Effect of diet type and added copper on growth performance, carcass characteristics, energy digestibility, gut morphology, and mucosal mRNA expression of finishing pigs

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ABSTRACT: A total of 757 pigs (PIC 337 × 1050; initially 27.6 kg BW) were used in a 117-d experiment to determine the effects of added Cu from tribasic copper chloride and diet type on growth performance, carcass characteristics, energy digestibility, gut morphology, and mucosal mRNA expression of finishing pigs. Pens of pigs were allotted to 1 of 4 dietary treatments, balanced on average pen weight in a randomized complete block design with 26 to 28 pigs per pen and 7 replications per treatment. Treatments were arranged in a 2 × 2 factorial with main effects of diet type, a corn-soybean meal-based diet (**corn-soy**) or a high by-product diet (**by-product**) with 30% distillers dried grains with solubles (**DDGS**) and 15% bakery meal, and added Cu (0 or 150 mg/kg added Cu). There were no Cu × diet type interactions for growth performance. Overall, neither added Cu nor diet type influenced growth performance. However, caloric efficiency was decreased ($P = 0.001$) for pigs fed the by-product diet compared to the corn-soy diet. Pigs fed the by-product diet had decreased ($P < 0.05$) carcass yield and carcass G:F) and marginally decreased ($P < 0.07$) HCW and carcass ADG compared to pigs fed the corn-soy diet. A Cu × diet type interaction ($P < 0.05$) existed for DM and GE digestibility during the early finishing period as added Cu improved ($P < 0.05$) digestibility of DM and GE in the corn-soy diet, but not in the by-product diet. During the late finishing period, added Cu marginally increased ($P = 0.060$) DM and GE digestibility while pigs fed the by-product diet had decreased DM and GE digestibility ($P = 0.001$) compared to those fed the corn-soy diet. For gut morphology, pigs fed added Cu had decreased crypt depth ($P = 0.017$) in the distal small intestine compared to those fed no added Cu. Furthermore, relative mRNA expression of intestinal fatty acid binding protein (*iFABP*) was decreased ($P = 0.032$) in pigs fed added Cu compared to those fed no added Cu. In summary, adding 150 mg/kg added Cu or including 30% DDGS and 15% bakery meal into a corn-soy diet did not influence growth performance. However, HCW ADG and HCW G:F were reduced in pigs fed the by-product diet compared to the corn-soy diet. Only minor differences in gut morphology or mRNA expression were observed from feeding diets with high levels of Cu or by-products compared to a corn-soy diet.

Key words: by-products, copper, finishing pigs, gene expression, glucagon-like peptide 1

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INTRODUCTION

For many years, copper (Cu) has been supplemented in nursery and early finishing diets to

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improve growth performance. While feeding high levels of Cu has been shown to improve growth, the duration and degree of response have not always been consistent. Research has typically shown that added Cu impacts growth the most during the early finishing period but not late finishing period, but the response is variable (Davis et al., 2002; Hastad, 2002; Carpenter et al., 2017). Recently, Coble et al. (2017) reported that adding 150 mg/kg Cu in finishing diets tended to increase ADFI and G:F during the late finishing period.

It has been postulated that the growth-promoting effects of Cu are partly due to its impact on tissue repair in the small intestine and its ability to stimulate the synthesis of digestive enzymes, resulting in a better digestion and absorption of nutrients (Hedemann et al., 2006). Lou and Dove (1996) report that nursery pigs fed 250 mg/kg Cu had improved fat digestibility. Rochelle et al. (2017) reported an improvement in AA digestibility in low Lys diets with added Cu in chicks, and Gonzales-Eguia et al. (2009) reported an improvement in OM and fat digestibility with added Cu in 30 to 60 kg growing pigs. Although the strategies for using Cu have not changed much over the years, the types of diets that are used in current commercial production are different in ingredient composition from diets utilized in the original researches with Cu. It has yet to be investigated if ingredient usage and diet formulation are an important factor to consider when adding Cu to improve growth performance. Therefore, the objective of this study was to determine the effects of added Cu and diet type on growth performance, carcass characteristics, energy digestibility, gut morphology, and mucosal gene expression of finishing pigs.

MATERIALS AND METHODS

All experimental procedures and animal care were approved by the Kansas State University Institutional Animal Care and Use Committee.

The experiment was conducted in a commercial research facility in southwestern Minnesota. The facility was double-curtain-sided with completely slatted concrete flooring. The barn contained pens (3.05 × 5.49 m) with 26 to 28 pigs (similar number of barrows and gilts) per pen. Each pen was equipped with a 4-hole conventional dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer providing ad libitum access to feed and water. A computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) delivered and recorded daily feed additions of specific diets to each pen.

Animals and Diets

A total of 757 pigs (PIC 337 × 1050; Genus PIC, Hendersonville, TN; initially 27.6 kg BW) from 28 pens were used in a 117-d experiment. Before day 0, all pigs were fed a common diet with 205 mg/kg Cu from tribasic copper chloride (**TBCC**, Intellibond C; Micronutrients, Inc., Indianapolis, IN). On day 0, pens of pigs were weighed, blocked by average pen weight, and allotted to 1 of 4 dietary treatments. There were 7 replications per treatment. Treatments were arranged in a 2 × 2 factorial and included 2 diet types, a corn-soybean meal-based diet (**corn-soy**) or a high by-product diet with 30% distillers dried grains with solubles (**DDGS**) and 15% bakery meal (**by-product**), and 2 levels of added Cu (0 or 150 mg/kg; Table 1).

