Journal of Animal Science, 2021, Vol. 99, No. 6, 1–8

doi:10.1093/jas/skab127
Advance Access publication April 21, 2021
Received: 6 February 2021 and **Accepted:** 15 April 2021
Non Ruminant Nutrition

NON RUMINANT NUTRITION

Inclusion of dicopper oxide instead of copper sulfate in diets for growing–finishing pigs results in greater final body weight and bone mineralization, but reduced accumulation of copper in the liver

Laia Blavi,[†] David Solà,[†] Alessandra Monteiro,[‡] J. Francisco Pérez,[†] and Hans H. Stein[‡,‖,1]

[†]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain, [‡]Animine, Annecy 74960, France, [‖]Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801, USA

[1]Corresponding author: hstein@illinois.edu

ORCiD numbers: 0000-0001-8365-340X (D. Solà); 0000-0001-8853-8945 (J. F. Pérez).

Abstract

An experiment was conducted to test the hypothesis that inclusion of Cu oxide (Cu_2O) in diets for growing–finishing pigs improves body weight (**BW**) and bone mineralization, and reduces accumulation of Cu in the liver compared with pigs fed diets containing Cu sulfate ($CuSO_4$). Two hundred growing pigs (initial BW: 11.5 ± 0.98 kg) were allotted to a randomized complete block design with 2 blocks of 100 pigs, 5 dietary treatments, 5 pigs per pen, and a total of 8 pens per treatment. Treatments included the negative control (**NC**) diet that contained 20 mg Cu/kg, and 4 diets in which 125 or 250 mg Cu/kg from $CuSO_4$ or Cu_2O were added to the NC diet. The experiment was divided into 4 phases and concluded when pigs reached market weight. Pig weights were recorded on day 1 and at the end of each phase and feed provisions were recorded throughout the experiment. On the last day of phases 1 and 4, 1 pig per pen was sacrificed to obtain samples of liver and spleen tissue, and the right metacarpal was collected. Results indicated that pigs fed diets containing 250 mg Cu/kg from $CuSO_4$ had greater BW at the end of phases 1 and 2 than pigs fed NC diets. Pigs fed diets containing 250 mg Cu/kg from Cu_2O had greater ($P < 0.05$) BW at the end of phases 1, 2, 3, and 4 compared with pigs fed NC diets, and these pigs also had greater BW at the end of phases 3 and 4 than pigs fed all other diets. Pigs fed the diets with 250 mg Cu/kg tended to have greater ($P < 0.10$) feed intake than pigs fed the NC diet at the end of phase 2, and for the overall experimental period, pigs fed diets containing 250 mg Cu/kg from Cu_2O had greater ($P < 0.05$) feed intake than pigs on all other treatments. However, no differences in gain:feed ratio were observed among treatments. Copper accumulation in liver and spleen increased with Cu dose, but at the end of phase 1, pigs fed 250 mg Cu/kg from $CuSO_4$ had greater ($P < 0.05$) Cu concentration in liver and spleen than pigs fed 250 mg Cu/kg from Cu_2O. Pigs fed diets containing 250 mg Cu/kg from Cu_2O had greater ($P < 0.05$) quantities of bone ash and greater ($P < 0.05$) concentrations of Ca, P, and Cu in bone ash than pigs fed NC diets or the 2 diets containing $CuSO_4$, but Zn concentration in bone ash was less ($P < 0.05$) in pigs fed diets containing 250 mg Cu/kg from Cu_2O. To conclude, supplementing diets for growing pigs with Cu_2O improves growth performance and bone mineralization with less Cu accumulation in liver compared with pigs fed diets containing $CuSO_4$.

Key words: bone mineralization, copper, copper oxide, copper sulfide, growth performance, pigs

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AEE	acid hydrolyzed ether extract
BW	body weight
CP	crude protein
DM	dry matter
FTU	phytase units
G:F	gain-to-feed ratio
NC	negative control

Introduction

Copper is an essential micro mineral for pigs and is involved in iron transport and metabolism, hematopoiesis, bone formation, and immune function, and Cu can also enhance the antioxidant capacity (Ewing and Charlton, 2007; Scheiber et al., 2013; Espinosa and Stein, 2021). The minimum requirement for Cu by growing–finishing pigs (11 to 135 kg body weight, **BW**) is 3.0 to 5.0 mg/kg (NRC, 2012), but supplementing diets with pharmacological doses of Cu (125 to 250 mg/kg per diet) improves growth performance (Hill et al., 2000; Pérez et al., 2011; Shelton et al., 2011) and reduces the prevalence of diarrhea in weanling pigs (Pérez et al., 2011; Espinosa et al., 2017). To assure adequate dietary Cu to meet requirements for body functions and growth promotion, Cu may be supplied in the forms of sulfates, oxides, chlorides, or chelates. However, the most common source is Cu sulfate ($CuSO_4 \cdot 5H_2O$), because of its high solubility in water and acid solutions (Park and Kim, 2016) and relatively low cost compared with other sources.

Absorption of Cu primarily occurs in the small intestine where Cu passes from enterocytes to the interstitial fluids and then to the hepatic portal vein. Absorbed Cu is rapidly deposited in the liver by albumin (Linder and Hazegh-Azam, 1996; Espinosa and Stein, 2021), and liver concentrations of Cu, therefore, can be used to asses bioavailability of Cu. High doses of Cu from Cu sulfate (250 to 500 mg Cu/kg) results in a linear increase in Cu accumulation in the liver of pigs (Izquierdo and Baker, 1986), poultry (Hamdi et al., 2018), and ruminants (Arnhold et al., 1998), which may result in generation of hydroxyl radicals in the liver (Luza and Speisky, 1996). However, a new source of Cu, dicopper oxide (Cu_2O; Animine, Annecy, France) results in lower accumulation of Cu in the liver of weanling pigs and broiler chickens compared with Cu sulfate (Bikker et al., 2018; Hamdi et al., 2018), which may be because Cu_2O has lower solubility than $CuSO_4$. Soluble sources of Cu interact in the intestinal digesta with phytate and may form Zn-Ca-Cu-phytate or Cu-Ca-phytate complexes (Oberleas, 1973) that are resistant to the hydrolytic activity of phytases (Persson et al., 1998). Indeed, Cu sulfate decreases apparent P retention (Banks et al., 2004), and Cu sulfate also reduces phytase activity more than dicopper oxide (Hamdi et al., 2018). Formation of the mineral–phytate complexes and the reduction of phytase activity by Cu sulfate may reduce bone mineralization, especially if Cu sulfate is provided for a long period of time. There is, however, limited information about using therapeutic levels of Cu on Cu accumulation in tissues and bone in growing–finishing pigs. Therefore, the objective of this experiment was to test the hypothesis that adding therapeutic levels of Cu as Cu_2O to diets for growing–finishing pigs will result in increased bone mineralization and reduced accumulation of Cu in the liver compared with pigs fed diets in which Cu is provided as $CuSO_4$.

