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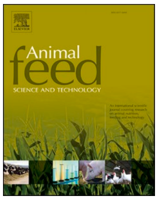

Copper hydroxychloride improves gain to feed ratio in pigs, but this is not due to improved true total tract digestibility of acid hydrolyzed ether extract

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ABSTRACT

Two experiments were conducted to test the hypothesis that copper (Cu) from Cu hydroxychloride improves gain to feed ratio (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 20, 40, or 60 g/kg 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 20 g/kg added CWG. Diets were fed for 4 weeks. Results indicated that supplementation of diets with either CWG or Cu hydroxychloride improved ($P < 0.05$) G:F of pigs, and the improvement obtained by Cu hydroxychloride supplementation was similar to the improvement in G:F obtained by adding 28–38 g/kg CWG to the diets. In experiment 2, 64 pigs (18.22 ± 1.80 kg) were housed individually in metabolism crates and randomly allotted to 8 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 150, 300, or 450 g/kg DDGS to both basal diets at the expense of cornstarch and corn bran. Feces were collected using the marker-to-marker approach with 5-day adaptation and 4-day collection periods. Supplementation of Cu hydroxychloride to diets improved ($P < 0.05$) the ATTD of acid hydrolyzed ether extract (AEE), but did not affect ATTD of dry matter or gross energy (GE). Supplementation of Cu hydroxychloride to diets also reduced ($P < 0.05$) 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 experiment 1 as a result of Cu supplementation to diets was not due to improved ATTD of GE, 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.

Abbreviations: ADF, acid detergent fiber; ADFI, average daily feed intake; ADG, average daily gain; AEE, acid hydrolyzed ether extract; ATTD, apparent total tract digestibility; BW, body weight; Cu, copper; CWG, choice white grease; DDGS, distillers dried grains with solubles; DM, dry matter; GE, gross energy; G:F, gain to feed ratio; NDF, neutral detergent fiber; SBM, soybean meal; TTTD, true total tract digestibility.

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1. 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 lipids to diets usually improves the gain to feed ratio (G:F) of pigs due to the high metabolizable energy 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; Frobish et al., 1970), 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 copper (Cu) to diets fed to weanling pigs at 100–250 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 50 g/kg animal fat improved growth performance, and it was

Table 1
Composition of experimental diets, experiment 1.

	Choice white grease, g/kg				Cu[1], 150 mg/kg	
Item	0	20	40	60	0 CWG	20 g/kg CWG
Ingredient, g/kg						
Ground corn	385.5	385.5	385.5	385.5	385.22	385.22
Soybean meal	225.0	225.0	225.0	225.0	225.0	225.0
Corn DDGS[1]	300.0	300.0	300.0	300.0	300.0	300.0
Cornstarch	60.0	40.0	20.0	–	60.0	40.0
Choice white grease	–	20.0	40.0	60.0	–	20.0
Ground limestone	15.3	15.3	15.3	15.3	15.3	15.3
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0
Copper hydroxychloride, 540 g/kg Cu	–	–	–	–	0.28	0.28
L-Lysine HCl, 780 g/kg Lysine	5.5	5.5	5.5	5.5	5.5	5.5
DL-Methionine	0.3	0.3	0.3	0.3	0.3	0.3
L-Threonine	0.8	0.8	0.8	0.8	0.8	0.8
Phytase[2]	0.1	0.1	0.1	0.1	0.1	0.1
Salt	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix[3]	1.5	1.5	1.5	1.5	1.5	1.5
Analyzed composition						
Dry matter, g/kg	871.8	872.8	867.5	873.1	869.0	866.2
Ash, g/kg	48.1	51.4	48.4	47.2	49.6	48.0
Gross energy, MJ/kg	16.8	17.2	17.8	18.3	16.8	17.1
Metabolizable energy[4], MJ/kg	13.9	14.2	14.6	15.0	13.9	14.2
Crude protein, g/kg	221.6	210.7	214.8	214.8	222.5	217.6
Acid hydrolyzed ether extract, g/kg	42.3	62.0	80.3	99.7	42.2	62.0
Calcium, g/kg	6.7	6.8	7.3	6.8	7.6	6.7
Phosphorus, g/kg	5.7	5.4	5.5	5.5	5.6	5.7
Manganese, mg/kg	57.4	59.9	65.5	54.1	70.2	70.3
Iron, mg/kg	198.0	189.0	190.0	153.0	187.0	200.0
Zinc, mg/kg	147.0	154.0	150.0	130.0	156.0	146.0
Copper, mg/kg	22.1	27.1	20.8	31.8	165.0	150.0
Amino acids, g/kg						
Arginine	12.1	11.8	12.4	12.3	12.6	12.4
Histidine	5.9	5.8	6.0	6.0	6.1	6.0
Isoleucine	9.1	8.9	9.3	9.2	9.5	9.3
Leucine	19.9	19.5	19.9	20.3	20.4	20.1
Lysine	14.2	14.5	14.2	14.0	14.8	14.6
Methionine	3.6	3.6	3.7	3.5	3.7	3.7
Methionine + Cysteine	3.7	3.5	3.7	3.6	3.9	3.8
Phenylalanine	10.4	10.2	10.6	10.7	10.9	10.7
Threonine	8.5	12.2	8.5	8.5	8.9	8.8
Tryptophan	2.4	2.3	2.4	2.0	2.5	2.5
Valine	10.5	10.3	10.8	10.7	10.9	10.7

