Kansas Agricultural Experiment Station Research Reports

Volume 1
Issue 7 *Swine Day*

Article 31

2015

Effect of Diet Type and Added Copper on Growth Performance, Carcass Characteristics, Energy Digestibility, Gut Morphology, and Mucosal mRNA Expression of Finishing Pigs

K. F. Coble
Kansas State University, Manhattan, kcoble@k-state.edu

D. D. Burnett
Kansas State University, derrisburn@ksu.edu

R. D. Goodband
Kansas State University, Manhattan, goodband@k-state.edu

J. M. Gonzales
Kansas State University, Manhattan, johngonz@k-state.edu

See next page for additional authors

Effect of Diet Type and Added Copper on Growth Performance, Carcass Characteristics, Energy Digestibility, Gut Morphology, and Mucosal mRNA Expression of Finishing Pigs

Abstract

A total of 757 pigs (PIC 337 × 1050, initially 60.8 lb) were used to determine the effects of added Cu (TBCC, tribasic copper chloride, IntelliBond C; Micronutrients, Inc., Indianapolis, IN) 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 as a 2 × 2 factorial with main effects of diet type, a corn-soybean meal-based diet or a high by-product diet with 30% distillers dried grains with solubles (DDGS) and 15% bakery meal (by-product), and with or without added Cu (0 or 150 ppm added Cu). There were no Cu × diet type interactions for growth performance. Overall, neither added Cu nor diet type influenced growth performance. Pigs fed the by-product diet had decreased carcass yield ($P = 0.007$) and HCW F/G ($P = 0.013$), and tended to have decreased HCW ($P = 0.067$) and HCW ADG ($P = 0.056$) compared to pigs fed the corn-soybean meal-based diet. A Cu × diet type interaction ($P < 0.05$) existed for DM and GE digestibility during the early finishing period as added Cu improved digestibility of DM and GE in the corn-soybean mealbased diet, but not in the by-product diet. During the late finishing period, added Cu increased DM and GE digestibility ($P = 0.060$), while pigs fed the by-product diet had decreased DM and GE digestibility ($P = 0.001$). For gut morphology, pigs fed added Cu had decreased crypt depth ($P = 0.017$) in the distal small intestine. Relative mRNA expression of intestinal fatty acid binding protein (iFABP) was decreased ($P = 0.032$) in pigs fed added Cu. In summary, adding 150 ppm added Cu or including 30% DDGS and 15% bakery meal into a corn-soybean meal-based diet did not influence growth performance. However, HCW ADG and HCW G/F were reduced in pigs fed the by-product diet. Only minor differences in gut morphology or mRNA expression were observed from pigs fed diets with high levels of Cu or by-products compared to those fed a corn-soybean meal-based diet.

Keywords

by-product, copper, finishing pigs, gene expression

Cover Page Footnote

Appreciation is expressed to New Horizon Farms (Pipestone, MN) for the use of pigs and facilities, Micronutrients (Indianapolis, IN) and the National Pork Board (Des Moines, IA) for funding of this project.

Authors

K. F. Coble, D. D. Burnett, R. D. Goodband, J. M. Gonzales, J. Usry, M. D. Tokach, J. R. Pluske, J. M. DeRouchey, J. C. Woodworth, S. S. Dritz, J. R. Flohr, and M. A. Vaughn

Effect of Diet Type and Added Copper on Growth Performance, Carcass Characteristics, Energy Digestibility, Gut Morphology, and Mucosal mRNA Expression of Finishing Pigs[1]

K. F. Coble, D. D. Burnett, R. D. Goodband, J. M. Gonzales, J. Usry[2], M. D. Tokach, J. R. Pluske, J. M. DeRouchey, J. C. Woodworth, S. S. Dritz[3], J. R. Flohr, M. A. Vaughn

Summary

A total of 757 pigs (PIC 337 × 1050, initially 60.8 lb) were used to determine the effects of added Cu (TBCC, tribasic copper chloride, IntelliBond C; Micronutrients, Inc., Indianapolis, IN) 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 as a 2 × 2 factorial with main effects of diet type, a corn-soybean meal-based diet or a high by-product diet with 30% distillers dried grains with solubles (DDGS) and 15% bakery meal (by-product), and with or without added Cu (0 or 150 ppm added Cu). There were no Cu × diet type interactions for growth performance. Overall, neither added Cu nor diet type influenced growth performance. Pigs fed the by-product diet had decreased carcass yield ($P = 0.007$) and HCW F/G ($P = 0.013$), and tended to have decreased HCW ($P = 0.067$) and HCW ADG ($P = 0.056$) compared to pigs fed the corn-soybean meal-based diet. A Cu × diet type interaction ($P < 0.05$) existed for DM and GE digestibility during the early finishing period as added Cu improved digestibility of DM and GE in the corn-soybean meal-based diet, but not in the by-product diet. During the late finishing period, added Cu increased DM and GE digestibility ($P = 0.060$), while pigs fed the by-product diet had decreased DM and GE digestibility ($P = 0.001$). For gut morphology, pigs fed added Cu had decreased crypt depth ($P = 0.017$) in the distal small intestine. Relative mRNA expression of intestinal fatty acid binding protein (iFABP) was decreased ($P = 0.032$)

[1] Appreciation is expressed to New Horizon Farms (Pipestone, MN) for the use of pigs and facilities, Micronutrients (Indianapolis, IN) and the National Pork Board (Des Moines, IA) for funding of this project.

