Effects of dietary chlortetracycline, *Origanum* essential oil, and pharmacological Cu and Zn on growth performance of nursery pigs

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ABSTRACT: Two 47-d experiments were conducted with 21-d-old weaned pigs (PIC 1050, initially 6.1 kg) to determine the effects of feeding low or high doses of chlortetracycline (**CTC**) and antibiotic alternatives (Cu, Zn, and essential oil [EO]), alone or in combination, on growth performance. On d 5 postweaning, pens of 5 pigs were allotted to diet treatments with 8 (exp. 1) or 7 (exp. 2) replicate pens per treatment. In exp. 1, treatments were fed from d 5 to 26 postweaning and arranged in a 2 × 3 factorial with main effects of added ZnO (0 vs. 2,500 ppm of Zn) and CTC (0, 55, or 441 ppm). In exp. 2, treatments were fed from d 5 to 33 and structured in a (2 × 2 × 2) + 2 factorial with main effects of added $CuSO_4$ (0 vs. 125 ppm Cu), added ZnO (0 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. 0.1% Regano EX containing 5% *Origanum* oil). The 2 additional treatments were subtherapeutic (55 ppm) and therapeutic (441 ppm) levels of CTC. Following the treatment period, a common diet without antimicrobial was fed until d 47. All diets contained 16.5 ppm Cu and 110 ppm Zn from the trace mineral premix. In exp. 1, no ZnO × CTC interactions were observed. Feeding ZnO increased ($P < 0.05$) ADG, ADFI, and BW during the treatment period and increased ($P < 0.05$) ADG and ADFI overall (d 5 to 47). Pigs fed CTC had increased (linear, $P < 0.05$) ADG, ADFI, and BW during the treatment period and had marginally significant increases (linear, $P < 0.10$) in overall ADG and ADFI, but overall G:F tended (quadratic, $P = 0.070$) to increase then decrease as CTC increased. During the treatment period in exp. 2, EO did not affect ADG or ADFI, whereas pharmacological levels of Cu, Zn, and CTC increased ($P < 0.05$) ADG with coinciding increases ($P = 0.055$, 0.006, and linear 0.079, respectively) in ADFI. Copper, Zn, and CTC did not affect G:F. EO decreased ($P = 0.009$) G:F. Diet treatments had minimal carryover effects on subsequent nursery pig growth performance. Overall from d 5 to 47, Cu increased ($P = 0.018$) ADG, Zn increased ($P < 0.05$) ADG and ADFI, and EO tended to decrease ($P = 0.086$) G:F. In conclusion, increased dietary Cu, Zn, or CTC improved weanling pig performance while EO elicited no growth benefits. The benefits of added Zn from ZnO and CTC were additive and could be included together in diets to maximize growth performance of weaned pigs.

Key words: Cu, chlortetracycline, growth performance, nursery pigs, oregano oil, Zn

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INTRODUCTION

Dietary inclusion of feed-grade antimicrobials is a common practice in food animal agriculture

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since 1950s. They have been particularly useful in swine production to improve growth rate and feed efficiency in piglets. With the growing public health concern on antimicrobial resistance, there is always a need for safe and effective alternatives to antibiotics intended for swine production. The broad-spectrum antibiotic chlortetracycline (**CTC**) has long been shown to improve the rate of gain and feed efficiency of pigs (Taylor and Rowell, 1957; NCR-89, 1984). Yet, alternative means of improving young pig growth performance via feed additives have been sought particularly with growing concern about antimicrobial resistance (Turner et al., 2001).

Feeding pharmacological levels of Zn from ZnO or Cu from $CuSO_4$ consistently improves feed intake and growth rate of nursery pigs (Pérez et al., 2011; Shelton et al., 2011). Essential oils (EOs) have also been evaluated as alternatives to dietary antibiotics. The major constituents of oregano (*Origanum vulgare*) EO are the bioactive phenolic compounds carvacrol and thymol (Burt, 2004) which have antioxidant properties and antimicrobial action against both Gram-positive and Gram-negative bacteria (Windisch et al., 2008). EOs have been proposed to improve growth through establishment of a healthier gastrointestinal microbiota with fewer pathogenic bacteria, less microbial fermentation, and thus an intestinal environment with enhanced digestive and absorptive capacity (Windisch et al., 2008).

Limited research has assessed whether pharmacological Zn influences the pigs' response to CTC and also whether dietary supplements commonly fed as antibiotic alternatives have interactive effects on weaned pig growth performance. Thus, 2 experiments were conducted to 1) evaluate whether a high level of dietary Zn fed in combination with low dosage or therapeutic levels of CTC have interactive effects on the performance of nursery pigs; and 2) compare the growth performance of pigs fed CTC with that of pigs fed pharmacological levels of Cu, pharmacological levels of Zn, and oregano EO, alone or in combination.