All diets contained a basal level of 17 mg/kg Cu from $CuSO_4$ provided by the trace mineral premix. Treatment diets were fed in 5 dietary phases (from day 0 to 21, 21 to 45, 45 to 68, 68 to 94, and 94 to 117 for phases 1 to 5, respectively) in meal form and formulated on a standardized ileal digestible (**SID**) Lys basis to meet or exceed requirements (NRC, 2012). Diets were balanced on a SID Lysine:NE ratio across all treatments within phase to insure Lys was not a limiting factor for growth. Nutrient values for the ingredients were based on the NRC (2012), with the exception of the DDGS. The NE value (2,634 kcal/kg) for DDGS were calculated based upon the oil content, as described by Graham et al. (2014a). Treatment diets were collected from each treatment during each phase from multiple feeders 2 d after the beginning of a phase and 2 d before ending a phase. The 2 samples were combined to form a composite sample for each treatment within each phase and analyzed, in duplicate, for DM (930.15, AOAC International, 2000), CP (990.03, AOAC International, 2000), NDF (Van Soest et al., 1991), crude fiber (978.10, AOAC International, 2000), ether extract (2003.05, AOAC International, 2000), ash (942.05, AOAC International, 2000), Ca, P, and Cu (985.01, AOAC International, 2000) at a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD; Table 2). Bulk density (mass per unit volume, g/liter) was also measured for the complete feed with a grain density cup (Seedburo Model 8800; Seedburo Equipment, Chicago, IL).

Pens of pigs were weighed and feed disappearance was recorded on day 0, 21, 45, 68, 94, and 117 to determine ADG, ADFI, G:F, and caloric efficiency on ME and NE basis. Caloric efficiency was calculated by dividing the product of total feed

Table 1. Diet formulation (as-fed basis)[1]

	Phase 1		Phase 2		Phase 3		Phase 4		Phase 5	
Diet type:	Corn-soy	By-product	Corn-soy	By-product	Corn-soy	By-product	Corn-soy	By-product	Corn-soy	By-product
Ingredient, %										
Corn	69.43	37.91	75.51	44.3	79.76	47.27	83.32	47.36	86.1	47.45
Soybean meal	27.83	14.19	21.88	7.92	17.7	5.15	14.17	5.32	11.36	5.41
DDGS[2]	–	30	–	30	–	30	–	30	–	30
Bakery meal	–	15	–	15	–	15	–	15	–	15
Monocalcium P, 21% P	0.6	0.1	0.6	0.13	0.65	0.13	0.6	0.08	0.65	0.05
Limestone	1.25	1.55	1.13	1.4	1.03	1.3	1.03	1.28	1	1.25
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[3]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Trace mineral premix[4]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-Lys HCl	0.225	0.56	0.238	0.575	0.24	0.52	0.25	0.4	0.25	0.3
DL-Met	0.075	0.02	0.055	–	0.04	–	0.035	–	0.03	–
L-Thr	0.055	0.1	0.05	0.095	0.045	0.065	0.055	0.015	0.065	–
L-Trp	–	0.032	0.001	0.041	0.005	0.035	0.011	0.017	0.014	0.002
Phytase[5]	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013
TBCC[6]	±	±	±	±	±	±	±	±	±	±
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis										
Standardized ileal digestible (SID) AA, %										
Lys	1.05	1.105	0.912	0.958	0.81	0.846	0.73	0.756	0.66	0.68
Ile:Lys	65	59	63	56	63	58	61	66	61	74
Met:Lys	31	29	31	28	30	31	31	34	31	38
Met + Cys:Lys	56	56	56	56	56	61	57	68	58	76
Thr:Lys	62	62	62	62	62	62	63	63	65	68
Trp:Lys	19.7	19	19	19	19	19	19	19	19	19
Val:Lys	69	69	69	69	69	73	69	82	69	91
Total Lys, %	1.19	1.29	1.03	1.13	0.92	1.01	0.83	0.93	0.75	0.85
ME, kcal/kg	3,289	3,351	3,300	3,362	3,305	3,366	3,311	3,369	3,314	3,369
NE, kcal/kg	2,449	2,571	2,487	2,610	2,511	2,627	2,533	2,628	2,551	2,628
SID Lys:NE, g/Mcal	4.29	4.29	3.67	3.67	3.22	3.22	2.88	2.88	2.59	2.59
CP, %	19	21.1	16.6	18.6	14.9	17.4	13.5	17.3	12.3	17.2
Ca, %	0.66	0.66	0.59	0.59	0.55	0.55	0.53	0.53	0.52	0.52
P, %	0.51	0.49	0.48	0.46	0.47	0.45	0.45	0.44	0.44	0.44
Available P, %	0.36	0.36	0.35	0.35	0.34	0.34	0.33	0.33	0.33	0.33

[1]Phase 1 diet fed from day 0 to 21, phase 2 fed from day 21 to 45, phase 3 diets fed from day 45 to 68, phase 4 diets fed from day 68 to 94, and phase 5 diets fed from day 94 to 117. All diets were provided in meal form.

[2]Distillers dried grains with solubles.

[3]Provided per kilogram of premix: 7,054,720 IU vitamin A; 1,102,300 IU vitamin D_3; 35,274 IU vitamin E; 3,527 mg vitamin K; 6,173 mg riboflavin; 22,046 mg pantothenic acid; 39,683 mg niacin; and 26 mg vitamin B_{12}.

[4]Provided per kilogram of premix: 17 g Mn from manganese oxide, 110 g Fe from ferrous sulfate, 110 g Zn from zinc oxide, 17 g Cu from copper sulfate, 331 mg I from ethylenediamine dihydroiodide, and 300 mg Se from sodium selenite.

