Effects of Copper Sulfate, Zinc Oxide, and NeoTerramycin on Weanling Pig Growth and Antibiotic Resistance Rate for Fecal *Escherichia coli*

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Summary

A total of 180 weanling pigs (PIC TR4 ×1050, initially 11.1 lb and 21 d of age) were used in a 42-d growth trial to compare the effects of supplemental zinc, copper, and in-feed antimicrobial on weanling pig growth and antibiotic resistance of fecal *Escherichia coli*. There were 5 dietary treatments with 6 pens per treatment and 5 pigs per pen. Pens were assigned to dietary treatments in a randomized complete block design. Treatments were arranged in a 2 × 2 factorial design with main effects of copper sulfate (0 or 125 ppm) and zinc oxide (0 or 3,000 ppm for 14 d and 0 or 2,000 for 28 d). The fifth treatment was in-feed antimicrobial (50 g/ton neomycin sulfate and 50 g/ton oxytetracycline HCl). All diets were supplemented with165 ppm zinc and 16.5 ppm copper from the trace mineral premix. Fecal samples were collected from 3 pigs per pen on d 14 and 42 to determine total coliform and *E. coli* counts as well as *E. coli* antibiotic resistance rates.

Pigs fed added zinc oxide had increased ($P < 0.04$) ADG and tended to have improved ($P < 0.09$) ADFI and F/G from d 0 to 14. From d 14 to 42, pigs fed added zinc oxide had poorer ($P < 0.007$) F/G than those with no added zinc oxide, and pigs fed added copper sulfate had improved ($P < 0.07$) F/G compared with those fed no added copper sulfate. Over the entire 42-d trial, a trend for a copper × zinc interaction was detected ($P < 0.09$) for ADG as pigs fed the addition of copper sulfate or zinc oxide had increased ADG over the control; however, when zinc and copper were combined, growth rate was similar to that when each was added singularly. Therefore, no additive effects were observed in this experiment from feeding a combination of high levels of dietary copper and zinc.

Dietary addition of copper sulfate, zinc oxide, or in-feed antibiotic had no effect ($P > 0.22$) on total coliform or *E. coli* concentrations on d 14 or 42. For d-14 isolates, zinc supplementation had no effect ($P > 0.43$) on *E. coli* resistance rate to chlortetracycline, neomycin, oxytetracycline, or tiamulin; however, copper supplementation tended to increase ($P < 0.10$) resistance to chlortetracycline and oxytetracycline. A copper × zinc interaction was detected ($P < 0.02$) for *E. coli* resistance to chlortetracycline and neomycin from isolates on d 42. These interactions were related to a significant decrease in resistance when copper sulfate was fed alone.

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High levels of zinc oxide improved performance in the early postweaning period, whereas high levels of copper sulfate offered numeric advantages in the later phase. Although the resistance rate varied with dietary treatment, no clear pattern was detected.

Key words: bacterial sensitivity, copper, zinc

Introduction

Pharmacological levels of dietary zinc and copper have often been used to increase growth in weanling pigs. Nursery studies have demonstrated that increased dietary zinc can promote growth rates and decrease diarrhea in weanling pigs. Zinc oxide (ZnO) is the most commonly used form of zinc in diets for nursery pigs. Dietary copper also has been shown to enhance growth rates in weanling pigs, and copper sulfate ($CuSO_4$) is the most commonly used form. Previous research indicates that using both ZnO and $CuSO_4$ in the diet results in growth rates similar to those when ZnO is used alone. However, Shelton et al. (2008)[3] observed additive growth responses to feeding both ZnO and $CuSO_4$. Another unresolved question related to the addition of pharmacological levels of copper and zinc is the potential effects on antibiotic sensitivity. Research has shown links between feeding increased levels of copper and resistance of *Enterococci* to copper as well as to vancomycin and erythromycin. Therefore, the objective of this trial was to determine the effects of pharmacological levels of copper and zinc or an in-feed antibiotic combination on weanling pig performance and antibiotic resistance of fecal *Escherichia coli*.

Procedures

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS.

