

A Comparison of Copper Methionine, Tribasic Copper Chloride and Copper Sulfate as Copper Sources for Swine

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Abstract: Two experiments were conducted to investigate the effects of added copper on pig performance, tissue copper concentrations and fecal copper excretion. In experiment one, 18 crossbred (Yorkshire x Landrace) barrows weighing an average of 14.27 ± 1.39 kg were housed in stainless metabolism cages for 14 weeks and fed one of three corn-soybean meal based diets ($n=6$) supplemented with either 0, 125 or 250 ppm copper from copper sulfate. The total fecal collection method was used to calculate daily fecal copper excretion. All pigs were slaughtered and samples of tissue were collected from the central right liver lobe, left kidney cortex and left longissimus muscle at the 10th rib. Copper excretion increased with increasing levels of dietary copper addition ($p<0.01$) and with the age of the pigs ($p<0.01$). Copper levels in muscle, kidney and liver were significantly ($p<0.01$) higher for pigs fed 250 ppm copper compared with pigs fed 0 or 125 ppm copper. In experiment two, 126 crossbred (Yorkshire x Landrace) barrows weighing 13.06 ± 1.36 kg were used in a 3×3 factorial experiment in which the pigs were fed diets supplemented with 0, 125 or 250 ppm copper fed in the form of either copper sulfate, copper methionine or tribasic copper chloride for 24 days. The pigs were housed in groups of three with six pens of pigs fed each diet. During the first 12 days of the experiment, pigs fed tribasic copper chloride had significantly improved feed conversion compared with pigs fed copper sulfate or copper methionine. However, during the next 12 days and overall, there was no difference in daily gain, feed intake or feed conversion for pigs fed the three copper sources. Over the 24-day experiment, pigs fed 125 ppm copper had the highest weight gain ($p=0.03$) while pigs fed 250 ppm copper had the best feed conversion ($p<0.01$). Fecal copper was highest for pigs fed tribasic copper chloride ($p=0.02$). Fecal copper levels increased with increasing level of dietary copper ($p<0.01$). The overall results of this experiment indicate little difference in the performance of pigs fed copper sulfate, copper methionine or tribasic copper chloride. From an environmental standpoint, there may be advantages to choosing copper sulfate or copper methionine as these sources resulted in lower fecal copper excretion than tribasic copper chloride.

Key words: Pig, performance, copper sulfate, copper methionine, tribasic copper chloride

INTRODUCTION

The growth promoting effects of high levels of dietary copper (100 to 250 ppm copper as copper sulfate) have been well documented for both weanling^[1,2] and growing-finishing pigs^[3-5]. However, increased dietary copper is associated with increased fecal copper concentration with the increase in fecal copper largely originating from the diet (Roof and Mahan^[6]).

One means to allay the detrimental effects caused by high copper addition to swine diets is to decrease the dietary copper supply (Jondreville *et al.*^[7]). Since Zhou *et al.*^[8] reported that intravenously injected copper improved performance in pigs to the same extent as dietary copper, it can be concluded that copper sources with a higher absorption rate would have growth-

promoting effects at lower inclusion levels and be more beneficial in terms of environmental protection than copper sulfate.

Copper methionine was more efficiently absorbed than the inorganic form under certain conditions in chicks (Aoyagi and Baker^[9]). In addition, copper was more available from tribasic copper chloride than from copper sulfate in chicks (Luo *et al.*^[10]). For pigs, tribasic copper chloride was reported to be nearly as effective as copper sulfate (Cromwell *et al.*^[11]). Whether copper methionine and tribasic copper chloride are as effective in promoting animal growth and at the same time possess superiorities in terms of environmental protection compared with copper sulfate still needs to be explored. The experiments described herein were designed to investigate the changes in copper excretion in the feces of pigs fed