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

[2] Quantum Blue 5 G; AB Vista, Marlborough, United Kingdom (5000 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 DL-alpha tocopheryl acetate, 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] Metabolizable energy values were calculated (NRC, 2012) rather than analyzed.

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). Dietary Cu also reduces microbial protein excretion from pigs indicating reduced microbial fermentation in the hindgut (Espinosa et al., 2019). 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 in diets based on corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS) improves G:F of pigs by increasing the ATTD and TTTD of fat.

2. Materials and methods

Protocols for 2 experiments were submitted to the Institutional Animal Care and Use Committee at the University of Illinois, Urbana-Champaign, USA, and both protocols were approved prior to initiation of the experiments. Pigs used in experiments 1 and 2 were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used. In both experiments, antibiotic growth promoters were not included in the diets, and pharmacological levels of zinc were also not used.

2.1. Experimental design and sample collection

2.1.1. Experiment 1: Effects of Cu on growth performance

A total of 144 growing pigs with an initial body weight (BW) of 15.40 ± 2.39 kg were used in a 4-week experiment. Pigs were randomly allotted to 6 dietary treatments with 2 pigs per pen (1 barrow and 1 gilt) for a total of 12 replicate pens per treatment. Pens (0.9 × 1.8 m) had fully slatted concrete floors, a feeder, and a cup waterer. Feed and water were available at all times. Diets were formulated based on corn, SBM, and DDGS to meet current estimates for nutrient requirements for 11–25 kg pigs (Table 1; NRC, 2012). A basal diet was formulated based on corn, SBM, and DDGS and this diet contained no choice white grease (CWG). Three diets with increasing concentrations of extracted fat were formulated by adding 20, 40, or 60 g/kg CWG (> 985 g/kg crude fat; Darling Ingredients, Mason City, IL, USA) to the basal diet. Two additional diets were formulated by adding 150 mg/kg of Cu as Cu hydroxychloride (540 g/kg Cu; IntelliBond C$^{\text{II}}$; Micronutrients, Indianapolis, IN, USA) to the diet without added CWG and to the diet with 20 g/kg added CWG.

Individual pig weights were recorded at the beginning of the experiment, after 2 weeks, 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 weeks and at the conclusion of the experiment. At the end of the 28-day experiment, data were summarized to calculate ADG, ADFI, and G:F within each pen and treatment group. Data were summarized for the initial 14 days, the final 14 days, and for the entire experiment.

Table 2

Chemical composition of corn, soybean meal (SBM), distillers dried grains with solubles (DDGS), and corn bran, experiment 2.