A total of 180 weanling pigs (PIC TR4 ×1050, initially 11.1 lb and 21 d of age) were used in a 42-d growth trial to compare the effects of supplemental zinc, copper, and an in-feed antibiotic on weanling pig growth and antibiotic resistance of fecal *E. coli*. Pigs were allotted to pens by initial BW, and pens were assigned to treatments in a randomized complete block design with both weight and location in the nursery serving as blocking factors. There were 6 pens per treatment with 5 pigs per pen. Treatments were arranged as a 2 × 2 factorial design with main effects of added copper from $CuSO_4$ (0 or 125 ppm) and added zinc from ZnO (0 or 3,000 ppm from d 0 to 14 and 0 or 2,000 ppm from d 14 to 42) along with an additional treatment with an in-feed antibiotic that providing neomycin (50 g/ton) and oxytetracycline (50 g/ton). The trace mineral premix supplied a base level of 165 ppm zinc and 16.5 ppm copper in all diets. The diets were fed in 2 phases: Phase 1 from d 0 to 14 and Phase 2 from d 14 to 42 (Table 1). Phase 1 and 2 diets were fed in meal form and formulated to contain 1.41% and 1.31% standard ileal digestible lysine, respectively. Phase 1 diets contained 15% spray-dried whey and 3.75% fish meal, and Phase 2 diets were corn-soybean meal based. Treatment diets were prepared by replacing cornstarch with ZnO, $CuSO_4$, or in-feed antibiotic.

[3] Shelton et al., Swine Day 2008, Report of Progress 1001, pp. 62-73.

Each pen contained a 4-hole dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Pens had wire-mesh floor and allowed for approximately 3 ft^2/pig. Weights and feed disappearance were measured every 14 d to determine ADG, ADFI, and F/G.

On d 14 and 42, fecal samples were collected from 3 randomly selected pigs per pen. Fecal samples were diluted, plated, and subsequently counted to determine the number of colony forming units per gram of sample for both *E. coli* and total coliforms. One colony per sample was then isolated and retained for further analysis. Minimum inhibitory concentrations (MIC) of antibiotics were then determined on each isolate by the micro-broth dilution method (CLSI, 2002[4]). The antibiotics evaluated included chlortetracycline, neomycin, oxytetracycline, and tiamulin. The MIC for each isolate was compared with published MIC values to determine whether each isolate was resistant or susceptible. Isolates were classified as resistant if the MIC was 16 μg/mL or higher for oxytetracycline, chlortetracycline, and neomycin and 32 μg/mL or higher for tiamulin. Finally, a pen resistant rate was calculated on the basis of the resistance for each pen's 3 isolates.

Pen was used as the experimental unit for all analyses, and data were analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). Main effects and potential interactions for added dietary copper and zinc were tested using contrast statements. Bacterial counts were log transformed to achieve normality. Pair-wise comparison was also used to test the difference between the control and antibiotic treatment.

Results

Over the first phase (d 0 to 14), pigs fed added dietary ZnO had improved ($P < 0.02$) ADG (Table 2). Dietary zinc additions also tended to increase ($P < 0.09$) ADFI and improve ($P < 0.06$) F/G. The addition of $CuSO_4$ did not affect ($P > 0.19$) ADG or ADFI, but a trend was detected for poorer ($P < 0.06$) F/G from d 0 to 14 compared with pigs fed no added copper. Also, no improvements ($P > 0.59$) in ADG, ADFI, or F/G were observed for pigs supplemented with in-feed antibiotics compared with pigs fed no added zinc or copper.

From d 14 to 28, no improvements in ADG or F/G were observed ($P > 0.14$) from supplementing dietary copper or zinc. However, a trend for a copper × zinc interaction was detected ($P < 0.07$). This interaction was due to increases in ADFI over the control when either copper or zinc were used independently; however, when copper and zinc were used in combination, ADFI was intermediate of that when either was singularly. In-feed antibiotic supplementation also increased ($P < 0.01$) ADFI and tended to increase ($P < 0.10$) ADG over that of pigs fed no added zinc or copper.