Table 1: Ingredient and chemical composition [%] of basal diets, as-fed basis

Item	Phase	Experiment 1			Experiment 2
		1	2	3	
Ingredients					
Ground yellow corn		57.00	66.00	72.00	60.38
Soybean meal		24.20	22.00	18.50	23.00
Spray-dried whey		5.00	0.00	0.00	5.00
Fish meal		7.50	5.50	3.00	6.00
Wheat bran		0.00	0.00	0.00	1.50
Soybean oil		3.50	3.50	3.30	1.00
Ground limestone		0.92	1.00	1.10	0.70
Dicalcium phosphate		0.65	0.79	0.91	1.20
Iodized salt		0.25	0.25	0.25	0.25
L-Lysine HCl		0.25	0.23	0.21	0.25
Antioxidant		0.03	0.03	0.03	0.02
Vitamin premix*		0.20	0.20	0.20	0.20
Trace mineral premix [†]		0.50	0.50	0.50	0.50
Chemical composition (% as fed)					
Crude protein		20.02	18.15	15.58	19.82
Calcium		1.02	0.89	0.82	0.87
Phosphorous		0.66	0.60	0.55	0.72
Copper, ppm		27.50	9.40	6.90	11.00
Lysine		1.55	1.40	1.13	1.61
Methionine		0.59	0.56	0.40	0.48
Tryptophan		0.18	0.16	0.15	0.18
Threonine		0.93	0.80	0.71	0.86

*Vitamin premix provided per kilogram of basal diet: 5,512 IU of vitamin A acetate; 551 IU vitamin D₃; 66.1 IU from dl- α -tocopheryl acetate; 27.6 ig of vitamin B₁₂; 5.5 mg of riboflavin; 13.8 mg of pantothenic acid as D-calcium pantothenate; 30.3 mg of niacin; 400 mg of choline from choline chloride. †Trace mineral premix provided per kilogram of basal diet: 20 mg of copper as CuSO₄ only for phase 1 of experiment 1; 120 mg of Fe as FeSO₄; 120 mg of Zn as ZnSO₄; 32 mg of Mn as MnSO₄; 0.3 mg Se as Na₂SeO₃; 0.2 mg I as Ca(IO₃)₂

different levels of copper as copper sulfate from growing through to market weight and then to compare copper methionine and tribasic copper chloride with copper sulfate for their effects on grower pig performance and fecal copper excretion.

MATERIALS AND METHODS

General: These experiments were conducted according to the “Guidelines for the Care and Use of Animals” in place at China Agricultural University. Tribasic copper chloride was provided by the Micronutrients Division of Heritage Technologies (Indianapolis, IN) and copper methionine was supplied by Beijing ZNBT Bio-Hightech Co., Ltd (Beijing, P. R. China).

Experiment one: Eighteen crossbred (Yorkshire and Landrace) barrows with an average initial BW of 14.27±1.39 kg were used for a 14-week experiment which was divided into three phases (phase 1, weeks 0 to 4; phase 2, weeks 5 to 8; phase 3, weeks 9 to 14). Three diets (Table 1) supplemented with 0, 125 and 250 ppm copper from copper sulfate were each fed to six pigs. The diets were analyzed to contain 27.5, 104.7 and 195.5 ppm copper for phase one, 9.4, 124.8 and 250.3 ppm for phase two and 6.9, 126.5 and 243.7 ppm for phase three. The diets (Table 1) contained sufficient other nutrients to meet or exceed the recommendations of the National Research Council (1998).

The pigs were individually housed in stainless metabolism cages (1.4 m x 0.5 m) in a temperature controlled metabolism room located in the Metabolic Unit of China Agricultural University. The average high and low temperatures were 24 and 20°C for phase one, 28 and 16°C for phase two and 24 and 20°C for phase three. The relative humidity was maintained between 55 and 75%. The pigs were fed three times a day at 0800, 1300 and 1900 h. During each feeding, the barrows were provided with free access to feed for 30 min and subsequently to water for another 30 min. A total collection of feces was made for each pig during the last five days of the 2nd, 4th, 6, 8, 10 and 12th week.

At the end of experiment one, all pigs were slaughtered at a commercial slaughter plant and samples of tissue were collected from the central right liver lobe, the left cortex of the kidney and the left longissimus muscle at the 10th rib. The tissue samples were stored at 20°C for later copper analysis.

Experiment two: Experiment two was conducted in the Ninghe Pig Breeding Farm (Tianjin, P. R. China). A total of 126 crossbred (Yorkshire x Landrace) barrows were used for a 24-day experiment that was divided into phase one and phase two (12 days per phase). The initial bodyweight of the pigs was 13.09 kg. In the control group, no extra copper was added to the basal diet containing 11 ppm copper. Copper sulfate, copper methionine or tribasic

copper chloride was added to the basal diet to provide 125 and 250 ppm copper. The analyzed total dietary levels of copper were 126 and 253 for the copper sulfate diets, 126 and 239 for the copper methionine diets and 129 and 252 ppm for the tribasic copper chloride diets, respectively. Copper supplementation was made at the expense of corn. DL-methionine was included in all diets to equalize the total methionine content. The diets (Table 1) contained sufficient other nutrients to meet or exceed the recommendations of the National Research Council (1998).

