Effect of Copper Source (Cupric Citrate vs Cupric Sulfate) and Level on Growth Performance and Copper Metabolism in Pigs[1,2]

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ABSTRACT : Two experiments were conducted to evaluate the efficacy of cupric citrate (Cu-citrate) relative to cupric sulfate ($CuSO_4$) as a Cu source for weanling and grow-finish pigs. In addition, the use of liver and bile Cu concentrations as indices of the bioavailability of Cu sources was investigated. Experiment one consisted of a nursery phase (35 d; initial BW=6.4 kg, final BW=21.4 kg) followed by a grow-finish phase (103 d; initial BW=21.5 kg, final BW=111.7 kg). Experiment two only consisted of a nursery phase (35 d; initial BW=6.3 kg, final BW=18.6 kg). Dietary treatments were identical for both experiments and consisted of: control (10 ppm $CuSO_4$); control+66 or 225 ppm $CuSO_4$; control+33, 66, or 100 ppm Cu-citrate. An antibiotic was included in diets for Exp. 1 but not Exp. 2. In both experiments, growth performance variables were similar for pigs receiving Cu-citrate and $CuSO_4$; however, growth performance was not improved by high concentrations of $CuSO_4$. Liver and bile Cu were increased ($p<0.05$) by 225 ppm $CuSO_4$; however, lower dietary concentrations of Cu from either $CuSO_4$ or Cu-citrate did not affect the Cu concentration of liver or bile relative to that observed in the control pigs. Irrespective of Cu source, there was no linear ($p>0.10$) increase in plasma Cu with increasing Cu concentrations in the diet for both experiments. However, the plasma Cu concentrations were highest ($p<0.10$) in pigs receiving diets supplemented with 225 ppm $CuSO_4$. Sixteen randomly chosen pigs per treatment in Exp. 1 were continued through the grow-finish phase. Body weight gain and feed intake were improved ($p<0.10$) by 66 ppm $CuSO_4$, but other dietary Cu treatments did not alter pig performance compared to the control diet. Plasma Cu concentrations were increased ($p<0.10$) by 225 ppm $CuSO_4$ in the growing phase and by 225 ppm $CuSO_4$ and 100 ppm Cu-citrate in the finishing phase. These data reveal no consistent effect of $CuSO_4$ on performance; therefore, it is difficult to assess the efficacy of these two Cu sources. In addition, these studies demonstrate that liver and bile Cu are not good indicators of Cu bioavailability in pigs fed adequate to pharmacological concentrations of Cu. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1154-1161*)

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INTRODUCTION

The copper (Cu) requirement for a weanling or a grow-finish pig is 3 to 6 mg Cu/kg of diet (NRC, 1998). However, pharmacological levels of supplemental Cu (100 to 250 ppm) in the form of cupric sulfate ($CuSO_4 \cdot 5H_2O$) have been shown to improve the growth rate of pigs (Barber et al., 1955; Bunch et al., 1961; Hawbaker et al., 1961). Cromwell et al. (1989) calculated from one experiment that growth rate was maximized in weanling pigs when $CuSO_4$ was supplemented at 242 ppm.

The growth stimulatory effects of other forms of Cu have been studied, such as: Cu-carbonate (Bunch et al., 1965), Cu-lysine (Coffey et al., 1994; Apgar et al., 1995), tribasic Cu-chloride (Cromwell et al., 1998), and organic Cu chelates (Zoubek et al., 1975; Stansbury et al., 1990). These studies indicate that the aforementioned Cu compounds appear to be as effective as $CuSO_4$. However, Coffey et al. (1994) and Zhou et al. (1994) did report an improvement in growth performance with Cu-lysine compared to $CuSO_4$.

Research in broiler chickens indicated that an organic form of Cu, cupric citrate ($C_6H_4Cu_2O_7$), was effective in stimulating growth at lower concentrations than $CuSO_4$ (Pesti and Bakalli, 1996; Ewing et al., 1998). Reducing dietary Cu concentrations without adversely affecting growth is appealing, because of current environmental concerns regarding the excretion of Cu in animal waste.

