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## Effects of Increasing Copper from Tri-basic Copper Chloride or a Copper-Amino Acid Complex on Growth Performance of Nursery Pigs

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## Abstract

A total of 665 pigs [Group 1; 350 barrows (DNA 200 × 400; initially 14.1 lb)] and [Group 2; 315 barrows and gilts (DNA 241 × 600; initially 11.4 lb)] were used to determine the effects of added Cu source and level on nursery pig performance. There were 5 pigs per pen and 10 replications per treatment in group 1 and 5 pigs per pen and 9 replications per treatment in group 2. Pens of pigs were allotted by BW to 1 of 7 dietary treatments arranged as a 2 × 3 factorial plus a control diet, with main effects of Cu source (IntelliBond-C; Micronutrients, Indianapolis, IN or Mintrex-Cu; Novus, St. Charles, MO) and Cu level (75, 150, or 225 ppm). Diets were corn-soybean meal-based and were fed in meal form in 2 phases (d 0 to 14 and 14 to 35). All diets contained a trace mineral premix formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> in the complete diet.

Overall (d 0 to 35), there were no Cu source × level interactions observed. Increasing Cu increased ADG (linear,  $P = 0.048$ ) and final BW (linear,  $P = 0.019$ ). The increase in ADG with no effect on ADFI resulted in a tendency for improved F/G (linear,  $P = 0.091$ ) with increasing added Cu in the diet. There were no effects of Cu source on growth performance. Because the growth effects were linear, it is unknown from our study if increasing added Cu beyond 225 ppm would further improve growth.

## Keywords

nursery pig, copper, copper amino acid-complex, tri-basic copper chloride

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## Cover Page Footnote

The authors would like to express appreciation to Micronutrients, Indianapolis, IN, for partial funding.

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## Effects of Increasing Copper from Tri-basic Copper Chloride or a Copper-Amino Acid Complex on Growth Performance of Nursery Pigs<sup>1</sup>

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### Summary

A total of 665 pigs [Group 1; 350 barrows (DNA 200 × 400; initially 14.1 lb)] and [Group 2; 315 barrows and gilts (DNA 241 × 600; initially 11.4 lb)] were used to determine the effects of added Cu source and level on nursery pig performance. There were 5 pigs per pen and 10 replications per treatment in group 1 and 5 pigs per pen and 9 replications per treatment in group 2. Pens of pigs were allotted by BW to 1 of 7 dietary treatments arranged as a 2 × 3 factorial plus a control diet, with main effects of Cu source (IntelliBond-C; Micronutrients, Indianapolis, IN or Mintrex-Cu; Novus, St. Charles, MO) and Cu level (75, 150, or 225 ppm). Diets were corn-soybean meal-based and were fed in meal form in 2 phases (d 0 to 14 and 14 to 35). All diets contained a trace mineral premix formulated to contribute 17 ppm of Cu from CuSO<sub>4</sub> in the complete diet.

Overall (d 0 to 35), there were no Cu source × level interactions observed. Increasing Cu increased ADG (linear,  $P = 0.048$ ) and final BW (linear,  $P = 0.019$ ). The increase in ADG with no effect on ADFI resulted in a tendency for improved F/G (linear,  $P = 0.091$ ) with increasing added Cu in the diet. There were no effects of Cu source on growth performance. Because the growth effects were linear, it is unknown from our study if increasing added Cu beyond 225 ppm would further improve growth.

Key words: nursery pig, copper, copper amino acid-complex, tri-basic copper chloride

<sup>1</sup> The authors would like to express appreciation to Micronutrients, Indianapolis, IN, for partial funding.

