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Non Ruminant Nutrition

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Effects of copper hydroxychloride and distillers dried grains with solubles on intestinal microbial concentration and apparent ileal and total tract digestibility of energy and nutrients by growing pigs[1]

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Abstract

An experiment was conducted to test the hypothesis that Cu hydroxychloride improves nutrient digestibility and alters the concentration of microbial protein in the small intestine or large intestine by pigs fed a corn-soybean meal diet or a diet based on corn, soybean meal, and distillers dried grains with solubles (**DDGS**). Twenty-four barrows (33.3 ± 3.4 kg) that had a T-cannula installed in the distal ileum were allotted to a 2 × 2 factorial design with 2 levels of DDGS (0% or 45%) and 2 levels of supplemental Cu from Cu hydroxychloride (0 or 150 mg/kg). A 2-period switch back design with the 4 diets and 6 replicate pigs per diet in each period was used resulting in 12 replicate pigs per diet for the 2 periods. The initial 9 d of each period was considered an adaptation period to the experimental diets. For each period, feces were collected on days 10, 11, and 12, and ileal digesta were collected for 8 h on days 13 and 14. Results indicated that inclusion of 45% DDGS to diets reduced ($P < 0.05$) the apparent ileal digestibility (AID) of AA and the AID and the apparent total tract digestibility (ATTD) of dry matter, gross energy, and crude protein. In contrast, inclusion of DDGS to diets increased ($P < 0.05$) the AID and the ATTD of acid hydrolyzed ether extract and the concentration of microbial protein in the hindgut ($P < 0.05$). However, the total concentration of volatile fatty acids (VFA) in ileal digesta and in feces from pigs fed the DDGS diets were not different from concentrations in pigs fed diets without DDGS. The AID and ATTD of dry matter, gross energy, and crude protein were not affected by dietary Cu concentrations, but the AID and ATTD of acid hydrolyzed ether extract were greater ($P < 0.05$) in diets supplemented with Cu hydroxychloride compared with diets without Cu hydroxychloride. There was also a reduction ($P < 0.05$) in the concentration of microbial protein and a tendency for a reduction ($P < 0.10$) in the total concentration of VFA in feces when diets were supplemented with Cu hydroxychloride. In conclusion, supplementation of Cu hydroxychloride to diets improved AID and ATTD of acid hydrolyzed ether extract and reduced the concentration of microbial protein in the large intestine and this effect was observed in diets containing DDGS as well as in diets without DDGS.

Key words: copper, copper hydroxychloride, digestibility, distillers dried grains with solubles, microbial protein, pigs

Introduction

Copper is involved in several metabolic reactions including cellular respiration, tissue pigmentation, and connective tissue development (Turnlund, 1998; Gaetke and Chow, 2003), and is an essential component of several metalloenzymes such as cytochrome C oxidase and lysyl oxidase. The requirement for Cu for normal metabolism by weanling pigs is usually 5 to 6 mg/kg (NRC, 2012), but it is common practice to include additional Cu in diets for pigs to enhance growth performance (Ma et al., 2015). Several modes of action for the improved growth performance have been proposed such as the ability of Cu to alter microbial activity (Højberg et al., 2005) and subsequently decrease deamination and decarboxylation of AA (Dierick et al., 1986). Copper may, therefore, reduce microbial degradation of AA, which may increase AA absorption (Dierick et al., 1986). The effect of dietary Cu has also been attributed to its bacteriostatic and bactericidal properties (Stahly et al., 1980), because clostridium, salmonella, total anaerobe bacteria, and coliform populations were reduced in the small intestine, as well as in the cecum and colon of pigs fed Cu-supplemented diets (Ma et al., 2006, 2007; Song et al., 2013). Apparent total tract digestibility (**ATTD**) of acid hydrolyzed ether extract (**AEE**) was also improved if Cu hydroxychloride was supplemented to diets containing distillers dried grains with solubles (**DDGS**; Espinosa et al., 2019). It is, therefore, possible that pigs consuming diets with Cu hydroxychloride have reduced fermentation in the small intestine, which may increase AA and fat absorption. Therefore, an experiment was conducted to test the hypothesis that inclusion of 150 mg/kg of Cu from Cu hydroxychloride to diets without or with DDGS and fed to growing pigs improves apparent ileal digestibility (**AID**) and ATTD of dry matter (DM), crude protein (CP), gross energy (GE), and AEE, and the AID of CP and AA. The second objective was to test the hypothesis that supplementing diets with Cu hydroxychloride reduces the concentration of microbial protein in the small intestine or in the large intestine of pigs.