Material and Methods

The Institutional Animal Care and Use Committee at the University of Illinois, USA, reviewed and approved the protocol for the experiment. The experiment was a collaborative project between the University of Illinois and Universitat Autònoma de Barcelona, Bellaterra, Spain, and the animal part of the experiment was conducted at the University of Illinois, Urbana-Champaign, IL, USA. Pigs used in the experiment were the offspring of L 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals, housing, and experimental design

A total of 200 growing pigs (100 barrows and 100 gilts) originating from 2 weaning groups and with an average initial BW of 11.5 ± 0.98 kg were used in the experiment. Pigs were allotted to a randomized complete block design with 2 blocks of 100 pigs with weaning group being the blocking factor. There were 5 dietary treatments, 5 pigs per pen, and 4 replicate pens per treatment in each block. Thus, there were a total of 8 replicate pens per treatment in the experiment. Pigs were housed in pens with fully slatted floors and a dry feeder and a nipple drinker were installed in each pen.

Diets and feeding

A 4-phase feeding program was used (Table 1); therefore, a total of 20 diets based on corn and soybean meal were formulated. Diets used in phases 1, 2, and 3 contained 500 units of phytase (**FTU**) per kilogram (Quantum Blue, AB Vista Feed Ingredients, Malborough, UK). Dietary treatments consisted of the negative control (**NC**) diet with 20 mg Cu/kg, and 4 diets in which 125 or 250 mg Cu/kg from either $CuSO_4$ or Cu_2O was added to the NC diet. Pigs were fed experimental diets for 116 d with phase 1 lasting 26 d, phases 2 and 3 lasting 35 d, and phase 4 lasting 20 d. Feed was provided on an ad libitum basis with water being available at all times. All diets were formulated to meet current estimates for nutrient requirements for growing–finishing pigs (NRC, 2012) and all diets were prepared in a meal form.

Data recording and sample collection

Pig weights were recorded at the start of the experiment and on the last day of each phase. The amount of feed offered to each pen was recorded daily and the amount of feed left in the feeder was recorded on the last day of each phase. On the last day of phase 1, the pig in each pen that had a BW closest to the pen average (4 barrows and 4 gilts per treatment) was sacrificed to obtain liver, spleen, and bile samples and the right metacarpal was collected as well from each pig. The weights of these tissues were recorded and samples were analyzed for Cu and Zn. Liver dry matter (**DM**) was determined in duplicate on a separate aliquot (3 g) after drying for 5 hr at 103 °C in a forced air oven. Bile was collected from the gallbladder and stored at −20 °C for analysis of Cu. Metacarpals were cleaned of adhering tissue, dried at 105 °C for 24 hr, defatted (Blavi et al., 2019), and ashed in a muffle furnace at 600 °C for 20 hr. Total ash weight was recorded, percentage of ash in the fat free bone was calculated, and Ca, P, Cu, and Zn in bone ash were analyzed.

On the last day of the experiment, 1 barrow or gilt in each pen with a BW that was closest to the pen average was transported to the Meat Science Laboratory at the University of Illinois (4 barrows and 4 gilts per treatment), where pigs were euthanized after an overnight fast. Samples collected from these pigs were

Table 1. Ingredient composition of the control diet in phases 1, 2, 3, and 4, as fed-basis[1]

Ingredients, %	Phase 1	Phase 2	Phase 3	Phase 4
Ground corn	59.75	67.64	75.43	78.77
Soybean meal, 48% CP	26.00	27.00	19.50	16.00
Dried whey	5.00	—	—	—
Fish meal, select Menhaden, 64% CP	3.00	—	—	—
Soybean oil	3.00	2.70	2.50	2.50
Ground limestone	0.86	0.85	0.80	0.74
Dicalcium phosphate, 19.5 % P	0.55	0.90	0.77	0.65
L-Lysine HCL, 78% Lys	0.35	0.11	0.18	0.13
DL-Met, 98% Met	0.09	—	—	—
L-Threonine, 98% Thr	0.10	—	0.02	0.01
Salt	0.40	0.40	0.40	0.40
Vitamin–mineral premix[2]	0.30	0.30	0.30	0.30
Phytase premix[3]	0.10	0.10	0.10	—
Titanium dioxide	0.50	—	—	0.50

[1]Four additional diets in each phase were formulated by adding 125 or 250 mg Cu/kg from copper sulfate pentahydrate (25% Cu) or 125 or 250 mg Cu/kg from copper (I) oxide (75% Cu) to the control diet used in each phase. The 2 copper sources were added at the expense of ground corn.

[2] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-α tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate and 10 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.15 mg as sodium selenite and 0.15 mg as selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]Phytase premix was prepared by mixing 900 g ground corn and 100 g Quantum Blue 5000 G (AB Vista Feed Ingredients, Marlborough, UK) to provide 500 phytase units per kilogram complete diet.

identical to the samples collected at the end of phase 1, and the same procedures for sample collection were used. In addition, hot carcass weight, fat depth, loin depth, dressing percentage, and lean percentage were determined using standard procedures (Overholt et al., 2016).