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## Introduction

The NRC (2012<sup>4</sup>) reports weanling pigs have a nutritional Cu requirement of 6 ppm. However, according to Flohr et al. (2015<sup>5</sup>), many U.S. swine nutritionists formulate nursery pig diets to contain as low as 11 ppm and as high as 250 ppm Cu. Feeding high levels of added Cu (125 to 250 ppm) above that provided by the trace mineral premix has resulted in increased ADFI and ADG. Research has shown that despite dissimilar chemical structure of copper sources, nursery pig growth performance will be similar. Huang et al. (2015<sup>6</sup>) compared two inorganic sources [tri-basic copper chloride (TBCC) and CuSO<sub>4</sub><sup>4</sup>] of added Cu that ranged from 11 to 327 ppm in nursery pig diets and found growth benefits of feeding added Cu but no difference in growth between sources was observed.

Organic Cu sources are argued to be more bioavailable to the young pig due to their chemical structure, compared to inorganic sources. It has also been documented that both TBCC and Cu-AA Cu are more bioavailable than that of more typically used sources of Cu (Spears et al. 1997<sup>7</sup> and Wang et al. 2009<sup>8</sup>). Tri-basic copper chloride and Mintrex-Cu (Cu-AA) differ in their chemical characteristics. Tri-basic copper chloride is an inorganic mineral source, which is non-hygroscopic and poorly soluble in water but highly soluble in acidic conditions (Miles et al., 1998<sup>9</sup>). Mintrex-Cu is an organic form of Cu [Cu(HMTBa)<sub>2</sub>] which has been shown to be more bioavailable to the pig because of decreased binding activity with other dietary constituents, therefore suggesting less supplementation required compared with inorganic minerals in nursery pigs (Zhao et al., 2009<sup>10</sup>). Currently, to our knowledge, there are no data available that directly compare the effects of increasing added Cu from TBCC or Cu-AA on growth performance of nursery pigs. Therefore, our study was designed to investigate the effects of increasing Cu from either TBCC or Cu-AA added Cu source and level on growth performance of nursery pigs.

## Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used for these studies. Two groups of pigs were used for the experiment. Group 1 pigs were housed at the K-State Segregated Early Weaning Facility in Manhattan, KS. Group 2 pigs were housed at the K-State Swine Teaching and Research Center

<sup>4</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

<sup>5</sup> Flohr, J. R.; Tokach, M. D.; Woodworth, J. C.; DeRouchey, J. M.; Dritz, S. S.; and Goodband, R. D. (2015) Vitamin and Trace Minerals: A Survey of Current Feeding Regimens, Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.

<sup>6</sup> Y. L. Huang, M. S. Ashwell, R. S. Fry, K. E. Lloyd, W. L. Flowers, and J. W. Spears. 2015. Effect of dietary copper amount and source on copper metabolism and oxidative stress of weanling pigs in short-term feeding. *J. Anim. Sci.* 93:2948–2955.

<sup>7</sup> Spears, J. W., E. B. Kegley, L. A. Mullis, and T. A. Wise, 1997. Bioavailability of copper chloride in cattle. *J. Anim. Sci.* 75 (Suppl. 1):265. (Abstr.)

<sup>8</sup> Wang, Z., S. Cerrate, C. Coto, F. Yan, and P. W. Waldroup. 2007. Evaluation of Mintrex copper as a source of copper in broiler diets. *Int. J. Poult. Sci.* 6:308-313.

<sup>9</sup> Miles, R. D., S. F. O'Keefe, P. R. Henry, C. B. Ammerman, and X. G. Luo. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and prooxidant activity. *Poult. Sci.* 77:416–425. doi:10.1093/ps/77.3.416

<sup>10</sup> J. R. Zhao, J. Harrell, G. Allee, B. Hinson, P. Winkelbauer, C. Atwell, J. D. Richards, and M. Vazquez-Anon. 2009. Effect of an organic copper source on growth performance and tissue copper concentration in nursery pigs. Mid-west Animal Science Meeting, Des Moines, IA.