If cupric citrate could be used at considerably lower concentrations than $CuSO_4$, this may allow swine producers to feed moderate concentrations of Cu throughout the nursery, growing, and finishing phases while maintaining the growth promoting effects of pharmacological concentrations of Cu. Therefore, the objectives of this study were to evaluate the effectiveness of $CuSO_4$ relative to cupric citrate on growth performance and to evaluate the use of liver and bile Cu concentrations as indicators of Cu bioavailability in pigs.

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MATERIALS AND METHODS

All experimental procedures, care, and handling of animals were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Experiment 1

1) Nursery trial

One hundred ninety-two crossbred ([Landrace x Yorkshire] x [Hampshire x Duroc]) gilts and barrows (96 gilts and 96 barrows) were weaned at 18 to 22 d of age, with an average initial weight of 6.4 kg. The gilts and barrows were allotted to 24 pens based on weaning weight and litter origin. The animals were blocked by weaning weight. Pigs were housed eight per pen in an environmentally controlled nursery, and the sexes were evenly distributed with four gilts and four barrows per pen. Pens were randomly assigned to one of six dietary treatments, and each treatment was replicated four times. Treatments consisted of: 1) control (10 ppm Cu as $CuSO_4$), 2) control+66 ppm Cu as $CuSO_4$, 3) control+225 ppm Cu as $CuSO_4$, 4) control+33 ppm Cu as Cu-citrate (Griffin Corporation, Casa Grande, AZ, USA; Cu-citrate=33.6% Cu), 5) control+ 66 ppm Cu as Cu-citrate, and 6) control+100 ppm Cu as Cu-citrate.

The nursery phase lasted a total of 35 d, and the final average weight was 21.4 kg. During the experimental period, a two-phase feeding regimen was implemented and all diets were offered in meal form. The basal diet contained a vitamin-trace mineral premix that provided all the essential vitamins and trace minerals except for Cu. Copper was supplemented according to the assigned treatments, and 10 ppm Cu as $CuSO_4$ was supplemented to the basal diet of each dietary treatment at the expense of corn. The phase I basal diet was fed for the first 14 d postweaning, and the phase II basal diet was fed from d 14 to 35 (table 1). The phase I and II basal diets contained an antibiotic, carbadox (Mecadox®, Pfizer, Inc., Terre Haute, IN, USA). The diets were formulated to meet or exceed the requirements for all nutrients (NRC, 1988) for weanling pigs. Pigs had ad libitum access to feed and water throughout the entire nursery phase.

Animal weights and feed intake measurements were recorded at the end of the phase I period (d 14) and at the completion of the trial (d 35). On d 28 of the experiment, venous blood samples were obtained from two randomly selected pigs per pen for the determination of plasma Cu concentrations. Plasma was obtained by centrifugation (1670×*g*) of the blood samples at 5℃ for 30 minutes. At the termination of the nursery phase (d 35), two randomly selected pigs per pen were stunned by electrocution and killed by exsanguination. Liver samples were obtained for the determination of Cu concentrations; in addition, bile was aspirated from the gall bladder with a 10 cc syringe and a 20 gauge needle for the determination of Cu concentrations. Liver, bile, and plasma samples were stored at -20℃ until they were analyzed for Cu concentrations.

Table 1. Composition of basal diets (as-fed basis) for weanling pigs

Item	Experiment 1		Experiment 2	
Phase	I[a]	II[b]	I[a]	II[b]
	%			
Ground corn	50.4	62.0	50.9	62.5
Dehulled soybean meal	17.0	30.0	17.0	30.0
Dried whey[c]	20.0	-	20.0	-
Fish meal	5.3	-	5.3	-
Porcine plasma	1.0	-	1.0	-
Poultry fat	4.0	4.0	4.0	4.0
Dicalcium phosphate	1.0	2.2	1.0	2.2
Calcium carbonate	0.25	0.51	0.25	0.51
Salt	0.10	0.35	0.10	0.35
Vit., trace min. mix[d]	0.25	0.25	0.25	0.25
L-Lysine · HCl	0.20	0.20	0.20	0.20
Antibiotic[e]	0.50	0.50	-	-