The diets were prepared into two equal parts. Chromic oxide was added to one part of all the diets at a concentration of 0.25% for the determination of apparent absorption values for copper, using the indicator method during phase two. Representative fecal samples were collected during the last five days of the experiment. Pigs were housed in an environmentally controlled ($28 \pm 2^\circ\text{C}$ and 55 to 75% relative humidity) nursery and placed in 42 elevated pens (1.20 m x 1.20 m) with plastic slotted floors. Each pen, accommodating three pigs, was equipped with a stainless steel self-feeder and a nipple waterer. Each diet was fed to six pens of pigs. At the end of experiment two, one pig was randomly selected from each pen and blood was collected via the anterior vena cava. Blood was allowed to clot and remained at 4°C for 2 h after which samples were centrifuged ($3,000 \times g$) for 10 min. The serum samples were stored at -20°C . Each fecal collection was weighed, recorded, mixed, sampled and then dried in a forced-air, drying oven (55°C). Air-dried samples were ground using a stainless steel mill and stored at -20°C for later copper analyses. Dry matter, crude protein, calcium and phosphorus content were analyzed according to the procedures of the AOAC^[12]. The diets, feces and untreated tissue samples were wet ashed in a 4:1 (v/v) mixture of nitric and perchloric acid. The serum concentration of copper was analyzed directly. All the copper and chromium determinations were conducted with a flame atomic absorption spectrophotometer (Hitachi Z-5000, Tokyo, Japan). The amino acid contents were analyzed according to the procedures used by Song *et al.*^[13].

Statistical analysis: The tissue copper concentration in experiment one was analyzed as a randomized complete block design using the GLM procedure of SAS (SAS Institute INC^[14]) with the pig as the experiment unit. Differences among the treatments were tested by the Least-Significant-Difference method. The fecal copper concentrations and daily fecal copper excretion in experiment one were analyzed with the Proc Mixed procedure of SAS as a randomized complete block design

with repeated measures over time on each experimental unit (Littell *et al.*^[15]). The model included treatment, time and treatment x time for the fixed effects and block was considered a random effect. Comparisons between times were made using the Slice option. The significance level was $p < 0.05$. The figures were drawn using Microsoft Excel version 11.6359.6360 (Microsoft, 2003). The data in experiment two were analyzed as a 3 x 3 factorial with the factors in the model consisting of source of copper, level of copper and their interaction. Pen was the experimental unit for all variables tested.

RESULTS

Experiment one: With increasing dietary copper concentration, the muscle, kidney and liver copper concentration increased ($p < 0.01$). Values for pigs fed 125 ppm copper did not differ significantly from the control while values for pigs fed 250 ppm copper were significantly higher ($p < 0.01$) than those for pigs fed the other two diets (Table 2). There were significant effects of treatment and time, as well as a significant treatment x time interaction ($p < 0.01$) for both fecal copper concentration and daily fecal copper excretion (Fig. 1 and 2). The 125 ppm and 250 ppm copper treatments greatly elevated the fecal concentration of copper relative to the control group.

For pigs fed the control diet, fecal concentrations of copper decreased during the first six weeks and then remained relatively stable during the following weeks. For the pigs fed 125 ppm copper, an increase and then decrease in fecal copper concentration appeared during the first six weeks before a relative steady concentration occurred. For pigs fed 250 ppm copper, the fecal concentration of copper increased up to the 4th week and then remained relatively static at a higher concentration and then began to decline during the 10th week.

For pigs fed the control diet (Table 2), the total amount of copper excreted from feces decreased gradually during the first 6 weeks and then increased slowly during the following 6 weeks. The total amount of copper excreted from feces for the pigs fed 125 ppm and 250 ppm copper increased over time with the highest levels observed at 12 weeks.

Experiment two: During phase one (0-12 days), feed conversion was significantly higher for pigs fed tribasic copper chloride than the other two copper sources (Table 3). However, during phase 2 and overall, there were no significant differences in performance for pigs fed any of the three copper sources. During the 24-day experiment, pigs fed 125 ppm copper had the highest

Table 2: Effect of dietary copper on tissue copper concentrations [ppm] (Dry matter basis, experiment 1)^{†‡}

Item	Copper			SEM*
	0	125	250	
Muscle	3.64 ^a	4.38 ^a	18.70 ^b	2.91
Kidney	19.99 ^a	23.76 ^a	40.18 ^b	3.02
Liver	25.34 ^a	50.34 ^a	520.04 ^b	51.22