[2] Micronutrients, Indianapolis, IN

[3] Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine, Kansas State University.

in pigs fed added Cu. In summary, adding 150 ppm added Cu or including 30% DDGS and 15% bakery meal into a corn-soybean meal-based diet did not influence growth performance. However, HCW ADG and HCW G/F were reduced in pigs fed the by-product diet. Only minor differences in gut morphology or mRNA expression were observed from pigs fed diets with high levels of Cu or by-products compared to those fed a corn-soybean meal-based diet.

Key words: by-product, copper, finishing pigs, gene expression

Introduction

For many years, copper (Cu) has been supplemented in nursery and early finishing diets to improve growth performance. While feeding high levels of Cu has been shown to improve growth, the duration and degree of response has not always been consistent. Research typically shows that added Cu affects growth the most during the early — but not late — finishing period. Recently, Coble et al. (2014[4]) reported that adding 150 ppm Cu in finishing diets tended to increase ADFI and improve F/G 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 better digestion and absorption of nutrients (Hedemann et al., 2006[5]). Lou and Dove (1996[6]) report that nursery pigs fed 250 ppm Cu had improved fat digestibility. Rochell et al. (2015[7]) reported an improvement in AA digestibility in low Lysine diets with added Cu in chicks, and Gonzales-Eguia et al. (2009[8]) reported an improvement in fat digestibility with added Cu in 66- to 132-lb growing pigs. Although the strategies for using Cu have not changed much over the years, the ingredient composition of diets used in commercial production is different from diets utilized in the original Cu research. It has yet to be investigated if ingredient usage and diet formulation are important factors to consider when adding Cu to improve growth performance. 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.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. 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 48 pens with 26 to 28 pigs (similar number of barrows and gilts) in each, equipped with a 4-hole conventional

[4] Coble et al., Swine Day 2013, Report of Progress 1092, pages 168-180.

[5] Hedemann, M. S., B. B. Jensen, and H. D. Poulsen. 2006. Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. J. Anim. Sci. 84:3310-3320.

[6] Lou, X. G., and C. R. Dove. 1996. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities, and tissue mineral levels in weanling pigs. J. Anim. Sci. 74: 1888-1896.

[7] Rochell, S., T. Parr, J. Usry, C. Parson, and R. Dilger. 2015. Effects of dietary amino density and tribasic copper chloride supplementation in *Eimeria acervulina*-infected chicks. International Poultry Science Forum. M106 (Abstr.).

[8] Gonzales-Equia, A., C. Fu, F. Lu, and T. Lien. 2009. Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. Livestock Sci. 126:122-129.

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.

A total of 757 pigs (PIC 337 × 1050; PIC, Hendersonville, TN; initially 60.8 lb) were used in a 117-d experiment. Before d 0, all pigs were fed a common diet with 205 ppm Cu from tribasic copper chloride (TBCC, Intellibond C; Micronutrients, Inc., Indianapolis, IN). On d 0, pens of pigs were weighed, ranked by average pen BW, and allotted to 1 of 4 dietary treatments in a 2 × 2 factorial arrangement with average pig BW balanced across each treatment. There were 7 replications per treatment. Treatments included 2 diet types, a corn-soybean meal-based diet or a high by-product diet with 30% distillers grains with solubles (DDGS) and 15% bakery meal (by-product), with or without added Cu (0 or 150 ppm) from TBCC (Tables 1 to 3).

All diets contained a basal level of 17 ppm added Cu from $CuSO_4$ provided by the trace mineral premix. Treatment diets were fed in 5 dietary phases 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 Lys: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 (1,194 kcal/lb) for DDGS was calculated based upon the oil content, as described by Graham et al. (2014[9]). Samples of each diet were collected 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 (Table 4).

Pens of pigs were weighed and feed disappearance was recorded approximately every 3 wk to determine ADG, ADFI, F/G, and caloric efficiency on an ME and NE basis. Caloric efficiency was calculated by dividing the sum of total feed intake and diet calorie content by total gain. On d 94, the 3 heaviest pigs in each pen were weighed and sold 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 individual carcass measurements to be recorded. On d 117, final pen weights were taken and feed disappearance was recorded. A subsample of two gilts per pen, representing the mean individual weight of the pen, were transported 67 miles to a commercial packing plant for processing, intestinal sampling, and data collection (Packing Plant #1; Natural Foods Holdings, Sioux Center, IA). All remaining pigs were transported 59 miles on d 118 to a commercial packing plant (Packing Plant #2; JBS Swift and Company, Worthington, MN) for processing and carcass data collection. Hot carcass weight was measured immediately after evisceration and each carcass 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

[9] Graham, A. B., R. D. Goodband, M. D. Tokach, S. S. Dritz, J. M. DeRouchey, S. Nitikanchana, and J. J. Updike. 2014a. The effects of low-, medium-, and high-oil distillers dried grains with solubles on growth performance, nutrient digestibility, and fat quality in finishing pigs. J. Anim. Sci. 92:3610-3623.