Chemical analysis

All diets were analyzed for DM (method 930.15; AOAC Int., 2007), and ash (Method 942.05: AOAC Int., 2007). Nitrogen was analyzed by combustion (method 999.03; AOAC Int., 2007) using a Leco FP 628 apparatus (LECO Corporation, Saint Joseph, MI) with aspartic acid being the calibration standard; crude protein (CP) was calculated as N × 6.25. Acid-hydrolyzed ether extract (**AEE**) was analyzed using the acid hydrolysis filter bag technique and 3N HCl (Ankom HCl Hydrolysis System, AnkomTechnology, Macedon, NY). Diets were also analyzed for phytase activity (Phytex Method, Version 1; Eurofins, Des Moines, IA). The detection limit of phytase activity was 70 units/kg. Copper was analyzed in diets, liver, spleen, bile, and bone ash; Zn was analyzed in diets, liver, spleen, and bone ash; and Ca and P were analyzed in diets and bone ash. All minerals were analyzed using inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B(b); AOAC Int., 2007).

Calculations and statistical analyses

The average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**) were calculated for each pen and treatment group. Bone ash percentage was calculated by dividing the quantity of bone ash by the quantity of fat-free, dried bone, and multiplied by 100. The quantity of bone P and Ca in grams, and Cu and Zn in micrograms was calculated by multiplying the bone Ca, P, Cu, or Zn concentration by the quantity of bone ash and dividing by 100.

Normality of residuals was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC) and outliers were identified using PROC ROBUSTREG of SAS. Growth performance data were analyzed as a randomized complete block design, using the PROC MIXED of SAS with a model that included treatment and block as main effects, and BW group nested within block as random effect. Mineral concentration in liver, spleen, and bile, bone mineralization, and carcass characteristics data were also analyzed as a randomized complete block design, using the PROC MIXED of SAS with a model that included treatment as main effect and block as random effect. Mean values were calculated using the LSMeans statement. Pen was the experimental unit for all analysis. An α value of 0.05 was used to assess significance among means and tendencies were considered at $0.05 \leq P < 0.10$.

Results

Analyzed values for DM, ash, CP, Lys, AEE, phytase activity, and minerals in diets were in agreement with formulated values (Tables 2).

Growth performance

There were no differences in initial BW among treatments (Table 3). At the end of phases 1 and 2, pigs fed 250 mg Cu/kg from $CuSO_4$ or Cu_2O had greater ($P < 0.05$) BW than pigs fed the NC diet. Addition of 250 mg/kg of $CuSO_4$ or Cu_2O to the NC diet also increased ADG ($P < 0.05$) in phase 1, and 250 mg/kg of Cu_2O increased ($P < 0.05$) ADG in phase 2. Pigs fed 250 mg Cu/kg from $CuSO_4$ or Cu_2O also tended ($P = 0.08$) to have greater ADFI in phase 2 compared with pigs fed the NC diet.

At the end of phases 3 and 4, pigs fed 250 mg Cu/kg from Cu_2O had greater ($P < 0.05$) BW compared with pigs from all other treatments, and pigs fed 250 mg Cu/kg from Cu_2O tended ($P = 0.08$) to have greater ADG in phase 3 compared with pigs fed 250 mg Cu/kg from $CuSO_4$. In phase 3, pigs fed 250 mg Cu/kg from Cu_2O had the greatest ($P < 0.05$) ADFI and pigs fed 125 mg Cu/kg had the least ADFI. Therefore, pigs fed 125 mg Cu/kg from Cu_2O had greater ($P < 0.05$) G:F compared with pigs fed the other diets. However, in phase 4, no differences among treatments were observed for ADFI and G:F.

For the entire period, pigs fed 250 mg Cu/kg from Cu_2O tended ($P = 0.09$) to have greater ADG compared with pigs fed the NC diet or the diet containing 250 mg Cu/kg from $CuSO_4$, and they also had greater ($P < 0.05$) ADFI compared with pigs fed all other diets. There were no differences among treatments in ending live weight, hot carcass weight, dressing percentage, 10th-rib fat depth, loin muscle area, or estimated carcass lean (Table 4).

Table 2. Analyzed composition of experimental diets

	DM, %	Ash, %	CP, %	Lys, %	Fat, %	Ca, %	P, %	Cu, mg/kg	Zn, mg/kg
Phase 1									
Control	87.19	4.07	17.88	1.38	5.50	0.81	0.54	26.5	116
125 mg/kg $CuSO_4$	87.05	5.11	17.93	1.29	4.79	0.79	0.52	142	129
250 mg/kg $CuSO_4$	87.02	5.47	16.94	1.38	4.65	0.73	0.56	245	132
125 mg/kg Cu_2O	86.76	5.05	17.98	1.49	5.33	0.72	0.53	128	134
250 mg/kg Cu_2O	87.16	5.62	18.23	1.31	5.52	0.81	0.54	254	123
Phase 2									
Control	86.40	3.99	16.47	1.04	6.22	0.61	0.44	29.1	141
125 mg/kg $CuSO_4$	86.29	4.08	17.15	1.05	5.68	0.66	0.41	198	141
250 mg/kg $CuSO_4$	86.30	4.03	16.61	0.92	4.90	0.61	0.41	303	167
125 mg/kg Cu_2O	86.21	4.19	17.04	1.03	5.38	0.74	0.41	165	146
250 mg/kg Cu_2O	86.26	4.01	17.56	0.99	5.09	0.69	0.44	279	166
Phase 3									
Control	86.57	3.71	13.14	0.88	5.60	0.63	0.42	20.5	137
125 mg/kg $CuSO_4$	86.53	3.38	13.68	0.85	5.06	0.65	0.35	162	135
250 mg/kg $CuSO_4$	86.53	3.51	14.79	0.91	4.37	0.59	0.38	276	134
125 mg/kg Cu_2O	86.61	3.72	12.53	0.89	5.97	0.64	0.37	168	129
250 mg/kg Cu_2O	86.48	3.77	12.81	0.86	4.81	0.54	0.35	262	141
Phase 4									
Control	87.64	3.90	11.81	0.78	5.60	0.46	0.36	24.1	133
125 mg/kg $CuSO_4$	87.70	4.36	11.86	0.85	4.77	0.48	0.31	161	129
250 mg/kg $CuSO_4$	87.66	4.25	13.50	0.83	4.45	0.47	0.31	274	137
125 mg/kg Cu_2O	87.45	4.06	12.39	0.80	5.07	0.47	0.34	141	127
250 mg/kg Cu_2O	87.61	4.10	11.56	0.84	4.49	0.45	0.31	248	128