From d 28 to 42, ZnO and $CuSO_4$ supplementation did not increase ($P > 0.18$) ADG or ADFI. However, a trend for improved F/G was observed ($P < 0.06$) with $CuSO_4$ addition, and a trend for worsened F/G was observed ($P < 0.09$) with zinc addition. Adding the in-feed antibiotic also had no effect ($P > 0.71$) on pig ADG, ADFI, or F/G compared with pigs fed the control diet.

[4] Clinical and Laboratory Standard Institute (CLSI). 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Guideline-2nd ed. CLSI Document M31-A2. CLSI, Wayne, PA.

Over the entire Phase 2 (d 14 to 42), pigs fed the additional ZnO had poorer ($P < 0.007$) F/G and tended to have increased ($P < 0.07$) ADFI compared with those not receiving additional ZnO. Pigs fed supplemental $CuSO_4$ had improved ($P < 0.04$) F/G without increased ($P > 0.13$) ADG or ADFI from d 14 to 42. Antibiotic addition did not improve ($P > 0.28$) in ADG, ADFI, or F/G compared with control pigs.

A trend for a copper × zinc interaction was detected ($P < 0.09$) for ADG over the entire 42-d trial. The addition of copper or zinc increased ADG over the control; however, when copper and zinc were combined, pigs had reduced growth compared with that achieved when feeding each independently. Pharmacological levels of zinc also increased ($P < 0.04$) ADFI. Over the entire trial, the in-feed antimicrobial did not improve ($P > 0.28$) ADG, ADFI, or F/G.

Coliform and *E. coli* counts were not affected ($P > 0.22$) by dietary addition of $CuSO_4$, ZnO, or in-feed antimicrobials (Table 3). For d-14 isolates, dietary ZnO supplementation had no effect ($P > 0.43$) on the percentage of *E. coli* isolates classified as resistant for chlortetracycline, neomycin, oxytetracycline, or tiamulin. However, from d-14 isolates, $CuSO_4$ tended to increase ($P < 0.10$) the percentage of isolates resistant to chlortetracycline and oxytetracycline. Also, the in-feed antimicrobial tended to increase ($P < 0.10$) the percentage of isolates resistant to chlortetracycline and oxytetracycline compared with the controls. A copper × zinc interaction was detected ($P < 0.02$) for *E. coli* resistance to chlortetracycline and neomycin from isolates on d 42. These interactions were related to a significant decrease in the percentage of isolates classified as resistant when copper was fed alone. In-feed antibiotic and $CuSO_4$ dietary additions also tended to increase ($P < 0.10$) the percentage of *E. coli* isolates resistant to tiamulin on d 42.

Discussion

Results from this trial agree with previous research that showed that benefits from additional dietary zinc and copper were not additive in nature. The improvement in ADG and ADFI with ZnO supplementation from d 0 to 14 agrees with other research that shows that zinc improves growth early postweaning. Only marginal improvements were observed from adding cooper to the diet. Many other studies have shown a greater response to copper, which is usually apparent later in the nursery stage (d 14 to 42), than this study did. These results are in contrast with those of Shelton et al. (2008).

The copper × zinc interaction for *E. coli* resistance to chlortetracycline and neomycin from isolates on d 42 is an interesting observation from this study. We cannot explain a biological reason why resistance would drop dramatically when additional dietary copper was fed alone. It may have been an effect of sampling, as only 3 isolates per pen were used. Although the resistance rate varied with dietary treatment, no clear pattern was detected. Additional research is warranted to evaluate the effects of high levels of dietary copper and zinc additions on antibiotic resistance. In addition, more research is needed to understand the factors that may be affecting the effectiveness of high dietary levels of copper and zinc supplementation fed to increase growth rates of weanling pigs.