*SEM: standard error of mean. [†]Means with different superscript letters indicate significant differences ($p < 0.05$). [‡]Data obtained from 6 pigs per treatment

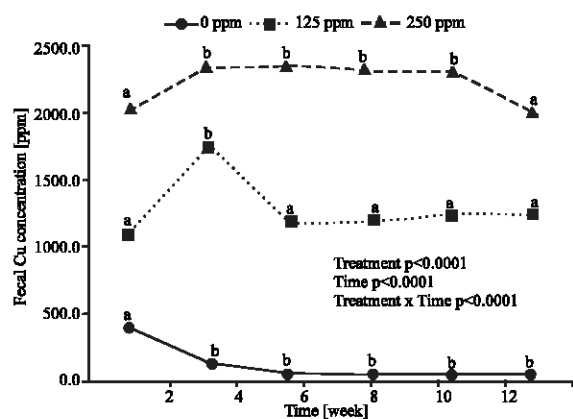


Fig. 1: Effect of dietary copper from copper sulfate on fecal copper concentration for the pigs at different ages. The data points with different superscripts differ significantly for each dietary copper addition level at $p < 0.001$ (Each data point represents 6 pigs per treatment experiment 1)

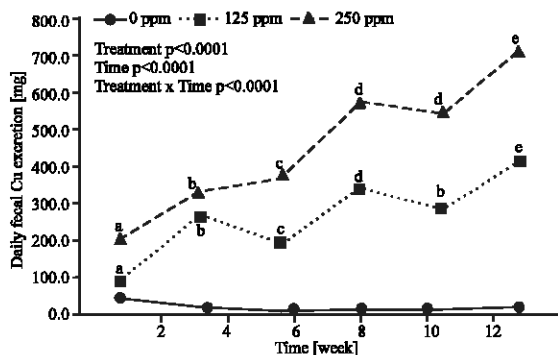


Fig. 2: Effect of dietary copper from copper sulfate on daily fecal copper excretion for the pigs at different ages. The data points with different superscripts differ significantly for each dietary copper addition level at $p < 0.001$ (Each data point represents 6 pigs per treatment, experiment 1)

weight gain ($p = 0.03$) while feed conversion was best for pigs fed 250 ppm copper ($p < 0.01$). The serum concentrations of copper were not significantly affected by source of dietary copper (Table 3). Pigs fed the 125 ppm level copper had significantly lower serum concentrations of copper compared with the other

treatments. Fecal copper concentrations were higher for pigs fed tribasic copper chloride than for pigs fed the other two copper sources ($p = 0.02$). Fecal copper was increased ($p < 0.01$) by copper addition and the absorption of copper was decreased ($p < 0.01$).

DISCUSSION

The growth-stimulative effect of copper at 125 ppm and 250 ppm has well been documented for weanling^[1,2] and growing-finishing pigs^[3-5]. The results of experiment two are consistent with previous research with significant improvements noted with the addition of 125 and 250 ppm copper.

Braude^[16] suggested that the 250 ppm copper level should be the supplementary level of choice compared with other addition levels based on a large number of previous studies that showed that this level maximized growth rate and feed conversion. Cromwell *et al.*^[2] calculated the greatest growth rate was obtained at 242 ppm of supplemental copper with the 125 ppm level being approximately 75% as effective in stimulating growth as the 250 ppm level.

The results of our study showed that the growth rate was higher at 125 than 250 ppm copper. This apparent discrepancy may be attributed to the new copper sources adopted in our study as a significant interaction between copper source and level was observed. In support of the current study, Coffey *et al.*^[17] concluded that 100 ppm of copper was as effective in growth stimulation as 200 ppm of copper regardless of source in a study to determine the relative availability of copper lysine relative to copper sulfate.

Feed conversion was consistently best for pigs fed 250 ppm copper during both phase 1 and 2 as well as overall, which supports the findings of Braude^[16]. The improvement in feed conversion coincided with a significant reduction in feed intake during phase two and overall. This finding does not support the conclusion of Zhou *et al.*^[18] who believed that the growth-stimulative effect of copper was dependent on a simultaneous increase in feed intake.

