

# Comparative Effects of Dietary Copper, Zinc, Essential Oils, and Chlortetracycline on Nursery Pig Growth Performance<sup>1</sup>

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## Summary

A total of 350 weaned pigs (PIC 1050; initially 13.3 lb) were used in a 47-d study to compare the effects of feeding antibiotic alternatives (copper, zinc, and essential oils), alone or in combination, on nursery pig performance. Pigs were allotted to pens at weaning (d 0) and fed a common starter diet with no antimicrobial for 5 d before the start of the experiment. On d 5, pens of 5 pigs were allotted to 1 of 10 dietary treatments in a randomized complete block design with 7 replications per treatment. Dietary treatments were arranged in a  $2 \times 2 \times 2 + 2$  factorial with main effects of added copper sulfate ( $\text{CuSO}_4$ ; 0 vs. 125 ppm Cu), added zinc oxide ( $\text{ZnO}$ ; none vs. 3,000 ppm Zn from d 5 to 12 and 2,000 ppm Zn from d 12 to 33), and Regano EX (0 vs. 45 g/ton essential oils blend; Ralco Animal Nutrition, Marshall, MN). The 2 additional treatments were growth-promoting and therapeutic levels of chlortetracycline (CTC at 50 or 400 g/ton). Pigs were fed experimental diets from d 5 to 33 followed by a common corn-soybean meal–based diet without any antimicrobial, essential oils, or pharmacological levels of Cu or Zn from d 33 to 47. To comply with FDA guidelines, CTC was removed on d 19 from the diet of pigs fed 400 g/ton CTC, then added again from d 20 to 33. All diets contained 16.5 ppm Cu and 165 ppm of Zn from the trace mineral premix. Essential oils had no effect on daily gain, but feeding CTC or pharmacological levels of Cu or Zn improved the growth rate of nursery pigs. Carryover effects from any of these dietary treatments on subsequent nursery growth performance were minimal. Although there were no improvements in feed efficiency due to Cu or Zn, the inclusion of an essential oils blend worsened feed and caloric efficiencies.

Key words: chlortetracycline, nursery pig, antibiotic, essential oil, copper, zinc

## Introduction

As alternatives to dietary antibiotics are increasingly sought, essential oils are one type of feed additive being explored. Essential oils were once thought to improve feed palatability and intake due to their taste and strong aroma; however, the potential for essential oils to improve nursery pig growth performance may lie in enhancing immune function, exercising their antimicrobial properties in the gastrointestinal tract of the pig, or improving protein digestibility, thereby improving F/G. The nursery growth benefits obtained by feeding pharmacological levels of copper (Cu) and zinc (Zn) are

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well established, but a direct comparison of feed antimicrobials, pharmacologic levels of Cu and Zn, and essential oils is lacking. Therefore, the objective of this experiment was to compare the performance of nursery pigs fed diets containing chlortetracycline (CTC) and different dietary supplements that are commonly fed as antibiotic alternatives (Cu, Zn, and oregano essential oils), alone or in combination with each other.

### Procedures

This trial was conducted in collaboration with the Kansas State University College of Veterinary Medicine Department of Diagnostic Medicine/Pathology with the primary objective of evaluating the potential impact of different types of feed additives used as antibiotic alternatives on antimicrobial resistance of enteric bacteria in pigs. This report describes the growth performance of these same pigs, whereas the impact on antimicrobial resistance will be reported elsewhere.

The protocol for this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the K-State Segregated Early Weaning Facility in Manhattan, KS.

A total of 350 nursery pigs (PIC 1050; initially 13.3 lb BW) were used in a 47-d study with 5 pigs per pen and 7 replications per treatment. Each pen had metal tri-bar flooring, one 4-hole self-feeder, and a cup waterer to provide ad libitum access to feed and water. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW to achieve equal average pen weights across all pens (d 0). During the time period in which the pigs were obtained, the sow farm of origin was experiencing active swine influenza circulation. Weaned pigs also exhibited clinical signs of influenza infection upon entry into the barn, and we believe this contributed to the elevated 4% removal rate during the study. To remove the confounding effect of concurrent diet treatment with injectable antimicrobial, pigs with clinical signs for which injectable treatment was deemed necessary were removed from the test, which contributed to the elevated removal rate. Removal rate was not influenced by dietary treatment.