in Manhattan, KS. The research facilities were environmentally controlled. In group 1, each pen (3.9 × 4 ft) had tri-bar flooring and contained one 4-hole dry self-feeder and one cup waterer to provide ad libitum access to feed and water. For group 2, each pen (4 × 5 ft) had tri-bar flooring and contained one 4-hole dry self-feeder and one nipple waterer to provide ad libitum access to feed and water. Dietary treatments for each group were manufactured at the O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

In group 1, 350 barrows (DNA 200 × 400; initially 14.1 lb) were weaned at approximately 21 d of age and allotted to pen based on initial BW with 5 pigs per pen and 10 replicate pens per treatment. In group 2, 315 barrows and gilts (DNA 241 × 600; initially 11.4 lb) were weaned and allotted to pens based on initial BW and age. Age block 1 consisted of 4 replicate pens per treatment and pigs ranged in age from 16 to 20 d. Age block 2 consisted of 5 replicate pens per treatment and pigs ranged in age from 21 to 24 d. Group 1 and 2 pigs were fed a common starter diet for 7 and 5 d, respectively. On d 7 and 5 post-weaning for group 1 and 2, respectively, pens were allotted by BW to 1 of 7 dietary treatments arranged as a 2 × 3 factorial plus one control diet, with main effects of Cu source (IntelliBond-C; Micronutrients, Indianapolis, IN or Mintrex-Cu; Novus, St. Charles, MO) and Cu level (75, 150, or 225 ppm). Diets were corn-soybean meal-based and fed in meal form in 2 phases (d 0 to 14 and 14 to 35; Table 1). The trace mineral premix added to all diets provided complete diets with 17 ppm Cu from CuSO<sub>4</sub>.

In group 1 and 2, complete diet samples were collected from a minimum of 6 feeders and combined to make 1 composite sample per treatment and phase. Each sample was then split, ground, and sent to a commercial lab for analysis of DM, CP, Ca, P, ether extract, ash, and Cu concentrations. In group 1, all diets were analyzed at Cumberland Valley Analytical Services (Hagerstown, MD) and Ward Laboratories Inc. (Kearney, NE). Final Cu concentrations were determined by averaging 3 individual analyzed values, 2 from Cumberland Valley and 1 from Ward Labs. In group 2, all diets were analyzed at Cumberland Valley Analytical Services (Hagerstown, MD). Final Cu concentrations were determined by averaging 2 individual analyzed values.

For each group, pigs and feeders were weighed on d 0, 7, 14, 21, 28, and 35 to calculate ADG, ADFI, and F/G. Data were combined and analyzed as a randomized complete block design using PROC GLIMMIX (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and dietary treatment as the fixed effect. Random effects of group and block within group were also used in the model. The main effects of source and level as well as their interaction were considered significant with  $P < 0.05$  and a tendency with  $P < 0.10$  and  $\geq 0.05$ ).

## Results and Discussion

The chemical analyses of the complete diets were similar to the intended formulation (Tables 2 and 3); however, the chemical analysis for Cu concentration was slightly higher than expected for diets formulated to contain 75 and 150 ppm of added Cu from Cu-AA (AAFCO, 2014<sup>11</sup>). Analytical variation for Cu is listed at 25% (AAFCO,

<sup>11</sup> Association of American Feed Control Officials (AAFCO). 2014. Official Publication. Assoc. Am. Feed Cont. Off., Champaign, IL.

2014). Total Ca and P concentrations were similar among diets across each dietary phase.

All other total Cu values for each diet in each group were within the acceptable analytical limits described by the Association of American Feed Control Officials (2014) given that 17 ppm of Cu from  $\text{CuSO}_4$  was provided by the trace mineral premix and accounting for the Cu provided by ingredients used in formulation. Corn (yellow dent) and soybean meal can contain on average 17 and 50 ppm Cu, respectively (NRC, 2012<sup>12</sup>). Based on these Cu concentrations, corn and soybean meal may have contributed up to 12 ppm to the complete diet in our study. Thus, some of the variation observed in the Cu analysis may partially be explained by the Cu concentrations provided by major ingredients used in formulation.

