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[1]

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ABSTRACT: Three experiments were conducted to determine effects of Cu hydroxychloride on DE and 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 (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 Cu/kg from Cu hydroxychloride to the control diet. Both diets were fed for 4 wk. Results indicated that ADG, G:F, and final BW were greater ($P \leq 0.05$) and fecal scores were reduced ($P \leq 0.05$) for pigs fed the diet containing150 mg Cu/kg as 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 Cu/kg. Two additional diets were formulated by adding 100 or 200 mg 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 GE 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 used in Exp. 2. 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 BW on d 28 were greater ($P \leq 0.05$) for pigs fed diets containing 100 or 200 mg Cu/kg supplemented by Cu hydroxychloride compared with pigs fed the control diet. Pigs fed the diets supplemented with Cu hydroxychloride also had reduced ($P \leq 0.05$) overall fecal scores and diarrhea frequency 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 pigs 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

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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 levels (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 ADG and

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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 CuSO4 (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 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 for 4 wk (Table 1). The control diet contained 20 mg of Cu from $CuSO_4$ and CuCl, 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, 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 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 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 were analyzed on a Hitachi AA Analyzer (model L8800; Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard, and Cu was analyzed by inductively coupled plasma (**ICP**) 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 χ^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 GE 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 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. Two additional diets were formulated by adding 100 or 200 mg Cu/kg from Cu hydroxychloride 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

Table 1. Ingredient and analyzed nutrient composition of diets used in Exp. 1

	Phase 1 diets		Phase 2 diets	
Item	Control	Cu[1]	Control	Cu[1]
Ingredient, %				
Ground corn	47.95	47.95	49.69	49.69
Soybean meal, 48% CP	26.50	26.50	33.50	33.50
Whey, dried	15.00	15.00	10.00	10.00
Fishmeal	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-Lysine HCl, 78%	0.35	0.35	0.37	0.37
DL-Methionine	0.13	0.13	0.13	0.13
L-Threonine	0.10	0.10	0.09	0.09
Sodium chloride	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				
DM, %	88.34	88.41	88.16	88.34
Ash, %	6.50	6.42	6.08	5.85
GE, kcal/kg	3995	3998	4034	4074
CP, %	22.44	22.71	19.97	22.42
Cu, mg/kg	20	171	14	154
AA, %				
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]Cu from Cu hydroxychloride.

[2]Provided the following quantities of vitamins and microminerals 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-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 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, 10 mg as copper sulfate and 10 mg as 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 2. Ingredient and analyzed nutrient composition of diets used in Exp. 2 and 3

	Diet		
Item	Control	100 mg Cu/kg,[1]	200 mg Cu/kg,[1]
Ingredient, %			
Ground corn	58.80	58.80	58.80
Soybean meal, 48% CP	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-Lysine HCl, 78% Lys	0.41	0.41	0.41
DL-Methionine	0.12	0.12	0.12
L-Threonine	0.12	0.12	0.12
Sodium chloride	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			
DM, %	87.74	87.83	88.37
Ash, %	5.01	4.99	5.04
GE, kcal/kg	4125	4142	4137
AEE,[4] %	7.13	6.91	7.02
CP, %	20.70	21.11	21.32
Ca, %	0.78	0.75	0.77
P, %	0.54	0.53	0.51
Fe, mg/kg	160	144	143
Cu, mg/kg	13	120	205
AA, %			
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.

[2]Provided the following quantities of vitamins and microminerals 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-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 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, 10 mg as copper sulfate and 10 mg as 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 complete diet.

[4]Acid hydrolyzed ether extract.

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 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 was calculated for each diet and collection period, and the DE and 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, 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 vena puncture. One of the 3 blood samples was collected in vacutainers without 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. An ANOVA was conducted, and treatment means were calculated

Table 3. Growth performance of pigs fed diets without or with 150 mg Cu/kg (Exp. 1)[1]

