RESEARCH ARTICLE

J Anim Sci Technol 2022;64(5):885-896
https://doi.org/10.5187/jast.2022.e48

JAST
Journal of Animal Science and Technology
pISSN 2672-0191 eISSN 2055-0391

Effects of dietary copper sources and levels on growth performance, copper digestibility, fecal and serum mineral characteristics in growing pigs

Byeonghyeon Kim, Jin Young Jeong, Seol Hwa Park, Hyunjung Jung and Minji Kim*

Animal Nutrition & Physiology Division, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

Abstract

This experiment was conducted to investigate the effects of three different copper (Cu) sources (one inorganic and two organics) and levels (0, 50, and 100 mg/kg) on the growth performance, Cu digestibility, fecal mineral excretion, serum mineral concentration, jejunal morphology, and serum biochemical profile of growing pigs. A total of 42 male, growing pigs (31.08 ± 1.82 kg) were randomly assigned to seven treatments consisting of one negative control (0 mg/kg of added Cu level) and treatments with copper sulfate (CuSO4), Cu-amino acid complex (CuAA), and Cu-hydroxy-4-methylthio butanoate chelate complex (CuHMB) at 50 and 100 mg/kg each for 28 d. Pigs fed 50 or 100 mg/kg of Cu showed improved ($p < 0.05$) average daily gain and feed intake. Although Cu excretion decreased ($p < 0.01$) in pigs fed 100 mg/kg of organic Cu sources compared to those fed $CuSO_4$, there was no difference between the Cu sources in pigs fed 50 mg/kg. However, the apparent total tract digestibility of Cu increased ($p < 0.01$) in pigs fed organic Cu sources compared with that in pigs fed $CuSO_4$. The addition of CuHMB increased ($p < 0.01$) serum phosphorus and sulfur concentrations; however, there were no effects of source and level on jejunal morphology and serum biochemical profile. These results suggest that the inclusion (50 mg/kg) of organic Cu sources (CuAA and CuHMB) in the growing pig diet could be beneficial for growth performance and Cu availability and may reduce environmental pollution.

Keywords: Copper, Cu excretion, Growing pigs, Growth performance

Received: May 24, 2022
Revised: Jun 7, 2022
Accepted: Jun 8, 2022

***Corresponding author**
Minji Kim
Animal Nutrition & Physiology Division,
National Institute of Animal Science,
Rural Development Administration,
Wanju 55365, Korea.
Tel: +82-63-238-7469
E-mail: mjkim00@korea.kr

ORCID
Byeonghyeon Kim
https://orcid.org/0000-0003-4651-6857
Jin Young Jeong
https://orcid.org/0000-0002-8670-7036
Seol Hwa Park
https://orcid.org/0000-0002-7218-8212
Hyunjung Jung
https://orcid.org/0000-0002-7004-2017
Minji Kim
https://orcid.org/0000-0003-2106-1921

Competing interests
No potential conflict of interest relevant to this article was reported.

INTRODUCTION

Copper (Cu), a trace element in the porcine diet, is essential for the growth and optimum health of growing pigs [1]. Several studies have shown the effects of Cu on growth performance, intestinal morphology, and blood characteristics of swine [2–4]. The addition of dietary Cu improved growth performance in weaned [3,5,6] and grower-finisher pigs [3,7]. However, these results vary depending upon the Cu source (organic or inorganic) used in the diet [4,7].

Chelated organic Cu sources have relatively higher bioavailability than inorganic sources such as copper sulfate ($CuSO_4$), owing to a difference in their chemical structures [1]. The ring structure formed by the chelating reaction in organic minerals protects organic Cu against chemical reactions at low pH, and renders it more stable in the gastrointestinal tract of animals [8,9]. Furthermore, organic Cu has the advantage of minimizing the formation of bonds between minerals and phytic acid, thereby increasing its absorption in the small intestine [10]. It has also been suggested that organic Cu may increase the absorption of phosphorus (P) by decreasing the formation of complexes between P and other minerals in a corn and soybean meal (SBM) diet [10]. Thus, organic Cu has been suggested as an alternative to $CuSO_4$ in swine diets to decrease fecal Cu excretion and thereby reduce environmental pollution [11,12].

From an environmental perspective, water and soil pollution due to excessive Cu excretion from the swine industry is a worldwide concern [1]. The continuous pig manure application by the high Cu content as a feed additive can accumulate on the top soil and it causes a high concentration in sediment, resulting in soil pollution and consequently in flora [13]. Korea recently reduced the authorized maximum level of Cu in swine feed to 60 mg/kg of feed. Therefore, in this study, we aimed to evaluate the effects of different dietary Cu sources (inorganic and organic) and levels (50 and 100 mg/kg) on growth performance, Cu digestibility, fecal mineral excretion, serum mineral concentrations, jejunal morphology, and hematological characteristics in growing pigs.