[5]Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided 1,102 phytase units (FTU)/kg, with a release of 0.10% available P.

[6]Tribasic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN) provided 150 mg/kg Cu and was added at the expense of corn.

intake and diet calorie content, by total gain. On day 94, the 3 heaviest pigs in each pen were weighed and marketed according to standard farm procedures. These pigs were used in calculation of pen growth performance, but not carcass characteristics. Prior to marketing, the remaining pigs in the barn were individually tattooed with a pen identification number to allow for carcass measurements to be recorded on an individual pen basis. On day 117, final pen weights were taken and feed disappearance was recorded. A subsample of 2 gilts per pen, representing the mean individual weight of the pen, were transported 108 km to a commercial packing plant (packing plant #1; Natural Foods Holdings, Sioux Center, IA) for processing, intestinal sampling, and data collection. All remaining pigs were transported 95 km on day 118 to a commercial packing plant (packing plant #2; JBS Swift

Table 2. Chemical analysis of diets (as-fed)[1]

	Added Cu,[2] mg/kg:	0		150	
Item	Diet type:	Corn-soy	By-product	Corn-soy	By-product
Phase 2					
DM, %		85.90	88.00	86.00	88.00
CP, %		15.98	18.30	15.74	18.48
NDF, %		6.61	14.87	8.00	15.05
Crude fiber, %		2.06	4.05	2.32	4.22
Ether extract, %		2.14	4.63	1.94	4.76
Ash, %		4.70	5.10	4.21	4.73
Ca, %		0.74	0.74	0.73	0.69
P, %		0.46	0.51	0.47	0.49
Cu, mg/kg		54	44	211	190
Bulk density, g/liter		665	614	644	606
Phase 3					
DM, %		86.30	88.40	86.30	88.20
CP, %		13.81	16.53	13.72	15.88
NDF, %		6.82	15.20	7.25	14.02
Crude fiber, %		1.98	3.98	2.16	3.79
Ether extract, %		1.96	4.95	2.07	4.64
Ash, %		3.71	4.66	4.03	5.01
Ca, %		0.72	0.72	0.81	0.78
P, %		0.43	0.50	0.47	0.49
Cu, mg/kg		41	33	209	204
Bulk density, g/liter		662	596	651	597
Phase 4					
DM, %		85.80	87.50	85.80	87.60
CP, %		11.50	15.93	12.70	15.94
NDF, %		8.24	14.70	9.87	14.37
Crude fiber, %		2.40	4.03	2.83	3.94
Ether extract, %		2.36	4.38	1.99	4.55
Ash, %		3.35	4.20	3.53	4.70
Ca, %		0.67	0.57	0.63	0.68
P, %		0.42	0.51	0.46	0.48
Cu, mg/kg		58	30	159	187
Bulk density, g/liter		624	596	610	590
Phase 5					
DM, %		85.80	87.20	85.50	87.40
CP, %		11.93	15.52	11.54	15.47
NDF, %		7.89	14.04	7.35	14.95
Crude fiber, %		2.23	4.01	2.05	3.67
Ether extract, %		1.68	4.59	1.98	4.71
Ash, %		3.73	4.68	3.62	5.26
Ca, %		0.72	0.72	0.71	0.75
P, %		0.49	0.54	0.47	0.54
Cu, mg/kg		48	39	209	203
Bulk density, g/liter		632	604	630	603

[1]Values represent means from 1 composite sample, analyzed in duplicate. Phase 1 diets were not available for analysis.

[2]Tribasic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN).

and Company, Worthington, MN) for processing and carcass data collection. Hot carcass weight was measured immediately after evisceration and each carcass was evaluated for carcass yield, backfat depth, loin depth, and percentage lean.

Carcass yield was calculated by dividing the HCW at the plant by the live weight at the farm before transport. Fat depth and loin depth were measured with an optical probe inserted between the third and fourth last rib (counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline. An assumed yield of 75% was used to calculate initial HCW at the beginning of the experiment. Hot carcass weight ADG

was calculated by subtracting initial HCW from the final HCW obtained at the plant, then divided by 117 d on test. Hot carcass weight G:F was calculated by dividing HCW gain by feed intake over the 117-d experiment.

Gross Energy Digestibility

Feed and fecal grab samples were collected from each pen over a 2-d period during phases 2 (day 25 to 26) and 4 (day 74 and 75) to determine DE content of the experimental diets (Beaulieu et al., 2009). Acid insoluble ash (Celite 545; Univar Inc., Redmond, WA) was included in phase 2 and 4 diets at 1.0% to allow for a 7-d adaptation period and 2-d collection period at the beginning of the phases 2 and 4. Feed and fecal samples from each pen and day were dried at both 50 and 100 °C, using a 2-step drying process. Samples across days within pen were then pooled together for a composite sample for analysis (Jang et al., 2014). To determine the acid insoluble ash content of the feed and feces, samples were heated to 600 °C for 18 h, then boiled in 100 mL of HCl (2 mol/liter) for 5 min, filtered, and heated again to 600 °C for 18 h, as explained by Atkinson et al. (1984). Gross energy values for the feed and feces were determined by oxygen bomb calorimetry (Model 1341EB; Parr Instrument Company, Moline, IL), according to Galyean (2010). Dry matter and GE digestibilites were calculated using the index method, according to Adeola (2001), using the following equation where AIA is acid insoluble ash:

$$\text{Digestibility, \%} = 100 - \left[100 \times \left(\frac{\%\,\text{AIA in feed} \times \%\,\text{component in feces}}{\%\,\text{AIA in feces} \times \%\,\text{component in feed}}\right)\right]$$