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 F/G was calculated by dividing HCW gain by feed intake over the 117 d experiment.

Feed and fecal grab samples were collected from each pen over a 2 d period during phases 2 (d 25 to 26) and 4 (d 74 and 75) to determine DE content of the experimental diets.

Prior to transportation to the packing plant #1, blood was collected from the 2 gilts per pen identified to be subsampled for intestinal collection that represented the mean weight of the pen. 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).

On d 117, tissue samples and mucosal scrapings were collected from the two pre-identified gilts per pen for small intestinal (SI) mucosal gene expression and gut morphology at packing plant #1. Measurements included villus height, crypt depth, and villus height to crypt depth ratio.

Ileal mucosal RNA was isolated and transcribed to cDNA to measure the relative mRNA gene expression of intestinal fatty acid binding protein (iFABP), copper transporter 1 (CTR1), and glucagon-like peptide 1 (GLP-1R). Five nanogram equivalents of total RNA were amplified with gene-specific primers (Table 5), DNA polymerase, and SYBR green chemistry (Perfecta Sybr fast mix; Quanta Biosciences, Gaithersburg, MD) in a Realplex PCR System (Eppendorf North America, Hauppauge, NY).

At the conclusion of the study, an economic analysis was calculated on both constant days on feed or constant market weight basis to determine the value of feeding TBCC in the two diet types by the two difference scenarios. Because of the negative impact that high-fiber ingredients have on carcass yield and that producers are paid for their pigs on a carcass basis, economics were calculated on a carcass basis. For calculating economics on a constant days-on-feed basis, economics were calculated using the treatment means from the experiment. To determine economics on a constant carcass weight basis, carcass feed efficiency was adjusted to a common carcass weight by a factor of 0.005 per lb of final weight, also accounting for the change in carcass yield.

For the constant days on feed and constant weight economic evaluation, total feed cost per pig, cost per lb of gain, gain value, and income over feed cost (IOFC) were calculated. Feed cost was calculated by multiplying ADFI by the feed cost per lb and the number of days in each respective period, then taking the sum of those values for each period to calculate the total feed cost per pig. Cost per lb of gain was calculated by dividing the total feed cost per pig by the total carcass pounds gained overall. The value of the carcass weight gained during the experiment (gain value) was calculated by subtracting the product of initial carcass weight from the final HCW, times $88.44/LCWT. Income over feed cost was calculated by subtracting total feed cost from gain value. The income over feed and facilities cost (IOFFC) was calculated for the constant market weight evaluation because pigs with faster growth rates will reach a 210-lb carcass sooner, therefore decreasing the cost of housing the pigs. Facility cost was calculated by multiplying the number of overall days the pigs need to reach a 210-lb carcass based on their respective growth rate by $0.10 per head per day facility cost.

Experimental data were analyzed in a randomized complete-block design using the PROC 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 were collected within an experimental unit (pen). Contrasts were used to evaluate the interaction between added Cu and diet type and main effects of added Cu or 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 the gene expression criteria, values were ranked using the PROC 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 hot carcass weight. Results from the experiment were considered significant and $P \leq 0.05$ and a tendency between $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

The chemical analyses of the complete diets were similar to the intended formulation (Table 4). 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-soybean meal-based diet as anticipated. Total Ca and P levels were similar between diet types across each dietary phase. The total analyzed Cu levels ranged from 30 to 58 ppm in the diets without added Cu, and ranged from 159 to 211 ppm for the diets with 150 ppm added Cu. These values are within the acceptable analytical limits according to the Association of American Feed Control Officials (AAFCO, 2014[10]), given 17 ppm of Cu was provided by the trace mineral premix and the Cu provided by that of the 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-soybean meal-based diet.

During the early finishing period (d 0 to 45), there were no Cu × diet type interactions. Feeding pigs 150 ppm added Cu tended to increase ADG ($P = 0.076$) by 2.4% compared to pigs fed no added Cu, which resulted in a tendency for a heavier BW on d 45 for pigs fed diets with added Cu ($P = 0.082$; Table 6). During the late finishing period (d 45 to 117), diet type tended to influence the response to Cu for F/G (Cu × diet type interaction, $P = 0.058$). This was the result of a poorer F/G for pigs fed the by-product diet compared to the corn-soybean meal-based diet when added Cu was fed, while pigs fed no added Cu had a slight improvement in F/G when fed the by-product diet compared to the corn-soybean meal-based diet. Overall (d 0 to 117) added Cu did not influence growth performance.