Item	Corn	SBM	DDGS	Corn bran
Dry matter, g/kg	873.3	897.3	875.5	913.6
Ash, g/kg	10.4	56.5	56.7	5.0
Gross energy, MJ/kg	16.2	17.6	18.9	17.2
Crude protein, g/kg	76.8	446.6	272.7	58.4
AEE[1], g/kg	41.8	15.1	107.4	15.4
NDF[1], g/kg	70.1	81.4	362.6	659.4
ADF[1], g/kg	19.9	51.1	137.3	153.2
Calcium, g/kg	0.1	3.1	0.2	0.1
Phosphorus, g/kg	2.4	6.0	9.2	0.7
Copper, mg/kg	1.4	20.8	5.8	2.3
Iron, mg/kg	36.0	184.0	59.5	13.4
Manganese, mg/kg	4.5	33.2	11.5	10.4
Zinc, mg/kg	46.6	66.5	57.8	10.9
Amino acids, g/kg				
Arginine	3.0	31.0	12.6	1.9
Histidine	2.0	11.3	7.0	1.2
Isoleucine	2.6	20.7	10.8	1.7
Leucine	8.0	33.6	28.5	4.6
Lysine	2.5	27.4	7.4	3.0
Methionine	1.5	5.7	4.4	0.8
Methionine + cysteine	3.2	11.8	9.2	1.8
Phenylalanine	3.4	22.5	12.8	2.3
Threonine	2.5	16.6	9.7	3.2
Tryptophan	0.6	5.9	2.0	0.4
Valine	3.3	21.4	13.6	2.4

[1] AEE, acid hydrolyzed ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber. Values for ADF and NDF were determined inclusive of residual ash.

2.1.2. 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 8 diets (Table 2). Two basal diets based on corn, SBM, corn bran, cornstarch, and casein were formulated to meet requirements for all nutrients for 11–25 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 20 g/kg acid hydrolyzed ether extract (AEE; Tables 3 and 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 (540 g/kg Cu; IntelliBond C^{II}; Micronutrients, Indianapolis, IN, USA). Vitamins and minerals were included in both diets to meet or exceed current requirement estimates (NRC, 2012). Six additional diets were formulated by adding 150, 300, or 450 g/kg DDGS to both basal diets at the expense of cornstarch and corn bran. Because of the intrinsic fat in DDGS, the diets containing 150, 300, or 450 g/kg DDGS contained approximately 30, 40, and 50 g/kg AEE, respectively. The concentration of corn bran was reduced as DDGS was increased to maintain a constant concentration of neutral detergent fiber (NDF) among diets (Kim et al., 2013). Likewise, inclusion of crystalline amino acids was adjusted to maintain constant concentrations of standardized ileal digestible amino acids among diets. Thus, there were 4 diets without Cu hydroxychloride and 4 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 64 growing pigs (initial BW: 18.22 ± 1.80 kg; 32 barrows and 32 gilts) were randomly allotted to the 8 diets with 8 replicate pigs per diet. Pigs were assigned to treatment groups using a randomized complete block design with 2 blocks of 32 pigs and 4 replicate pigs per diet in each block. For each treatment, there were 4 gilts and 4 barrows. Weaning group (age) was the blocking factor. Pigs were placed in individual metabolism crates (0.71 × 0.84 m) 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., 0.824 MJ/kg × body weight $^{0.60}$; 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 days 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 (Adeola, 2001). Fecal samples were stored at −20 °C immediately after collection.

2.2. Chemical analyses

2.2.1. Experiment 1: Effects of Cu on growth performance

All diet samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas

Scientific, Swedesboro, NJ, USA) 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, USA). Acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis

Table 3
Ingredient composition of experimental diets, experiment 2.

Item	Control	150 g/kg DDGS[1]	300 g/kg DDGS	450 g/kg DDGS	Control + Cu[1]	150 g/kg DDGS + Cu	300 g/kg DDGS + Cu	450 g/kg DDGS + Cu
Ingredient, g/kg								
Ground corn	305.8	305.8	305.8	305.8	305.52	305.52	305.52	305.52
Soybean meal	217.0	217.0	217.0	217.0	217.0	217.0	217.0	217.0
Corn DDGS	–	150.0	300.0	450.0	–	150.0	300.0	450.0
Corn bran	273.0	182.0	92.0	–	273.0	182.0	92.0	–
Cornstarch	122.8	82.5	40.5	–	122.8	82.5	40.5	–
Casein	56.3	37.4	19.0	–	56.3	37.4	19.0	–
Ground limestone	8.8	12.0	14.9	15.6	8.8	12.0	14.9	15.6
Dicalcium phosphate	6.5	3.0	–	–	6.5	3.0	–	–
Copper hydroxychloride, 540 g/kg Cu	–	–	–	–	0.28	0.28	0.28	0.28
L-Lysine HCl, 780 g/kg Lysine	2.0	3.0	4.0	5.0	2.0	3.0	4.0	5.0
DL-Methionine	0.7	0.3	–	–	0.7	0.3	–	–
L-Threonine	0.5	0.4	0.2	–	0.5	0.4	0.2	–
Phytase[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix[3]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

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

[2] Quantum Blue 5 G; 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 DL-alpha tocopheryl acetate, 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 4
Analyzed composition of experimental diets, experiment 2.