MATERIALS AND METHODS

General Methodologies

The protocols for these experiments were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC #3135). The experiments were conducted at the Kansas State University's Segregated Early Weaning Facility in Manhattan, KS. Each pen (3.2 m^2) 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. To avoid any potentially confounded treatment responses due to postweaning lags in feed intake, all pigs were fed a common pelleted starter diet for the first 5 d after weaning. On d 5 postweaning, pens of 5 pigs each were randomly allotted to dietary treatments in a randomized complete block design with blocks based on location within barn.

The initial common diet contained no antimicrobial, no EO, nor any added Cu or Zn above that contained in the trace mineral premix (Table 1). The test ingredients were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1). Diet samples were collected periodically throughout the study. Pooled samples of each diet were analyzed for DM (method 935.29; AOAC, 2012); CP (method 990.03; AOAC, 2012); crude fat using method 920.39a (AOAC, 2012) for preparation and ANKOM solvent extraction procedure (ANKOM, 2004) with ANKOM XT20 Fat Analyzer (Ankom Technology; Fairport, NY); crude fiber (CF) using method 978.10 (AOAC, 2012) for preparation and ANKOM CF determination procedure (ANKOM, 2005) with ANKOM 2000 Fiber Analyzer (Ankom Technology; Fairport, NY); ash (method 942.05; AOAC, 2012); minerals and metals (in duplicate) with sample preparation according to method 968.08b (AOAC, 2012) and analysis using an iCAP 6500 series ICP Emission Spectrometer (Thermo Electron Corp., Marietta, OH) (Ward Laboratories, Inc., Kearney, NE; Table 2). Dietary ME and NE values were derived from feed ingredient energy values based on those in the NRC (2012).

Experiment 1

Animals and management. A total of 240 nursery pigs (PIC 1050; initially 6.08 kg BW) were used in a 47-d study with 5 pigs per pen and 8 replications per treatment. Treatment diets were fed from d 5 to 26 postweaning at which time all pigs received a common diet and growth performance was monitored for an additional 3 wk to d 47 postweaning to assess potential carryover effects of dietary treatment. Average daily gain, ADFI, and G:F were determined by weighing pigs and measuring feed disappearance on d 5, 26, and 47.

Diet composition. The 6 dietary treatments were arranged in a 2 × 3 factorial with main effects of added Zn from ZnO (0 vs. 2,500 ppm

Table 1. Diet composition (as-fed basis)[a]

	Phase 1 common diet (d 0 to 5)	Phase 2 experimental diets (d 5 to 26 or 33)	Phase 3 common diet (d 26 or 33 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[b], 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-Lys HCL	0.225	0.300	0.300
DL-Met	0.150	0.175	0.115
L-Thr	0.085	0.150	0.115
Trace mineral premix[c]	0.150	0.150	0.150
Vitamin premix[d]	0.250	0.250	0.250
Choline chloride, 60%	0.035	—	—
Phytase[e]	—	0.015[d]	0.015[d]
$CuSO_4$, ZnO, Regano EX, CTC-50 additives[f]	—	0 to 0.965	—
Total	100.00	100.00	100.000
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lys	1.40	1.35	1.22
Ile:Lys	56	58	63
Leu:Lys	128	125	129
Met:Lys	32	35	33
Met & Cys:Lys	57	58	57
Thr:Lys	63	64	63
Trp:Lys	19	18	19
Val:Lys	71	69	69
Total Lys, %	1.57	1.50	1.37
CP, %	22.2	22.2	21.4
ME, kcal/kg	3,470	3,291	3,272
NE, kcal/kg	2,599	2,429	2,410
SID Lys: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[g]	0.39[7]

[a] Common phase 1 diet was fed from d 0 to 5 after weaning, experimental phase 2 diets were fed from d 5 to 26 (exp. 1) or 33 (exp. 2), and the common phase 3 diet was fed from d 26 (exp. 1) or 33 (exp. 2) to 47.

[b] Distillers dried grains with solubles.

[c] Provided per kg of diet: 33 mg Mn from manganese oxide, 110 mg Fe from iron sulfate, 110 mg Zn from zinc sulfate, 16.5 mg Cu from copper sulfate, 0.30 mg I from calcium iodate, and 0.30 mg Se from sodium selenite.