[10] Association of American Feed Control Officials (AAFCO). 2014. Official Publication. Assoc. Am. Feed Cont. Off., Champaign, IL.

From d 0 to 45, pigs fed the by-product diet had decreased BW on d 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-soybean meal-based diet. However, from d 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 fed high-fiber, by-product diets not equalized for dietary energy. Although in this 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-soybean meal-based diet, as they required more Mcal of energy per lb of gain on both the ME and NE basis. This is partly due to both the numerical reduction in F/G for pigs fed the by-product diet compared to the corn-soybean meal-based diet, as well as the potential overvaluing of the energy content of either the DDGS, bakery meal, or both, during formulation.

Due to the differences in growth response observed in other studies from added Cu in early and late finishing, two 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[11]). In this study, diet type influenced the response to Cu (Cu × diet type interaction, $P < 0.05$) for both DM and GE digestibility during early finishing (Table 7). Pigs fed the by-product diet had a greater decrease in DM and GE digestibility compared to those fed the corn-soybean meal-based diet when Cu was added to the diet as compared to when Cu was not added to the diet. Despite the interaction, pigs fed the by-product diet had decreased ($P < 0.05$) DM and GE digestibility compared to pigs fed the corn-soybean meal-based diet during the early and late finishing periods. Adding Cu tended to increase the digestibility of DM ($P = 0.060$) and GE ($P = 0.003$) in the late finishing period.

For carcass characteristics, pigs fed the by-product diet compared to the corn-soybean meal-based diet tended to have decreased HCW ($P = 0.067$), and a significant reduction in carcass yield ($P = 0.007$, Table 8). As a result of the decrease in HCW, HCW ADG also tended to be decreased ($P = 0.056$) for pigs fed the by-product diet compared to the corn-soybean meal-based diet. The numerical reduction in F/G and significant reduction in carcass yield for pigs fed the by-product diet compared to the corn-soybean meal-based diet also led to a decrease in HCW F/G ($P = 0.013$) for pigs fed the by-product diet compared to the corn-soybean meal-based diet. Added Cu did not increase HCW or HCW ADG, which is not consistent with previous research completed by Coble et al. (2014). However, the reduction in HCW and carcass yield for pigs fed the by-product diet compared to those fed the corn-soybean meal-based diet is consistent with most published literature (Asmus et al., 2014).

There were no Cu × diet type interactions for any of the calculated economic scenarios (Table 9). When economics were calculated on a constant days basis, pigs fed the by-product diet had decreased ($P = 0.001$) feed cost and cost per lb of carcass gain, as well as a tendency ($P = 0.056$) for a decrease in carcass gain value compared to pigs fed the corn-soybean meal-based diet. Neither reduction resulted in a difference in IOFC.

---

[11] Dove, C. R., and K. D. Haydon. 1992. The effect of copper and fat addition to the diets of weanling swine on growth performance and serum fatty acids. J. Anim. Sci. 70:805-810.

When economics were calculated on a constant carcass weight basis, the adjusted carcass F/G was increased ($P = 0.010$) for pigs fed the by-product diet compared to the corn-soybean meal-based, as expected. However, facility cost tended to increase ($P = 0.069$) for pigs fed the by-product compared to those fed the corn-soybean meal-based diet, as it would take those pigs longer to reach the common carcass weight of 210 lb.

As a result of the variability in proposed modes of action for Cu, other areas of research have evolved to suggest that Cu may potentially improve growth performance by acting on specific neurological pathways shown to be associated with feed intake. Glucagon-like peptide 1 (GLP-1) is an incretin hormone that is released by the L-cells in the intestine in response to food ingestion, stimulating insulin production. 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[12]; Daily and Moran, 2013[13]). In addition, GLP-2 is a hormone secreted in a 1 to 1 ratio with GLP-1 that is shown to improve intestinal health through increasing the growth and functionality of mucosa in the small intestine (Janssen et al., 2013[14]). In the current experiment, there was no evidence for a difference in serum concentrations of glucagon-like peptide-1 (GLP-1) or glucagon like peptide-2 (GLP-2, Table 10) between diet types or with added Cu. Factors that could have affected this could be that the current experiment measured the concentrations in serum versus plasma, the age of the pig, and time of collection. Both GLP-1 and GLP-2 have a relatively short half-life (7–17 min) after being released into the blood stream, and feed intake was not controlled across each animal since all pigs were allowed ab libitum intake.

Research has demonstrated that added Cu and diet type potentially affect gut morphology. In this study, in the proximal section of the small intestine, neither villus height, crypt depth, nor villus height to crypt depth ratio was influenced by added Cu or diet type (Table 11). 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 ($P = 0.017$).