[a] Calculated to contain 19.2% CP, 1.34% lysine, 0.83% Ca, 0.77% P, and analyzed to contain 18 ppm Cu.
[b] Calculated to contain 19.9% CP, 1.27% lysine, 0.80% Ca, 0.78% P, and analyzed to contain 18 ppm Cu.
[c] Edible grade, spray-dried, whole whey.
[d] Supplied per kilogram of diet: vitamin A, 11,002 IU; vitamin D, 2,750 IU; vitamin E, 33 IU; menadione, 5.5 mg; riboflavin, 6.6 mg; d-pantothenic acid, 26.4 mg; niacin, 88 mg; vitamin B_{12}, 33.1 μg; Zn, 150 mg; Fe, 150 mg; Mn, 50 mg; I, 1.3 mg; and Se, .3 mg.
[e] Mecadox® (Pfizer, Inc., Terre Haute, IN, USA).

Plasma samples were diluted (1:2 vol/vol) with deionized water and analyzed for Cu concentrations by flame atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Norwalk, CT, USA). Liver samples were dried at 100℃ for 48 h, weighed, and wet-ashed according to the procedure of Ward et al. (1996). Bile samples were wet-ashed with trace metal grade nitric acid, hydrogen peroxide, and hydrochloric acid. Copper concentrations of wet-ashed liver and bile samples were analyzed by flame atomic absorption spectrophotometry.

2) Grow-finish trial

Two randomly selected barrows and two randomly selected gilts per nursery pen for a total of 16 pigs per treatment (average initial weight of 21.5 kg), with four replicate pens per treatment, were continued on their respective experimental diets for 103 d (average final weight was 111.7 kg). Ingredient composition of

the growing and the finishing diets are shown in table 2. The diets were offered in meal form, and were formulated to meet or exceed the requirements for growing and finishing pigs (NRC, 1988). The basal diet contained a vitamin-trace mineral premix that provided all the essential vitamins and trace minerals except for Cu. Copper was supplemented at the same concentrations described for the nursery trial. Throughout the growing and the finishing phase, all pigs had *ad libitum* access to feed and water. Pigs were changed from the growing to the finishing diet after 42 d (average BW of 52.8 kg).

Table 2. Composition of basal diets (as-fed basis) for grow-finish pigs

Item	Growing[a]	Finishing[b]
	%	
Ground corn	69.7	79.3
Dehulled soybean meal	22.7	15.5
Poultry fat	4.0	1.7
Dicalcium phosphate	1.75	1.25
Calcium carbonate	0.60	1.00
Salt	0.35	0.35
Antibiotic[c]	0.50	0.50
L-Lysine · HCl	0.15	0.15
Vitamin, trace mineral mix[d]	0.25	0.25

[a] Calculated to contain 16.6% CP, 0.87% lysine, 0.71% Ca, 0.68% P, and analyzed to contain 17 ppm Cu.
[b] Calculated to contain 13.9% CP, 0.68% lysine, 0.74% Ca, 0.56% P, and analyzed to contain 19 ppm Cu.
[c] CTC-50® (Alpharma, Animal Health Division, Fort Lee, NJ, USA).
[d] Supplied per kilogram of diet: vitamin A, 10,989 IU; vitamin D, 2,640 IU; vitamin E, 39.6 IU; menadione, 4.4 mg; riboflavin, 8.8 mg; d-pantothenic acid, 35.2 mg; niacin, 70.4 mg; vitamin B_{12}, 55 μg; Zn, 100 mg; Fe, 100 mg; Mn, 40 mg; I, 2 mg; and Se, 0.3 mg.

Pigs were weighed and feed intake was measured at the completion of the growing phase and the finishing phase. Blood samples were obtained from two randomly selected pigs per pen on d 42 and on d 84 of the grow-finish trial for the determination of plasma Cu concentrations.