MATERIALS AND METHODS

Animals, experimental design, and diets

The Institutional Animal Care and Use Committee of the Rural Development Administration approved this study (Approval No. NIAS-2020-1849), which proceeded at the swine facility of the National Institute of Animal Science of Korea. A total of 42 male growing pigs [(Landrace × Yorkshire) × Duroc] with an average initial body weight of 31.08 ± 1.82 kg were randomly assigned to seven dietary treatments for a 28-d experimental period. In this experiment, the pigs were penned individually and a 3 × 2 + 1 treatment structure was used. The dietary treatments consisted of a control (CON) diet (basal corn-SBM diet) without additional Cu and six other dietary treatments consisting of CON diets with $CuSO_4$, Cu-amino acid complex (CuAA), and Cu-hydroxy-4-methylthio butanoate chelate complex (CuHMB) as additives at concentrations of 50 and 100 mg/kg each. Each treatment was carried out on six pigs. All diets were formulated to meet or exceed the dietary requirements of growing pigs (Table 1) [14]. Feed and water were provided ad libitum throughout the trial.

Sampling and measurements

Pigs were weighed individually on days 0 and 28, and feed intake was measured to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). During the last week of the experiment, chromic oxide (Cr_2O_3) was added at 0.2% to the diet as an indigestible marker to calculate the apparent total tract digestibility (ATTD) of Cu. On the last day, fecal samples were collected from all pigs and stored at −20℃ for the analyses of fecal Cr_2O_3 and mineral concentrations. Blood samples were collected from all pigs into serum-separating tubes and allowed to clot at room temperature for 1 h. Serum was obtained after centrifugation at 1,800×g for 15 min and frozen at −20℃ for analysis of mineral concentrations and serum characteristics. All pigs were then slaughtered and 5-cm-long segments from the central part of the jejunum were washed and fixed in 10% buffered formalin for studying the intestinal morphology.

Funding sources
This study was carried out with the support of the "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01493601)", Rural Development Administration, Korea.

Acknowledgements
This study was supported by the 2022 RDA Fellowship Program of the National Institute of Animal Science, Rural Development Administration, Korea. We are grateful for the direct or indirect help provided by all division members during the course of this study.

Availability of data and material
Upon a reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Jung H, Kim M.
Data curation: Kim B, Kim M.
Methodology: Kim B, Jeong JY, Park SH, Kim M.
Software: Kim B, Park SH.
Validation: Jung H, Kim M.
Investigation: Kim B, Jeong JY, Kim M.
Writing - original draft: Kim B.
Writing - review & editing: Kim B, Jeong JY, Park SH, Jung H, Kim M.

Ethics approval and consent to participate
This study was approved by IACUC of Rural Development Administration (No. NIAS-2020-1849).

Table 1. Ingredients and chemical composition of the basal diet (as-fed basis) for growing pigs

Ingredient	%
Corn	64.60
Soybean meal (45%)	20.50
Wheat bran	8.90
Soybean oil	2.00
Molasses	1.50
Limestone	0.75
Tricalcium phosphate	0.55
L-Lysine	0.40
Salt	0.30
Vitamin-mineral premix[1]	0.50
Calculated composition	
ME (kcal/kg)	3,300
Crude protein (%)	16.00
Crude fat (%)	4.80
Lysine (%)	1.01
Methionine + cysteine (%)	0.48
Calcium (%)	0.53
Phosphorus (%)	0.46
Ash (%)	4.47

[1] Provided per kg of diet: Vit A, 5,000,000 IU; Vit D_3, 1,000,000 IU; Vit E, 1,000 mg; Vit B_1, 150 mg; Vit B_2, 300 mg; Vit B_{12}, 1,500 mg; niacinamide, 1,500 mg; DL-calcium pantothenate, 1,000 mg; folic acid, 200 mg; Vit H, 10 mg; choline chloride, 2,000 mg; manganese, 3,800 mg; zinc, 1,500 mg; iron, 4,000 mg; copper, 500 mg; iodine, 250 mg; cobalt, 100 mg; magnesium, 200 mg.

ME, metabolizable energy.

Apparent total tract digestibility of Cu

The diets and freeze-dried fecal samples were ground into a fine powder before further analysis. The diets and fecal samples were analyzed for dry matter, and the ATTD of Cu was calculated according to the following equation [15]: 100 – 100 × [(% Cu in the feces/% Cu in the diet) × (Cr_2O_3 in the diet/ Cr_2O_3 in the feces)]. The concentration of Cr_2O_3 in the diet and fecal samples was determined using graphite furnace absorption spectrometry.

Mineral concentrations in diet, feces, and serum

In brief, the diet, fecal, and serum samples were digested with nitric acid and hydrogen peroxide using a microwave digestion system. The digested solutions were diluted with deionized water and the mineral concentrations in the samples were measured using inductively coupled plasma-mass spectrometry (Agilent 7700x, Agilent Technologies, Santa Clara, CA, USA).

Jejunal morphology

Jejunal morphology was determined as described previously [16]. The fixed samples were dehydrated, embedded in paraffin, and cut into 5-µm-thick sections using a rotary microtome (Leica RM 2245, Leica Biosystem, Tokyo, Japan). The sections were fixed on glass slides and stained with hematoxylin and eosin. The stained slides were scanned and captured to measure the villus height (VH), crypt depth (CD), and VH:CD ratio of a minimum of 10 well-orientated villi in each jejunal segment using the ImageJ program (National Institute of Health, Bethesda, MD, USA).

Serum biochemistry

Serum samples were analyzed to measure glucose, creatinine (CREA), blood urea nitrogen (BUN), BUN/CREA, total protein (TP), albumin (ALB), globulin (GLOB), ALB/GLOB, alanine aminotransferase (ALT), alkaline phosphatase (ALKP), total bilirubin (TBIL), and cholesterol (CHOL) levels using a chemistry analyzer (Catalyst Dx, IDEXX Labs, Westbrook, ME, USA).