To further investigate the different modes of action for Cu, the relative ileal mucosal mRNA expression of proteins involved with digestion was measured. In combination with the GE digestibility measurements, intestinal fatty acid binding protein (iFABP) mRNA expression was measured because of its importance in 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 an important protein involved in Cu transport across the cell wall, copper transporter protein-1 (CTR1), was also measured. For relative mucosal mRNA expression, there was no evidence for any diet type × Cu interactions for the measured genes in the proximal or distal small intestine (Table 12). There was no evidence of a difference for the relative mRNA expression of iFABP, CTR1, or GLP-1R in the mucosal layer of the

[12] Tang-Christensen, M., P. J. Larsen, R. Goke, A. Fink-Jensen, D. Jessop, M. Moller, and S. P. Sheikh. 1996. Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. Am. J. Physiol. 274:848-856.

[13] Daily, M. J., and T. H. Moran. 2013. Glucagon-like peptide 1 and appetite. Trend. Endocr. Met. 24(2):85-91.

[14] Janssen, P., A. Rotondo, F. Mule, and J. Tack. 2013. Review article: a comparison of glucagon-like peptides 1 and 2. Aliment. Pharmacol. Ther. 37:18-36.

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. A decrease in iFABP of the distal small intestine mucosa of pigs fed added Cu would suggest that the gene responsible for iFABP transcription is possibly down regulated with added Cu. If fat digestibility is truly increased, we would expect this to be upregulated.

In conclusion, adding 150 ppm Cu to the diet during the early finishing period tended to increase ADG, but growth performance for the overall study was not influenced by added Cu. Pigs fed the by-product diet compared to the corn-soybean meal-based 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. The changes in growth performance typically observed in finishing pigs fed added Cu does not appear to be related to the changes in serum metabolite profile for GLP-1 and GLP-2 concentrations or relative mRNA expression of GLP-1R, or CTR1. However, more research is need to clarify the impacts that added Cu has on DM and GE digestibility, especially since Cu was observed to influence energy digestibility during the late finishing period.

Table 1. Composition of diets for phases 1 and 2 (as-fed basis)[1]

	Phase 1		Phase 2	
Item	Corn-soy	By-product	Corn-soy	By-product
Ingredient, %				
Corn	69.43	37.91	75.51	44.30
Soybean meal, 46.5% CP	27.83	14.19	21.88	7.92
Distillers dried grains with solubles	---	30.00	---	30.00
Bakery meal	---	15.00	---	15.00
Monocalcium P, 21% P	0.60	0.10	0.60	0.13
Limestone	1.25	1.55	1.13	1.40
Salt	0.35	0.35	0.35	0.35
Vitamin premix	0.08	0.08	0.08	0.08
Trace mineral premix[2]	0.10	0.10	0.10	0.10
L-Lysine HCl	0.225	0.560	0.238	0.575
DL-methione	0.075	0.020	0.055	---
L-threonine	0.055	0.100	0.050	0.095
L-tryptophan	---	0.032	0.001	0.041
Phytase[3]	0.013	0.013	0.013	0.013
TBCC[4]	±	±	±	±
Total	100.0	100.0	100.0	100.0
Calculated analysis				
Standardized ileal digestible (SID) Lys:NE, g/Mcal	4.29	4.29	3.67	3.67
SID AA, %				
Lys	1.050	1.105	0.912	0.958
Ile:lys	65	59	63	56
Met:lys	31	29	31	28
Met + cys:lys	56	56	56	56
Thr:lys	62	62	62	62
Trp:lys	19.7	19.7	19.0	19.0
Val:lys	69	69	69	69
Total Lys, %	1.19	1.29	1.03	1.13
ME, kcal/lb	1,492	1,520	1,497	1,525
NE, kcal/lb	1,111	1,166	1,128	1,184
CP, %	19.0	21.1	16.6	18.6
Ca, %	0.66	0.66	0.59	0.59
P, %	0.51	0.49	0.48	0.46
Available P, %	0.36	0.36	0.35	0.35
Base diet cost[5], $/ton	228.14	216.57	215.43	204.49

[1] Phase 1 diets were fed from d 0 to 21, phase 2 diets were fed from d 21 to 45, and both were provided in meal form.
[2] Trace mineral premix provided 17 ppm Cu in the form of $CuSO_4$ to each diet.
[3] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided 500 phytase units (FTU)/lb, with a release of 0.10% available P.
[4] Tribasic copper chloride (Intellibond C, Micronutrients, Inc., Indianapolis, IN) provided 150 ppm Cu and was added at the expense of corn.
[5] Cost of corn = $4.14/bushel; soybean meal = $355/ton; DDGS = $201/ton; bakery = $120/ton; L-Lys = $0.64/lb; TBCC = $3.85/lb. The diet cost including TBCC in the base diet is the base diet cost, plus $2.02/ton.