Pigs were fed a common pelleted starter diet for the first 5 d after weaning. This diet contained no antimicrobial, no essential oils, nor any added Zn or Cu above that contained in the trace mineral premix. On d 5, pens of pigs were weighed and randomly allotted to 1 of 10 dietary treatments in blocks by barn location. The 10 dietary treatments consisted of a corn-soybean meal-based diet and were arranged as a  $2 \times 2 \times 2 + 2$  factorial with main effects of added Cu from copper sulfate ( $\text{CuSO}_4$ ; 0 vs. 125 ppm Cu), added Zn from zinc oxide ( $\text{ZnO}$ ; 0 vs. 3,000 ppm Zn from d 5 to 12 and 2,000 ppm Zn from d 12 to 33), or Regano EX (0 vs. 45 g/ton essential oils blend; Ralco Animal Nutrition, Marshall, MN). The 2 additional treatments were CTC at growth-promoting (50 g/ton) or therapeutic (400 g/ton) levels. The treatment ingredients were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1).

The experimental diets were fed from d 5 to 33. Food and Drug Administration regulations prohibit the continuous feeding of therapeutic levels of CTC longer than 14 d.<sup>3</sup>

<sup>3</sup> Code of Federal Regulations. Title 21; Volume 6; Sec. 558.128. Accessed at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcr/CFRSearch.cfm?fr=558.128> on August 14, 2014.

Thus, on d 19 of the study, the feeders from pens assigned to the 400 g/ton CTC diet were emptied, and pigs were fed the control diet for 1 d. The normal treatment diet containing CTC at 400 g/ton was then re-added on d 20 and fed for the remainder of the 28-d period. From d 33 to 47, a common corn-soybean meal–based diet without any antimicrobial, essential oils, or pharmacological levels of Cu or Zn was fed to all pigs to evaluate any carryover effects from the treatment diets.

All diets were prepared at the K-State O.H. Kruse Feed Technology Innovation Center and contained 16.5 ppm Cu and 165 ppm of Zn from the trace mineral premix. Diet samples were collected periodically throughout the study, and pooled samples of each diet were submitted to Ward Laboratories, Inc. (Kearney, NE) for near-infrared reflectance (NIR) spectrometry analysis of Cu and Zn (Table 2). As determined by analysis, the Phase 1 common diet contained 114 ppm Zn and 24 ppm Cu, whereas the Phase 3 common diet contained 144 ppm Zn and 24 ppm Cu. Average daily gain, ADFI, and F/G were determined by weighing pigs and measuring feed disappearance on d 5, 12, 19, 33, and 47.

Growth data were analyzed as a randomized complete block design using PROC MIXED in SAS (v9.3, SAS Institute Inc., Cary, NC) with pen as the experimental unit and barn location as a blocking factor. The main effects of Cu from  $\text{CuSO}_4$ , Zn from ZnO, essential oils from Regano EX essential oils blend, and CTC from CTC-50, as well as any interactions, were tested using preplanned CONTRAST statements. Linear and quadratic contrasts were used for the CTC treatments. Differences between treatments were determined by using least squares means, with results considered significant at  $P \leq 0.05$  and a trend at  $P \leq 0.10$ . Analysis of studentized residual values revealed a geographic cluster of four pens, each on a different treatment (essential oils, Cu+Zn, Cu+essential oils, Cu+Zn+essential oils), which had ADG or feed efficiency observations greater than three standard deviations from the mean. Taking this as evidence for data outliers, these pens were removed from the dataset used for analysis.