[d] Provided per kg of diet: 11,023 IU vitamin A, 1,653 IU vitamin D_3, 44 IU vitamin E, 4.4 mg vitamin K, 8.3 mg riboflavin, 27.6 mg pantothenic acid, 50 mg niacin, and 0.04 mg vitamin B_{12}.

[e] Diets in exp. 1 contained 0.0125% Phytase 600 (Phyzyme; Danisco Animal Nutrition, St Louis, MO), providing 750.7 phytase units (FTU)/kg and an estimated release of 0.12% available P. Diets in exp. 2 contained 0.015% HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 406.3 phytase units (FTU)/kg and an estimated release of 0.10% available P.

[f] Exp. 1 treatment diets contained added ZnO at 0 or 0.347% and CTC-50 at 0, 0.05, or 0.4%. Exp. 2 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% EO 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.

[g] Available P (%) levels were calculated as 0.47 and 0.41% for exp. 1 phase 2 and 3 diets, respectively.

Table 2. Analyzed dietary composition and mineral concentrations of phase 2 treatment diets (as-fed basis)[a]

Diets	DM, %	CP, %	CF, %	Fat, %	Ash, %	Ca, %	P, %	Zn, ppm	Cu, ppm
Experiment 1									
0 CTC	90.78	23.0	2.0	2.2	7.18	1.52	0.62	148	53
55 CTC	90.59	22.5	1.5	2.1	6.27	1.21	0.65	317	24
441 CTC	90.40	23.7	1.7	2.4	5.59	0.93	0.60	186	22
0 CTC + Zn	90.79	21.9	1.5	2.0	6.09	1.12	0.60	2,918	23
55 CTC + Zn	90.77	22.4	1.7	2.3	6.45	1.12	0.64	2,946	20
441 CTC + Zn	90.79	22.3	1.4	1.8	6.57	1.19	0.59	2,823	27
Experiment 2									
Control	90.96	23.0	1.8	2.5	5.00	0.86	0.67	140	16
Cu	90.27	23.2	1.9	2.6	5.01	0.89	0.64	115	109
Zn[c]									
d 5 to 12	90.32	22.9	1.8	2.7	5.31	0.88	0.63	2,110	20
d 12 to 33	90.41	22.9	1.7	2.7	5.49	0.92	0.74	1,632	25
EO[d]	90.18	22.8	1.9	2.7	5.00	0.96	0.69	177	25
Cu + Zn[c]									
d 5 to 12	90.34	22.7	1.7	2.7	5.65	0.96	0.70	2,254	166
d 12 to 33	90.45	23.0	1.7	2.6	5.45	0.98	0.68	1,778	135
Cu + EO	89.92	22.4	1.7	2.8	5.00	1.03	0.69	385	161
Zn + EO[c]									
d 5 to 12	89.66	23.2	1.9	2.8	5.36	0.96	0.66	2,166	19
d 12 to 33	90.55	22.9	1.6	2.9	5.63	0.98	0.66	1,780	21
Cu + Zn + EO[c]									
d 5 to 12	90.44	22.6	1.8	2.7	5.56	0.96	0.66	2,181	120
d 12 to 33	90.14	22.5	1.9	2.9	5.32	0.93	0.65	1,701	137
55 CTC	90.75	23.2	2.0	2.8	5.02	0.90	0.64	219	22
441 CTC	90.26	22.9	1.9	2.9	5.06	0.93	0.66	205	22

[a] Analysis was performed by Ward Laboratories, Inc. (Kearney, NE) on pooled diet samples. All diets were formulated to contain 16.5 ppm Cu and 110 ppm of Zn from the trace mineral premix.

[b] Phase 2 treatment diets were fed from d 5 to 26 (exp. 1) or to d 33 (exp. 2), whereas a phase 1 common was fed to all pigs from d 0 to 5 and a phase 3 common diet was fed to all pigs from d 26 (exp. 1) or 33 (exp. 2) to d 47.

[c] In exp. 2 only, pharmacologic 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.

[d] From Regano EX (Ralco Animal Nutrition, Marshall, MN).

of added Zn) and CTC (0, 55, or 441 ppm). United States Food and Drug Administration regulations (Code of Federal Regulations) prohibit the continuous feeding of therapeutic levels of CTC longer than 14 d. Thus, on study d 15, the feeders from pens assigned to the 441 ppm CTC diets were emptied and pigs were fed the control diet with or without the 2,500 ppm of added Zn. The normal treatment diet containing CTC at 441 ppm was then re-added to the feeders on d 16 and fed for the remainder of the 21-d period. Treatment diets were corn-soybean meal-based and contained 10% dried whey, 1.25% fish meal, and 1.25% blood cells. From d 26 to 47, a common corn-soybean meal-based diet with no added ZnO and no CTC was fed to all pigs to evaluate any carryover effects from the treatment diets. All diets contained 110 ppm of Zn from the trace mineral premix. As determined by analysis, the common diet fed for the first 5 d postweaning contained 166 ppm Zn and 29 ppm Cu, whereas the common diet fed after the treatment period contained 160 ppm Zn and 21 ppm Cu.