Table 3. Body weight and growth performance of pigs fed diets without Cu supplementation (control) or with 125, 250 mg/kg of Cu from $CuSO_4$ or Cu_2O[1]

		$CuSO_4$		Cu_2O			*P*-value
Item	Control	125	250	125	250	SEM	Treatment
BW							
Initial BW, kg	11.55	11.52	11.51	11.48	11.50	0.217	0.998
Phase 1[2], kg	28.46^{b}	29.52^{ab}	29.96^{a}	29.13^{ab}	30.05^{a}	0.744	0.036
Phase 2[2], kg	58.77^{c}	62.13^{ab}	62.79^{ab}	59.88^{bc}	64.31^{a}	1.551	0.007
Phase 3[2], kg	96.74^{b}	97.99^{b}	96.84^{b}	97.68^{b}	103.29^{a}	2.100	0.027
Phase 4[2], kg	117.21^{b}	117.94^{b}	115.96^{b}	117.44^{b}	123.23^{a}	2.268	0.032
ADG							
Phase 1, kg/d	0.66^{b}	0.69^{ab}	0.71^{a}	0.68^{ab}	0.71^{a}	0.015	0.042
Phase 2, kg/d	0.87^{b}	0.93^{ab}	0.93^{ab}	0.88^{b}	0.98^{a}	0.027	0.026
Phase 3, kg/d	1.08^{x}	1.02^{xy}	1.01^{y}	1.07^{x}	1.09^{x}	0.035	0.083
Phase 4 kg/d	1.00	0.87	0.96	1.03	0.96	0.044	0.142
Overall, kg/d	0.89^{y}	0.91^{xy}	0.90^{y}	0.91^{xy}	0.95^{x}	0.019	0.092
ADFI							
Phase 1, kg/d	1.02	1.08	1.07	1.05	1.08	0.026	0.173
Phase 2, kg/d	1.82^{y}	1.96^{xy}	1.99^{x}	1.89^{xy}	2.02^{x}	0.062	0.085
Phase 3, kg/d	2.68^{ab}	2.50^{c}	2.55^{bc}	2.52^{c}	2.76^{a}	0.065	0.005
Phase 4, kg/d	2.83	2.88	2.76	2.94	2.92	0.086	0.300
Overall, kg/d	2.17^{b}	2.16^{b}	2.14^{b}	2.17^{b}	2.33^{a}	0.055	0.034
G:F							
Phase 1	0.649	0.651	0.660	0.648	0.651	0.0071	0.674
Phase 2	0.475	0.476	0.475	0.466	0.486	0.0058	0.170
Phase 3	0.404^{b}	0.404^{b}	0.394^{b}	0.426^{a}	0.394^{b}	0.0088	0.035
Phase 4	0.331	0.310	0.349	0.351	0.329	0.0178	0.303
Overall	0.421	0.421	0.419	0.421	0.409	0.0059	0.356

[1]Data are means of 8 observations per treatment.
[2]Phase 1, days 1 to 26; phase 2, days 26 to 61; phase 3, days 61 to 96; and phase 4, days 96 to 116.
[a–c]Values within a row without a common superscript are different ($P < 0.05$).
[xy]Values within a row without a common superscript tend to be different ($P < 0.10$).

Mineral concentration in the body

There were no differences in liver and spleen weight among treatments at the end of phases 1 and 4 (data not shown). At the end of phases 1 and 4, pigs fed 250 mg Cu/kg from $CuSO_4$ had greater ($P < 0.05$) concentration of Cu in liver, bile, and spleen compared with pigs fed the NC diets (Table 5), and pigs fed 250 mg Cu/kg from Cu_2O had greater ($P < 0.05$) concentration of Cu in liver and bile than pigs fed the NC diets. Liver concentration of Cu was less ($P < 0.05$) for pigs fed diets containing 125 mg Cu/kg compared with pigs fed 250 mg Cu/kg, regardless of source. The concentration of Cu was greater ($P < 0.01$) in liver and spleen from pigs fed 250 mg Cu/kg from $CuSO_4$ than from pigs fed 250 mg Cu/kg from Cu_2O at the end of phase 1, but not at the end of phase 4. However, at the end of phase 4, a greater ($P < 0.05$) concentration of Cu was observed in bile from pigs fed 250 mg Cu/kg from Cu_2O compared with pigs fed 250 mg Cu/kg from $CuSO_4$. No differences among dietary treatments were observed for Zn concentration in liver and spleen at the end of phases 1 and 4 (data not shown).

Bone mineralization

At the end of phase 1, pigs fed 250 mg Cu/kg from Cu_2O had greater ($P < 0.05$) quantity (g) of bone ash compared with pigs fed the NC diet or the diet containing 250 mg Cu/kg from $CuSO_4$ (Table 6), but the percentage (%) of bone ash was not different among treatments. The percentages of Ca and P in bone ash were also not affected by dietary treatments, but the quantity (g) of Ca and P was greater ($P < 0.05$) in bone ash from pigs fed the diet supplemented with 250 mg Cu/kg from Cu_2O than in bone ash from pigs fed the diet containing 250 mg Cu/kg from $CuSO_4$. The concentration (mg/kg) and quantity (mg) of Cu in bone ash was greater ($P < 0.05$) in pigs fed diets containing 250 mg Cu/kg from Cu_2O compared with pigs fed all other diets, with the exception that Cu concentration in bone ash at the end of phase 1 from pigs fed 250 mg Cu/kg from $CuSO_4$ did not differ from that of pigs fed 250 mg Cu/kg from Cu_2O. The concentration (mg/kg) of Zn in bone ash at the end of phase 1 was greater ($P < 0.05$) for pigs fed the NC diet compared with pigs fed 250 mg Cu/kg from $CuSO_4$ or 125 or 250 mg Cu/kg from Cu_2O, but no differences among treatments were observed for the quantity (mg) of Zn in bone ash.