Item	Control	150 g/kg DDGS[1]	300 g/kg DDGS	450 g/kg DDGS	Control + Cu[1]	150 g/kg DDGS + Cu	300 g/kg DDGS + Cu	450 g/kg DDGS + Cu
Dry matter, g/kg	873.4	884.9	879.6	872.3	885.5	883.8	878.5	870.6
Ash, g/kg	32.3	41.5	45.0	59.5	33.3	43.8	49.8	58.1
Gross energy, MJ/kg	16.6	17.0	17.2	17.3	16.6	16.8	17.0	17.3
Crude protein, g/kg	177.5	209.0	227.2	241.1	172.0	200.9	227.7	235.2
AEE[2], g/kg	18.4	25.1	36.1	49.1	20.2	26.7	40.2	47.5
NDF[2], g/kg	209.6	209.7	197.8	200.7	214.9	193.4	195.6	188.8
Calcium, g/kg	5.7	6.1	6.3	7.9	5.0	6.8	6.0	7.2
Phosphorus, g/kg	4.1	4.7	5.2	6.6	3.9	4.6	5.3	6.6
Mn, mg/kg	70.0	67.0	65.0	68.0	73.0	70.0	73.0	65.0
Iron, mg/kg	233.0	197.0	168.0	186.0	201.0	203.0	174.0	197.0
Zinc, mg/kg	157.0	153.0	160.0	161.0	156.0	164.0	168.0	169.0
Copper, mg/kg	24.0	23.0	21.0	23.0	151.0	156.0	148.0	170.0
Amino acids, g/kg								
Arginine	9.9	11.0	12.1	13.1	9.4	10.7	11.9	13.5
Histidine	5.2	5.7	6.2	6.6	5.2	5.8	6.1	6.7
Isoleucine	8.5	9.0	9.8	10.1	8.6	9.4	9.7	10.3
Leucine	16.0	18.4	20.8	22.6	16.1	18.7	20.5	23.2
Lysine	13.3	13.8	14.9	14.8	13.3	14.7	14.3	14.5
Methionine	4.1	3.8	3.7	3.9	3.9	4.1	3.8	3.9
Methionine + Cysteine	6.8	6.7	7.3	8.1	6.2	7.2	7.5	8.1
Phenylalanine	2.5	2.9	3.6	4.2	2.3	3.1	3.7	4.2
Threonine	9.2	10.1	11.0	11.6	9.2	10.3	10.9	11.9
Tryptophan	8.1	8.4	8.7	8.9	7.9	8.5	8.6	9.2
Valine	2.4	2.5	2.6	2.6	2.3	2.4	2.6	2.4

[1] DDGS, distillers dried grains with solubles; Cu, 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond C^{II}; Micronutrients, Indianapolis, IN, USA).
[2] AEE, acid hydrolyzed ether extract; NDF, neutral detergent fiber. Values for NDF were determined inclusive of residual ash.

System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA), and crude protein was determined using the combustion procedure (Method 990.03; AOAC Int., 2007) on a Leco FP628 protein analyzer (Leco Corporation, St. Joseph, MI, USA). Amino acids were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) 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].

2.2.2. *Experiment 2: Digestibility of gross energy and acid hydrolyzed ether extract*

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 experiment 1. Diets and ingredients were also analyzed for ash, crude protein, amino acids, and minerals as described for experiment 1. Ingredient samples were analyzed for NDF inclusive of residual ash and acid detergent fiber (ADF) inclusive of residual ash using fiber bags (Ankom2000 fiber analyzer; Ankom Technology, Macedon, NY, USA) followed by an adaptation of the procedure described by Van Soest et al. (1991). 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).