Materials and Methods

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved prior to initiation of the experiment. Twenty-four barrows (33.3 ± 3.4 kg) that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were surgically fitted with a T-cannula in the distal ileum (Stein et al., 1998). After surgery, pigs were housed individually in pens (1.2 × 1.5 m) that had a fully slatted tribar floor, a feeder, and a nipple drinker. Pigs were allotted to a 2-period switch back design with 4 diets and 6 replicate pigs per diet in each period resulting in 12 replicate pigs per diet for the 2 periods. Feed and water were available at all times. Pigs were randomly allotted to a 2 × 2 factorial design with 2 levels of DDGS (0% or 45%) and 2 levels of supplemental Cu from Cu hydroxychloride (0 or 150 mg/kg; IntelliBond C^{II}, Micronutrients USA, Indianapolis, IN). Two diets based on corn and soybean meal were formulated to meet the nutrient requirements for 25- to 50-kg pigs (Table 1; NRC, 2012). The only difference between the 2 diets was that one diet contained no Cu hydroxychloride, whereas the other diet contained 150 mg/kg of Cu from Cu hydroxychloride. Two additional diets were formulated based on corn, soybean meal, and DDGS without and with Cu hydroxychloride. All diets contained 0.40% titanium dioxide as an indigestible marker.

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

For analysis of concentrations of volatile fatty acids (VFA), approximately 5 g of feces and ileal digesta were collected on days

Table 1. Ingredient composition of experimental diets

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

[1]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond C^{II}; Micronutrients USA, Indianapolis, IN).
[2]The phytase premix contained phytase (5,000 FTU/g of Quantum Blue 5G; AB Vista, Marlborough, United Kingdom) mixed with corn. The premix was formulated to provide 500 units of phytase per kilogram of complete diet if included at 1% in the diet.
[3]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

10 and 13, respectively. After collection, ileal digesta and fecal samples were placed in small wide-mouth plastic jars and samples were stabilized in 2N HCl and stored at –80 °C until analyzed for concentrations of VFA. Additional samples of approximately 10 g of feces and 10 g of ileal digesta were also collected on days 10 and 13, respectively, and immediately stored at –80 °C. These samples were later analyzed for concentrations of microbial protein.

At the conclusion of the experiment, fecal samples and ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected for analysis. Digesta samples were lyophilized and fecal samples were dried in a 50 °C forced air drying oven prior to analysis. Dried ileal digesta and fecal samples were finely ground through a 1-mm screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ). Diets, ileal digesta, and fecal samples were analyzed for titanium following the procedure of Myers et al. (2004). All diets, ileal digesta, and fecal samples were analyzed for GE using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL), DM (Method 930.15; AOAC Int., 2007), and CP (Method 990.03; AOAC Int., 2007) using the combustion procedure on a Leco FP628 protein apparatus (Leco Corp., St Joseph, MI). Samples were analyzed for AEE using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction using petroleum ether (Method 2003.06, AOAC Int., 2007) on an Ankom fat analyzer (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). All diets and ileal digesta samples were also analyzed for AA (Method 982.30 E (a, b, c); AOAC Int., 2007) on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Diets were analyzed for ash (Method 942.05; AOAC Int., 2007) and were also analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 (AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Total dietary fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber. Following analysis, the ATTD of DM, AEE, GE, and CP, and the AID of DM, AEE, GE, CP, and AA were calculated for all diets as described previously (Stein et al., 2007).

Concentrations of VFA (acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate) were analyzed using a Hewlett-Packard (Hewlett Packard, Avondale, PA) Model 5890A gas chromatograph equipped with a flame ionization detector on a column (1.8 m × 4 mm i.d.) packed with GP 10% SP-1200/1% H3P04 on 80/100 chromosorb W/AW (Chromosorb W/AW-DMCS, Supelco, Bellefonte, PA). The carrier gas (N) was used with a flow rate of 45 mL/min. The oven, injection port, and detector port temperatures were 125, 175, and 180 °C, respectively.