At the end of phase 4, no differences among treatments were observed for total quantity or concentration of bone ash, Ca, P, or Zn. However, pigs fed diets containing 250 mg Cu/kg from $CuSO_4$ or Cu_2O had greater ($P = 0.05$) Cu concentration in bone ash than pigs fed the NC diet.

Discussion

The observation that 250 mg Cu/kg from $CuSO_4$ increased BW and ADG at the end of phase 1 is in agreement with published data (Hill et al., 2000; Pérez et al., 2011). However, it was surprising that neither 125 nor 250 mg Cu/kg from $CuSO_4$ increased BW at the end of phase 4 because in other experiments, Cu fed during the entire growing–finishing period increased final BW (Bowler et al., 1955; Davis et al., 2002). It is not clear why $CuSO_4$ failed to increase final BW at the end of phase 4 in this

Table 4. Carcass characteristics of pigs fed diets without Cu supplementation (control) or with 125, 250 mg/kg of Cu from $CuSO_4$ or Cu_2O[1]

		$CuSO_4$		Cu_2O			P-value
Item	Control	125	250	125	250	SEM	Treatment
Ending live weight, kg	107.73	110.73	110.96	113.33	115.41	2.498	0.217
Hot carcass weight, kg	83.66	86.27	86.55	87.12	90.63	1.971	0.194
Dressing percentage%	77.65	77.88	77.99	78.07	78.05	0.34	0.886
10th-rib fat depth, cm	1.65	1.84	1.62	1.68	1.49	0.136	0.499
Loin muscle area, cm	49.46	52.16	51.26	50.21	52.71	1.202	0.264
Estimated carcass lean, %	55.94	55.29	56.21	56.01	56.69	0.795	0.807

[1]Data are means of 8 observations per treatment.

Table 5. Concentrations of copper at the end of phase 1 and phase 4 in liver, bile, and spleen of pigs fed experimental diets[1]

		$CuSO_4$		Cu_2O			P-value
Item	Control	125[2]	250	125[2]	250	SEM	Treat
Liver							
Cu phase 1[2], mg/kg DM	19.8^{c}	24.5^{c}	473.3^{a}	22.8^{c}	339.9^{b}	47.63	0.001
Cu phase 4[2], mg/kg DM	26.5^{b}	172.3^{b}	897.1^{a}	99.3^{b}	695.2^{a}	92.75	0.001
Bile							
Cu phase 1, mg/kg	2.1^{b}	-	10.6^{a}	-	10.0^{a}	0.90	0.001
Cu phase 4, mg/kg	0.9^{c}	-	6.9^{b}	-	8.2^{a}	0.42	0.001
Spleen							
Cu phase 1, mg/kg	0.76^{b}	0.77^{b}	0.90^{a}	0.78^{b}	0.79^{b}	0.021	0.001
Cu phase 4, mg/kg	0.82^{bc}	0.81^{bc}	0.93^{a}	0.80^{c}	0.89^{ab}	0.033	0.016

[1]Data are means of 8 observations per treatment.
[2]Phase 1, day 26; phase 4, day 116.
[2]Cu analysis in bile was not performed in pigs fed 125 mg/kg of Cu from $CuSO_4$ and Cu_2O.
[a–d]Values within a row without a common superscript are different ($P < 0.05$).

Table 6. Bone mineralization at the end of phase 1 and at the end of phase 4 for pigs fed experimental diets[1]

		$CuSO_4$		Cu_2O			P-value
Item	Control	125	250	125	250	SEM	Treat
Bone[2] ash							
Phase 1[3], %[4]	54.3	54.7	53.7	56.3	56.7	1.27	0.423
Phase 4[3], %	66.7	65.1	66.0	67.6	68.2	3.31	0.960
Phase 1, g/bone	3.95^{bc}	3.94^{bc}	3.86^{c}	4.22^{ab}	4.31^{a}	0.113	0.031
Phase 4, g/bone	17.10	17.27	17.71	17.84	18.93	0.845	0.472
Bone Ca							
Phase 1, %	37.6	37.1	36.2	37.1	38.1	0.57	0.178
Phase 4, %	37.9	38.0	37.5	38.9	38.1	0.65	0.621
Phase 1, g/bone	1.49^{abc}	1.47^{bc}	1.40^{c}	1.56^{ab}	1.62^{a}	0.052	0.039
Phase 4, g/bone	6.43	6.62	6.60	6.60	7.10	0.264	0.463
Bone P							
Phase 1, %	18.6	18.3	17.9	18.3	18.4	0.28	0.549
Phase 4, %	17.7	18.6	17.7	18.3	17.6	0.37	0.368
Phase 1, g/bone	0.75^{ab}	0.72^{bc}	0.69^{c}	0.76^{ab}	0.79^{a}	0.023	0.036
Phase 4, g/bone	3.09	3.03	3.10	3.10	3.27	0.123	0.718
Bone Cu							
Phase 1, mg/kg	2.7^{b}	2.4^{b}	3.3^{ab}	2.1^{b}	4.2^{a}	0.44	0.015
Phase 4, mg/kg	1.0^{c}	1.3^{abc}	1.6^{a}	1.2^{bc}	1.5^{ab}	0.15	0.050
Phase 1, mg/bone	0.11^{b}	0.10^{b}	0.13^{b}	0.08^{b}	0.18^{a}	0.020	0.006
Phase 4, mg/bone	0.18	0.23	0.22	0.20	0.26	0.031	0.296
Bone Zn							
Phase 1, mg/kg	297.3^{a}	286.1^{ab}	263.9^{c}	271.3^{bc}	264.9^{c}	7.46	0.012
Phase 4, mg/kg	207.4	216.4	203.8	219.6	214.0	7.02	0.341
Phase 1, mg/bone	11.76	11.29	10.18	11.46	11.47	0.500	0.228
Phase 4, mg/bone	35.51	37.00	35.15	40.06	39.84	2.327	0.327

$^{a-c}$Means within a row without a common superscript are different ($P < 0.05$).
[1]Data are means of 8 observations per treatment.
[2]The bone used was the right metacarpal.
[3]Phase 1, day 26; phase 4, day 116.
[4]Bone ash as percent of the weight of dried, defatted bone.

experiment, but it is possible that the accumulation of Cu in the liver, especially at the end of phase 1, that was observed for pigs fed $CuSO_4$ prevented pigs from reaching their growth potential because feeding high levels of Cu for a long period of time may be toxic for pigs (NRC, 2012; Espinosa and Stein, 2021). However, for the overall experimental period there was not a strong correlation between liver Cu accumulation and growth performance of pigs.