From d 0 to 14, there was a tendency for a source  $\times$  level interaction (quadratic,  $P = 0.086$ ; Table 4) for ADG with maximal ADG at 150 ppm Cu with the Cu-AA complex, but at 225 ppm with TBCC. For Cu main effects, increasing Cu increased (linear,  $P \leq 0.004$ ) ADG, ADFI, and d 14 BW with no changes in F/G.

From d 14 to 35, there were no Cu source  $\times$  level interactions observed. Neither Cu source nor level influenced ADG or ADFI; however, increasing Cu improved (linear,  $P = 0.035$ ) F/G.

Overall (d 0 to 35), there were no Cu source  $\times$  level interactions observed. Increasing Cu increased ADG (linear,  $P = 0.048$ ) and final BW (linear,  $P = 0.019$ ). The increase in ADG combined with no differences ADFI resulted in a tendency for improved F/G (linear,  $P = 0.091$ ).

It has been generally shown that increasing dietary Cu for nursery pigs increases ADG by increasing ADFI. Overall, increasing Cu increased ADG and tended to improve F/G but without any differences in ADFI. Interestingly, previous research has shown inconsistent performance differences in pigs fed different Cu sources. Unlike our study, previous research that compared TBCC or  $\text{CuSO}_4$  at increasing levels from 26 to 224 ppm found no response to the source or level of the Cu (De Jong et al., 2015<sup>13</sup>). The authors speculated their lack of response may have been related to the high levels of Zn that were fed in the diet immediately prior to their study; however, our study does not support this speculation. In each group of pigs for the study herein, pharmacological levels of Zn were fed prior to the pigs being fed experimental diets and a growth response was observed, particularly from d 0 to 14. Furthermore, because De Jong et al. (2015) did not observe any Cu response, they were unable to determine if one Cu source affected growth differently from the other.

In contrast, some earlier research reported improvements in ADG and F/G when pigs (initially 68.9 lb. BW), were fed either  $\text{CuSO}_4$  or TBCC compared with a control diet

<sup>12</sup> NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

<sup>13</sup> De Jong, J. A.; Bailey, L.; DeRouchey, J. M.; Tokach, M. D.; Goodband, R. D.; and Dritz, S. S. (2015). Effects of Copper Sources and Levels on Nursery Pig Growth Performance, Kansas State University Swine Day 2015. Kansas Agricultural Experiment Station Research Reports. Vol. 1: Iss. 7.



(Hastad et al., 2001<sup>14</sup>). In their study, the response to Cu was evident during the first 14 d, but not in the later part of the trial. The authors used increasing added Cu that ranged from 50 to 200 ppm and observed added Cu from either TBCC or CuSO<sub>4</sub> increased ADG compared with pigs fed the control diet; however, they observed growth was not linear as added Cu increased.

In our study, increasing Cu increased ADG and ADFI in the early nursery period but not the late nursery period. However, in each period the growth response magnitude to increasing level of Cu was numerically similar. In the early and late nursery period the ADG advantage was 0.05 and 0.04 lb/d, respectively, compared to the pigs fed the control diet. It appears that the ADG response to increasing levels of Cu was more difficult to detect in the late nursery period, which may be attributed to the increased amount of variation observed in that particular growth period. Additionally, because we observed a F/G response with no differences in feed intake during the late nursery period and increasing added Cu increased d 35 BW, this may help support that although not significant, there may be potential for a growth advantage to increasing added Cu during the late nursery period.

In summary, our study suggests that increasing TBCC or Cu-AA improves growth in nursery pigs. It appears the effect on ADG was more detectable during the early nursery period, with only a F/G improvement found during the second phase. The improved ADG led to a heavier BW both on d 14 and d 35. However, no differences between sources were observed. Because the growth effects were linear, it is unknown if increasing added Cu beyond 225 ppm would provide any further benefit for growth promotion above those observed in the current study.