Serum Collection and Protein Analysis

Prior to transportation to the packing plant #1, blood samples from the 2 sample gilts per pen were collected via jugular venipuncture into sterile vacutainer tubes (Tyco Health Care Group LP, Mansfield, MA) and immediately placed on ice until processed. Whole blood was centrifuged (2,000 × *g* for 15 min at 4 °C) and serum was removed and frozen at −80 °C until analysis. Mammalian specific ELISA kits (EMD Millipore Corp., Billerica, MA) were used to determine serum concentrations of glucagon-like peptide 1 (**GLP-1**; Cat. # EZGLP1T-36K) and glucagon-like peptide 2 (**GLP-2**; Cat. # EZGLP2-37-K). Prior to completing the assay, kits were validated for parallelism and recovery of added mass. Fluorescence was measured at 450 nm with a 96-well microplate spectrophotometer (Eon; BioTek, Winooski, VT). The limit of detection for GLP-1 and GLP-2 was 1.4 pM and 0.562 ng/mL, respectively. For samples with values below the detectable limit, the lowest detectable limit was reported.

Intestinal Collection

On day 117, intestinal tissue samples and mucosal scrapings were collected from the 2 sample gilts per pen at packing plant #1 for the analyses of small intestinal (**SI**) mucosal gene expression and gut morphology. Approximately 15 min after the pigs were slaughtered, the entire viscera was collected and segregated. The small intestine was dissected from the stomach 2 cm distal from the pyloric sphincter of the stomach and 2 cm proximal the ileocaecal junction. From each intestine, two, 5 cm samples were collected from the proximal (2 m from the proximal end of the SI-duodenum) and distal (2 m from the distal end of the SI-ileum) sections of the SI. A mucosal scrape was collected from one of the samples by using a sterile plastic slide to scrape the intestinal cells off the lining of the lumen. Scrapings were placed in a sterile Whirl-Pak bag (Fisher Scientific, Hampton, NH), snap chilled, and stored in liquid N until all samples were collected. These samples were utilized for mRNA analysis and were maintained at −80 °C until analysis. To preserve samples for histological analysis, samples were placed in 4% formaldehyde solution in a 50 mL conical tube for transport back to Kansas State University.

Small Intestine Histology

Approximately 48 h after samples were placed in the formaldehyde solution, samples were embedded in paraffin and 4 µm cross sections were cut and stained with hematoxylin and eosin (H & E) for histological examination of gut morphology, as described by Hedemann et al. (2006). Measurements included villus height, crypt depth, and villus height to crypt depth ratio. Slides were viewed using a Nikon Eclipse TI-U inverted microscope with 4× working distance magnification (Nikon Instruments Inc., Melville, NY). Villus height and crypt depth were measured using NIS Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc.) and calibrated to the 4× objective. Measurements were recorded in µm and determined in real-time.

Ileal Mucosal Gene Expression

Ileal mucosal RNA was isolated and transcribed to cDNA as described by Gonzalez et al. (2014) and Paulk et al. (2015). Five nanogram equivalents of total RNA were amplified with gene-specific primers (Table 3), DNA polymerase, and SYBR green chemistry (Perfecta Sybr fast mix; Quanta Biosciences, Gaithersburg, MD) in a Realplex^2 S PCR System (Eppendorf North America, Hauppauge, NY). Thermal cycling parameters included an initial heating step of 50 °C for 2 min and an initial denaturing step of 95 °C for 10 min, followed by 50 cycles of 15 s at 95 °C, an annealing step for 30 s at the appropriate temperature for each primer, and an extension step of 20 s at 68 °C. A final dissociation step was included at 95 °C for 15 s followed by annealing at 60 °C for 15 s. Melting temperature analysis was then conducted between 60 and 95 °C using a 20 min ramp time and continuous fluorescence detection to determine primer specificity for each reaction. Normalized expression (ΔCt) for each sample was determined using the *Ribosomal protein L4* (*RPL4*) as an endogenous gene. Relative gene expression levels were calculated as $2^{-\Delta\Delta Ct}$, where ΔΔCt represents ΔCt sample minus ΔCt calibrator, where a pooled sample representing all treatment groups served as the calibrator sample (Livak and Schmittgen, 2001).

Statistical Analysis

Experimental data were analyzed in a randomized complete block design using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC) with pen serving as the experimental unit and initial BW serving as the blocking factor. The random effect of pen within treatment was included in the model when multiple observations (subsamples) were collected within an experimental unit (pen). Contrasts were used to evaluate the interaction between added Cu and diet type and the main effects of added Cu and diet type. Residual assumptions were checked using standard diagnostics on studentized residuals. The assumptions were reasonably met with the exception of gene expression data. For gene expression, the values were ranked using the RANK procedure prior to analysis. Degrees of freedom were estimated using the Kenward–Roger's approach. Backfat depth, loin depth, and lean percentage were adjusted to a common HCW. Results from the experiment were considered significant and $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Table 3. Sequences, annealing temperatures, amplicon length, and efficiency of primers used for real-time PCR quantification of gene expression

Item	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	T_m,[1] °C	Amplicon length	Efficiency
Small intestine genes					
Copper transport protein -1	CCATGATGATGCCTATGACCTT	ATAGAACATGGCTAGTAAAAACACC	60.5	131	1.12
Glucan-like peptide-1	TACTTCTGGCTGCTGGTGGAG	ACCCCAGCCTATGCTCAGGTA	62.4	104	1.11
Intestinal fatty acid binding protein	CCTCGCAGACGGAACTGAAC	GTCTGGACCATTTCATCCCCG	64.5	135	1.03
Normalizing gene					
Ribosomal protein L4	AGGAGGCTGTTCTGCTTCTG	TCCAGGGATGTTTCTGAAGG	60.5	184	1.06

[1]T_m = melting temperature.