Table 1. Composition of diets[1]

Ingredient, %	Phase 1[2]	Phase 2[3]
Corn	48.72	60.74
Soybean meal (46.5% CP)	29.01	35.00
Spray-dried whey	15.00	---
Select menhaden fish meal	3.75	---
Monocalcium P (21% P)	1.05	1.60
Limestone	0.70	1.10
Salt	0.33	0.33
Vitamin premix	0.25	0.25
Trace mineral premix	0.15	0.15
Lysine HCl	0.30	0.30
DL-methionine	0.175	0.125
L-threonine	0.125	0.110
Cornstarch[4]	0.435	0.307
Total	100	100
Calculated analysis		
SID[5] amino acids, %		
Lysine	1.41	1.31
Isoleucine:lysine	60	63
Leucine:lysine	120	129
Methionine:lysine	36	33
Met & Cys:lysine	58	58
Threonine:lysine	62	62
Tryptophan:lysine	17	18
Valine:lysine	65	69
Total lysine, %	1.55	1.45
ME, kcal/lb	1,495	1,495
SID lysine:ME, g/Mcal	4.28	3.97
CP, %	22.3	21.9
Ca, %	0.88	0.85
P, %	0.78	0.75
Available P, %	0.50	0.42
Available P:calorie, g/Mcal	1.51	1.26

[1] A total of 180 weanling pigs (PIC, initially 11.1 lb and 21 d of age) were used in a 42-d experiment with 6 pens per treatment and 5 pigs per pen.
[2] Pigs were fed Phase 1 from d 0 to 14.
[3] Pigs were fed Phase 2 from d 14 to 42.
[4] Cornstarch was replaced with ZnO at 7.7 lb/ton in Phase 1 and 5.1 lb/ton in Phase 2, $CuSO_4$ at 1 lb/ton, or 5 lb/ton of Neo/Oxy 10/10 (Penfield Animal Health, Omaha, NE) to create treatment diets.
[5] Standardized ileal digestible.

Table 2. Effects of zinc oxide, copper sulfate, and antibiotic combination on weanling pig growth performance[1]

	Treatment[2]						Probability, $P<$			
	Control	Cu	Zn	Cu and Zn	Antibiotic	SEM	Zinc × Copper	Zinc	Copper	Antibiotic vs. Control
Initial wt, lb	11.1	11.1	11.1	11.1	11.1	0.049	0.82	0.86	0.91	0.89
d 0 to 14										
ADG, lb	0.37[a]	0.41[ab]	0.48[b]	0.46[b]	0.40[ab]	0.041	0.45	0.02	0.84	0.60
ADFI, lb	0.42[a]	0.49[ab]	0.50[ab]	0.52[b]	0.44[ab]	0.039	0.46	0.09	0.20	0.71
F/G	1.12[ab]	1.18[b]	1.03[a]	1.12[ab]	1.09[ab]	0.037	0.84	0.06	0.06	0.66
wt on d 14, lb	16.1[a]	16.6[ab]	17.7[b]	17.4[ab]	16.5[ab]	0.63	0.45	0.03	0.86	0.60
d 14 to 28										
ADG, lb	0.92	1.01	1.00	0.99	1.00	0.039	0.13	0.38	0.24	0.10
ADFI, lb	1.26[a]	1.36[ab]	1.42[b]	1.37[b]	1.40[b]	0.048	0.07	0.03	0.52	0.01
F/G	1.41	1.38	1.45	1.42	1.44	0.032	0.99	0.15	0.33	0.46
d 28 to 42										
ADG, lb	1.51	1.60	1.55	1.57	1.49	0.055	0.40	0.89	0.19	0.72
ADFI, lb	2.24	2.30	2.37	2.33	2.22	0.087	0.45	0.23	0.93	0.83
F/G	1.51[ab]	1.46[a]	1.55[b]	1.50[ab]	1.51[ab]	0.028	0.89	0.09	0.06	0.90
d 14 to 42										
ADG, lb	1.21[a]	1.30[b]	1.27[ab]	1.27[ab]	1.24[ab]	0.039	0.10	0.63	0.14	0.45
ADFI, lb	1.74[a]	1.82[ab]	1.89[b]	1.84[ab]	1.81[ab]	0.059	0.15	0.07	0.69	0.29
F/G	1.46[ab]	1.41[a]	1.50[b]	1.47[b]	1.47[b]	0.021	0.66	0.007	0.04	0.56
d 0 to 42										
ADG, lb	0.92[a]	1.00[b]	1.01[b]	0.99[ab]	0.95[ab]	0.034	0.09	0.15	0.21	0.39
ADFI, lb	1.29[a]	1.37[ab]	1.42[b]	1.40[b]	1.34[ab]	0.047	0.14	0.04	0.41	0.29
F/G	1.42	1.39	1.43	1.42	1.42	0.019	0.47	0.14	0.30	0.78
Final wt, lb	50.1	53.0	53.2	52.7	51.8	1.58	0.15	0.24	0.32	0.30

[1] A total of 180 weanling pigs (PIC, initially 11.1 lb and 21 d of age) were used in a 42-d experiment with 6 pens per treatment and 5 pigs per pen.