Experiment 2

The performance data in Exp. 1 revealed no effect ($p>0.10$) of Cu on growth during the nursery phase. Therefore, in order to determine if the growth promoting effects of Cu were being obscured by the growth stimulating effects of an antibiotic, 192 crossbred gilts and barrows (96 gilts and 96 barrows) were weaned at 18 to 22 d of age and allotted to 24 pens based on weaning weight and litter origin. The animals were blocked by weaning weight. The average initial weight was 6.3 kg, and the average final weight was 18.6 kg. All experimental procedures, treatments, and diets were identical to the nursery trial in Exp. 1, except there was no antibiotic added to either the phase I or phase II basal diets.

Statistical analyses

Data were analyzed by analysis of variance using the general linear model procedure (PROC GLM) of SAS (1993). Pen was considered the experimental unit. Performance data, liver Cu concentrations, bile Cu concentrations, and plasma Cu concentrations were analyzed using a statistical model that contained the effects of treatment, block, and treatment × block. Significance was declared at $p<0.10$, and differences in the least squares means among treatments were determined by the Student's t-test in the predicted difference (PDIFF) option of the GLM procedure. There were no treatment × block interactions ($p>0.10$).

RESULTS AND DISCUSSION

The supplementation of Cu as $CuSO_4$ at concentrations of 125 to 250 ppm has been demonstrated to stimulate growth and improve feed efficiency in weanling (Bunch et al., 1965; Stahly et al., 1980; Cromwell et al., 1989) and grow-finish (Hawbaker et al., 1961; Cromwell et al., 1978; Prince et al., 1979) pigs. Therefore, the addition of 225 ppm Cu as $CuSO_4$ was used as a positive control, in order to stimulate maximum growth in weanling pigs. However, in phase I, phase II, and over the entire nursery phase of Exp. 1, the addition of 225 ppm Cu as $CuSO_4$ resulted in no improvement ($p>0.10$) over the control animals with respect to ADG (table 3). In addition, there was no effect ($p>0.10$) on ADG in Exp. 1 between any of the treatments during the nursery phase (table 3).

During phase I of Exp. 1, the animals receiving 66 ppm Cu as $CuSO_4$ had greater ($p<0.10$) ADFI than animals consuming diets containing 33 ppm Cu as Cu-citrate (table 3). During phase II and over the entire nursery period, animals receiving 66 ppm Cu as $CuSO_4$ had increased ($p<0.10$) ADFI when compared to animals receiving 225 ppm Cu as $CuSO_4$ and 33 ppm Cu as Cu-citrate (table 3). Gain:feed was not different ($p>0.10$) between treatments during phase I and II; however, over the entire nursery phase, animals consuming diets containing 66 ppm Cu as $CuSO_4$ had depressed ($p<0.10$) gain:feed compared to pigs fed all concentrations of Cu-citrate or the high concentration of $CuSO_4$ (table 3). Gain:feed in control pigs did not differ ($p>0.10$) from any of the Cu treatments (table 3).

Previous research has speculated that the growth promoting effects of Cu may be due to the antibiotic-like action of Cu in the gastrointestinal tract

Table 3. Effects of dietary copper concentration and source on growth performance of weanling pigs fed diets with an antibiotic (Exp. 1)[a]

Item	Cu added, ppm:	10	66	225	33	66	100	
	Cu source:	Sulfate	Sulfate	Sulfate	Citrate	Citrate	Citrate	SEM
ADG (kg)								
0~14 d		0.29	0.30	0.31	0.28	0.29	0.29	0.020
14~35 d		0.52	0.54	0.50	0.50	0.53	0.52	0.019
0~35 d		0.42	0.44	0.42	0.41	0.43	0.43	0.014
ADFI (kg)								
0~14 d		0.44[bc]	0.48[b]	0.47[bc]	0.42[c]	0.46[bc]	0.45[bc]	0.019
14~35 d		0.90[bc]	0.97[b]	0.85[c]	0.86[c]	0.90[bc]	0.90[bc]	0.032
0~35 d		0.72[bc]	0.77[b]	0.70[c]	0.68[c]	0.72[bc]	0.72[bc]	0.025
Gain:feed (kg:kg)								
0~14 d		0.66	0.63	0.66	0.67	0.63	0.64	0.028
14~35 d		0.58	0.56	0.59	0.58	0.59	0.58	0.015
0~35 d		0.58[bc]	0.57[b]	0.60[c]	0.60[c]	0.60[c]	0.60[c]	0.009

[a] Means represent 4 pens per treatment and 8 pigs per pen.
[b,c] Means in a row without a common superscript differ ($p<0.10$).