	Barrow		Gilt			*P*-value		
Item	Control	Cu[2]	Control	Cu[2]	SEM	Sex	Diet	Sex × diet
BW, kg								
Initial BW	7.10	7.13	6.41	6.56	1.21	0.002	0.479	0.821
d 14	7.61	8.55	7.99	8.58	2.01	0.462	0.015	0.718
d 28	12.84	16.12	15.36	15.59	3.46	0.063	0.004	0.095
ADFI, g								
d 0 to 14	114	226	212	203	0.022	0.056	0.012	0.037
d 14 to 28	753	845	774	817	0.050	0.937	0.135	0.658
d 0 to 28	432	536	495	510	0.033	0.508	0.048	0.251
ADG, g								
d 0 to 14	37	104	112	142	0.020	0.007	0.010	0.408
d 14 to 28	382	534	517	507	0.031	0.032	0.006	0.039
d 0 to 28	211	318	314	326	0.023	0.011	0.005	0.090
G:F								
d 0 to 14	0.238	0.425	0.510	0.702	0.098	0.009	0.033	0.983
d 14 to 28	0.530	0.620	0.655	0.633	0.029	0.046	0.280	0.090
d 0 to 28	0.502	0.592	0.628	0.636	0.026	0.013	0.089	0.177

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

[2]Cu from Cu hydroxychloride.

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 1). Greater ($P \leq 0.05$) BW was observed for pigs fed diets with 150 mg Cu/kg as hydroxychloride compared with pigs fed the control diet on d 14 and on d 28 (Table 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 ($P \leq 0.05$) 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 Cu/kg as hydroxychloride had greater ($P \leq 0.05$) 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 ($P \leq 0.05$) between sex and Cu as hydroxychloride was observed, with barrows having increased ($P \leq 0.05$) ADG with the 150 mg Cu/kg hydroxychloride diet, whereas no difference between the 2 diets was observed for gilts. Pigs fed the diet with 150 mg Cu/kg as Cu hydroxychloride also had greater ($P \leq 0.05$) G:F in phase 1 compared with pigs fed the control diet.

In phase 2 and overall, a reduction ($P \leq 0.05$) in diarrhea scores was observed for pigs fed the diets with 150 mg Cu/kg as hydroxychloride compared with pigs fed the control diet (Table 4). Reduction ($P \leq 0.05$) in diarrhea frequency was also observed for pigs fed diets with 150 mg Cu/kg as hydroxychloride compared with pigs fed the control diet in both phases of the experiment and for the overall experimental period.

Table 4. Diarrhea score and frequency of diarrhea of pigs fed diets without or with 150 mg Cu/kg (Exp. 1)[1]

	Treatment			
Item	Control	Cu[2]	SEM	*P*-value
Diarrhea score[3]				
d 0 to14	2.3	2.0	0.11	0.076
d 14 to28	2.6	2.2	0.10	0.008
d 0 to 28	2.5	2.1	0.09	0.011
Frequency of diarrhea				
d 0 to14				
Pen days[4]	70	70		
Frequency[5]	52.86	32.86	—	0.017
d 14 to28				
Pen days[4]	70	70		
Frequency[5]	55.71	27.14	—	<0.001
Overall, d 0 to 28				
Pen days[4]	140	140		
Frequency[5]	54.29	30.00	—	<0.001

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

[2]Cu from Cu hydroxychloride.

[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.

Table 5. Effect of feeding period and Cu hydroxychloride on the apparent total tract digestibility (ATTD) of GE and nutrients and on the concentration of DE and ME (as-fed basis) 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,[2] 200 mg/kg	SEM	*P*-value
GE intake, kcal/d	2,707[c]	3,594[b]	4,253[a]	77.5	<0.001	3,458	3,553	3,542	119.7	0.829
Fecal output, g/d	65[c]	81[b]	90[a]	2.8	<0.001	78	80	78	4.8	0.879
Fecal GE loss, kcal/d	312[c]	395[b]	437[a]	13.8	<0.001	375	397	428	20.2	0.909
DE of diet, kcal/kg	3,685[b]	3,701[b]	3,734[a]	11.1	<0.001	3,688	3,713	3,720	14.6	0.270
ME of diet, kcal/kg	3,504[b]	3,538[a]	3,544[a]	12.7	0.011	3,506	3,543	3,538	16.7	0.245
ATTD, GE, %	88.6[b]	89.0[b]	89.8[a]	0.3	<0.001	88.99	89.1	89.3	0.4	0.850
ATTD, Ash, %	77.8[b]	79.7[a]	80.5[a]	0.7	0.021	78.90	79.3	79.8	0.7	0.713
ATTD, AEE, %	80.0	79.5	80.3	0.4	0.394	79.68	80.3	79.9	0.5	0.658

[a–c]Means within a row that do not have a common superscript tend to differ ($P < 0.05$).