Samples that were collected for analysis for microbial protein were fractionated using differential centrifugation following the procedure of Metges et al. (1999). Samples were centrifuged first at 250 relative centrifugal force for 15 min at 4 °C, which resulted in fractions that were expected to contain undigested feed particles and porcine cells in the precipitate and supernatant, respectively (Miner-Williams et al., 2009). The supernatant was then centrifuged at 14,500 relative centrifugal force for 30 min at 4 °C to result in a precipitate that was expected to contain microbial cells (Miner-Williams et al., 2009). These cells were then subjected to a lysis buffer, which contained 100 mM of tris(hydroxymethyl)aminomethane at pH 7.2, 0.5% sodium dodecyl sulfate, and 0.5% sodium deoxycholate. The protein concentration of the lysed microbial cells was then analyzed using Pierce Bicinchoninic Acid Assay Kit (ThermoFisher Scientific, Waltham, MA).

Data were analyzed as a randomized complete block design in a 2 × 2 factorial arrangement using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. Fixed effects included Cu, DDGS, and the interaction between Cu and DDGS. Random effects included pig and period. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

Results

Diet analyses indicated that the intended concentrations of AEE, total dietary fiber, AA, and Cu were present in all diets (Table 2). Inclusion of 45% DDGS in the diet without added Cu resulted in a greater reduction in AID and ATTD of DM

Table 2. Analyzed composition of experimental diets

	No added Cu[1]		150 mg/kg Cu[1]	
Item	No DDGS	45% DDGS	No DDGS	45% DDGS
Dry matter, %	87.98	87.75	88.20	87.29
Ash, %	5.75	5.85	5.79	5.91
Gross energy, kcal/kg	3,829	4,091	3,837	4,086
Crude protein, %	17.90	23.33	18.18	23.47
Acid hydrolyzed ether extract, %	2.36	4.16	2.57	4.43
Insoluble dietary fiber, %	12.10	20.80	13.10	20.60
Soluble dietary fiber, %	1.40	2.40	1.10	2.65
Total dietary fiber, %	13.50	23.12	14.20	23.25
Minerals				
Ca, %	0.65	0.72	0.63	0.73
P, %	0.41	0.61	0.42	0.62
Na, %	0.19	0.30	0.18	0.31
Mg, %	0.13	0.21	0.13	0.20
K, %	0.78	1.09	0.78	1.07
S, %	0.19	0.27	0.19	0.27
Mn, mg/kg	75	76	71	80
Fe, mg/kg	234	203	232	205
Zn, mg/kg	152	179	143	179
Cu, mg/kg	29	34	189	220
Indispensable amino acids, %				
Arg	1.14	1.22	1.07	1.25
His	0.47	0.59	0.44	0.60
Ile	0.79	0.96	0.75	0.97
Leu	1.51	2.18	1.42	2.19
Lys	1.08	1.15	1.05	1.13
Met	0.28	0.37	0.25	0.36
Met + Cys	0.56	0.75	0.50	0.73
Phe	0.90	1.13	0.85	1.14
Thr	0.69	0.87	0.63	0.86
Trp	0.22	0.22	0.21	0.23
Val	0.86	1.14	0.82	1.14
Dispensable amino acids, %				
Ala	0.88	1.30	0.84	1.32
Asp	1.69	1.85	1.62	1.85
Cys	0.28	0.38	0.25	0.37
Glu	3.13	3.48	2.90	3.49
Gly	0.75	0.97	0.71	0.97
Ser	0.78	0.95	0.72	0.95
Tyr	0.63	0.83	0.60	0.83
All amino acids, %	17.59	21.40	16.54	21.55

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

compared with the diet containing 45% DDGS and 150 mg/kg of Cu from Cu hydroxychloride (interaction, $P < 0.05$; Table 3). Likewise, inclusion of 45% DDGS in the diet without added Cu resulted in a tendency for greater reduction in AID and ATTD of GE compared with the diet containing 45% DDGS and 150 mg/kg of Cu from Cu hydroxychloride (interaction; $P < 0.10$). Greater ($P < 0.01$) AID and ATTD of AEE was observed in the DDGS diets compared with diets without DDGS, but a reduction ($P < 0.01$) in AID and ATTD of CP was observed in diets containing DDGS compared with diets without DDGS. No impact of Cu hydroxychloride was observed for AID and ATTD of CP, GE, and DM, but Cu hydroxychloride improved ($P < 0.01$) the AID and ATTD of AEE. The AID of all AA was less ($P < 0.01$) in diets containing DDGS compared with diets without DDGS (Table 4), but supplementation of Cu hydroxychloride to the diets did not affect AID of AA except for a reduction ($P < 0.05$) in the AID of Cys.