Table 4. Effects of dietary copper concentration and source on growth performance of weanling pigs fed diets without an antibiotic (Exp. 2)[a]

Item	Cu added, ppm:	10	66	225	33	66	100	SEM
	Cu source:	Sulfate	Sulfate	Sulfate	Citrate	Citrate	Citrate	
ADG (kg)								
0~14 d		0.19	0.23	0.23	0.21	0.20	0.23	0.022
14~35 d		0.46	0.48	0.44	0.45	0.46	0.46	0.029
0~35 d		0.35	0.38	0.36	0.35	0.36	0.37	0.026
ADFI (kg)								
0~14 d		0.35[bc]	0.39[b]	0.38[bc]	0.33[c]	0.38[bc]	0.37[bc]	0.018
14~35 d		0.86[bc]	0.96[b]	0.81[c]	0.94[b]	0.89[bc]	0.87[bc]	0.045
0~35 d		0.65[b]	0.73[c]	0.64[b]	0.69[bc]	0.68[bc]	0.67[bc]	0.031
Gain:feed (kg:kg)								
0~14 d		0.54	0.59	0.61	0.64	0.53	0.62	0.055
14~35 d		0.53	0.50	0.54	0.48	0.52	0.53	0.030
0~35 d		0.54	0.52	0.56	0.51	0.53	0.55	0.033

[a] Means represent 4 pens per treatment and 8 pigs per pen.
[b,c] Means in a row without a common superscript differ ($p<0.10$).

of pigs (Bunch et al., 1961; Hawbaker et al., 1961). When Cu is supplemented to a nursery diet with an antibiotic present, the growth of animals is greater than with diets only containing supplemental Cu (Stahly et al., 1980; Roof and Mahan, 1982; Edmonds et al., 1985). Therefore, it was hypothesized that the Cu effects upon growth may have been concealed in Exp. 1 by the growth stimulating effects of the antibiotic. However, in Exp. 2 there was no effect ($p>0.10$) on ADG with the supplementation of 225 ppm Cu as $CuSO_4$ during phase I, phase II, or over the entire nursery phase (table 4). In Exp. 2, as seen in Exp. 1, there were no differences ($p>0.10$) among the treatments with respect to ADG (table 4). Therefore, it is difficult to evaluate the effectiveness of Cu-citrate relative to $CuSO_4$, because the positive control (225 ppm Cu as $CuSO_4$) did not stimulate growth in weanling pigs in either experiment.

In phase I of Exp. 2, the animals receiving 66 ppm Cu as $CuSO_4$ had greater ($p<0.10$) ADFI than animals receiving 33 ppm Cu as Cu-citrate (table 4). During phase II, animals consuming diets that contained 66 ppm Cu as $CuSO_4$ or 33 ppm Cu as Cu-citrate had higher ($p<0.10$) ADFI than animals receiving 225 ppm Cu as $CuSO_4$ (table 4). Over the entire nursery phase, animals consuming diets supplemented with 66 ppm Cu as $CuSO_4$ had greater ($p<0.10$) ADFI than the control pigs or animals receiving 225 ppm Cu as $CuSO_4$ (table 4). During phase I, phase II, or over the entire nursery phase in Exp. 2, there was no effect of treatment ($p>0.10$) on gain:feed (table 4).