Table 2. Composition of diets for phases 3 and 4 (as-fed basis)[1]

	Phase 3		Phase 4	
Item	Corn-soy	By-product	Corn-soy	By-product
Ingredient, %				
Corn	79.76	47.27	83.32	47.36
Soybean meal, 46.5% CP	17.70	5.15	14.17	5.32
Distillers dried grains with solubles	---	30.00	---	30.00
Bakery meal	---	15.00	---	15.00
Monocalcium P, 21% P	0.65	0.13	0.60	0.08
Limestone	1.03	1.30	1.03	1.28
Salt	0.35	0.35	0.35	0.35
Vitamin premix	0.08	0.08	0.08	0.08
Trace mineral premix[2]	0.10	0.10	0.10	0.10
L-Lysine HCl	0.240	0.520	0.250	0.400
DL-methionine	0.040	---	0.035	---
L-threonine	0.045	0.065	0.055	0.015
L-tryptophan	0.005	0.035	0.011	0.017
Phytase[3]	0.013	0.013	0.013	0.013
TBCC[4]	±	±	±	±
Total	100.0	100.0	100.0	100.0
Calculated analysis				
Standardized ileal digestible (SID) Lys:NE, g/Mcal	3.22	3.22	2.88	2.88
SID AA, %				
Lys	0.810	0.846	0.730	0.756
Ile:lys	63	58	61	66
Met:lys	30	31	31	34
Met + cys:lys	56	61	57	68
Thr:lys	62	62	63	63
Trp:lys	19.0	19.0	19.0	19.0
Val:lys	69	73	69	82
Total Lys, %	0.92	1.01	0.83	0.93
ME, kcal/lb	1,499	1,527	1,502	1,528
NE, kcal/lb	1,139	1,192	1,149	1,192
CP, %	14.9	17.4	13.5	17.3
Ca, %	0.55	0.55	0.53	0.53
P, %	0.47	0.45	0.45	0.44
Available P, %	0.34	0.34	0.33	0.33
Base diet cost[5], $/ton	207.12	196.66	200.54	191.49

[1] Phase 3 diets were fed from d 45 to 68 , phase 4 diets were fed from d 68 to 94, and both provided in meal form.
[2] Trace mineral premix provided 17 ppm Cu in the form of $CuSO_4$ to each diet.
[3] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided 500 phytase units (FTU)/lb, with a release of 0.10% available P.
[4] Tribasic copper chloride (Intellibond C, Micronutrients, Inc., Indianapolis, IN) provided 150 ppm Cu and was added at the expense of corn.
[5] Cost of corn = $4.14/bushel; soybean meal = $355/ton; DDGS = $201/ton; bakery = $120/ton; L-Lys = $0.64/lb; TBCC = $3.85/lb. The diet cost including TBCC in the base diet is the base diet cost, plus $2.02/ton.

Table 3. Composition of diets for phase 5 (as-fed basis)[1]

	Phase 5	
Item	Corn-soy	By-product
Ingredient, %		
Corn	86.10	47.45
Soybean meal, 46.5% CP	11.36	5.41
Distillers dried grains with solubles	---	30.00
Bakery meal	---	15.00
Monocalcium P, 21% P	0.65	0.05
Limestone	1.00	1.25
Salt	0.35	0.35
Vitamin premix	0.08	0.08
Trace mineral premix[2]	0.10	0.10
L-Lysine HCl	0.250	0.300
DL-methionine	0.030	---
L-threonine	0.065	---
L-tryptophan	0.014	0.002
Phytase[3]	0.013	0.013
TBCC[4]	±	±
Total	100.0	100.0

continued

Table 3. Composition of diets for phase 5 (as-fed basis)[1]

	Phase 5	
Item	Corn-soy	By-product
Calculated analysis		
Standardized ileal digestible (SID) Lys:NE, g/Mcal	2.59	2.59
SID AA, %		
Lys	0.66	0.68
Ile:Lys	61	74
Met:Lys	31	38
Met + cys:lys	58	76
Thr:lys	65	68
Trp:lys	19.0	19.0
Val:lys	69	91
Total lys, %	0.75	0.85
ME, kcal/lb	1,503	1,528
NE, kcal/lb	1,157	1,192
CP, %	12.3	17.2
Ca, %	0.52	0.52
P, %	0.44	0.44
Available P, %	0.33	0.33
Base diet cost[5], $/ton	195.53	187.79

[1] Phase 5 diets were fed from d 94 to 117 and were provided in meal form.
[2] Trace mineral premix provided 17 ppm Cu in the form of $CuSO_4$ to each diet.
[3] Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided 1,102 phytase units (FTU)/kg, with a release of 0.10% available P.
[4] Tribasic copper chloride (Intellibond C, Micronutrients, Inc., Indianapolis, IN) provided 150 ppm Cu and was added at the expense of corn.
[5] Cost of corn = $4.14/bushel; soybean meal= $355/ton; DDGS = $201/ton; bakery = $120/ton; L-Lys = $0.64/lb; TBCC = $3.85/lb. The diet cost including TBCC in the base diet is the base diet cost, plus $2.02/ton.