Inclusion of DDGS or Cu hydroxychloride to diets did not affect the concentration of microbial protein in ileal digesta (Table 5). However, fecal microbial protein concentration increased ($P < 0.01$) if diets contained DDGS, whereas Cu supplementation reduced ($P < 0.05$) the concentration of microbial protein in feces.

The proportion of butyrate in ileal digesta was less ($P < 0.01$) in diets containing DDGS compared with diets without DDGS, whereas the proportions of isobutyrate and valerate were greater ($P < 0.05$) in ileal digesta from pigs fed DDGS-containing diets. However, the total concentration of VFA in ileal digesta was not affected by inclusion of DDGS in the diets. Dietary Cu concentrations did not affect the total concentration and proportions of VFA produced in ileal digesta. The proportion of acetate in feces was greater ($P < 0.01$) from pigs fed DDGS diets compared with pigs fed no DDGS. However, reduced ($P < 0.01$) proportions of propionate and isobutyrate were observed in feces from pigs fed diets containing DDGS compared with diets without DDGS and the proportion of valerate in feces tended to be less ($P < 0.10$) if pigs were fed DDGS diets than diets without DDGS. The proportion of acetate tended to be less ($P < 0.10$) in feces from pigs fed diets with Cu hydroxychloride compared with diets without Cu hydroxychloride. This resulted in a tendency for a reduction ($P < 0.10$) in the total concentration of VFA in feces when Cu hydroxychloride was added to diets.

Discussion

Distillers dried grains with solubles is a co-product from ethanol production, which contains 30% to 35% total dietary fiber (NRC, 2012; Espinosa and Stein, 2018). Therefore, inclusion of 45% DDGS in diets resulted in diets with greater concentration of total dietary fiber compared with diets without DDGS. Dietary fiber includes nonstarch polysaccharides, resistant starch, and oligosaccharides that are resistant to enzymatic hydrolysis in the small intestine (Bindelle et al., 2008), and therefore, can affect a wide range of physiological processes in pigs. The observation that the AID and ATTD of DM and GE were reduced as the concentration of total dietary fiber increased in the diets is likely due to the insoluble portion of dietary fiber in DDGS, which are poorly utilized by pigs (Lindberg, 2014). Fermentation of dietary fiber results in synthesis of methane and microbial biomass, and this may reduce the efficiency of energy utilization from dietary fiber (Noblet and Le Goff, 2001; Bindelle et al., 2008). The observation that the AID and ATTD of CP decreased as total dietary fiber increased in the diets is in agreement with previous data (Urriola and Stein, 2010; Zhang et al., 2013). This is likely a result of increased endogenous secretions of CP due to the insoluble portion of dietary fiber in DDGS (Eggum, 1992).

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

The improved AID and ATTD of AEE in the DDGS diets is due to the greater concentration of AEE in diets containing DDGS because increased dietary AEE reduces the relative contribution of endogenous fat in the ileal or fecal output (Jørgensen et al., 1993), which results in increased calculated values for AID and ATTD of AEE. This response is in agreement with published data that indicated that the ATTD of fat increased upon inclusion of increased concentrations of soybean oil or corn oil to a diet with a low concentration of fat (Jørgensen et al., 1993; Kil et al., 2010).

Increased concentration of TDF in diets results in increased fermentation by microbes in the gastrointestinal tract.

Table 3. Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of energy and nutrients in experimental diets[1]

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

[1]Data are least squares means of 12 observations for treatments without DDGS and 11 observations for treatments with DDGS.
[2]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond C[II]; Micronutrients USA, Indianapolis, IN).
[3]DDGS, distillers dried grains with solubles; AEE, acid hydrolyzed ether extract.

Table 4. Apparent ileal digestibility (AID) of amino acids (AA) in experimental diets[1]

