F of pigs fed the copper hydroxychloride diet is in agreement with previous data (Cromwell et al., 1989, 1998; Hill et al., 2000; Ma et al., 2015; Espinosa et al., 2017), but there is limited data on 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). However, addition of 150 mg Cu/kg from copper hydroxychloride did not improve apparent total tract digestibility of energy or true total tract digestibility of fat (Espinosa et al., 2017, 2019), but Gonzalez-Esquerra et al. (2019) demonstrated that addition of 150 mg Cu/kg increased the mRNA expression of ghrelin, which may partially explain the observed improvement in growth performance. 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 125 to 280 mg Cu/kg reduced the concentration of serum polyunsaturated fatty acids, increased iodine value of back fat, and increased proportion of unsaturated fatty acids in the outer back fat, inner back fat, and perinephric back fat of pigs with BWs of 26 to 70 kg (Elliot and Bowland, 1968; Dove, 1993; Zhao et al., 2007; Wu et al., 2018). The addition of 150 mg Cu/kg reduces the concentration of intestinal microbial protein and total volatile fatty acids (Espinosa et al., 2019). Thus, it appears that copper hydroxychloride, in addition to modulating microbial populations in the hindgut of pigs, may impact certain aspects of energy metabolism. Based on these results, we hypothesized that dietary Cu improves the growth performance of nursery pigs by influencing the expression of specific genes involved in lipid metabolism.

To determine if dietary Cu influences postabsorptive metabolism of lipids in pigs, the abundance of genes involved in the uptake, transport, synthesis, and oxidation of fatty acids was analyzed. Tested genes involved in lipogenesis include *ACC* and *FAS*. Acetyl CoA carboxylase catalyzes the irreversible carboxylation of acetyl CoA to malonyl CoA (Coutanson et al., 2004), whereas *FAS* 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 *LPL*, which hydrolyzes the triacylglycerol component of 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 *CPT1*, *HSL*, and *PPAR-α*. *CPT1* is the rate-limiting enzyme in the β-oxidation of fatty acids and facilitate the transfer of fatty acids into the mitochondria through formation of acylcarnitines (Brown et al., 1997), whereas *HSL* is an intracellular lipase that catalyzes the hydrolysis of stored triacylglycerols, diacylglycerols, and cholesteryl esters to free fatty acids for energy provision (Kraemer and Shen, 2002). *PPAR-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 *PPAR-α* and *PPAR-γ*. *PPAR-γ* 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 includes *FABP*, *FATP*, and *CD36*. *FATP* and *FABP* 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). A cluster of differentiation 36 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 three 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 abundance of *LPL* 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 *LPL*. *LPL* is synthesized in the parenchymal cells of adipose tissue, cardiac muscle, and skeletal muscle (Wang and Eckel, 2009). After synthesis, *LPL* is transported to the luminal surface of capillary endothelial cells where it is attached by heparin sulfated proteoglycans (Braun and Severson, 1992), and the activation of *LPL* is influenced by apolipoprotein C-II and divalent cations (Srinivasan et al., 1975). Therefore, the observed increase in *LPL* abundance upon addition of copper hydroxychloride to the diet may have promoted *LPL* activation in a similar manner as divalent cations. Lau and Klevay (1982) demonstrated that *LPL* activity was reduced by 40% when rats were fed diets deficient in Cu, which demonstrates the importance of Cu in lipoprotein lipase activity. The increased mRNA abundance of *LPL* that was observed for pigs fed copper hydroxychloride, therefore, may indicate an increased efficiency in the uptake and utilization of fatty acids from hydrolysis of the triacylglycerol component of chylomicrons. The fatty acids derived from *LPL* may be used for uptake by other tissues such as heart, liver, and muscle. The increased abundance of *FABP4* in pigs fed the copper hydroxychloride diet is likely a result of increased concentration of fatty acids released by *LPL*. 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 *CPT1B* tended to increase when pigs were fed the copper hydroxychloride diet is in agreement with data indicating that dietary addition of Cu at 45 mg/kg increased the abundance of *CPT1* in the adipose tissue of rabbits (Lei et al., 2017). The observed increase in *CPT1B* 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 copper hydroxychloride diet compared with pigs fed the control diet.