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<sup>14</sup> Hastad, C. W. 2002. Phosphorus requirements of grow-finish pigs reared in commercial environments. MS Thesis. Kansas State University, Manhattan.

**Table 1. Diet composition (as-fed basis)<sup>1</sup>**

Item	Phase	
	1	2
Ingredient, %		
Corn	48.47	57.30
Soybean meal (47.7% CP)	27.68	33.73
Dried whey	10.00	---
Choice white grease	5.00	5.00
Limestone	0.85	0.85
Monocalcium P (21% P)	1.60	1.70
Salt	0.30	0.35
L-Lys-HCl	0.33	0.33
L-Thr	0.15	0.16
HMB <sup>2</sup>	0.22	0.18
HP-300 <sup>3</sup>	5.00	---
Vitamin premix	0.15	0.15
Trace mineral premix	0.25	0.25
Cu source <sup>4,5</sup>	---	---
Total	100.00	100.00
Calculated analysis		
Standardized ileal digestible (SID) AA, %		
Lys	1.30	1.25
Ile:Lys	63	62
Leu:Lys	122	124
Met: Lys	36	35
Met + Cys: Lys	58	58
Thr: Lys	65	65
Trp: Lys	18.4	18.4
Val: Lys	67	67
Total Lys, %	1.45	1.40
ME, kcal/lb	1,597	1,577
NE, kcal/lb	1,199	1,181
SID Lys:ME, g/Mcal	3.69	3.59
CP, %	21.7	21.3
Ca, %	0.85	0.80
P, %	0.78	0.75
Available P, %	0.49	0.44

<sup>1</sup>In each group of pigs, Phases 1 and 2 were fed from d 0 to 14 and 14 to 35, respectively. Dietary treatments were formed by adding 75, 150, or 225 ppm of Cu from either TBCC or Cu-AA at the expense of corn. The trace mineral premix was formulated to contribute 17 ppm of Cu in the complete diet.

<sup>2</sup>Hydroxymethylthio-butanoic acid, Novus International (Saint Charles, MO).

<sup>3</sup>HP-300, Hamlet Protein, Findlay, OH, formulated with 3.25% SID lysine.

<sup>4</sup>Mintrex Cu, copper methionine hydroxy analogue (St. Charles, MO).

<sup>5</sup>IntelliBond-C, TBCC (Micronutrients, Indianapolis, IN).



**Table 2. Chemical analysis of diets, (Groups 1 and 2, as-fed basis)<sup>1</sup>**

Item	Phase 1							Phase 2						
	Added Cu, ppm							Added Cu, ppm						
	Control	TBCC, ppm <sup>2</sup>			Cu-AA, ppm <sup>3</sup>			Control	TBCC, ppm			Cu-AA, ppm		
	0	75	150	225	75	150	225	0	75	150	225	75	150	225
DM, % <sup>4</sup>	88.9	88.1	88.9	89.0	89.0	89.0	88.9	88.9	88.4	88.2	88.6	88.4	88.6	88.6
CP, % <sup>4</sup>	24.1	24.3	24.3	24.5	24.7	24.8	24.0	24.3	23.8	24.3	32.9	24.4	24.0	23.4
Crude fiber, % <sup>4</sup>	2.5	2.5	2.4	2.5	2.8	2.7	2.6	2.6	3.0	2.7	2.6	2.5	2.5	2.3
Ether extract, % <sup>4</sup>	6.0	6.7	6.4	7.2	6.9	6.8	7.3	7.3	7.1	7.0	7.0	7.3	7.1	6.6
Ash, % <sup>4</sup>	6.6	6.6	6.6	6.8	6.5	6.4	6.4	5.9	6.1	6.0	7.2	5.7	5.7	5.3
Ca, % <sup>4</sup>	1.06	0.97	1.01	1.01	0.94	0.95	1.03	1.02	0.93	0.93	1.01	0.93	0.99	0.93
P, % <sup>4</sup>	0.86	0.85	0.84	0.89	0.84	0.88	0.88	0.82	0.81	0.82	0.84	0.79	0.84	0.76
Cu, ppm <sup>4</sup>	24	86	179	248	134	227	316	28	90	144	246	114	177	283