## Results and Discussion

During the d 5 to 33 treatment period, increasing CTC increased (linear,  $P = 0.03$ ) ADG, resulting in a tendency for pigs fed increasing CTC to have greater d-33 BW (linear,  $P = 0.07$ ; see Tables 3 and 4). During this period, there was also a tendency for a linear increase in ADFI ( $P = 0.08$ ) with increasing CTC. When the pigs ceased consuming CTC and were on the common diet from d 33 to 47, there was a tendency for a linear reduction in ADG for pigs previously fed CTC ( $P = 0.10$ ). Consequently, CTC had no effect on overall ADG or ADFI from d 5 to 47. Although CTC failed to affect F/G during either the treatment period or the succeeding common period, increasing CTC level had a tendency to improve (quadratic,  $P = 0.08$ ) overall F/G and caloric efficiency from d 5 to 47, with pigs fed CTC at 50 g/ton having the best feed efficiency.

During the treatment period from d 5 to 33, there was a tendency for a 3-way interaction between essential oils, Cu, and Zn ( $\text{Cu} \times \text{EO} \times \text{Zn}$ ,  $P = 0.10$ ), by which feeding all three in combination resulted in a lower ADG than would have been expected if improvements were additive. Both Cu and Zn increased ( $P < 0.01$ ) ADG, resulting in greater ( $P < 0.05$ ) BW on d 33 at the end of the treatment period. Furthermore, feeding

Zn increased ADFI, while Cu tended to do the same ( $P < 0.01$  and  $P = 0.06$ , respectively). Although essential oils had no effect on feed intake during the treatment period, a Cu (regardless of Zn inclusion)  $\times$  essential oils interaction ( $P = 0.03$ ) was observed due to poorer than expected F/G of pigs when Cu was fed in combination with essential oils. The interaction thus facilitated the main effect of essential oils worsening F/G ( $P = 0.01$ ) during the treatment period. This interaction and main effect of essential oils were also observed when considering efficiency on a caloric basis for both ME and NE (Cu  $\times$  EO,  $P = 0.03$ ; main effect of EO,  $P = 0.02$ ) during the treatment period. A tendency for Zn to improve caloric efficiency ( $P = 0.09$  for ME and 0.08 for NE) also was observed from d 5 to 33.

Despite a tendency for greater ADG (Cu  $\times$  Zn,  $P = 0.10$ ) of pigs previously fed diets with both Cu and Zn relative to growth of pigs previously fed diets with either Cu or Zn alone, during the 14-d common period from d 33 to 47, there was no effect of previous Zn, Cu, or essential oils dietary treatment on subsequent nursery pig growth performance. Overall from d 5 to 47, Zn increased ( $P < 0.05$ ) ADG and ADFI, while Cu improved ( $P = 0.02$ ) ADG, resulting in greater ending d-47 BW ( $P = 0.03$  for Zn, 0.10 tendency for Cu). Although essential oils had no observed effect on overall ADG or ADFI, essential oils tended to have an adverse effect on overall F/G ( $P = 0.10$ ).

In summary, feeding CTC or pharmacological levels of Cu or Zn improved the growth rate of nursery pigs with coinciding increases in feed intake. Feeding essential oils had no effect on daily gain but resulted in poorer feed and caloric efficiencies during the treatment period. In addition, carryover effects from any of the dietary treatments on subsequent nursery growth performance were minimal. Previous research at K-State found no effects of feeding essential oils, whereas other research has reported improved feed efficiency from feeding essential oils. This inconsistency in feed efficiency responses warrants further research to better characterize the effects of essential oils on feed efficiency amongst pigs with differing health statuses. In closing, this study further demonstrates the positive effects of added Zn, Cu, or CTC on the growth performance of weaned pigs.