Statistical analysis

Data analysis was performed using the general linear model (GLM) procedure in the SAS software (SAS Institut, Cary, NC, USA), with the individual pig as the experimental unit. Tukey's multiple comparison test (SAS Institute) was used to determine the statistical differences among treatments (for interaction effects). The results are expressed as mean ± standard error of the mean. Significance was considered at $p < 0.05$.

RESULTS

Growth performance

The growth performance of growing pigs is presented in Table 2. The increase in the final body weight (FBW) was higher ($p < 0.05$) in the growing pigs fed 100 mg/kg of Cu compared with that in pigs fed 0 mg/kg of Cu, and there was no difference in the FBW between the pigs fed 50 and

Table 2. Effect of different dietary copper (Cu) sources and levels on growth performance in growing pigs (n = 6/treatment)

Cu source	Added Cu level (mg/kg)	IBW (kg)	FBW (kg)	ADG (kg)	ADFI (kg)	G:F
$CuSO_4$	0	30.60	48.70^{b}	0.65	1.94	0.33
	50	30.44	50.24^{ab}	0.71	2.07	0.34
	100	30.72	49.94^{ab}	0.69	2.02	0.34
CuAA	0	30.60	48.70^{b}	0.65	1.94	0.33
	50	30.94	51.66^{ab}	0.74	2.03	0.37
	100	31.28	51.80^{ab}	0.73	2.00	0.37
CuHMB	0	30.60	48.70^{b}	0.65	1.94	0.33
	50	31.44	51.62^{ab}	0.72	2.02	0.36
	100	32.14	52.88^{a}	0.74	1.99	0.37
SEM		0.84	1.33	0.04	0.04	0.02
p-value for source × level		0.147	0.044	0.150	0.840	0.166
Main effect of source						
$CuSO_4$		30.59	49.63	0.68	2.01	0.34
CuAA		30.94	50.72	0.71	1.99	0.36
CuHMB		31.39	51.07	0.70	1.98	0.35
SEM		0.46	0.80	0.02	0.02	0.01
p-value for source		0.467	0.418	0.690	0.622	0.481
Main effect of level						
	0	30.60	48.70^{b}	0.65^{b}	1.94^{b}	0.33
	50	30.94	51.17^{ab}	0.72^{a}	2.04^{a}	0.35
	100	31.38	51.54^{a}	0.72^{a}	2.00^{a}	0.36
SEM		0.46	0.74	0.02	0.02	0.01
p-value for level		0.491	0.019	0.015	0.001	0.299

[a,b] Values with different superscripts in the same column are significantly different ($p < 0.05$).

IBW, initial body weight; FBW, final body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed; $CuSO_4$, copper sulfate; CuAA, Cu-amino acid complex; CuHMB, Cu-hydroxy-4-methylthio butanoate chelate complex.

100 mg/kg of Cu. The FBW of growing pigs fed 100 mg/kg of CuHMB had higher (p < 0.05) than those fed 0 mg/kg of CuHMB; however, there were no differences in growing pigs fed 100 mg/kg of $CuSO_4$ and CuAA than in those fed 0 mg/kg of $CuSO_4$ and CuAA. Growing pigs fed 50 and 100 mg/kg of Cu showed higher (p < 0.05) ADG and ADFI than those fed the CON diet. However, there were no significant differences in growth performance based on the source.

Apparent total tract digestibility of Cu

As presented in Table 3, the ATTD of growing pigs fed 50 mg/kg Cu was higher (p < 0.05) than those fed 0 mg/kg Cu. Growing pigs fed CuAA and CuHMB had higher ATTD (p < 0.05) than those fed $CuSO_4$. Furthermore, the ATTD in growing pigs fed 50 and 100 mg/kg of CuAA and CuHMB was higher (p < 0.05) than that in pigs fed 50 and 100 mg/kg of $CuSO_4$; however, there was no difference between the growing pigs fed 50 mg/kg of $CuSO_4$ and CuHMB.

Fecal mineral concentration

The fecal mineral concentrations are summarized in Table 4. The fecal concentration of Cu increased (p < 0.05) in growing pigs fed 100 mg/kg of Cu compared with that in pigs fed 0 and 50 mg/kg of Cu in their diets. However, there were no significant differences among the different source treatments. Although the fecal Cu concentrations of growing pigs fed 50 mg/kg of $CuSO_4$, CuAA, and CuHMB were not significantly different, those of growing pigs fed 100 mg/kg of

Table 3. Effect of different dietary copper (Cu) sources and levels on apparent total tract digestibility (ATTD) of Cu in growing pigs (n = 6/treatment)

Cu source	Added Cu level (mg/kg)	ATTD (%)
$CuSO_4$	0	34.03^{bc}
	50	34.79^{bc}
	100	28.60^{c}
CuAA	0	34.03^{bc}
	50	54.19^{a}
	100	45.89^{ab}
CuHMB	0	34.03^{bc}
	50	46.30^{ab}
	100	48.96^{a}
SEM		2.99
p-value for source × level		< 0.0001
Main effect of source		
$CuSO_4$		32.47^{b}
CuAA		44.70^{a}
CuHMB		43.10^{a}
SEM		2.22
p-value for source		0.001
Main effect of level		
	0	34.03^{b}
	50	45.09^{a}
	100	41.15^{ab}
SEM		2.33
p-value for level		0.006

[a-c] Values with different superscripts in the same column are significantly different (p < 0.01).