	No added Cu[2]		150 mg/kg Cu[2]			P-value		
Item	–DDGS[3]	45% DDGS	–DDGS	45% DDGS	SEM	DDGS	Cu	DDGS × Cu
Crude protein, %	77.8	65.6	78.0	66.2	1.3	<0.001	0.674	0.820
Indispensable AA, %								
Arg	88.5	80.7	88.3	81.1	0.6	<0.001	0.869	0.583
His	84.6	70.5	83.6	69.3	1.1	<0.001	0.210	0.905
Ile	81.1	70.6	79.6	70.9	0.9	<0.001	0.524	0.305
Leu	81.5	75.0	79.6	74.6	1.0	<0.001	0.183	0.347
Lys	85.4	71.3	84.8	71.5	1.1	<0.001	0.857	0.659
Met	83.4	74.7	81.2	74.4	1.0	<0.001	0.149	0.282
Phe	82.2	73.3	80.8	73.7	0.9	<0.001	0.520	0.233
Thr	74.6	60.6	71.8	61.1	1.3	<0.001	0.266	0.127
Trp	81.4	69.6	83.1	68.9	0.9	<0.001	0.576	0.194
Val	78.3	66.7	77.2	67.0	1.2	<0.001	0.640	0.441
Total	82.4	72.0	81.2	72.1	1.0	<0.001	0.488	0.421
Dispensable AA, %								
Ala	74.6	66.8	75.1	67.5	1.4	<0.001	0.567	0.914
Asp	78.4	64.1	78.6	63.6	1.2	<0.001	0.854	0.734
Cys	64.6	47.9	61.6	39.5	2.2	<0.001	0.010	0.213
Glu	83.5	72.2	82.8	71.6	1.4	<0.001	0.619	0.940
Gly	64.7	50.3	64.7	48.5	2.1	<0.001	0.645	0.625
Ser	80.0	69.8	80.0	70.6	1.2	<0.001	0.754	0.532
Tyr	81.6	75.6	81.0	75.7	0.8	<0.001	0.745	0.607
Total	78.7	67.0	78.4	66.4	1.4	<0.001	0.685	0.922
All AA	80.6	69.5	79.8	69.3	1.2	<0.001	0.762	0.762

[1]Data are least squares means of 12 observations for treatments without DDGS and 11 observations for treatments with DDGS.
[2]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond C[II]; Micronutrients USA; Indianapolis, IN).
[3]DDGS, distillers dried grains with solubles.

Table 5. Intestinal microbial protein concentrations (mg/g, DM basis) and rate of fermentation of volatile fatty acids in ileal digesta and feces of pigs fed experimental diets[1]

	No added Cu[2]		150 mg/kg Cu[2]			P-value		
Item	–DDGS[3]	45% DDGS	–DDGS	45% DDGS	SEM	DDGS	Cu	DDGS × Cu
Ileal digesta								
Microbial protein	23.38	19.91	19.09	19.37	2.36	0.501	0.312	0.430
Volatile fatty acids (µmol/100 µmol)								
Acetate	93.13	93.49	91.22	94.91	1.86	0.200	0.868	0.276
Propionate	2.54	3.66	3.88	2.88	1.08	0.954	0.797	0.329
Butyrate	3.63	1.59	3.93	1.08	0.69	<0.001	0.847	0.453
Isobutyrate	0.53	0.78	0.68	0.74	0.07	0.019	0.434	0.139
Isovalerate	0.16	0.37	0.28	0.27	0.07	0.170	0.874	0.123
Valerate	0.01	0.11	0.01	0.12	0.07	0.010	0.885	0.897
Total [VFA[3]], µmol/g, DM basis	7.88	9.05	7.88	7.00	1.10	0.866	0.229	0.230
Feces								
Microbial protein	169.32	195.15	142.08	182.07	9.63	0.002	0.046	0.469
Volatile fatty acids (µmol/100 µmol)								
Acetate	69.66	75.70	61.75	75.10	1.73	0.006	0.096	0.277
Propionate	20.27	16.17	25.08	16.69	2.52	0.005	0.215	0.317
Butyrate	1.15	1.09	1.25	1.12	0.10	0.384	0.523	0.718
Isobutyrate	5.56	4.15	7.48	4.12	0.72	0.002	0.195	0.180
Isovalerate	1.34	1.18	1.60	1.27	0.18	0.168	0.342	0.644
Valerate	2.02	1.71	2.84	1.70	0.42	0.067	0.300	0.280
Total [VFA], µmol/g, DM basis	12.36	10.68	10.06	10.42	0.67	0.333	0.065	0.140

[1]Data are least squares means of 12 observations for treatments without DDGS and 11 observations for treatments with DDGS.
[2]Diets containing added Cu were fortified with 150 mg/kg of Cu as Cu hydroxychloride (IntelliBond C[II]; Micronutrients USA, Indianapolis, IN).
[3]DDGS, distillers dried grains with solubles; VFA, volatile fatty acids.