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**Table 1. Diet composition (as-fed basis)**

	Phase 1 common diet (d 0 to 5)	Phase 2 experimental diets (d 5 to 26)	Phase 3 common diet (d 26 to 47)
Ingredient, %			
Corn	37.54	54.73	63.83
Soybean meal (47.7% CP)	19.86	29.53	32.86
Spray-dried blood cells	1.25	1.25	---
Spray-dried animal plasma	4.00	---	---
Corn DDGS <sup>1</sup> , 6 - 9% oil	5.00	---	---
Select menhaden fish meal	1.25	1.25	---
Spray-dried whey	25.00	10.00	---
Choice white grease	3.00	---	---
Monocalcium phosphate	0.90	0.80	1.00
Limestone	1.00	1.10	1.03
Salt	0.30	0.30	0.35
L-lysine HCL	0.225	0.300	0.300
DL-methionine	0.150	0.175	0.115
L-threonine	0.085	0.150	0.115
Trace mineral premix	0.150	0.150	0.150
Vitamin premix	0.250	0.250	0.250
Choline chloride, 60%	0.035	---	---
Phytase <sup>2</sup>	---	0.015	0.015
CuSO <sub>4</sub> , ZnO, Regano EX, CTC-50 additives <sup>3</sup>	---	0 to 0.965	---
Total	100.00	100.00	100.000

*continued*

**Table 1. Diet composition (as-fed basis)**

	Phase 1 common diet (d 0 to 5)	Phase 2 experimental diets (d 5 to 26)	Phase 3 common diet (d 26 to 47)
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lysine	1.40	1.35	1.22
Isoleucine:lysine	56	58	63
Leucine:lysine	128	125	129
Methionine:lysine	32	35	33
Met & Cys:lysine	57	58	57
Threonine:lysine	63	64	63
Tryptophan:lysine	19	18	19
Valine:lysine	71	69	69
Total lysine, %	1.57	1.50	1.37
CP, %	22.2	22.2	21.4
ME, kcal/lb	1,574	1,493	1,484
NE, kcal/lb <sup>4</sup>	1,179	1,102	1,093
SID lysine:ME, g/Mcal	4.0	4.1	3.7
Ca, %	0.85	0.80	0.70
P, %	0.73	0.63	0.61
Available P, %	0.51	0.44	0.39

<sup>1</sup> Dried distillers grains with solubles.

<sup>2</sup> HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 184.3 phytase units (FTU)/lb and an estimated release of 0.10% available P.

<sup>3</sup> Treatment diets contained zinc oxide added at 0 or 0.415% from d 5 to 12 and at 0 or 0.28% from d 12 to 33, copper sulfate added at either 0 or 0.05%, Regano EX (Ralco Animal Nutrition, Marshall, MN) containing approximately 5% essential oils blend added at either 0 or 0.1%, and CTC-50 added at 0, 0.05, or 0.4%. Additions of treatment ingredients were made in place of an equivalent amount of corn in respective experimental diets.

<sup>4</sup> NE values for ingredients were derived from NRC (2012).

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**Table 2. Analyzed dietary mineral concentrations (as-fed basis)<sup>1</sup>**

Diets	Phase 2 treatment diets <sup>2</sup>				Analyzed composition	
	Added Copper, ppm	Added Zinc, ppm	Essential oils blend, g/ton <sup>3</sup>	Chlortetracycline (CTC), g/ton	Zn, ppm	Cu, ppm
Control	-	-	-	-	140	16
Cu	125	-	-	-	115	109
Zn <sup>4</sup>						
d 5 to 12	-	3,000	-	-	2,110	20
d 12 to 33	-	2,000	-	-	1,632	25
Essential oils (EO)	-	-	45	-	177	25
Cu + Zn <sup>4</sup>						
d 5 to 12	125	3,000	-	-	2,254	166
d 12 to 33	125	2,000	-	-	1,778	135
Cu + EO	125	-	45	-	385	161
Zn + EO <sup>4</sup>						
d 5 to 12	-	3,000	45	-	2,166	19
d 12 to 33	-	2,000	45	-	1,780	21
Cu + Zn + EO <sup>4</sup>						
d 5 to 12	125	3,000	45	-	2,181	120
d 12 to 33	125	2,000	45	-	1,701	137
CTC 50	-	-	-	50	219	22
CTC 400	-	-	-	400	205	22

<sup>1</sup> Analysis was performed by Ward Laboratories, Inc. (Kearney, NE) on pooled diet samples.