$CuSO_4$, copper sulfate; CuAA, Cu-amino acid complex; CuHMB, Cu-hydroxy-4-methylthio butanoate chelate complex.

Table 4. Effects of different dietary copper (Cu) sources and levels on fecal excretion (mg/kg) of minerals in growing pigs (n = 6/treatment)

Cu source	Added Cu level (mg/kg)	Cu	Fe	Zn	P	S
$CuSO_4$	0	51.44^{d}	810.66	629.79	15,800.00	842.70
	50	171.14^{c}	851.10	626.23	16,433.33	935.90
	100	338.12^{a}	826.49	608.69	15,350.00	964.53
CuAA	0	51.44^{d}	810.66	629.79	15,800.00	842.70
	50	134.30^{c}	841.93	727.71	15,433.33	902.13
	100	257.43^{b}	784.77	597.33	16,483.33	875.57
CuHMB	0	51.44^{d}	810.66	629.79	15,800.00	842.70
	50	140.37^{c}	730.66	532.22	15,966.67	921.49
	100	227.96^{b}	697.54	569.61	15,250.00	844.47
SEM		8.29	75.02	39.02	651.94	72.10
p-value for source × level		0.0004	0.141	0.097	0.692	0.661
Main effect of source						
$CuSO_4$		186.90	829.42	621.57	15,861.11	914.38
CuAA		147.72	812.45	651.61	15,905.56	873.47
CuHMB		139.92	746.28	577.20	15,672.22	869.55
SEM		23.10	41.42	23.27	366.65	40.25
p-value for source		0.313	0.333	0.085	0.892	0.685
Main effect of level						
	0	51.44^{c}	810.66	629.79	15,800.00	842.70
	50	148.60^{b}	807.90	628.72	15,944.44	919.84
	100	274.50^{a}	769.60	591.88	15,694.44	894.86
SEM		8.24	42.08	24.04	366.63	39.79
p-value for level		< 0.0001	0.744	0.452	0.890	0.383

$^{a-d}$Values with different superscripts in the same column are significantly different ($p < 0.001$).

$CuSO_4$, copper sulfate; CuAA, Cu-amino acid complex; CuHMB, Cu-hydroxy-4-methylthio butanoate chelate complex.

CuAA and CuHMB were lower ($p < 0.05$) than those of growing pigs fed $CuSO_4$. There were no significant differences in other minerals, such as iron (Fe), zinc (Zn), P, and sulfur (S).

Serum mineral concentration

Table 5 shows the effects of the Cu sources and levels on serum mineral concentrations. The Cu concentration was higher ($p < 0.05$) in groups fed 50 mg/kg Cu than in those fed 0 mg/kg Cu. The groups fed 50 and 100 mg/kg Cu had lower ($p < 0.05$) Zn concentrations than those fed 0 mg/kg Cu and the growing pigs fed 50 mg/kg of $CuSO_4$ had higher ($p < 0.05$) Zn concentration than those fed organic Cu sources. Furthermore, the concentrations of P and S increased ($p < 0.05$) after adding 100 mg/kg of Cu to the diets. Growing pigs fed 50 and 100 mg/kg of CuHMB showed higher ($p < 0.05$) P and S concentrations than those in the groups fed $CuSO_4$ and CuAA.

Jejunal morphology and serum characteristics

Tables 6 and 7 show the jejunal morphology and serum characteristics. There were no significant differences in VH, CD, or VH:CD among treatments. No significant differences were observed in serum characteristics, except for TP and GLOB. TP was lower ($p < 0.05$) in pigs fed 100 mg/kg of CuHMB than in those fed CuAA; however, there was no difference between groups fed $CuSO_4$ and CuHMB at 100 mg/kg. GLOB was lower ($p < 0.05$) in pig fed 100 mg/kg of CuHMB than in those fed 100 mg/kg of $CuSO_4$ and CuAA.

Table 5. Effects of different dietary copper (Cu) sources and levels on mineral concentrations (mg/L) in serum of growing pigs (n = 6/treatment)

Cu source	Added Cu level (mg/kg)	Cu	Fe	Zn	P	S
$CuSO_4$	0	1.49	2.04	0.53^{a}	123.81^{b}	883.80^{c}
	50	1.64	2.50	0.52^{a}	119.97^{b}	904.06^{c}
	100	1.64	1.81	0.50^{ab}	125.54^{b}	907.96^{c}
CuAA	0	1.49	2.04	0.53^{a}	123.81^{b}	883.80^{c}
	50	1.68	1.14	0.45^{b}	125.99^{b}	927.88^{c}
	100	1.56	2.06	0.48^{ab}	133.53^{b}	941.55^{bc}
CuHMB	0	1.49	2.04	0.53^{a}	123.81^{b}	883.80^{c}
	50	1.70	2.26	0.45^{b}	162.38^{a}	1,067.39^{b}
	100	1.70	1.17	0.46^{b}	182.12^{a}	1,206.14^{a}
SEM		0.09	0.43	0.02	5.30	28.96
p-value for source × level		0.255	0.287	0.002	< 0.0001	< 0.0001
Main effect of source						
$CuSO_4$		1.59	2.12	0.52	123.11^{b}	898.60^{b}
CuAA		1.57	1.71	0.49	127.78^{b}	917.74^{b}
CuHMB		1.63	1.81	0.48	156.10^{a}	1,052.44^{a}
SEM		0.05	0.26	0.01	4.49	24.55
p-value for source		0.729	0.520	0.097	< 0.0001	< 0.0001
Main effect of level						
	0	1.49^{b}	2.04	0.53^{a}	123.81^{b}	883.80^{b}
	50	1.67^{a}	1.97	0.47^{b}	136.11^{ab}	966.44^{ab}
	100	1.63^{ab}	1.66	0.48^{b}	147.06^{a}	1,018.55^{a}
SEM		0.05	0.25	0.01	5.23	26.40
p-value for level		0.026	0.558	0.004	0.011	0.003