2.3. *Statistical analyses*

2.3.1. *Experiment 1: Effects of Cu on growth performance*

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., 2016). 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 150 mg/kg Cu hydroxychloride with the diet containing no CWG and no Cu hydroxychloride. A similar analysis was used to compare the diet containing 20 g/kg CWG and 150 mg/kg of Cu hydroxychoride and the diet containing 20 g/kg CWG and no Cu hydroxychloride. The CWG equivalency of Cu from Cu hydroxychloride was estimated using the prediction equation derived from the regression analysis (Nitikanchana et al., 2015). Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

2.3.2. *Experiment 2: Digestibility of gross energy and acid hydrolyzed ether extract*

Data were analyzed as a randomized complete block design in a 4 × 2 factorial arrangement with the pig as the experimental unit. Homogeneity of the variances was confirmed using the UNIVARIATE procedure in PROC MIXED. The model included amount of DDGS, Cu, and the interaction between DDGS and Cu as the main effects. After confirming linearity of the responses to DDGS on ATTD of AEE in diets without and with Cu hydroxychloride supplementation, 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 the 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 standard error 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.

3. Results

3.1. *Effects of Cu on growth performance*

Average daily feed intake of pigs linearly decreased ($P < 0.05$) as the concentration of CWG increased in the diets from day 15–28 and in the overall experimental period (Table 5). A tendency for a quadratic decrease ($P < 0.10$) in ADFI was also observed from day 1–14 and from day 15–28, 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 day 1–14, and a linear increase ($P < 0.05$) in G:F was observed from day 15–28 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 day 15–28 and for the entire experimental period. Supplementation of 150 mg/kg of Cu from Cu hydroxychloride to the diet with 20 g/kg added CWG reduced ($P < 0.05$) ADFI, but improved ($P < 0.05$) G:F of pigs from day 15–28 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 6), which indicated that the intercept and slope were 0.526 and 0.0022, respectively. Therefore, G:F increases by 0.0022 for each g change of CWG inclusion per kg of complete diet. From these prediction equations, it was calculated that the improvement obtained by Cu hydroxychloride supplementation for the overall experimental unit was similar to the improvement in G:F obtained by adding 28–38 g/kg CWG to the diets (Table 7).

3.2. *Digestibility of gross energy and acid hydrolyzed ether extract*

All pigs readily consumed their diets and remained healthy throughout the experiment. There was no DDGS × Cu interaction for any response variables (Table 8). Inclusion of 300 or 450 g/kg DDGS to diets improved ($P < 0.01$) the ATTD of DM and AEE. Likewise, the diet containing 450 g/kg DDGS had greater ($P < 0.01$) ATTD of GE compared with diets containing 0, 150, or 300 g/kg DDGS. The ATTD of DM and GE in diets containing Cu hydroxychloride was not different from values for diets without supplemental Cu

Table 5

Growth performance of pigs fed diets containing 0, 20, 40, or 60 g/kg choice white grease (CWG) and 150 mg/kg Cu from Cu hydroxychloride in diets without or with 20 g/kg CWG (experiment 1)[1].

	CWG, g/kg				Cu[2], 150 mg/kg			*P*-value			
	–	20	40	60	- CWG	20 g/kg CWG	Pooled SEM	Linear	Quadratic	- CWG vs. -CWG + Cu	20 g/kg CWG vs. 20 g/kg CWG + Cu
day 1 to 14											
Initial BW[3],kg	15.274	15.494	15.416	15.520	15.338	15.341	0.3571	0.109	0.279	0.765	0.465
ADG[3], kg	0.571	0.632	0.601	0.602	0.621	0.645	0.0265	0.557	0.214	0.172	0.706
ADFI[3], kg	1.049	1.130	1.045	1.012	1.092	1.083	0.0320	0.150	0.063	0.304	0.260
G:F[3]	0.548	0.561	0.574	0.593	0.568	0.597	0.0195	0.074	0.868	0.431	0.152
Final BW, kg	23.267	24.342	23.833	23.950	24.058	24.375	0.5692	0.312	0.163	0.199	0.955
day 15 to 28											
ADG, kg	0.784	0.836	0.838	0.831	0.865	0.799	0.0266	0.186	0.217	0.028	0.294
ADFI, kg	1.483	1.512	1.344	1.170	1.359	1.231	0.0581	<0.001	0.054	0.116	<0.001
G:F	0.530	0.555	0.637	0.733	0.645	0.666	0.0311	<0.001	0.250	0.009	0.010
Final BW, kg	34.242	36.050	35.567	35.583	36.136	35.562	0.7566	0.156	0.110	0.032	0.564
day 1 to 28											
ADG, kg	0.677	0.734	0.720	0.717	0.744	0.722	0.0200	0.248	0.136	0.020	0.661
ADFI, kg	1.266	1.321	1.195	1.091	1.225	1.157	0.0411	<0.001	0.032	0.450	0.003
G:F	0.536	0.557	0.608	0.666	0.609	0.632	0.0177	<0.001	0.320	0.003	0.003

[1] Data are least square means of 12 observations (pen was the experimental unit; 2 pigs per pen).