The observation that pigs fed 250 mg Cu/kg from Cu_2O had greater BW at the end of the experiment than pigs fed 250 mg Cu/kg from $CuSO_4$ indicates that pigs tolerated the high levels of Cu from Cu_2O better than from $CuSO_4$. Copper sulfate pentahydrate is soluble in both water (99%) and acidic solvents (Pang and Applegate, 2006), whereas Cu_2O is not soluble in water (Baker, 1999). In the presence of phytic acid, the solubility of Cu in Cu_2O at pH 4.5 is 80% to 90% and at pH 6.5, the solubility is ~40%, whereas the solubility of Cu in $CuSO_4$ is close to 100% regardless of pH (Hamdi et al., 2018). Copper is absorbed as Cu^+ ions (Lönnerdal, 2008), and absorption is influenced by the solubility of the compound in the small intestine because only soluble compounds can be absorbed (Wapnir, 1998). Results of an in vitro experiment indicated that $CuSO_4$ is more soluble than Cu lysinate and tribasic Cu chloride at pH 2.5, 5.5, and 6.5 (Pang and Applegate, 2006), and differences observed in growth performance among Cu sources may be a result of differences in solubility (Pang and Applegate, 2007). It is, therefore, possible that the greater solubility of $CuSO_4$ compared with Cu_2O may result in greater absorption of Cu from $CuSO_4$ as indicated by the greater accumulation of Cu in the liver at the end of phase 1 if pigs were fed diets containing $CuSO_4$ compared with pigs fed diets containing Cu_2O. This may also be the reason differences in growth performance between pigs fed diets supplemented with 125 mg Cu/kg of $CuSO_4$ or Cu_2O were not observed, whereas if 250 mg Cu/kg was included, pigs fed the Cu_2O supplemented diets had greater growth performance than pigs fed diets containing $CuSO_4$.

At intestinal pH, Cu and Zn have high affinity for phytic acid (Persson et al., 1998), which results in Zn-Ca-Cu-phytate and Cu-Ca-phytate complexes being formed (Oberleas, 1973). These complexes tend to be resistant to the hydrolytic activity of phytases (Selle and Ravindran, 2008) and Cu and Zn bound in these complexes can, therefore, not be absorbed. Soluble sources of Cu are likely to interact more with phytate and phytase than less soluble sources. As a consequence, the observation that pigs fed diets containing Cu_2O had greater quantities of Ca, P, and Cu in bone ash compared with pigs fed Cu sulfate may be the result of reduced formation of Cu-phytate complexes in the intestinal tract compared with pigs fed diets containing $CuSO_4$. Hamdi et al. (2018) reported that $CuSO_4$ precipitated more phytic phosphorus than Cu_2O at intestinal pH, which limited the bio-availability of phytate-bound P to the phytase. The effect of Cu on phytase activity is dependent on the source of Cu used and tribasic Cu chloride and Cu lysinate inhibit phytate P hydrolysis much less than $CuSO_4$, Cu chloride, or Cu citrate (Pang et al., 2009).

Copper bioavailability may be affected not only by Cu source but also by diet composition because the presence

of chelating agents (phytate), metal-ion interactions, fiber, and ascorbic acid may interfere with bioavailability. Likewise, growth performance, tissue concentrations of Cu, and the age of pigs may affect bioavailability of Cu (Baker and Ammerman, 1995). Liver Cu concentration has been used to compare the relative bioavailability of Cu from different sources in pigs, chickens, cattle, and rats (Baker and Ammerman, 1995). There is a positive correlation between dietary concentration of Cu and Cu accumulation in liver for most sources of Cu (Baker et al., 1991; Zhou et al., 1994). Therefore, the observation in the present experiment that pigs fed 250 mg Cu/kg from $CuSO_4$ had greater Cu concentration in the liver than pigs fed 250 mg Cu/kg from Cu_2O at the end of phase 1 indicates that Cu from Cu_2O is less available than Cu from $CuSO_4$. Greater Cu concentrations in the liver of animals fed diets containing $CuSO_4$ compared with those fed Cu_2O was also observed in weanling pigs (Roméo et al., 2018) and in poultry (Hamdi et al., 2018), further indicating reduced absorption if Cu_2O rather than $CuSO_4$ is provided due to the reduced intestinal solubilization of Cu_2O. However, in both trials the lower hepatic accumulation did not negatively affect growth performance, indicating that Cu in Cu_2O may have exerted its effect in the intestinal tract rather than in the liver or in other tissues. Indeed, the reason pigs fed the diets with Cu_2O had greater final BW compared with pigs fed the NC diets or diets containing $CuSO_4$, despite the reduced absorption of Cu as indicated by reduced Cu accumulation in liver and spleen, indicates that Cu requirements were met and that Cu primarily affects intestinal conditions. As a consequence, it is likely that the growth promoting effect of Cu in pigs primarily is a result of the impact of Cu on the intestinal microbiota because dietary Cu reduces intestinal concentrations of microbes, which may result in improved intestinal health of pigs (Espinosa et al., 2019).

Bile Cu concentration is correlated with the amount of Cu absorbed (Aoyagi and Baker, 1993a) and biliary excretion is the primary route by which Cu is excreted from the body (Davis and Mertz, 1987). Increasing dietary Cu increases Cu in bile of chickens (Aoyagi and Baker, 1993b; Aoyagi and Baker, 1993a) and pigs (Armstrong et al., 2000). The greater concentration of Cu in bile in finishing pigs fed diets containing Cu_2O compared with pigs fed $CuSO_4$ is difficult to explain and does not support the hypothesis of reduced absorption of Cu from pigs fed Cu_2O. Nevertheless, more Cu in bile results in more Cu from bile entering the duodenum, but the Cu from bile is in a form that is not available for absorption (Tao and Gitlin, 2003).