<sup>2</sup> Experimental Phase 2 treatment diets were fed from d 5 to 33, whereas a Phase 1 common diet (114 ppm Zn and 24 ppm Cu as per analysis) was fed to all pigs from d 0 to 5 and a Phase 3 common diet (144 ppm Zn and 24 ppm Cu as per analysis) was fed to all pigs from d 33 to 47.

<sup>3</sup> From Regano EX (Ralco Animal Nutrition, Marshall, MN).

<sup>4</sup> Pharmacological Zn diet treatments had an addition of 3,000 ppm Zn from added ZnO from d 5 to 12 and an addition of 2,000 ppm Zn from added ZnO from d 12 to 33.

**Table 3. Effects of dietary copper, zinc, essential oils, and chlortetracycline (CTC) on nursery pig growth performance<sup>1,2</sup>**

	Added Cu <sup>3</sup> :	-	+	-	-	+	+	-	+	-	-	
	Added Zn <sup>4</sup> :	-	-	+	-	+	-	+	+	-	-	
	Essential oil blend <sup>5</sup> :	-	-	-	+	-	+	+	+	-	-	
	CTC, g/ton:	-	-	-	-	-	-	-	-	50	400	SEM
BW, lb												
d 5	14.5	14.5	14.5	14.4	14.6	14.8	14.5	14.6	14.4	14.4	14.4	0.19
d 33	41.8	43.2	43.7	42.1	45.1	43.9	43.8	44.0	41.6	43.3	43.3	0.76
d 47	64.0	64.6	65.4	63.6	67.4	65.0	65.0	65.9	63.7	64.5	64.5	1.11
d 5 to 33												
ADG, lb	0.96	1.01	1.04	0.92	1.09	1.04	1.05	1.05	0.96	1.02	1.02	0.026
ADFI, lb	1.24	1.28	1.34	1.21	1.34	1.36	1.34	1.37	1.21	1.31	1.31	0.040
F/G	1.29	1.27	1.30	1.32	1.23	1.31	1.28	1.31	1.27	1.28	1.28	0.020
d 33 to 47												
ADG, lb	1.59	1.53	1.55	1.53	1.59	1.51	1.51	1.56	1.58	1.52	1.52	0.035
ADFI, lb	2.57	2.48	2.53	2.50	2.55	2.48	2.50	2.52	2.47	2.47	2.47	0.062
F/G	1.62	1.62	1.63	1.63	1.61	1.64	1.65	1.62	1.57	1.63	1.63	0.030
d 5 to 47												
ADG, lb	1.17	1.18	1.21	1.11	1.26	1.20	1.20	1.21	1.16	1.18	1.18	0.024
ADFI, lb	1.68	1.67	1.74	1.61	1.75	1.73	1.73	1.74	1.62	1.69	1.69	0.042
F/G	1.44	1.42	1.44	1.45	1.39	1.45	1.44	1.44	1.40	1.42	1.42	0.019
Caloric efficiency <sup>6</sup>												
d 5 to 33												
ME	1,933	1,888	1,929	1,968	1,836	1,952	1,903	1,947	1,889	1,902	1,902	27.6
NE	1,427	1,393	1,423	1,452	1,355	1,440	1,404	1,436	1,394	1,403	1,403	21.8
d 5 to 47												
ME	2,144	2,110	2,141	2,157	2,065	2,155	2,134	2,133	2,082	2,117	2,117	27.9
NE	1,580	1,555	1,578	1,589	1,522	1,589	1,573	1,572	1,535	1,560	1,560	20.6

<sup>1</sup> A total of 350 nursery pigs (PIC 1050, initially 13.3 lb BW) were used in a 47-d study with 5 pigs per pen and 7 replications per treatment except for 4 treatments (essential oils, Cu+Zn, Cu+essential oils, Cu+Zn+essential oils), which had 6 replications each.