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

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

Table 6
Regression coefficients used for estimating the gain to feed ratio (G:F) response to including choice white grease (CWG) in diets (experiment 1)[1].

		SE		*P*-value			
Dependent variable	Prediction equation	Intercept	Slope	Intercept	Slope	R^2	RMSE[2]
G:F, day 1 to 14	0.546 + 0.00075 × (CWG, g/kg)	0.017	0.0004	<0.001	0.099	0.058	0.069
G:F, day 15 to 28	0.510 + 0.00347 × (CWG, g/kg)	0.025	0.0007	<0.001	<0.001	0.366	0.104
G:F, day 1 to 28	0.526 + 0.00220 × (CWG, g/kg)	0.015	0.0004	<0.001	<0.001	0.402	0.061

[1] Data were subjected to linear regression analysis with the g/kg 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 g/kg point change of CWG included in the diet.
[2] RMSE, root mean square error.

Table 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 20 g/kg added CWG (experiment 1)[1].

	Cu, 150 mg/kg	
Items	- CWG	20 g/kg CWG
day 1 to 14		
Gain to feed ratio	0.568	0.597
CWG equivalency, g/kg[2]	29.3	48.0
day 15 to 28		
Gain to feed ratio	0.645	0.666
CWG equivalency, g/kg	38.9	24.8
day 1 to 28		
Gain to feed ratio	0.609	0.632
CWG equivalency, g/kg	37.7	28.2

[1] Data are least square means of 12 observations for all treatments.
[2] Derived from the prediction equation used for estimating gain to feed ratio in CWG.

hydroxychloride. However, supplementation of Cu hydroxychloride to diets improved ($P < 0.01$) the ATTD of AEE regardless of DDGS inclusion.

The estimated endogenous loss of fat for diets with supplemental Cu as Cu hydroxychloride was 7.14 g/kg DM intake, 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 DM intake; Table 9). However, values for the TTTD of AEE in diets without or with Cu hydroxychloride were not different.

4. Discussion

4.1. *Effects of Cu on growth performance*

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 days 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 weeks post-weaning, pigs gradually develop the ability to digest dietary lipids (Stahly, 1984; Jung et al., 2003), which was the reason pigs in the present experiments were 15 to 18 kg (4 weeks post-weaning) at the start of the experiment assuming they have maximum lipid digestibility at that time. However, the observation that the response to CWG was

Table 8
Apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), 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 (experiment 2)[1].

	No added Cu				150 mg/kg Cu[2]					*P*-value		
Item	0 g/kg DDGS	150 g/kg DDGS	300 g/kg DDGS	450 g/kg DDGS	0 g/kg DDGS	150 g/kg DDGS	300 g/kg DDGS	450 g/kg DDGS	Pooled SEM	DDGS	Cu	DDGS × Cu
ATTD of DM	0.822	0.819	0.834	0.849	0.815	0.821	0.832	0.844	0.0060	<0.001	0.399	0.882
ATTD of GE	0.823	0.817	0.828	0.844	0.816	0.817	0.824	0.834	0.0069	0.002	0.204	0.819
ATTD of AEE	0.414	0.385	0.536	0.640	0.527	0.550	0.637	0.700	0.0388	<0.001	<0.001	0.594

[1] Data are least square means of 8 observations (pen was the experimental unit; 1 pig per pen).
[2] Cu as Cu hydroxychloride (IntelliBond C^{II}; Micronutrients, Indianapolis, IN, USA).

Table 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 (experiment 2)[1].

Item	Regression equation	Slope SE	Slope *P*-value	Intercept SE	Intercept *P*-value	R^2	Estimated TTTD[2] of AEE	Estimated endogenous loss of fat[3]
DDGS	$y = 0.8282x - 11.23$	0.0660	<0.001	2.6737	<0.001	0.85	0.828	11.23^{y}
DDGS + Cu	$y = 0.8185x - 7.14$	0.0404	<0.001	1.6305	<0.001	0.94	0.819	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.

[3] Gram per kilogram dry matter intake.

greater from day 15 to 28 than from day 1 to 14 indicates that pigs may need time to adopt to changes in dietary fat concentration.