$^{a-c}$Values with different superscripts in the same column are significantly different ($p < 0.05$).

$CuSO_4$, copper sulfate; CuAA, Cu-amino acid complex; CuHMB, Cu-hydroxy-4-methylthio butanoate chelate complex.

DISCUSSION

Dietary Cu is generally used as a growth stimulator in the porcine industry. Although its mechanism remains unclear, it has been suggested that Cu can shift the intestinal microbiota through its antimicrobial effect [17,18]. Due to the beneficial effect of Cu, it has been added excessively to swine diets, although its retention rate is poor [10,12]. The Cu requirement in pigs decreases according to the increase in body weight: 5–10 mg/kg Cu for piglets and 3–6 mg/kg Cu for grower-finisher pigs [1,14]. The use of Cu in growing pig diets is inevitable for optimal growth performance in the swine production industry; however, further research efforts are needed to reduce environmental pollution by reducing Cu levels or using organic Cu sources.

In the present study, the inclusion of Cu in growing pig diets resulted in positive effects on growth performance, regardless of the Cu source. Also, both ADG and ADFI were increased by the addition of Cu at 50 and 100 mg/kg in growing pig diets, indicating improved growth performance similar to that observed in previous studies [3,6]. However, our results showed no differences in growth performance between different Cu sources (organic vs. inorganic). It has been established previously that weaned pigs fed an organic Cu source showed greater gain and feed efficiency compared with that pigs fed an inorganic Cu source [3,5,6]. Furthermore, although Zhao et al. [3] reported that a relatively lower organic Cu (CuHMB) source increases the ADG and carcass weight of finishing pigs compared with that in pigs fed inorganic Cu source [3], others

Table 6. Effects of different dietary copper (Cu) sources and levels on jejunal morphology in growing pigs (n = 6/treatment)

Cu source	Added Cu level (mg/kg)	Villus height (μm)	Crypt depth (μm)	Villus:crypt (μm/μm)
$CuSO_4$	0	510.89	184.17	2.96
	50	595.36	159.62	4.13
	100	459.72	178.99	2.69
CuAA	0	510.89	184.17	2.96
	50	525.43	174.98	3.08
	100	558.81	185.78	3.34
CuHMB	0	510.89	184.17	2.96
	50	600.48	187.03	3.22
	100	530.59	167.87	3.31
SEM		37.10	20.26	0.39
p-value for source × level		0.400	0.854	0.896
Main effect of source				
$CuSO_4$		521.99	174.26	3.26
CuAA		531.71	181.64	3.13
CuHMB		547.32	179.69	3.16
SEM		22.43	11.17	0.23
p-value for source		0.724	0.890	0.918
Main effect of level				
	0	510.89	184.17	2.96
	50	573.76	173.88	3.48
	100	516.37	177.54	3.11
SEM		21.49	11.15	0.23
p-value for level		0.082	0.804	0.261

$CuSO_4$, copper sulfate; CuAA, Cu-amino acid complex; CuHMB, Cu-hydroxy-4-methylthio butanoate chelate complex.

have reported that the Cu source did not affect weight gain during the growing and finishing phases [4,7]. Overall, both organic and inorganic Cu sources may have positive effects on growth performance in the overall growth stage of pigs. In the current study, low inclusion level (50 mg/kg) Cu in growing pig diets resulted in a similar growth performance to that of pigs fed high inclusion level (100 mg/kg) Cu. Thus, a 50 mg/kg dose, which is below the permissible level in Korea, was sufficient for optimal growth performance in growing pigs. Furthermore, our results suggest that the Cu source is irrelevant when a low inclusion level (50 mg/kg) of Cu is administered.

In young pigs, Cu is necessary for stimulating growth and the immune system [6]; however, it has been suggested that an organic Cu source can be used to reduce fecal Cu excretion concerning environmental pollution [4,6,11]. Furthermore, it was reported that organic Cu was absorbed more effectively and digested in pigs fed a corn-SBM diet containing phytic acid [10]. Phytic acid binds to inorganic Cu to form insoluble compounds, thereby making it unavailable for absorption [3]. In the present study, the digestibility of organic Cu sources (CuAA and CuHMB) was found to be higher than that of inorganic Cu in growing pigs who were fed a corn-SBM diet. As the inclusion level of Cu in the diet increased, so did the excretion of Cu; however, there was no difference in ATTD between pigs fed 50 and 100 mg/kg of Cu. Although the excretion of Cu was not different when organic Cu sources were used, that of Cu decreased when 100 mg/kg of organic Cu was used rather than inorganic Cu. These results indicate that extra Cu was excreted in pigs fed 100 mg/kg of Cu because of the lower digestibility of $CuSO_4$ than that of organic Cu sources, and the inclusion level (50 mg/kg) of organic Cu sources was more suitable for better digestibility and lower fecal Cu excretion. Therefore, organic