With the exception of an improvement in feed conversion in phase 1 for pigs fed tribasic copper

Table 3: Effect of source and level of copper on pig growth performance, serum and feces concentrations and absorption of copper (experiment 2)*^{1,2}

	Copper sources			Copper levels (ppm)			p-value			
	Copper sulphate	Copper methionine	Copper chloride	0	125	250	SEM	Source	Level	Source × Level
Phase 1 (0 to 12 days)										
ADG [g]	398	393	398	365 ^a	418 ^b	406 ^b	12.45	0.83	<0.01	0.04
ADFI [g]	687	659	656	658	683	662	24.58	0.25	0.43	0.06
Feed:gain	1.73 ^a	1.68 ^b	1.65 ^b	1.81 ^a	1.63 ^b	1.63 ^b	0.02	<0.01	<0.01	<0.01
Phase 2 (13-24 days)										
ADG [g]	534	548	566	557	556	535	19.49	0.15	0.29	0.25
ADFI [g]	984	1031	1040	1068 ^a	1032 ^a	953 ^b	31.16	0.07	<0.01	0.60
Feed:gain	1.84	1.88	1.84	1.92	1.86	1.79	0.07	0.74	0.10	0.52
Overall (0 to 24 days)										
ADG [g]	466	470	482	461 ^a	487 ^b	470 ^{ab}	11.94	0.24	0.03	0.05
ADFI [g]	835	845	848	863 ^a	858 ^a	808 ^b	20.95	0.74	<0.01	0.56
Feed:gain	1.79	1.78	1.78	1.86 ^a	1.77 ^{ab}	1.71 ^b	0.05	0.97	<0.01	0.84
Serum copper [ug/mL]	1.66	1.72	1.66	1.68 ^a	1.56 ^b	1.79 ^c	0.06	0.46	<0.01	0.12
Feces copper [ppm]	878 ^b	830 ^a	918 ^b	57 ^a	846 ^b	1723 ^c	37.99	0.02	<0.01	0.25
Absorption [%]	15.58	17.30	14.44	30.28 ^a	11.08 ^b	5.97 ^c	2.71	0.44	<0.01	0.68

¹SEM: standard error of mean

chloride, source of copper had little effect on pig performance. In previous literature, tribasic copper chloride was reported as being more available for broilers than copper sulfate by Luo *et al.*^[10], but less effective than copper sulfate in improving growth for weanling pigs by Cromwell *et al.*^[11]. A greater availability of copper methionine for weaning pigs, relative to that of copper sulfate, was reported by Bunch *et al.*^[19].

The fecal concentration of copper for the pigs fed low dietary copper level in experiment one decreased to a relatively stable level. This should be attributed to the adjustment of the endogenous excretion of copper in response to the low dietary copper level since the absorption rate of copper was not affected by the length of adaptation period to a copper added diet by Bowland *et al.*^[20].

Corresponding adjustments in fecal excretion were observed for pigs fed the medium and high copper diets, which were reflected by the changes of fecal copper concentration during the first several weeks. The gradual increases of daily copper excretion for the medium and high copper added treatments would mainly depend on an increased daily feed intake and the subsequently increased fecal excretion as the pigs aged. The decrease of fecal copper concentration at the end of the experiment for pigs fed the high copper diet may be attributed to a gradual lost in liver function to deal with the high copper inclusion in diet, although no symptoms of copper toxicity were found for any pig.

The serum concentration of copper did not show a consistently gradual increase coinciding with the increased copper concentration of the diets, which suggests that serum concentration of copper should not be a good indicator for comparing the bioavailability of different sources of copper at levels similar to our study.

This is in line with the results of Roof and Mahan^[6], but not with the results of Apgar *et al.*^[21]. The elevated liver and kidney concentration of copper as dietary levels increased agree with most other studies^[2,8,18,22,23]. However, the increased concentration of muscle copper is only consistent with the result of Bunch *et al.*^[20] and is contrary to most results showing that copper accumulation in muscle is not affected by high copper feeding^[3,5,24].

In the current study, the fecal concentration of copper increased and the apparent copper absorption rate decreased with increasing copper addition level. The apparent copper absorption rates in our experiment for pigs fed the control diet and the diet with 250 ppm added copper as copper sulfate are lower than the results of Roof and Mahan^[6]. This may be explained by the fact that lower copper absorption rates are obtained with the indicator method used in the current study compared with the total fecal collection method (Apgar and Kornegay,^[25]. The younger pigs used in our experiment may explain the higher apparent copper absorption obtained here compared with Apgar and Kornegay^[25] using the same method as in our study.

The overall results of this experiment indicate little difference in the performance of pigs fed copper sulfate, copper methionine or tribasic copper chloride. From an environmental standpoint, there may be advantages to choosing copper sulfate or copper methionine as these sources resulted in lower fecal copper excretion than tribasic copper chloride.

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