[2] Treatments were: control (basal diet with no added Cu or Zn), Cu (125 ppm of added Cu from $CuSO_4$), Zn (3,000 ppm from d 0 to 14 and 2,000 ppm from d 14 to 28 of added Zn from ZnO), Cu and Zn (125 ppm of added Cu from $CuSO_4$ and 3,000 ppm from d 0 to 14 and 2,000 ppm from d 14 to 28 of added Zn from ZnO), and antibiotic (55 ppm or 50 g/ton of neomycin and oxytetracycline from Neo/Oxy 10/10).

[ab] Within a row, means without a common superscript differ ($P < 0.05$).

Table 3. Effects of zinc oxide, copper sulfate, and antibiotic combination on fecal bacteria counts and *E. coli* and antibiotic resistance[1]

	Treatment[2]						Probability, *P*<			
	Control	Cu	Zn	Cu and Zn	Antibiotic	SEM	Zinc × Copper	Zinc	Copper	Antibiotic vs. Control
Coliform counts, Log_{10} CFU/g										
d 14	6.2	5.8	6.2	5.6	6.0	0.50	0.82	0.81	0.25	0.68
d 42	5.5	4.9	5.1	5.0	4.9	0.49	0.52	0.72	0.30	0.23
E. coli count, Log_{10} CFU/g										
d 14	5.9	5.3	5.9	5.4	5.6	0.53	0.91	0.95	0.25	0.62
d 42	4.7	4.2	4.8	4.6	4.3	0.52	0.78	0.59	0.38	0.49
Antibiotic-resistant *E. coli* isolates, %										
d-14 isolates										
Chlortetracycline[3]	56	89	61	78	92	14.1	0.57	0.85	0.10	0.09
Neomycin[3]	33	33	28	28	44	15.0	1.00	0.68	1.00	0.56
Oxytetracycline[3]	72^{ab}	94^{ab}	67^{a}	89^{ab}	100^{b}	11.2	1.00	0.63	0.07	0.10
Tiamulin[4]	100	94	100	100	94	3.5	0.44	0.44	0.44	0.28
d-42 isolates										
Chlortetracycline[3]	83^{b}	47^{a}	81^{b}	89^{b}	81^{b}	9.6	0.02	0.03	0.10	0.81
Neomycin[3]	78^{b}	25^{a}	67^{b}	83^{b}	81^{b}	11.2	0.01	0.05	0.13	0.87
Oxytetracycline[3]	94	72	86	89	94	8.4	0.16	0.63	0.27	1.00
Tiamulin[4]	90	100	94	100	100	3.7	0.59	0.59	0.06	0.09

[1] A total of 180 weanling pigs (PIC, initially 11.1 lb and 21 d of age) were used in a 42-d experiment with 6 pens per treatment and 5 pigs per pen.

[2] Treatments were: control (basal diet with no added Cu or Zn), Cu (125 ppm of added Cu from $CuSO_4$), Zn (3,000 ppm from d 0 to 14 and 2,000 ppm from d 14 to 28 of added Zn from ZnO), Cu and Zn (125 ppm of added Cu from $CuSO_4$ and 3,000 ppm from d 0 to 14 and 2,000 ppm from d 14 to 28 of added Zn from ZnO), and antibiotic (55 ppm or 50 g/ton of neomycin and oxytetracycline from Neo/Oxy 10/10).

[3] Isolates with a minimum inhibitory concentration of 16 µg/mL or higher for this antibiotic were considered resistant.

[4] Isolates with a minimum inhibitory concentration of 32 µg/mL or higher for this antibiotic were considered resistant.

[ab] Within a row, means without a common superscript differ ($P < 0.05$).