Table 7. Effects of different dietary copper (Cu) sources and levels on serum characteristics in growing pigs (n = 6/treatment)

Cu source	Added Cu level (mg/kg)	GLU (mg/dL)	CREA (mg/dL)	BUN (mg/dL)	BUN /CREA	TP (g/dL)	ALB (g/dL)	GLOB (g/dL)	ALB /GLOB	ALT (U/L)	ALKP (U/L)	TBIL (mg/dL)	CHOL (mg/dL)
$CuSO_4$	0	83.33	1.03	8.33	8.17	7.27^{ab}	3.20	4.07^{a}	0.80	72.67	170.00	0.42	75.83
	50	80.67	1.07	11.83	11.33	7.13^{ab}	3.27	3.87^{ab}	0.83	78.50	210.83	0.27	65.83
	100	77.17	1.05	11.50	10.83	7.32^{ab}	3.13	4.18^{a}	0.78	72.50	189.17	0.27	75.83
CuAA	0	83.33	1.03	8.33	8.17	7.27^{ab}	3.20	4.07^{a}	0.80	72.67	170.00	0.42	75.83
	50	83.17	1.03	10.50	10.33	7.12^{ab}	3.05	4.07^{a}	0.75	73.50	194.17	0.13	68.83
	100	85.00	0.97	10.67	11.67	7.53^{a}	3.48	4.05^{a}	0.87	64.00	201.83	0.48	77.67
CuHMB	0	83.33	1.03	8.33	8.17	7.27^{ab}	3.20	4.07^{a}	0.80	72.67	170.00	0.42	75.83
	50	82.50	1.05	9.00	8.67	6.93^{ab}	3.30	3.63^{ab}	0.90	88.67	170.50	0.30	77.50
	100	77.67	1.03	8.67	8.83	6.45^{b}	3.23	3.22^{b}	1.42	62.50	208.00	0.12	74.33
SEM		3.43	0.06	1.14	1.33	0.21	0.12	0.24	0.18	12.40	16.56	0.16	3.97
p-value for source × level		0.906	0.761	0.214	0.789	0.028	0.496	0.022	0.057	0.848	0.849	0.558	0.321
Main effect of source													
$CuSO_4$		80.39	1.05	10.56	10.11	7.24^{ab}	3.20	4.04	0.81	74.56	190.00	0.32	72.50
CuAA		83.83	1.01	9.83	10.06	7.31^{a}	3.24	4.06	0.81	70.06	188.67	0.34	74.11
CuHMB		81.17	1.04	8.67	8.56	6.88^{b}	3.24	3.64	1.04	74.61	182.83	0.28	75.89
SEM		1.93	0.03	0.68	0.77	0.13	0.07	0.14	0.11	6.93	9.79	0.09	2.32
p-value for source		0.422	0.718	0.147	0.281	0.047	0.880	0.061	0.211	0.868	0.860	0.876	0.590
Main effect of level													
	0	83.33	1.03	8.33	8.17	7.27	3.20	4.07	0.80	72.67	170.00	0.42	75.83
	50	82.11	1.05	10.44	10.11	7.06	3.21	3.86	0.83	80.22	191.83	0.23	70.72
	100	79.94	1.02	10.28	10.44	7.10	3.28	3.82	1.02	66.33	199.67	0.29	75.94
SEM		1.93	0.03	0.66	0.75	0.13	0.07	0.14	0.11	6.81	9.34	0.09	2.27
p-value for level		0.459	0.796	0.052	0.080	0.509	0.654	0.422	0.287	0.360	0.076	0.345	0.188

[a,b] Values with different superscripts in the same column are significantly different ($p < 0.05$).

GLU, glucose; CREA, creatinine, BUN, blood urea nitrogen; TP, total protein; ALB, albumin; GLOB, globulin; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; TBIL, total bilirubin; CHOL, cholesterol; $CuSO_4$, copper sulfate; CuAA, Cu-amino acid complex; CuHMB, Cu-hydroxy-4-methylthio butanoate chelate complex.

Cu could be an alternative to $CuSO_4$ for better Cu availability and lower fecal excretion.

In the body, Cu is mainly stored in the liver and kidneys, and the Cu content in these organs contributes to the Cu concentration in the blood [19]. It has been reported that liver Cu concentration is high in pigs fed a Cu-rich diet, and excessive Cu accumulation can lead to its leakage into the circulatory system [4,12]. The blood Cu concentrations in weaned and finishing pigs are reported to have increased after the intake of dietary Cu [4,6]. Furthermore, sustained Cu intake gradually increased Cu accumulation in the bile, kidney, lung, and liver; thus, blood Cu concentration could be an indicator of Cu accumulation in the porcine body [6,20]. However, in the present study, there was no source effect on serum Cu concentration, as reported in a previous study that Cu sources have a lower effect on Cu concentration in the blood than in the tissue [4]. In contrast, the serum Cu concentration in growing pigs fed 50 mg/kg Cu was higher than that in CON diets, suggesting that Cu accumulated in the tissues of growing pigs. However, there was no difference in serum Cu concentrations between pigs fed 50 and 100 mg/kg of Cu. The reason for this is unclear; however, it can be assumed that extra Cu was not used and excreted into the feces due to its limited availability in growing pigs fed 100 mg/kg of Cu. Furthermore, the Zn concentration decreased with the addition of 50 and 100 mg/kg of Cu into the diet. This result might be explained by the fact that increased dietary Cu intake can interfere with Zn absorption [21]. Interestingly, serum P and S concentrations were higher in growing pigs fed CuHMB than in those fed $CuSO_4$ and CuAA. It has been reported that HMB increases P absorption by reducing the formation of complexes between P and other minerals [10,22]. The increased P and S concentrations could be explained by the reducing effect of CuHMB on the formation of phytate and minerals in the corn-SBM diet.