Table 5. Effects of dietary copper concentration and source on growth performance of grow-finish pigs (Exp. 1) fed diets with an antibiotic[a]

Item	Cu added, ppm: / Cu source:	10 Sulfate	66 Sulfate	225 Sulfate	33 Citrate	66 Citrate	100 Citrate	SEM
ADG (kg)								
Grower		0.77[b]	0.85[c]	0.84[cd]	0.79[bd]	0.83[cd]	0.82[cd]	0.021
Finisher		0.98[bc]	1.01[b]	0.93[c]	1.01[b]	0.96[bc]	0.95[bc]	0.028
Total		0.90[b]	0.95[c]	0.89[b]	0.93[bc]	0.91[bc]	0.90[b]	0.018
ADFI (kg)								
Grower		1.65[b]	1.91[c]	1.78[bc]	1.67[b]	1.72[bc]	1.73[bc]	0.092
Finisher		3.00[bc]	3.13[b]	2.85[c]	3.07[b]	2.98[bc]	2.98[bc]	0.075
Total		2.48[b]	2.66[c]	2.42[b]	2.53[bc]	2.49[bc]	2.49[bc]	0.072
Gain:feed (kg:kg)								
Grower		0.47	0.45	0.47	0.47	0.48	0.47	0.011
Finisher		0.33	0.32	0.33	0.33	0.32	0.32	0.010
Total		0.36	0.36	0.37	0.37	0.37	0.36	0.010

[a] Means represent 4 pens per treatment and 4 pigs per pen.
[b,c,d] Means in a row without a common superscript differ ($p<0.10$).

The supplementation of Cu in the form of cupric citrate did not improve gain as hypothesized. This organic Cu compound has been shown to improve gain at lower dietary concentrations than $CuSO_4$ in broiler chickens (Pesti and Bakalli, 1996; Ewing et al., 1998). In addition, cupric citrate has been reported to stimulate growth in weanling pigs at concentrations half that required for maximum growth with $CuSO_4$ (Dove, 1998). It is unclear as to why there was no effect on body weight gain with the supplementation of Cu-citrate; however, there was also no effect due to $CuSO_4$ in either Exp. 1 or 2. This may be explained in part by the cleanliness of the nursery facilities. In this facility, litters were moved as a farrowing group to a sanitized all-in-all-out nursery, in an attempt to maximize animal health and cleanliness of the facilities. Kornegay et al. (1975) and Stansbury et al. (1990) indicated that Cu or antibiotics may have little effect in a clean facility with healthy animals.

Sixteen pigs per treatment from Exp. 1 were continued through the grow-finish phase. During the growing phase, animals receiving 66 ppm Cu as $CuSO_4$, 225 ppm Cu as $CuSO_4$, 66 ppm Cu as Cu-citrate, and 100 ppm Cu as Cu-citrate had increased ($p<0.10$) ADG when compared to the control animals (table 5). In the finishing phase, pigs supplemented with 66 ppm Cu as $CuSO_4$ and 33 ppm Cu as Cu-citrate had improved ($p<0.10$) ADG compared to the pigs receiving 225 ppm Cu as $CuSO_4$ (table 5). When the growing and finishing phases were combined, animals consuming diets supplemented with 66 ppm Cu as $CuSO_4$ had increased ($p<0.10$) ADG compared to the control pigs and those consuming 225 ppm Cu as $CuSO_4$ or 100 ppm Cu as Cu-citrate (table 5).

The animals receiving 66 ppm Cu as $CuSO_4$ had increased ($p<0.10$) ADFI compared to the control and the animals fed 33 ppm Cu as Cu-citrate during the growing phase (table 5). In the finishing phase, pigs receiving 66 ppm Cu as $CuSO_4$ and pigs receiving 33 ppm Cu as Cu-citrate had greater ($p<0.10$) ADFI than animals supplemented with 225 ppm Cu as $CuSO_4$ (table 5). Over the entire grow-finish phase, animals consuming diets supplemented with 66 ppm Cu as $CuSO_4$ had increased ($p<0.10$) ADFI compared to pigs fed the control or high $CuSO_4$ diets (table 5). In the grower, finisher, and throughout the entire grow-finish phase, there were no differences ($p>0.10$) in gain:feed (table 5).