In conclusion, supplementing diets for growing pigs with 250 mg Cu/kg of Cu_2O improved final BW and bone mineralization of pigs compared with pigs fed diets containing $CuSO_4$. At the end of phase 1, pigs fed diets containing Cu_2O also had reduced Cu concentration in the liver and spleen compared with pigs fed diets containing $CuSO_4$. Therefore, it appears that Cu_2O is at least as effective as $CuSO_4$ in improving growth and bone mineralization of pigs, but without resulting in the same accumulation of Cu in the liver in the early growing period.

Acknowledgment

The financial support from Animine, Annecy, France, is greatly appreciated.

Conflict of Interest Statement

A.M. is an employee at Animine, Annecy, France, a company that has commercial interests in mineral nutrition of food producing animals. Animine adheres to the principles of the European Code of Conduct for Research Integrity (Drenth, 2010). The other authors declare no real or perceived conflicts of interest.

Literature Cited

AOAC International. 2007. *Official methods of analysis of AOAC international*. 18th ed. Rev. 2. (W. Hortwitz and G. W. Latimer Jr., editors.). Gaithersburg, MD: AOAC International.

Aoyagi, S., and D. H. Baker. 1993a. Bioavailability of copper in analytical-grade and feed-grade inorganic copper sources when fed to provide copper at levels below the chick's requirement. *Poult. Sci.* **72**:1075–1083. doi:10.3382/ps.0721075.

Aoyagi, S., and D. H. Baker. 1993b. Biological efficacy of copper in chicken bile. *J. Nutr.* **123**:870–875. doi:10.1093/jn/123.5.870.

Armstrong, T. A., J. W. Spears, E. van Heugten, T. E. Engle, and C. L. Wright. 2000. Effect of copper source (cupric citrate vs. cupric sulfate) and level on growth performance and copper metabolism in pigs. *Asian-Australas. J. Anim. Sci.* **13**:1154–1161. doi:10.5713/ajas.2000.1154.

Arnhold, W., M. Anke, M. Glei, B. Rideout, I. Stalis, L. J. Lowenstine, M. Edwards, K. F. Schuppel, K. Eulenberger, and G. Nötzold. 1998. Determination of copper status in ruminants. *Trace Elem. Electrocytes*. **15**:65–69.

Baker, D. H. 1999. Cupric oxide should not be used as a copper supplement for either animals or humans. *J. Nutr.* **129**:2278–2279. doi:10.1093/jn/129.12.2278.

Baker, D. H., and C. B. Ammerman. 1995. Copper bioavailability. In: C. B. Ammerman, D. H. Baker, and A. J. Lewis, editors, *Bioavailability of nutrients for animals*. San Diego, CA: Academic Press; p. 127–156.

Baker, D. H., J. Odle, M. A. Funk, and T. M. Wieland. 1991. Research note: bioavailability of copper in cupric oxide, cuprous oxide, and in a copper-lysine complex. *Poult. Sci.* **70**:177–179. doi:10.3382/ps.0700177.

Banks, K. M., K. L. Thompson, P. Jaynes, and T. J. Applegate. 2004. The effects of copper on the efficacy of phytase, growth, and phosphorus retention in broiler chicks. *Poult. Sci.* **83**:1335–1341. doi:10.1093/ps/83.8.1335.

Bikker, P., S. Durosoy, A. Roméo, and J. van Baal. 2018. Both dietary copper (I) oxide and copper sulphate stimulate growth performance in pigs but differentially affect copper absorption and metal transporter genes. Abstract from 14th International Symposium on Digestive Physiology of Pigs (DPP2018), Brisbane, Australia; p. 65-65.

Blavi, L., C. J. Muñoz, J. N. Broomhead, and H. H. Stein. 2019. Effects of a novel corn-expressed E. coli phytase on digestibility of calcium and phosphorous, growth performance, and bone ash in young growing pigs. *J. Anim. Sci.* **97**:3390–3398. doi:10.1093/jas/skz190.

Bowler, R. J., R. Braude, R. C. Campbell, J. N. Craddock-Turnbull, H. F. Fieldsend, E. K. Griffiths, I. A. Lucas, K. G. Mitchell, N. J. Nickalls, and J. H. Taylor. 1955. High-copper mineral mixture for fattening pigs. *Br. J. Nutr.* **9**:358–362. doi:10.1079/bjn19550050.

Davis, G., and W. Mertz. 1987. Copper. In: W. Mertz, editor, *Trace elements in human and animal nutrition*. San Diego, CA: Academic Press; p. 301–364.

Davis, M. E., C. V. Maxwell, D. C. Brown, B. Z. de Rodas, Z. B. Johnson, E. B. Kegley, D. H. Hellwig, and R. A. Dvorak. 2002. Effect of dietary mannan oligosaccharides and(or) pharmacological additions of copper sulfate on growth performance and immunocompetence of weanling and growing/finishing pigs. *J. Anim. Sci.* **80**:2887–2894. doi:10.2527/2002.80112887x.

Drenth, P. J. D. 2010. A European code of conduct for research integrity. All European Academies. 14 pp.Available from https://allea.org/wp-content/uploads/2015/09/A-European-Code-of-Conduct-for-Research-Integrity_final.10.10.pdf – [accessed April 5, 2021]

Espinosa, C. D., and H. H. Stein. 2021. 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.* **12**:13. doi:10.1186/s40104-020-00533-3.

Espinosa, C. D., R. S. Fry, J. L. Usry, and H. H. Stein. 2017. Copper hydroxychloride improves growth performance and reduces diarrhea frequency of weanling pigs fed a corn–soybean meal diet but does not change apparent total tract digestibility of energy and acid hydrolyzed ether extract. *J. Anim. Sci.* **95**:5447–5454. doi:10.2527/jas2017.1702.