It was postulated that dietary Cu can improve intestinal morphology by increasing the VH:CD ratio and converting the energy required for maintenance of the gastrointestinal tract towards growth [23]. Although no differences in intestinal morphology were observed in this study, it has been previously reported that duodenal CD was decreased, and VH:CD was increased in the duodenum and jejunum after the administration of 200 ppm of organic Cu (Cu proteinate complex) in early-weaned pigs [23]. They also reported that 225 mg/kg of $CuSO_4$ in weaned pigs decreased the VH of the duodenum and proximal jejunum compared with that in pigs fed control and inorganic tribasic Cu chloride diets [24]; no differences were observed in VH, CD, and VH:CD in finishing pigs fed 205 mg/kg of tribasic Cu chloride [25]. Therefore, a high concentration of $CuSO_4$ in diets could induce oxidative stress in the small intestine of young pigs, but not in older pigs, resulting in adverse effects on intestinal morphology [26]. In this study, jejunal morphology in growing pigs who were fed Cu at different levels and from varying sources did not differ among the treatments. Lower Cu levels and different growth stages of the pigs may have caused this discrepancy.

Serum characteristics were determined as indicators of the physiological condition and health of growing pigs [27]. Although there was no significant difference among the different Cu source groups, the TP was decreased by about 12 % in pigs fed 100 mg/kg of CuHMB than that in pigs fed $CuSO_4$. A previous study showed that weaned pigs fed 650 g/t $CuSO_4$ in diet had higher serum CREA, ALT, aspartate aminotransferase, GLOB, total CHOL, TP, urea, direct-acting-bilirubin, and TBIL than pigs fed on control and 640 g/t of chelated Cu diets [12]. Our results showed no differences in serum parameters between treatments, except for TP and GLOB, and that Cu levels, regardless of the source, had no hazardous effects on growing pigs.

In conclusion, supplementing the diets of growing pigs with 50 or 100 mg/kg of Cu resulted in improved growth performance without any detrimental effects on their health. A high inclusion level (100 mg/kg) of Cu in the diet can lead to high fecal Cu excretion; therefore, a low inclusion level (50 mg/kg) of Cu in growing pig diets is recommended to reduce environmental pollution without detrimental effects on growth performance. Although the fecal Cu excretions in growing pigs fed 50 mg/kg of inorganic and organic Cu did not differ, the organic Cu sources improved Cu digestibility, and it was found that adding CuHMB may increase P and S availability in the corn-SBM diet. Therefore, the inclusion of these organic Cu sources could be more beneficial in terms of growth performance and Cu availability at 50 mg/kg, which is below the authorized maximum Cu level (60 mg/kg) in the Korean swine industry.

REFERENCES

1. Espinosa CD, Stein HH. Digestibility and metabolism of copper in diets for pigs and influence of dietary copper on growth performance, intestinal health, and overall immune status: a review. J Anim Sci Biotechnol. 2021;12:13. https://doi.org/10.1186/s40104-020-00533-3
2. Hedemann MS, Jensen BB, Poulsen HD. Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. J Anim Sci. 2006;84:3310-20. https://doi.org/10.2527/jas.2005-701
3. Zhao J, Allee G, Gerlemann G, Ma L, Gracia MI, Parker D, et al. Effects of a chelated copper as growth promoter on performance and carcass traits in pigs. Asian-Australas J Anim Sci. 2014;27:965-73. https://dx.doi.org/10.5713/ajas.2013.13416
4. Wen Y, Li R, Piao X, Lin G, He P. Different copper sources and levels affect growth performance, copper content, carcass characteristics, intestinal microorganism and metabolism of finishing pigs. Anim Nutr. 2022;8:321-30. https://doi.org/10.1016/j.aninu.2021.10.007
5. Carpenter CB, Woodworth JC, DeRouchey JM, Tokach MD, Goodband RD, Dritz SS, et al. Effects of increasing copper from tri-basic copper chloride or a copper-methionine chelate on growth performance of nursery pigs. Transl Anim Sci. 2019;3:369-76. https://dx.doi.org/10.1093/tas/txy091
6. Liao P, Shu X, Tang M, Tan B, Yin Y. Effect of dietary copper source (inorganic vs. chelated) on immune response, mineral status, and fecal mineral excretion in nursery piglets. Food Agric Immunol. 2018;29:548-63. https://doi.org/10.1080/09540105.2017.1416068
7. Carpenter CB, Woodworth JC, Derouchey JM, Tokach MD, Goodband RD, Dritz SS, et al. Effects of increasing copper from either copper sulfate or combinations of copper sulfate and a copper–amino acid complex on finishing pig growth performance and carcass characteristics. Transl Anim Sci. 2019;3:1263-9. https://doi.org/10.1093/tas/txz112
8. Mézes M, Erdélyi M, Balogh K. Deposition of organic trace metal complexes as feed additives in farm animals. Eur Chem Bull. 2012;1:410-3.
9. Sandström B. Micronutrient interactions: effects on absorption and bioavailability. Br J Nutr. 2001;85:S181-5. https://doi.org/10.1079/BJN2000312
10. Liu Y, Ma YL, Zhao JM, Vazquez-Añón M, Stein HH. Digestibility and retention of zinc, copper, manganese, iron, calcium, and phosphorus in pigs fed diets containing inorganic or organic minerals. J Anim Sci. 2014;92:3407-15. https://doi.org/10.2527/jas.2013-7080
11. Huang Y, Yoo JS, Kim HJ, Wang Y, Chen YJ, Cho JH, et al. The effects of different copper (inorganic and organic) and energy (tallow and glycerol) sources on growth performance, nutrient digestibility, and fecal excretion profiles in growing pigs. Asian-Australas J Anim Sci. 2010;23:573-9. https://doi.org/10.5713/ajas.2010.80436
12. Liao P, Li M, Li Y, Tan X, Zhao F, Shu X, et al. Effects of dietary supplementation with cupreous N-carbamylglutamate (NCG) chelate and copper sulfate on growth performance, serum biochemical profile and immune response, tissue mineral levels and fecal excretion of mineral in weaning piglets. Food Agric Immunol. 2017;28:1315-29. https://doi.org/10.1080/0