The basal diet was formulated to contain 60 g/kg cornstarch, and increasing concentrations of CWG were included in the diets at the expense of cornstarch. Therefore, this resulted in a calculated metabolizable energy of 13.9, 14.2, 14.6, and 15.0 MJ/kg in diets containing 0, 20, 40, and 60 g/kg CWG, respectively. 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 containing CWG. This effect was expected and have been demonstrated in previous research as well (De la Llata et al., 2001; Kil et al., 2011), and the magnitude of the increase in G:F in response to CWG was close to that reported by Kil et al. (2011). Therefore, the objective of including the 4 levels of CWG in the diets was to establish the regression equation that subsequently was used to calculate the response to Cu hydroxychloride on G:F. The reduced ADFI that was observed in experiment 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). Inclusion of dietary fat may also increase the uptake and utilization of fatty acids from hydrolysis of the triacylglycerol component of chylomicrons (Jump and Clarke, 1999). This may enhance fatty acid oxidation and subsequently increase ATP synthesis (Houten and Wanders, 2010) that may contribute to the observed increase in G:F for pigs fed diets with increasing concentration of CWG.

The observation that supplementation of Cu to diets without CWG resulted in increased ADG of pigs concurs with data indicating that addition of Cu at 125 to 250 mg/kg in diets for pigs containing 0 or 50 g/kg animal fat improved growth performance (Dove, 1993). The greater G:F of pigs fed diets with 0 or 20 g/kg 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; Espinosa et al., 2020). Addition of 45 mg/kg of Cu to diets 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 future research, the hypothesis that effects of added Cu hydroxychloride obtained in diets containing 0 or 20 g/kg CWG can also be obtained in diets with greater concentrations of CWG needs to be tested.

4.2. Digestibility of gross energy and acid hydrolyzed ether extract

In experiment 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). The observed increase in the ATTD of DM, GE, and AEE if 300 or 450 g/kg DDGS is included in the diets is likely due to the greater digestibility of dietary fiber and greater concentration of AEE in DDGS compared with corn bran (Gutierrez et al., 2014). The observed improvement in ATTD of AEE upon Cu supplementation is in agreement with previous data (Espinosa et al., 2019; Dove, 1995) which is likely a result of the ability of Cu to reduce the endogenous loss of fat due to reduced excretion of microbial fat (Espinosa et al., 2019).

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 experiment 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 in diets containing Cu hydroxychloride that was observed in this experiment. Indeed, inclusion of 150 mg/kg of Cu hydroxychloride in diets for growing pigs resulted in a reduction in microbial fat excretion in feces indicating reduced microbial activity in the hindgut of pigs fed diets containing elevated concentrations of Cu (Espinosa et al., 2019). The observation that there was no difference in TTTD of AEE between diets without or with added Cu further indicates that the effects of Cu on ATTD of AEE was caused by a reduction in endogenous loss of AEE rather than a change in absorption of AEE.

The observation from experiment 1 that Cu hydroxychloride improves G:F combined with the observation from experiment 2 that TTTD of AEE is not increased by Cu hydroxychloride indicates that the increased G:F observed in experiment 1 is a result of improved post-absorptive utilization of AEE. Therefore, the hypothesis that Cu hydroxychloride increases digestibility of fat was rejected. Instead, 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; Espinosa et al., 2020), 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.

5. Conclusion

Supplementation of 150 mg/kg Cu as Cu hydroxychloride to diets improved gain to feed ratio of 15–35 kg pigs. The improvement obtained by adding Cu hydroxychloride to the diets was similar to the improvement in gain to feed ratio obtained by adding 28–38 g/kg choice white grease to the diets. However, the improvements in gain to feed ratio obtained by adding Cu hydroxychloride to diets appears not to be a result of improved energy or acid hydrolyzed ether extract digestibility, because the true total tract digestibility of acid hydrolyzed ether extract was not improved by Cu. Instead, the increased gain to feed ratio may be a result of the effect of Cu on post-absorptive metabolism of energy and fat. The improved apparent total tract digestibility of acid hydrolyzed ether extract 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.

Author statement

CDE, JLU, RSF, and HHS conceptualized the experiment. CDE conducted the animal part of the experiment, analyzed the data, and wrote the first draft of the manuscript. JLU and RSF contributed with data interpretation. HHS supervised the project, CDE and HHS edited the final version of the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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