RESULTS AND DISCUSSION

Chemical Analysis

The chemical analyses of the complete diets were similar to the intended formulation (Table 2). The addition of 30% DDGS and 15% bakery meal increased the CP, NDF, crude fiber, ether extract, and ash concentrations in the by-product diet compared to the corn-soy diet as anticipated. Total Ca and P levels were similar between diet types across each dietary phase. The analyzed total Cu levels ranged from 30 to 58 mg/kg in the diets without added Cu, and ranged from 159 to 211 mg/kg for the diets with 150 mg/kg added Cu. These values are within the acceptable analytical limits according to the Association of American Feed Control Officials (AAFCO, 2014), given 17 mg/kg of Cu was provided by the trace mineral premix and the Cu provided by other ingredients used in formulation. For diet characteristics, the by-product diet decreased bulk density of the diet by an average of 7.4% compared to the corn-soy diet, which is similar to others whom fed diets containing 30% or more of by-product ingredients (Wang et al., 2007, Asmus et al., 2014; Wu et al., 2016).

Growth Performance

Added Cu effects. During the early finishing period (day 0 to 45), there were no Cu × diet type interactions ($P > 0.14$; Table 4). Feeding pigs 150 mg/kg added Cu increased ADG by 2.4% with a marginal significance ($P = 0.076$) compared to pigs fed no added Cu. During the late finishing period (day 45 to 117), diet type marginally influenced the G:F response to added Cu (Cu × diet type interaction, $P = 0.060$). This was the result of a tendency in decreased ($P = 0.055$) G:F for pigs fed the by-product diet compared to the corn-soy diet when added Cu was fed, while pigs fed no added Cu had no difference in G:F between by-product and corn-soy diets. Overall (day 0 to 117) added Cu did not influence growth performance.

The magnitude of increase for ADG during the early finishing period in the current study has been consistently observed in other experiments with similar levels of added Cu. For instance, Davis et al. (2002), Zhao et al. (2014), and Coble et al. (2017) observed that feeding 150 to 175 mg/kg added Cu increased overall ADG by 3% to 8%. However, the current study was unable to demonstrate an overall growth response to added Cu. Even with the numerous studies that have shown positive responses with added Cu during the finishing stage; other work has shown no evidence of an effect (Lauridsen et al., 1999; Carpenter et al., 2017). The lack of replication, difference in Cu sources, and individual housing could have been contributing factors for not observing a response to Cu. Furthermore, the G:F interaction during the late finishing period is partly due to the numerical increase in ADFI with added Cu in the by-product diets without an increase in ADG.

Diet type effects. From day 0 to 45, pigs fed the by-product diet had decreased BW on day 45 ($P = 0.004$) in response to a 3.5% decrease in ADG ($P = 0.001$) and 7.5% decrease in ADFI ($P = 0.009$) compared to the corn-soy diet. However, from day 45 to 117 and overall, growth performance and final BW were not influenced by diet type. The reduction in growth performance during the early finishing period was not surprising and is consistent with others whom have fed high-fiber, by-product diets not equalized for dietary energy. Coble (2015) reported that G:F was reduced by 2.3% over the entire finishing period for pigs fed a by-product diet, containing the same amounts of bakery meal and DDGS as the current study, compared to a corn-soy diet as a result of the lower amount of NE in the by-product diet. Salyer et al. (2012) reported that increasing the amount of wheat middlings from 0% to 20%, in diets containing 30% DDGS decreased ADG by 4%. Furthermore, Paulk et al. (2012) reported that ADG decreased as bakery meal increased from 0% to 15% in diets containing 15% to 50% DDGS.

Although in our study, overall growth performance was not affected by diet type, caloric efficiency was worse ($P < 0.05$) for pigs fed the by-product diet compared to the corn-soy diet as they required more Mcal of energy per kg of gain on both an ME and NE basis. This is partly due to both the numerical reduction in G:F for pigs fed the by-product diet compared to the corn-soy diet, as well as the potential overvaluing of the energy content of either the DDGS, bakery meal, or both, during formulation. To predict the energy content of the DDGS, an equation from Graham et al. (2014a) was used to determine the NE based on oil content. However, the ME and NE values for bakery meal are questionable, as reflected by the NRC (2012) which reported nutrient values for bakery meal that were determined from 1 observation. This is further supported by the fact that research published after the NRC (2012) reported the ME value for bakery meal was 600 kcal/kg less than the NRC (2012) value (Rojas et al., 2013). Therefore,

Table 4. Effect of added Cu and diet type on growth performance and carcass characteristics of finishing pigs[1]