Reports indicating a gain response to pharmacological concentrations of Cu are inconsistent. Early reports indicated that 250 ppm Cu as $CuSO_4$ improved gain in growing pigs (Bowler et al., 1955; Barber et al., 1955; Barber et al., 1957). Later studies with $CuSO_4$ demonstrated that 125 ppm Cu was as effective as 250 ppm Cu in stimulating gain during the growing phase; however, over the entire grow-finish phase, 250 ppm Cu was more effective in increasing gain compared to 125 ppm Cu (Bellis, 1961; Ritchie et al., 1963; Kline et al., 1973). In weanling pigs, some studies indicate an improved (Stahly et al., 1980; Coffey et al., 1994) response or an equivalent (Roof and Mahan, 1982; Cromwell et al., 1989) response in gain with 125 ppm compared to 250 ppm supplemental Cu.

Pigs receiving 225 ppm Cu from $CuSO_4$ had higher ($p<0.10$) plasma Cu concentrations than those fed the control, 66 ppm Cu as $CuSO_4$, 66 ppm Cu as Cu-citrate, and 100 ppm Cu as Cu-citrate diets in Exp. 1 (table 6). In addition, pigs fed 225 ppm Cu as $CuSO_4$ had higher ($p<0.05$) plasma Cu concentrations in Exp. 2 (table 7) than pigs in other treatments. In

Table 6. Effects of dietary copper concentration and source on plasma, liver, and bile copper concentrations of weanling pigs fed diets with an antibiotic (Exp. 1)[a]

Item	Cu added, ppm: 10	66	225	33	66	100	SEM
	Cu source: Sulfate	Sulfate	Sulfate	Citrate	Citrate	Citrate	
Plasma Cu, ppm	1.60[b]	1.59[b]	1.71[c]	1.62[bc]	1.55[b]	1.58[b]	0.044
Liver Cu, ppm	26.9[d]	31.9[d]	514.9[e]	30.9[d]	36.4[d]	38.6[d]	40.39
Bile Cu, ppm	1.7[d]	2.0[d]	8.6[e]	1.6[d]	1.7[d]	2.3[d]	0.70

[a] Means represent samples collected from 2 random pigs per pen with 4 pens per treatment.
[b,c] Means in a row without a common superscript differ ($p<0.10$).
[d,e] Means in a row without a common superscript differ ($p<0.01$).

Table 7. Effects of dietary copper concentration and source on plasma, liver, and bile copper concentrations of weanling pigs fed diets without an antibiotic (Exp. 2)[a]

Item	Cu added, ppm: 10	66	225	33	66	100	SEM
	Cu source: Sulfate	Sulfate	Sulfate	Citrate	Citrate	Citrate	
Plasma Cu, ppm	1.26[bd]	1.31[b]	1.55[c]	1.16[bd]	1.11[d]	1.20[bd]	0.078
Liver Cu, ppm	24.2[e]	20.2[e]	627.5[f]	34.7[e]	29.6[e]	26.6[e]	68.12
Bile Cu, ppm	1.8[e]	1.5[e]	11.9[f]	2.4[e]	1.3[e]	1.6[e]	0.85

[a] Means represent samples collected from 2 random pigs per pen with 4 pens per treatment.
[b,c,d] Means in a row without a common superscript differ ($p<0.05$).
[e,f] Means in a row without a common superscript differ ($p<0.01$).

Exp. 2, pigs fed 66 ppm Cu from $CuSO_4$ had higher ($p<0.05$) plasma Cu concentrations than those fed the same level of Cu from Cu-citrate (table 7). Irrespective of Cu source, there was no linear ($p>0.10$) increase in plasma Cu with increasing Cu concentration in the diet. During the growing phase, animals receiving 225 ppm Cu as $CuSO_4$ had increased ($p<0.10$) plasma Cu concentrations compared with the control animals (table 8). In the finishing phase, animals receiving 225 ppm Cu as $CuSO_4$ and 100 ppm Cu as Cu-citrate had greater ($p<0.10$) plasma Cu concentrations than pigs fed 66 ppm Cu as $CuSO_4$ or 33 ppm Cu as Cu-citrate (table 8). These results are in contrast to Apgar et al. (1995), where they reported an increase in serum Cu concentrations with supplemental dietary Cu concentrations of 100, 150, and 200 ppm. However, Roof and Mahan (1982) reported an increase in plasma Cu concentrations only at supplemental Cu concentrations of 375 and 500 ppm, but not at or below 250 ppm.