<sup>1</sup>For each group of pigs, multiple samples of each diet were collected, blended and sub-sampled, and analyzed (Cumberland Valley Analytical Services, Hagerstown, MD). The trace mineral premix was formulated to contribute 17 ppm of Cu in the complete diet.

<sup>2</sup>IntelliBond-C, TBCC (Micronutrients, Indianapolis, IN).

<sup>3</sup>Mintrex-Cu, copper methionine hydroxy analogue (St. Charles, MO).

<sup>4</sup>Values represent the mean of analytical values for each group of pigs.

**Table 3. Effects of increasing Cu from TBCC or Cu-AA on growth performance of pigs<sup>1</sup>**

Item	Added Cu, ppm							SEM	Probability, $P <$				
	Control	TBCC <sup>2</sup>			Cu-AA <sup>3</sup>				Cu Source	Cu level		Source × level	
		0	75	150	225	75	150			225	Linear	Quadratic	Linear
BW, lb													
d 0	12.82	12.81	12.82	12.81	12.81	12.80	12.81	1.322	0.960	0.974	0.964	0.956	0.965
d 14	20.15	20.08	20.78	21.22	20.53	21.19	20.93	2.226	0.431	0.002	0.886	0.502	0.108
d 35	46.41	46.24	47.85	48.53	47.62	48.88	48.04	6.105	0.315	0.019	0.624	0.619	0.130
d 0 to 14													
ADG, lb	0.53	0.52	0.56	0.59	0.55	0.59	0.57	0.071	0.289	0.004	0.854	0.631	0.086
ADFI, lb	0.69	0.68	0.76	0.77	0.74	0.75	0.75	0.046	0.456	0.001	0.921	0.203	0.139
F/G	1.33	1.32	1.38	1.34	1.37	1.29	1.36	0.100	0.820	0.772	0.989	0.515	0.494
d 14 to 35													
ADG, lb	1.25	1.24	1.29	1.30	1.26	1.30	1.28	0.196	0.852	0.143	0.957	0.564	0.431
ADFI, lb	1.88	1.86	1.89	1.92	1.90	1.91	1.85	0.276	0.872	0.831	0.702	0.181	0.147
F/G	1.51	1.51	1.47	1.48	1.52	1.48	1.47	0.027	0.895	0.035	0.866	0.683	0.569
d 0 to 35													
ADG, lb	0.96	0.95	1.00	1.01	0.97	1.01	0.99	0.146	0.712	0.048	0.976	0.560	0.296
ADFI, lb	1.41	1.39	1.44	1.46	1.43	1.44	1.40	0.184	0.939	0.305	0.808	0.150	0.144
F/G	1.47	1.47	1.45	1.44	1.49	1.43	1.44	0.039	0.943	0.091	0.923	0.565	0.887

<sup>1</sup>A total of 665 pigs [Group 1; 350 barrows (DNA 200 × 400; initially 14.1 lb)] and [Group 2; 315 pigs (DNA 241 × 600; initially 11.4 lb)] were used in two 35-d growth studies. Data were combined across the 2 groups with 5 pigs per pen and 10 replications per treatment in group 1 and 5 pigs per pen and 9 replications per treatment in group 2. The treatment design was the same across both groups of pigs. In both groups of pigs the trace mineral premix was formulated to contribute 17 ppm of Cu in the complete diet.

<sup>2</sup>IntelliBond-C, TBCC (Micronutrients, Indianapolis, IN).

<sup>3</sup>Mintrex Cu, copper methionine hydroxy analogue (St. Charles, MO).