Added Cu,[2] mg/kg:	0		150			Probability, $P <$		
Diet type:	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × diet type	Cu	Diet type
Growth performance								
BW, kg								
Day 0	27.6	27.6	27.6	27.6	0.63	1.000	0.954	0.988
Day 45	65.6	64.0	66.3	64.9	1.02	0.776	0.082	0.004
Day 117	125.4	125.6	127.7	126.1	1.42	0.419	0.237	0.567
Day 0 to 45								
ADG, kg	0.84	0.81	0.86	0.83	0.012	0.874	0.076	0.001
ADFI, kg	1.70	1.67	1.76	1.68	0.029	0.221	0.114	0.009
G:F	0.497	0.483	0.490	0.493	0.006	0.147	0.771	0.303
Day 45 to 117								
ADG, kg	0.85	0.87	0.88	0.87	0.015	0.207	0.504	0.551
ADFI, kg	2.68	2.72	2.70	2.75	0.039	0.881	0.519	0.224
G:F	0.318	0.321	0.324	0.315	0.004	0.060	0.946	0.414
Day 0 to 117								
ADG, kg	0.85	0.85	0.87	0.85	0.010	0.311	0.191	0.269
ADFI, kg	2.29	2.31	2.32	2.32	0.029	0.814	0.376	0.824
G:F	0.370	0.368	0.374	0.366	0.003	0.356	0.705	0.114
Caloric efficiency[4]								
ME	8.94	9.16	8.84	9.20	0.084	0.345	0.720	0.001
NE	6.80	7.13	6.72	7.16	0.064	0.346	0.723	0.001
Carcass characteristics								
HCW, kg	93.8	92.1	95.4	93.2	1.21	0.776	0.195	0.067
Carcass yield, %	74.28	73.12	74.37	73.26	0.370	0.953	0.752	0.007
Backfat depth,[5] mm	18.7	18.1	18.7	18.1	0.44	0.910	0.951	0.192
Loin depth,[5] mm	66.7	63.7	65.3	65.9	1.14	0.135	0.719	0.349
Lean,[5] %	55.48	55.50	55.24	55.76	0.282	0.389	0.900	0.435
Carcass performance								
HCW ADG, kg	0.63	0.61	0.64	0.62	0.009	0.766	0.173	0.056
HCW G:F	0.272	0.265	0.275	0.267	0.003	0.910	0.432	0.011

[1]A total of 757 pigs (PIC 337 × 1050; initially 27.6 kg BW) were used in a 117-d experiment with 26 to 28 pigs per pen and 7 replications per treatment.

[2]Tri-basic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN).

[3]Refers to a diet containing 30% distillers dried grains with solubles (DDGS) and 15% bakery meal.

[4]Caloric efficiency is expressed as kcal of intake per kg of live weight gain.

[5]Hot carcass weight was used as a covariate.

these data highlight the need for more research to determine the energy value for bakery meal.

Carcass Characteristics

For carcass characteristics, pigs fed the by-product diet compared to the corn-soy diet had marginally decreased HCW (P = 0.067), and a significant reduction in carcass yield (P = 0.007; Table 4). As a result of the decrease in HCW, HCW ADG also marginally decreased (P = 0.056) for pigs fed the by-product diet compared to the corn-soy diet. The numerical reduction in G:F and significant reduction in carcass yield for pigs fed the by-product diet compared to the corn-soy diet also led to a decrease in HCW G:F (P = 0.011) for pigs fed the by-product diet compared to the corn-soy diet. Added Cu did not increase HCW or HCW ADG, which is not consistent with previous research completed by Coble et al. (2017). However, the reduction in HCW and carcass yield for pigs fed the by-product diet compared to those fed the corn-soy diet is consistent with most published literature (Asmus et al., 2014; Graham et al., 2014b; Wu et al., 2016). By-product ingredients generally contain high amounts of dietary fiber and have a low

bulk density, which increases gut fill and weight in the large intestine (Turlington, 1984; Asmus et al., 2014).

Diet Digestibility

Diet type influenced the response to Cu (Cu × diet type interaction, $P < 0.05$) for both DM and GE digestibility during early finishing period (Table 5). Pigs fed the by-product diet had decreased ($P < 0.05$) DM and GE digestibility compared to corn-soy diet, and the magnitude of this response was more prominent when Cu was added in the diet. No Cu × diet type interaction ($P > 0.43$) was observed for DM and GE digestibility during late finishing period. Pigs fed the by-product diet had decreased ($P < 0.05$) DM and GE digestibility compared to pigs fed the corn-soy diet. However, adding Cu marginally increased the digestibility of DM ($P = 0.060$) and improved GE digestibility ($P = 0.003$) by 2.3% compared to pigs not fed added Cu.

Due to the differences in growth response previously seen from added Cu in early and late finishing, the 2 different time points for GE and DM digestibility were measured in this study. In understanding how Cu can affect diet digestibility, research has suggested that Cu potentially improves fat digestibility (Dove and Haydon, 1992). Dove (1995) reported that weanling pigs fed 5% added fat had greater improvements in ADG when 250 mg/kg added Cu from $CuSO_4$ was included in the diet compared to diets without added Cu. The improvement was possibly the result of increased intestinal lipase and phospholipase activity (Lou and Dove, 1996).

During early finishing period, added Cu influenced the response for DM and GE digestibility between the 2 diet types; however, no biological explanation exists to describe the response. Importantly though, the current experiment was successful at demonstrating the potential for Cu to improve GE digestibility in a higher total dietary fat diet containing by-products. This could possibly explain the improvement Davis et al. (2002) observed for G:F in both the early and late finishing periods with added Cu, as their diets contained 4% added fat. While our diets did not contain added fat, the high by-product diet had approximately 2.5% higher crude fat compared to the corn-soybean meal diet. However, because of the inconsistency in response between phases, more research is needed to clarify how Cu may impact fat digestibility in finishing swine and whether the response is different for fat contributed from the basal ingredients as compared to fat contributed from added oils or fat.