Measurements of Cu bioavailability have traditionally focused on liver Cu concentrations. Numerous studies have demonstrated that liver Cu accumulation is linear when the dietary Cu concentration is greater than 250 ppm in pigs (Hedges and Kornegay, 1973; Cromwell et al., 1989), chicks (Czarnecki et al., 1984; Baker et al., 1991), and rats (Milne and Weswig, 1968). However, at dietary concentrations below 250 ppm, some studies have revealed no increase in liver Cu concentrations (Hedges and Kornegay, 1973; Cromwell et al., 1989; Cromwell et al., 1998). In the present studies, liver Cu concentrations increased ($p<0.01$) with the addition of 225 ppm Cu as $CuSO_4$ in Exp. 1 and 2 (table 6 and 7). There was a 18-fold and a 25-fold increase in liver Cu concentrations from the control to 225 ppm Cu as $CuSO_4$ in Exp. 1 and 2, respectively (table 6 and 7). There was no change ($p>0.10$) in liver Cu concentrations relative to the control treatment in Exp. 1 or 2 with increasing dietary concentrations of Cu-citrate up to 100 ppm Cu or with 66 ppm Cu as $CuSO_4$ (table 6 and 7). Therefore, the current data suggest that liver Cu concentrations do not provide an accurate assessment of the bioavailability of Cu sources when dietary Cu is fed below 225 mg Cu/kg diet to pigs not nutritionally deficient in Cu.

Bile Cu concentrations have been used to evaluate the bioavailability of various Cu sources in Cu-deficient chicks fed between 0 and 2 mg Cu/kg diet (Aoyagi and Baker, 1993a, b, c). In these studies, biliary Cu increased linearly when Cu was supplemented at concentrations up to 2 ppm. Bowland et al. (1961) reported that biliary excretion of Cu is the primary route of excreting absorbed Cu in pigs. Therefore, biliary Cu excretion was also used to assess the bioavailability of Cu-citrate relative to $CuSO_4$. Bile Cu was higher ($p<0.01$) in animals receiving diets supplemented with 225 ppm Cu as $CuSO_4$ in Exp. 1 and 2 (table 6 and 7). The lower concentrations of supplemental Cu did not increase ($p>0.10$) bile Cu

Table 8. Effects of dietary copper concentration and source on plasma copper concentrations of grow-finish pigs fed diets with an antibiotic (Exp. 1)[a]

Item	Cu added, ppm: / Cu source:	10 Sulfate	66 Sulfate	225 Sulfate	33 Citrate	66 Citrate	100 Citrate	SEM
Plasma Cu, ppm								
Grower		1.36[b]	1.49[bc]	1.64[c]	1.53[bc]	1.44[bc]	1.52[bc]	0.10
Finisher		1.65[bcd]	1.59[bc]	1.81[d]	1.55[b]	1.72[cd]	1.77[d]	0.07

[a] Means represent samples collected from 2 random pigs per pen with 4 pens per treatment.
[b,c,d] Means in a row without a common superscript differ ($p<0.10$).

compared to the control treatment (table 6 and 7). These data agree with Czarnecki et al. (1984), who reported that an increase in biliary Cu occurred only when greater than 250 ppm Cu were included in swine diets.

As a test for bioavailability, bile Cu concentrations provided the best measure in Cu-deficient chicks fed between 0 and 2 mg Cu/kg diet (Aoyagi and Baker, 1993a, b, c). However, liver Cu concentrations provided the best measure of bioavailability when Cu was fed at concentrations greater than 250 ppm (Baker et al., 1991). According to the current data, neither liver or bile Cu concentrations provide an accurate means to assess bioavailability in pigs fed adequate to pharmacological concentrations of Cu.

These data reveal no consistent effect of either $CuSO_4$ or Cu-citrate on performance variables; therefore, it is difficult to make an assessment on the efficacy of Cu-citrate relative to $CuSO_4$ as a Cu supplement. In addition, liver and bile Cu concentrations were not reliable indicators of bioavailability of Cu sources in pigs when adequate to pharmacological concentrations of Cu were fed.

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