Table 4. Chemical analysis of diets (as-fed)[1]

	Added Cu,[2] ppm			
	0		150	
Item	Corn-soy	By-product	Corn-soy	By-product
Phase 2[3]				
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, ppm	54	44	211	190
Bulk density, lb/ft^3	41.5	38.3	40.2	38.6
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, ppm	41	33	209	204
Bulk density, lb/ft^3	41.3	37.2	40.6	37.3
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, ppm	58	30	159	187
Bulk density, lb/ft^3	40.0	37.2	38.1	36.8

continued

Table 4. Chemical analysis of diets (as-fed)[1]

	Added Cu,[2] ppm			
	0		150	
Item	Corn-soy	By-product	Corn-soy	By-product
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, ppm	48	39	209	203
Bulk density, lb/ft[3]	39.5	37.7	39.3	37.6

[1] Values represent means from one composite sample, analyzed in duplicate.
[2] Tribasic copper chloride (Intellibond C; Micronutrients, Inc., Indianapolis, IN).
[3] Phase 1 diets were not available for analysis.

Table 5. 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	CCATGATGAT-GCCTATGACCTT	ATAGAACATGGC-TAGTAAAAACACC	60.5	131	1.12
Glucan-like peptide-1	TACTTCTGGCT-GCTGGTGGAG	ACCCCAGCCTAT-GCTCAGGTA	62.4	104	1.11
Intestinal fatty acid binding protein	CCTCGCAGACG-GAACTGAAC	GTCTGGAC-CATTTCATCCCCG	64.5	135	1.03
Normalizing gene					
Ribosomal protein L4	AGGAGGCTGTTCT-GCTTCTG	TCCAGGGAT-GTTTCTGAAGG	60.5	184	1.06

[1] T_m = melting temperature

Table 6. Effect of added Cu and diet type on growth performance of finishing pigs[1]

	Added Cu,[2] ppm					Probability, $P <$		
	0		150					
	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × Diet Type	Cu	Diet Type
BW, lb								
d 0	60.8	60.8	60.7	60.8	1.40	1.000	0.954	0.988
d 45	144.6	141.0	146.1	143.1	2.25	0.776	0.082	0.004
d 117	276.3	277.0	281.5	278.0	3.13	0.419	0.237	0.567
d 0 to 45								
ADG, lb	1.86	1.78	1.90	1.82	0.027	0.874	0.076	0.001
ADFI, lb	3.75	3.68	3.87	3.70	0.063	0.221	0.114	0.009
F/G	2.02	2.07	2.04	2.03	0.025	0.147	0.758	0.303
d 45 to 117								
ADG, lb	1.88	1.93	1.93	1.91	0.033	0.207	0.504	0.551
ADFI, lb	5.91	6.00	5.95	6.07	0.086	0.881	0.519	0.224
F/G	3.15	3.11	3.09	3.18	0.035	0.058	1.000	0.390
d 0 to 117								
ADG, lb	1.87	1.87	1.92	1.87	0.023	0.311	0.191	0.269
ADFI, lb	5.06	5.08	5.12	5.12	0.064	0.814	0.376	0.824
F/G	2.71	2.72	2.67	2.74	0.025	0.342	0.737	0.110
Caloric efficiency[4]								
ME	4,056	4,153	4,009	4,174	37.9	0.345	0.720	0.001
NE	3,085	3,233	3,049	3,249	29.2	0.346	0.723	0.001

[1] A total of 757 pigs (PIC 337 × 1050; initially 60.8 lb) were used in a 117-d experiment with 26 to 28 pigs per pen and 7 replications per treatment.
[2] Tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, Indianapolis, IN).
[3] Refers to a diet containing 30% DDGS and 15% bakery meal.
[4] Caloric efficiency is expressed as kcal consumed per lb of live weight gain.

Table 7. Effect of added Cu and diet type on dry matter (DM) and gross energy (GE) digestibility of finishing pigs, %[1]

	Added Cu,[2] ppm							
	0		150			Probability, *P* <		
	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × Diet Type	Cu	Diet Type
Phase 2 digestibility[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
Phase 4 digestibility[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

[1] A total of 757 pigs (PIC 337 × 1050; initially 60.8 lb) were used in a 117-d experiment with 26 to 28 pigs per pen and 7 replications per treatment.
[2] Tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, Indianapolis, IN).
[3] Refers to a diet containing 30% DDGS and 15% bakery meal.
[4] Phase 2 fecal samples collected over a 2 d period from d 25 to 26.
[5] Phase 4 fecal samples collected over a 2 d period from d 74 to 75.

Table 8. Effect of added Cu and diet type on carcass characteristics of finishing pigs[1]

	Added Cu,[2] ppm							
	0		150			Probability, *P* <		
	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × Diet Type	Cu	Diet Type
Carcass characteristics								
HCW, lb	206.7	203.0	210.4	205.4	2.67	0.776	0.195	0.067
Yield, %	74.28	73.12	74.37	73.26	0.370	0.953	0.752	0.007
BF[4], in.	0.73	0.71	0.73	0.71	0.017	0.135	0.719	0.349
LD[4], in.	2.62	2.50	2.57	2.59	0.045	0.910	0.951	0.192
Lean[4], %	55.48	55.50	55.24	55.76	0.282	0.389	0.900	0.435
Carcass performance								
HCW ADG, lb	1.38	1.35	1.41	1.37	0.021	0.766	0.173	0.056
HCW F/G	3.68	3.78	3.64	3.76	0.043	0.863	0.342	0.013

[1] A total of 757 pigs (PIC 337 × 1050; initially 60.8 lb) were used in a 117-d experiment with 26 to 28 pigs per pen and 7 replications per treatment.
[2] Tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, Indianapolis, IN).
[3] Refers to a diet containing 30% DDGS and 15% bakery meal.
[4] Hot carcass weight was used as a covariate.