Therefore, the observation that the concentration of fecal microbial protein increased if DDGS was included in the diets is likely due to an increased growth of bacteria in the hindgut (Bach Knudsen and Hansen, 2007; Bindelle et al., 2008). The undigested portion of dietary carbohydrates serve as a substrate for intestinal microbes to hydrolyze and metabolize

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

Inclusion of pharmacological concentrations of dietary Cu improves growth performance of pigs (Cromwell et al., 1998; Hill et al., 2000; Ma et al., 2015), but the effect of dietary Cu on nutrient digestibility remains inconsistent. The lack of differences in the AID and ATTD of GE among diets containing 0 or 150 mg Cu/kg from Cu hydroxychloride is in agreement with data indicating that supplementation of Cu to diets for pigs did not improve energy digestibility (Castell and Bowland, 1968; Espinosa et al., 2017). However, this is in contrast with results by Gonzales-Eguia et al. (2009) and Coble et al. (2018), who reported that supplementation of 50 to 150 mg Cu/kg from Cu hydroxychloride or $CuSO_4$ resulted in an improved ATTD of GE. In the present experiment, we used diets both with a low and a high concentration of fiber to address the possibility that dietary fiber concentration may affect the response of Cu on digestibility of GE, but results do not indicate that fiber affects the response to Cu on GE digestibility. Further research is, therefore, needed to investigate why Cu sometimes appears to increase GE digestibility, whereas this effect was not observed in other experiments.

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

The improvement in AID and ATTD of AEE was observed when diets were supplemented with Cu concurs with previous data (Espinosa et al., 2019). Dietary fiber from corn, SBM, and DDGS facilitates microbial growth in the intestinal tract, which may result in increased endogenous loss of fat due to excretion of microbial fat (Kil et al., 2010). Therefore, the increased calculated values for AID and ATTD of AEE are possibly due to the effect of Cu on reducing microbial intestinal mass, and therefore, reducing excretion of microbial fat by pigs (Espinosa et al., 2019), which then results in increased calculated values for AID and ATTD of AEE. The observation that Cu did not increase AID and ATTD of GE, despite the observed increase in AID and ATTD of AEE, further indicates that the effects of Cu on AID and ATTD of AEE likely were a result of changed microbial excretion of fat rather than increased absorption of fat.

To the best of our knowledge, no data demonstrating the effects of Cu hydroxychloride on intestinal microbial protein concentrations of pigs have been reported. The observation that the concentration of microbial protein in feces was reduced when diets were supplemented with Cu hydroxychloride is likely a result of the bacteriostatic properties of dietary Cu. Dietary Cu may alter the microbial populations in the intestine and thereby affect the growth and community structure of microorganisms in the cecum and colon (Stahly et al., 1980; Højberg et al., 2005). Copper may also disrupt enzyme structures and functions of bacteria by binding to S or carboxylate-containing groups and amino groups of proteins (Sterritt and Lester, 1980). Therefore, it is possible that Cu hydroxychloride inhibited the growth of microbes and subsequently reduced the concentration of microbial protein in the hindgut. The observed response in the concentration of fecal microbial protein also supports the observed improvement in the ATTD of AEE upon supplementation of Cu hydroxychloride to diets.

The observed tendency for a reduction in the total concentration of VFA in feces from pigs fed diets with Cu hydroxychloride is in contrast with data indicating that the concentrations of VFA were greater in cecal contents from pigs fed diets supplemented with 250 mg Cu/kg from $CuSO_4$ (Mei et al., 2010). This observation is also in contrast with data reported by Huang et al. (2015) that indicated that supplementation of 225 mg Cu/kg from Cu hydroxychloride or $CuSO_4$ to diets for nursery pigs did not affect total concentration of VFA produced in the cecum. However, the observed response for VFA is in agreement with the observed reduction of microbial protein concentration in the hindgut. Copper may reduce the fermentation of fiber in the hindgut by altering the growth, activity, and metabolism of microbes, and therefore, may have reduced VFA synthesis in the hindgut of pigs.

In conclusion, inclusion of 45% DDGS in diets resulted in a reduced AID and ATTD of DM, GE, CP, and AA, which is likely due to increased endogenous losses of nutrients and reduced utilization of energy and addition of Cu hydroxychloride to the diets did not ameliorate this effect. Supplementation of Cu hydroxychloride to diets reduced the concentrations of total VFA and microbial protein in feces, indicating reduced microbial activity in the hindgut of pigs fed diets containing Cu hydroxychloride and these reductions were independent of the presence of fiber from DDGS in the diets. The improved AID and ATTD of AEE that was observed was, therefore, likely caused by reduced endogenous loss of fat due to reduced excretion of microbial fat.

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