Although the potential for Cu to improve GE digestibility in finishing pigs is less known, the reduction in DM and GE digestibility for pigs fed a by-product diet containing DDGS compared to those fed a corn-soy diet is more understood.

Table 5. Effect of added Cu and diet type on DM and GE digestibility and serum glucagon-like peptide (GLP) concentration of finishing pigs[1]

Added Cu,[2] mg/kg:	0		150			Probability, $P <$		
Diet type:	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × diet type	Cu	Diet type
Apparent total tract digestibility, %								
Early finishing[4]								
DM	94.31	92.09	95.02	91.45	0.306	0.029	0.906	0.001
GE	81.67	75.96	83.57	71.16	1.110	0.005	0.187	0.001
Late finishing[5]								
DM	95.72	93.14	96.47	93.46	0.280	0.435	0.060	0.001
GE	85.97	77.88	88.11	80.32	0.676	0.832	0.003	0.001
Serum GLP concentration[6]								
GLP-1, pM	12.97	14.07	14.54	12.24	2.659	0.530	0.960	0.825
GLP-2, ng/mL	1.92	1.67	1.78	1.68	0.354	0.831	0.854	0.616

[1]A total of 757 pigs (PIC 337 × 1050; initially 27.6 kg BW) were used in a 117-d growth study with 26 to 28 pigs per pen and 7 replications per treatment.

[2]Tri-basic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN).

[3]Refers to a diet containing 30% distillers dried grains with solubles (DDGS) and 15% bakery meal.

[4]Fecal samples collected over a 2-d period during phase 2 from day 25 to 26.

[5]Fecal samples collected over a 2-d period during phase 4 from day 74 to 75.

[6]Samples collected from 2 pigs per pen (totally 84 pigs).

Dietary fiber has been shown to decrease the digestibility of nutrients and energy (Degen et al., 2009; Urriola and Stein, 2010). Even though the by-product diet contained more GE and analyzed crude fat, the apparent digestibility of fat in by-products is lower than that of a supplemental oil source (Kil et al., 2010; Kim et al., 2013). The apparent total tract digestibility of DM and GE for diets containing 30% DDGS has been reported to be decreased by at least 6% compared to diets without DDGS (Liu et al., 2012). The GE digestibility has also been shown to vary among DDGS sources based on oil content (Graham et al., 2014a). This is mainly due to the reduction in apparent total tract digestibility of the insoluble fiber in DDGS (Stein and Shurson, 2009). The impact of bakery meal in this diet and its contribution to the GE digestibility is less known, as previously stated, and more research is needed to verify these results.

Serum Concentrations of Metabolites

As a result of the variability in proposed modes of action for Cu, other areas of research have evolved to suggest that Cu may improve growth performance by acting on specific neurological pathways associated with feed intake. Yang et al. (2011) reported an increase in relative mRNA expression for GhRH, which provides positive feedback to the hypothalamus to increase appetite. This increase in appetite comes as a result of increasing neuropeptide Y (NPY) concentration, a neurotransmitter in the brain that signals increased feed intake and also has been demonstrated to be increased as a result of feeding 250 mg/kg added Cu from $CuSO_4$ in swine (Li et al., 2008). In efforts to continue to expand our knowledge, we sought to investigate other areas of possible modes of action of Cu to increase feed intake that have been of high importance in human medicine.

Glucagon-like peptide 1 is an incretin hormone that is released by the L cells in the intestine in response to food ingestion, stimulating insulin secretion (Kieffer and Habener, 1999) and as a result, therapeutic administration of GLP-1 has been an important therapy for type-2 diabetic patients. However, in addition to GLP-1 mediating glucose levels, it also signals the brain to the slow the rate of digestion, and has been shown to directly influence feed intake (Tang-Christensen et al., 1996; Daily and Moran, 2013). In addition, GLP-2 is a hormone that is secreted in a 1 to 1 ratio with GLP-1, also from the L cells in the intestine (Janssen et al., 2013). Glucagon-like peptide 2 has been shown to improve intestinal health through increasing the growth and functionality of mucosa in the small intestine (Janssen et al., 2013). Thymann et al. (2014) reported that pigs under low sanitary conditions had an increase in small intestine weight, villus height, and crypt depth when GLP-2 was administered.

In the current experiment, there was no evidence for a difference in serum concentrations of GLP-1 or GLP-2 (Table 5) between diet types or with added Cu. The concentration of GLP-1 was greater than other reported values for pigs (Nagell et al., 2006); however, the circulating levels of GLP-2 were only slightly higher than those reported by Thymann et al. (2014; 1.54 ng/mL vs. current experiment levels of 1.76 ng/mL). The current experiment measured the concentrations in serum vs. plasma, age of the pig, and time of collection, which may explain the absence of the expected effects. Furthermore, both GLP-1 and GLP-2 have a relatively short half-life (7 to 17 min) after being released into the blood stream, and feed intake was not controlled across each animal since all pigs were allowed ad libitum intake.

Digestive Tract Morphology

Research has demonstrated that added Cu and diet type potentially impact gut morphology. Di Giancamillo et al. (2017) reported that 150 mg/kg $CuSO_4$ in an unprotected form increased duodenum villi length and crypt depths in nursery pigs relative to diets without added Cu. In contrast, Fry et al. (2012) reported that nursery pigs fed 225 mg/kg of added Cu in the form of $CuSO_4$ had reduced villus height in the duodenum and proximal jejunum compared to those fed no added Cu. However, they further reported that pigs fed 225 mg/kg of added Cu from TBCC did not have a difference in villus height when compared to pigs fed no added Cu. In our study, in the proximal section of the small intestine, neither villus height, crypt depth, nor villus height to crypt depth ratio were influenced by added Cu or diet type ($P > 0.10$; Table 6). In contrast, in the distal section of the small intestine, crypt depth was decreased in pigs fed added Cu compared to those not fed added Cu ($P = 0.017$). Hedemann et al. (2006) also observed that 175 mg/kg added Cu from $CuSO_4$ decreased crypt depth in both the proximal and distal small intestine, but no differences was observed from Radecki et al. (1992) who fed 250 mg/kg added Cu from $CuSO_4$. Limited research has been published that compares the gut morphology of pigs fed Cu from $CuSO_4$ vs. TBCC.