9540105.2017.1339668

13. Jondreville C, Revy PS, Dourmad JY. Dietary means to better control the environmental impact of copper and zinc by pigs from weaning to slaughter. Livest Prod Sci. 2003;84:147-56. https://doi.org/10.1016/j.livprodsci.2003.09.011
14. National Research Council. Nutrient requirements of swine. Washington, DC: National Academies Press; 2012.
15. Stein HH, Sève B, Fuller MF, Moughan PJ, de Lange CFM. Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J Anim Sci. 2007;85:172-80. https://doi.org/10.2527/jas.2005-742
16. Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I, et al. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J Anim Sci. 2009;87:2614-24. https://doi.org/10.2527/jas.2008-1512
17. Mei SF, Yu B, Ju CF, Zhu D, Chen DW. Effect of different levels of copper on growth performance and cecal ecosystem of newly weaned piglets. Ital J Anim Sci. 2010;9:e71. https://doi.org/10.4081/ijas.2010.e71
18. Kim M, Cho JH, Seong PN, Jung H, Jeong JY, Kim S, et al. Fecal microbiome shifts by different forms of copper supplementations in growing pigs. J Anim Sci Technol. 2021;63:1386-96. https://doi.org/10.5187/jast.2021.e118
19. Luo XG, Dove CR. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities, and tissue mineral levels in weanling pigs. J Anim Sci. 1996;74:1888-96. https://doi.org/10.2527/1996.7481888x
20. Gao Y, Yang W, Che D, Adams S, Yang L. Advances in the mechanism of high copper diets in restraining pigs growth. J Anim Physiol Anim Nutr. 2020;104:667-78. https://doi.org/10.1111/jpn.13213
21. Lönnerdal B. Dietary factors influencing zinc absorption. J Nutr. 2000;130:1378S-83S. https://doi.org/10.1093/jn/130.5.1378s
22. Liem A, Pesti GM, Edwards HM Jr. The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. Poult Sci. 2008;87:689-93. https://doi.org/10.3382/ps.2007-00256
23. Zhao J, Harper AF, Estienne MJ, Webb KE Jr, McElroy AP, Denbow DM. Growth performance and intestinal morphology responses in early weaned pigs to supplementation of antibiotic-free diets with an organic copper complex and spray-dried plasma protein in sanitary and nonsanitary environments. J Anim Sci. 2007;85:1302-10. https://doi.org/10.2527/jas.2006-434
24. Fry RS, Ashwell MS, Lloyd KE, O'Nan AT, Flowers WL, Stewart KR, et al. Amount and source of dietary copper affects small intestine morphology, duodenal lipid peroxidation, hepatic oxidative stress, and mRNA expression of hepatic copper regulatory proteins in weanling pigs. J Anim Sci. 2012;90:3112-9. https://doi.org/10.2527/jas.2011-4403
25. Coble KF, Burnett DD, DeRouchey JM, Tokach MD, Gonzalez JM, Wu F, et al. Effect of diet type and added copper on growth performance, carcass characteristics, energy digestibility, gut morphology, and mucosal mRNA expression of finishing pigs. J Anim Sci. 2018;96:3288-301. https://doi.org/10.1093/jas/sky196
26. Huang YL, Ashwell MS, Fry RS, Lloyd KE, Flowers WL, Spears JW. Effect of dietary copper amount and source on copper metabolism and oxidative stress of weanling pigs in short-term feeding. J Anim Sci. 2015;93:2948-55. https://doi.org/10.2527/jas.2014-8082
27. Hong J, Fang LH, Kim YY. Effects of dietary energy and lysine levels on physiological responses, reproductive performance, blood profiles, and milk composition in primiparous sows. J Anim Sci Technol. 2020;62:334-47. https://doi.org/10.5187/jast.2020.62.3.334