Table 9. Effect of added Cu and diet type on economics of finishing pigs[1]

	Added Cu,[2] ppm					Probability, $P <$		
	0		150					
	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × Diet Type	Cu	Diet Type
Constant days, $/pig								
Feed cost	61.45	58.89	62.98	60.06	0.768	0.799	0.072	0.001
Cost/lb gain carcass wt.	0.370	0.362	0.370	0.363	0.005	0.326	0.330	0.001
Carcass gain value[4]	142.52	139.20	145.80	141.33	2.151	0.766	0.173	0.056
IOFC[5]	81.07	80.31	82.82	81.27	1.744	0.803	0.396	0.469
Constant carcass weight[6] $/pig								
Adjusted carcass F/G[7]	3.69	3.81	3.64	3.77	0.047	0.832	0.348	0.010
Feed cost	62.31	61.30	62.26	61.46	0.745	0.881	0.935	0.215
Cost/lb gain carcass wt.	0.380	0.374	0.379	0.374	0.005	0.802	0.887	0.224
Carcass gain value[8]	145.41	145.41	145.43	145.42	0.927	---	---	---
IOFC[5]	83.10	84.10	83.16	83.96	1.094	0.892	0.956	0.256
Facility cost[9]	11.92	12.20	11.70	12.05	0.194	0.842	0.284	0.069
IOFFC[10]	71.18	71.91	71.47	71.91	1.143	0.873	0.872	0.512

[1] A total of 757 pigs (PIC 337 × 1050; initially 60.8 lb) were used in a 117-d experiment with 26 to 28 pigs per pen and 7 replications per treatment.
[2] Tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, Indianapolis, IN).
[3] Refers to a diet containing 30% DDGS and 15% bakery meal.
[4] Carcass gain value calculated using (HCW × $88.44/cwt) – (initial wt. × $88.44/cwt × assumed 75% yield).
[5] Income over feed cost = carcass gain value – feed cost.
[6] Adjusted to constant final carcass weight of 210 lb.
[7] Adjusted using a factor of 0.005 for 1 lb change in carcass weight × carcass yield.
[8] Adjusted gain value calculated using (final carcass wt. × $88.44/cwt) – (initial wt. × $88.44/cwt × assumed 75% yield).
[9] Facility cost at $0.10/hd/day.
[10] Income over feed and facility cost = IOFC – facility cost.

Table 10. Effect of added Cu and diet type on serum glucagon-like peptide 1 (GLP-1) and 2 (GLP-2) concentrations of finishing pigs[1]

	Added Cu,[2] ppm					Probability, $P <$		
	0		150					
	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × Diet Type	Cu	Diet Type
Serum Concentrations								
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 84 pigs (PIC 337 × 1050; initially 60.8 lb) were used in a 117-d experiment with 2 pigs per pen and 7 replications per treatment.
[2] Tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, Indianapolis, IN).
[3] Refers to a diet containing 30% distillers dried grains with solubles (DDGS) and 15% bakery meal.

Table 11. Effect of added Cu and diet type on small intestine (SI) villus height and crypt depth of finishing pigs, um[1]

	Added Cu,[2] ppm					Probability, $P <$		
	0		150					
	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × Diet Type	Cu	Diet Type
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

[1] A total of 84 pigs (PIC 337 × 1050; initially 60.8 lb) were used in a 117-d experiment with 2 pigs per pen and 7 replications per treatment.
[2] Tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, Indianapolis, IN).
[3] Refers to a diet containing 30% distillers dried grains with solubles (DDGS) and 15% bakery meal.

Table 12. Effect of added Cu and diet type relative mRNA gene expression of intestinal fatty acid binding protein (iFABP), copper transporter-1 (CTR1), and glucagon-like peptide 1 (GLP-1R) in the proximal and distal small intestinal of finishing pigs[1]

	Added Cu,[2] ppm					Probability, $P <$		
	0		150					
	Corn-soy	By-product[3]	Corn-soy	By-product[3]	SEM	Cu × Diet Type	Cu	Diet Type
Proximal SI[4]								
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 60.8 lb) were used in a 117-d experiment with 2 pigs per pen and 7 replications per treatment.
[2] Tri-basic copper chloride (TBCC; Intellibond C; Micronutrients, 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 −ΔΔCt, in which ΔΔCt represents ΔCt sample − ΔCt calibrator (Livak and Schmittgen, 2001).