<sup>2</sup> Experimental treatment diets were fed from d 5 to d 33. All diets contained 16.5 ppm Cu and 165 ppm of Zn from the trace mineral premix.

<sup>3</sup> Cu from CuSO<sub>4</sub> was added to treatment diets at either 0 or 125 ppm.

<sup>4</sup> Pharmacological Zn diet treatments had an addition of 3,000 ppm Zn from added ZnO from d 5 to 12 and an addition of 2,000 ppm Zn from added ZnO from d 12 to 33.

<sup>5</sup> Regano EX (Ralco Animal Nutrition, Marshall, MN) was added to treatment diets at either 0 or 45 g/ton.

<sup>6</sup> Caloric efficiency is expressed as kcal per pound of live weight gain.

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**Table 4. Statistical analysis of dietary copper, zinc, essential oils, and chlortetracycline (CTC) on nursery pig growth performance<sup>1</sup>**

	Probability, <i>P</i> <							CTC	
	Cu	Zn	Essential oils (EO)	Cu × Zn	Cu × EO	Zn × EO	Cu × Zn × EO	Linear	Quadratic
BW, lb									
d 0	0.461	0.804	0.704	0.207	0.362	0.849	0.106	0.100	0.036
d 5	0.250	0.976	0.685	0.830	0.442	0.710	0.519	1.000	0.723
d 33	0.022	0.009	0.965	0.437	0.689	0.331	0.463	0.074	0.739
d 47	0.099	0.034	0.514	0.796	0.945	0.516	0.544	0.590	0.808
d 0 to 5									
ADG, lb	0.170	0.985	0.695	0.675	0.547	0.692	0.741	0.737	0.944
d 5 to 33 <sup>2</sup>									
ADG, lb	0.003	<0.001	0.605	0.120	0.822	0.707	0.098	0.028	0.755
ADFI, lb	0.055	0.006	0.444	0.173	0.182	0.798	0.444	0.079	0.392
F/G	0.153	0.165	0.011	0.871	0.025	0.860	0.143	0.831	0.227
d 33 to 47									
ADG, lb	0.928	0.608	0.136	0.095	0.692	0.965	0.782	0.101	0.987
ADFI, lb	0.675	0.696	0.355	0.347	0.668	0.978	0.675	0.377	0.222
F/G	0.613	0.966	0.493	0.296	0.961	0.993	0.797	0.320	0.144
d 5 to 47									
ADG, lb	0.018	0.001	0.207	0.573	0.621	0.825	0.111	0.422	0.771
ADFI, lb	0.225	0.025	0.818	0.425	0.225	0.942	0.304	0.499	0.240
F/G	0.138	0.278	0.099	0.561	0.146	0.972	0.562	0.957	0.084
Caloric efficiency <sup>3</sup>									
d 5 to 33									
ME	0.138	0.089	0.015	0.870	0.025	0.858	0.144	0.645	0.226
NE	0.137	0.084	0.015	0.870	0.025	0.858	0.144	0.631	0.226
d 5 to 47									
ME	0.131	0.207	0.111	0.560	0.147	0.976	0.561	0.937	0.084
NE	0.131	0.202	0.111	0.560	0.147	0.977	0.560	0.930	0.084

<sup>1</sup>A total of 350 nursery pigs (PIC 1050; initially 13.3 lb BW) were used in a 47-d study with 5 pigs per pen and 7 replications per treatment except for 4 treatments (essential oils, Cu+Zn, Cu+essential oils, Cu+Zn+essential oils), which had 6 replications each.

<sup>2</sup> Experimental treatment diets were fed from d 5 to d 33. All diets contained 16.5 ppm Cu and 165 ppm of Zn from the trace mineral premix.

<sup>3</sup> Caloric efficiency is expressed as kcal per pound of live weight gain.