Table 6. Effect of added Cu and diet type on gut morphology and relative mRNA gene expression of intestinal fatty acid binding protein (iFABP), copper transporter protein-1 (CTR1), and glucagon-like peptide 1 (GLP-1R) in the proximal and distal small intestinal (SI) of finishing pigs[1]

Added Cu,[2] mg/kg:	0		150			Probability, $P <$		
Diet type:	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × diet type	Cu	Diet type
Gut morphology, µm								
Proximal SI								
Villus height	290	277	277	274	9.3	0.625	0.376	0.369
Crypt depth	244	244	214	221	16.7	0.843	0.102	0.874
Villus:crypt ratio	1.25	1.17	1.38	1.27	0.105	0.892	0.253	0.367
Distal SI								
Villus height	412	404	375	400	17.9	0.340	0.230	0.615
Crypt depth	227	223	202	212	7.6	0.330	0.017	0.683
Villus:crypt ratio	1.83	1.83	1.88	1.92	0.106	0.834	0.514	0.823
Relative mRNA gene expression[4]								
Proximal SI								
iFABP	0.289	0.360	0.316	0.325	0.072	0.442	0.870	0.741
CTR1	0.591	0.601	0.661	0.528	0.130	0.363	0.882	0.457
GLP-1R	0.800	0.802	1.364	0.957	0.420	0.623	0.391	0.685
Distal SI								
iFABP	1.143	0.726	0.664	0.571	0.178	0.283	0.032	0.258
CTR1	1.189	0.995	1.028	1.151	0.198	0.713	0.813	0.634
GLP-1R	2.336	1.592	2.088	1.718	0.566	0.575	0.664	0.382

[1]A total of 84 pigs (PIC 337 × 1050; initially 27.6 kg BW) were used in a 117-d experiment with 2 pigs per pen and 7 replications per treatment.

[2]Tri-basic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN).

[3]Refers to a diet containing 30% distillers dried grains with solubles (DDGS) and 15% bakery meal.

[4]All values indicate relative expression of genes. Normalized expression (ΔCt) for each sample was determined using ribosomal protein L4 as an endogenous control gene. The average normalized expression of the pooled control sample was used as the calibrator to calculate relative gene expression. For each sample, relative expression was calculated as $2^{-\Delta\Delta Ct}$, in which ΔΔCt represents ΔCt sample − ΔCt calibrator (Livak and Schmittgen, 2001).

Future research is desired to investigate if the discrepancies in gut morphology responses among studies could be attributed to Cu source. For diet type, Agyekum et al. (2012) reported that nursery pigs fed a diet containing 30% DDGS tended to have a decrease in villus height and villus height to crypt depth ratio.

Relative mRNA Gene Expression

In order to further investigate the different modes of action for Cu, the relative ileal mucosal mRNA expression of proteins involved with digestion was measured. Intestinal fatty acid binding protein (*iFABP*) mRNA expression was measured because of the importance it has on fatty acid transport across cell membranes. In addition to the serum concentration, *GLP-1R* mRNA expression was measured for the reasons mentioned previously. Furthermore, mRNA expression of copper transporter protein-1 (*CTR1*), an important protein involved in Cu transport across the cell wall (Hill and Link, 2008), was also measured.

For relative mucosal mRNA expression, there was no evidence ($P > 0.28$) for any diet type × Cu interactions for the measured genes in the proximal or distal small intestine (Table 6). Furthermore, there was no evidence of a difference for the relative mRNA expression of *iFABP*, *CTR1*, or *GLP-1R* in the mucosal layer of the proximal small intestine. However, relative mRNA expression of *iFABP* in the mucosal layer of the distal small intestine was decreased ($P = 0.032$) in pigs fed added Cu compared to those not fed added Cu, suggesting that the gene responsible for *iFABP* transcription is possibly downregulated with added Cu. If fat digestibility is truly increased, we would expect this to be upregulated. No data are available to compare the results of the relative mRNA expression of *GLP-1* or *iFABP* in response to diet composition type or added Cu in finishing pigs; however, Fry et al. (2012) reported that 225 mg/kg added Cu in the form of either $CuSO_4$ or TBCC did not affect *CTR-1* mRNA expression in the liver of weanling pigs, similar to the current experiment.

In conclusion, adding 150 mg/kg Cu to the diet during the early finishing period marginally increased ADG, but growth performance for the overall study period was not influenced by added Cu. Pigs fed the by-product diet compared to the

corn-soy diet had decreased ADG and ADFI during the early finishing period, but diet type did not affect overall growth performance even though pigs fed the by-product diet had a reduction in carcass yield and HCW. Added Cu did not affect serum metabolite profile for GLP-1 and GLP-2 concentrations or relative mRNA expression of *GLP-1R* or *CTR1*. However, more research is needed to clarify the impacts that added Cu has on DM and GE digestibility, especially since we observed that Cu influenced energy digestibility during the late finishing period.

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