Espinosa, C. D., R. S. Fry, M. E. Kocher, and H. H. Stein. 2019. Effects of copper hydroxychloride and distillers dried grains with solubles on intestinal microbiota concentration and apparent ileal and total tract digestibility of energy and nutrients by growing pigs. *J. Anim. Sci.* **97**:4904–4911. doi:10.1093/jas/skz340.

Ewing, W. N., and S. J. Charlton. 2007. *The minerals directory*. 2nd ed. Leicestershire, UK: Cont. Prod. Ltd.

Hamdi, M., D. Solà, R. Franco, S. Durosoy, A. Roméo, and J. F. Pérez. 2018. Including copper sulphate or dicopper oxide in the diet of broiler chickens affects performance and copper content in the liver. *Anim. Feed Sci. Technol.* **237**:89–97. doi:10.1016/j.anifeedsci.2018.01.014.

Hill, G. M., G. L. Cromwell, T. D. Crenshaw, C. R. Dove, R. C. Ewan, D. A. Knabe, A. J. Lewis, G. W. Libal, D. C. Mahan, G. C. Shurson, *et al.* 2000. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). *J. Anim. Sci.* **78**:1010–1016. doi:10.2527/2000.7841010x.

Izquierdo, O. A., and D. H. Baker. 1986. Bioavailability of copper in pig feces. *Can. J. Anim. Sci.* **66**:1145–1148. doi:10.4141/cjas86-127.

Linder, M. C., and M. Hazegh-Azam. 1996. Copper biochemistry and molecular biology. *Am. J. Clin. Nutr.* **63**:797S–811S. doi:10.1093/ajcn/63.5.797.

Lönnerdal, B. 2008. Intestinal regulation of copper homeostasis: a developmental perspective. *Am. J. Clin. Nutr.* **88**:846S–850S. doi:10.1093/ajcn/88.3.846s.

Luza, S. C., and H. C. Speisky. 1996. Liver copper storage and transport during development: implications for cytotoxicity. *Am. J. Clin. Nutr.* **63**:812S–820S. doi:10.1093/ajcn/63.5.812.

NRC. 2012. *Nutrient requirements of swine*. 11th ed. Washington, DC: National Academies Press.

Oberleas, D. 1973. Phytates. In: *Committee on Food Protection: Toxicants occurring naturally in foods*. 2nd ed. Washington, DC: National Academies Press; p. 363–371.

Overholt, M. F., E. K. Arkfeld, D. A. Mohrhauser, D. A. King, T. L. Wheeler, A. C. Dilger, S. D. Shackelford, and D. D. Boler. 2016. Comparison of variability in pork carcass composition and quality between barrows and gilts. *J. Anim. Sci.* **94**:4415–4426. doi:10.2527/jas.2016-0702.

Pang, Y., and T. J. Applegate. 2006. Effects of copper source and concentration on in vitro phytate phosphorus hydrolysis by phytase. *J. Agric. Food Chem.* **54**:1792–1796. doi:10.1021/jf052053b.

Pang, Y., and T. J. Applegate. 2007. Effects of dietary copper supplementation and copper source on digesta pH, calcium, zinc, and copper complex size in the gastrointestinal tract of the broiler chicken. *Poult. Sci.* **86**:531–537. doi:10.1093/ps/86.3.531.

Pang, Y., J. A. Patterson, and T. J. Applegate. 2009. The influence of copper concentration and source on ileal microbiota. *Poult. Sci.* **88**:586–592. doi:10.3382/ps.2008-00243.

Park, C. S., and B. G. Kim. 2016. In vitro solubility of Copper(II) sulfate and dicopper chloride trihydroxide for pigs. *Asian-Australas. J. Anim. Sci.* **29**:1608–1615. doi:10.5713/ajas.16.0189.

Pérez, V. G., A. M. Waguespack, T. D. Bidner, L. L. Southern, T. M. Fakler, T. L. Ward, M. Steidinger, and J. E. Pettigrew. 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiota of pigs after weaning. *J. Anim. Sci.* **89**:414–425. doi:10.2527/jas.2010-2839.

Persson, H., M. Türk, M. Nyman, and A.-S. Sandberg. 1998. Binding of Cu^{2+}, Zn^{2+}, and Cd^{2+} to inositol tri-, tetra-, penta-, and hexaphosphates. *J. Agric. Food Chem.* **46**:3194–3200. doi:10.1021/jf971055w.

Roméo, A., S. Durosoy, J. Van Baal, and P. Bikker. 2018. Effet de deux sources de cuivre sur les performances et le statut en cuivre de porcelets sevrés. *Proc. J. Rech. Porc.* **50**:131–136.

Scheiber, I., R. Dringen, and J. F. B. Mercer. 2013. Copper: effects of deficiency and overload. In: A. Sigel, H. Sigel, and R. Sigel, editors, *Interrelations between essential metal ions and human diseases*. The Netherlands, Dordrecht: Springer; p. 359–387.

Selle, P. H., and V. Ravindran. 2008. Phytate-degrading enzymes in pig nutrition. *Livest. Sci.* **113**:99–122. doi:10.1016/j.livsci.2007.05.014.

Shelton, N. W., M. D. Tokach, J. L. Nelssen, R. D. Goodband, S. S. Dritz, J. M. DeRouchey, and G. M. Hill. 2011. Effects of copper sulfate, tri-basic copper chloride, and zinc oxide on weanling pig performance1. *J. Anim. Sci.* **89**:2440–2451. doi:10.2527/jas.2010–3432.

Tao, T. Y., and J. D. Gitlin. 2003. Hepatic copper metabolism: insights from genetic disease. *Hepatology* **37**:1241–1247. doi:10.1053/jhep.2003.50281.

Wapnir, R. A. 1998. Copper absorption and bioavailability. *Am. J. Clin. Nutr.* **67**(5 Suppl.):1054S–1060S. doi:10.1093/ajcn/67.5.1054S.

Zhou, W., E. T. Kornegay, M. D. Lindemann, J. W. Swinkels, M. K. Welten, and E. A. Wong. 1994. Stimulation of growth by intravenous injection of copper in weanling pigs. *J. Anim. Sci.* **72**:2395–2403. doi:10.2527/1994.7292395x.