[1]Data are least squares means of 12 observations for all treatments. Period × treatment interactions were not significant; therefore, only main effects are indicated.

[2]Cu from Cu hydroxychloride.

Experiment 2

The intended concentrations of Cu were analyzed in all diets. 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 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 ($P \leq 0.01$) BW on d 14 were observed for pigs fed diets with 100 or 200 mg Cu/kg as Cu hydroxychloride compared with pigs fed the control diet (Table 6). No differences were observed in the ADFI of pigs fed the dietary treatments from d 0 to 14, for d 14 to 28, or for the entire experimental period. Greater ($P \leq 0.01$) final BW on d 28 was also observed for pigs fed diets containing 100 or 200 mg Cu/kg as Cu hydroxychloride compared with pigs fed the control diet. No differences were observed between the diet supplemented with 100 mg Cu/kg from Cu hydroxychloride and the diet supplemented with 200 mg Cu/kg from Cu hydroxychloride. Reductions ($P \leq 0.05$) in diarrhea scores were observed for pigs fed the Cu hydroxychloride diets compared with pigs fed the control diet (Table 7).

No differences among dietary treatments were observed in the concentrations of BUN, total protein, or albumin (Table 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, and these concentrations were also 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 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 are 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

Table 6. Growth performance of pigs fed diets with 0, 100, or 200 mg Cu/kg (Exp. 3)[1]

	Treatment				
Item	Control	Cu,[2] 100 mg/kg	Cu,[2] 200 mg/kg	SEM	*P*-value
BW, kg					
d 0	10.21	10.23	10.22	0.41	0.844
d 14	15.25[b]	16.73[a]	16.71[a]	0.57	0.001
d 28	23.78[b]	25.20[a]	25.89[a]	0.83	0.006
ADFI, g					
d 0 to 14	872	873	883	0.07	0.991
d 14 to 28	950	1,000	1,056	0.04	0.163
d 0 to 28	903	945	966	0.04	0.174
ADG, g					
d 0 to 14	359[b]	461[a]	464[a]	0.03	0.002
d 14 to 28	589	580	616	0.04	0.713
d 0 to 28	485	525	538	0.03	0.174
G:F					
d 0 to 14	0.414[b]	0.530[a]	0.531[a]	0.03	0.005
d 14 to 28	0.612	0.583	0.586	0.03	0.698
d 0 to 28	0.542	0.550	0.561	0.03	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.

Table 7. Diarrhea score and frequency of diarrhea of pigs fed diets with 0, 100, or 200 mg Cu/kg (Exp. 3)[1]

	Treatment				
Item	Control	Cu,[2] 100 mg/kg	Cu,[2] 200 mg/kg	SEM	*P*-value
Diarrhea score[3]					
d 0 to14	2.3	2.1	2.2	0.07	0.201
d 14 to28	2.2	2.1	2.1	0.04	0.129
d 0 to 28	2.2[a]	2.1[b]	2.1[b]	0.08	0.037
Frequency of diarrhea					
d 0 to14					
Pen days[4]	70	70	70		
Frequency[5]	25.71	12.86	21.43	—	0.153
d 14 to 28					
Pen days[4]	70	70	70		
Frequency[5]	11.43	2.86	7.14	—	0.144
Overall, d 0 to 28					
Pen days[4]	140	140	140		
Frequency[5]	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.

[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.

and Kalish, 1972), and the lack of differences among treatments indicates that dietary Cu concentrations have no effect on serum protein concentration. 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 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 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 is 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

Table 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 Cu/kg (Exp. 3)[1]

	Treatment				
Item	Control	Cu,[2] 100 mg/kg	Cu,[2] 200 mg/kg	SEM	*P*-value
BUN, mg/dL	11.4	12.6	11.7	0.69	0.452
Total protein, g/dL	5.7	5.7	5.5	0.11	0.459
Albumin, g/dL	3.4	3.5	3.5	0.07	0.271
TNF-α, pg/mL	59.1	53.9	58.3	7.52	0.846
IgA, mg/mL	0.5	0.4	0.6	0.06	0.255

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

[2]Cu from Cu hydroxychloride.

growth performance is a result of increased synthesis of neuropeptide Y in the brain (Li et al., 2008).

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.

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