The increase in mRNA abundance of *FABP1* and *CD36* in liver tissue that was observed in pigs fed the copper hydroxychloride diet indicates an increased uptake of fatty acids by hepatocytes.

Table 4. Growth performance for pigs fed diets containing 0 or 150 mg Cu/kg from copper hydroxychloride[1]

	Diet			
Item	Control	Control + Cu[2]	SEM	*P*-value
Days 0 to 15				
Initial BW, 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 BW, kg	23.825	24.757	0.510	0.150
Days 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 BW, kg	34.575	35.713	0.492	0.076
Days 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 eight observations for all treatments.
[2]The diet containing added Cu was fortified with 150 mg/kg of Cu from copper hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN).

Table 5. Least squares means (log_2-back-transformed) 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 copper 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
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-ϒ	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 seven or eight 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-γ*, peroxisome proliferator-activated receptor γ; *LPL*, lipoprotein lipase; *HSL*, hormone sensitive lipase.
[3]The diet containing added Cu was fortified with 150 mg/kg of Cu from copper hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN).

Table 6. Concentrations (% of total fat) of total fatty acids and free fatty acids in liver, skeletal muscle, and subcutaneous adipose tissue of pigs fed diets containing 0 or 150 mg Cu/kg from copper hydroxychloride[1]

	Diet			
Item	Control	Control + Cu[2]	SEM	*P*-value
Liver				
Total fatty acids, %	4.065	4.090	0.052	0.713
Free fatty acids, %	0.078	0.071	0.006	0.172
Skeletal muscle				
Total fatty acids, %	1.510	1.455	0.094	0.685
Free fatty acids, %	0.026	0.027	0.003	0.885
Subcutaneous adipose tissue				
Total fatty acids, %	60.320	61.001	0.849	0.405
Free fatty acids, %	0.042	0.041	0.004	0.860

[1]Data are least-squares means of eight observations for all treatments.
[2]The diet containing added Cu was fortified with 150 mg/kg of Cu from copper hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN).

Lipids in the liver may originate from non-esterified fatty acids and lipoprotein remnants formed from the action of *LPL* (Nguyen et al., 2008). Therefore, the increase in mRNA abundance of *LPL* 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 *FABP1* and *CD36* 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 ~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 *LPL*-mediated hydrolysis of chylomicrons and VLDL (Watt and Hoy, 2012). The observation that the mRNA expression of *PPAR-α* tended to increase in pigs fed the diet with copper hydroxychloride indicates an increased uptake of fatty acids in skeletal muscle by pigs fed the diet containing copper hydroxychloride. *PPAR-α* is a transcription factor that is 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 *PPAR-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 copper hydroxychloride to the diet improved fatty acid oxidation and subsequently increased ATP synthesis by increasing mRNA expression of *PPAR-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 of nursery pigs. Nursery pigs prioritize deposition of lean tissue over deposition of adipose tissue if the maintenance energy requirement has been met (Patience et al., 2015). However, as pigs grow older, this relationship changes and responses to dietary Cu may, therefore, be different in finishing pigs than in nursery pigs.

The lack of differences between the 2 diets in concentrations of total fatty acids and free fatty acids in the tissues seems in contrast to the hypothesis that copper hydroxychloride supplementation improves efficiency of fatty acid uptake and transport. However, these results only represent the fatty acid concentrations in analyzed samples, and the total quantity of fat in liver, skeletal muscle, and adipose tissues may have been different between the 2 diets. However, because total body composition was not analyzed in this experiment, further investigations are needed to address this hypothesis.

In conclusion, supplementation of Cu from copper 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 copper hydroxychloride in 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. This may partially explain the increased ADG and G:F of pigs fed the Cu-supplemented diet compared with pigs fed the control diet.

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