Experiment 2

Animals and management. A total of 350 nursery pigs (PIC 1050; initially 6.05 kg BW) were used in a 47-d study with 5 pigs per pen and 7 replications per treatment. Weaned pigs exhibited clinical signs of influenza infection upon entry into the barn. Pigs with clinical signs for which injectable treatment was deemed necessary were removed from the study which contributed to an elevated 4% removal rate during the study. Removal rate was not influenced by dietary treatment. Treatment diets were fed from d 5 to 33 postweaning at which time all pigs received a common diet and growth performance was monitored for an additional 2 wk to d 47 postweaning in order to assess potential carryover effects of dietary treatment. Average daily gain, ADFI, and G:F were determined by weighing pigs and measuring feed disappearance on d 5, 33, and 47.

Diet composition. The basal diet formulations used in exp. 1 were used for exp. 2. The 10 dietary treatments fed from d 5 to 33 were structured as a (2 × 2 × 2) + 2 factorial with main effects of added Cu from copper sulfate ($CuSO_4$; 0 vs. 125 ppm Cu), added Zn from zinc oxide (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. 0.1% Regano EX containing *Origanum* oil; Ralco Animal Nutrition, Marshall, MN). The 2 additional treatments were CTC at subtherapeutic (55 ppm) or therapeutic (441 ppm) levels. All diets contained 16.5 ppm Cu and 110 ppm of Zn from the trace mineral premix. 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.

Similar to exp. 1, in order to comply with United States Food and Drug Administration regulations, on d 19 of the study the feeders from pens assigned to the 441 ppm CTC diet were emptied and pigs were fed the control diet for 1 d. The normal treatment diet containing CTC at 441 ppm was then re-added on d 20 and fed for the remainder of the 28-d period (d 5 to 33). From d 33 to 47, a common corn-soybean meal-based diet without any antimicrobial, EO, or pharmacological levels of Cu or Zn was fed to all pigs to evaluate any carryover effects from the treatment diets.

Statistical Analysis

For each experiment, growth data were analyzed as a randomized complete block design with pen as the experimental unit. The PROC MIXED procedure of SAS (v9.3, SAS Institute Inc., Cary, NC) was used to model diet treatment as a fixed effect and barn location nested within barn as a random effect. The main effects of Zn, Cu, CTC, and EO, as well as any interactions, were tested using a priori orthogonal CONTRAST statements. Within the CTC treatments, linear and quadratic contrasts were used.

In exp. 2, analysis of studentized residual values revealed a geographic cluster of four pens, each on a different treatment (EO, Cu + Zn, Cu + EO, Cu + Zn + EO), which had ADG or feed efficiency observations greater than 3 SDs from the mean. Taking this as evidence for data outliers, these pens were removed from the data set used for analysis.

Results were considered statistically significant at $P \leq 0.05$; results with P-values > 0.05 and ≤0.10 were considered marginally significant.

RESULTS

In exp. 2, analyzed Zn concentrations were consistently less than calculated concentrations for all diets containing added ZnO (Table 2). Although analyzed Ca levels were consistent across treatment diets within each experiment, analyzed Ca levels were greater than formulated levels (0.8%; Table 1) across all phase 2 experimental diets and analyzed levels in exp. 1 diets were greater than those of diets in exp. 2 (Table 2). Further investigation and analysis failed to identify a single explanatory cause for the differences between calculated and reported analyzed Zn and Ca levels. However, high pharmacological levels of Zn were clearly achieved in the respective experimental diets. Analyzed levels of all other nutrients were similar to calculated levels and Cu concentrations of diets containing added $CuSO_4$ in exp. 2 were within the Association of American Feed Control Officials acceptable analytical variation range for Cu (AOAC, 2000).

Within each experiment, the growth rates of pigs did not differ among treatments during the first 5 d when a common starter diet was fed.

Experiment 1

In exp. 1, no ZnO × CTC interactions were observed for any response criteria in any period (Table 3). During the 21-d treatment period, added Zn increased ($P < 0.001$) ADG, ADFI, and BW on d 26 but did not affect G:F. Similarly, feeding CTC increased ($P \leq 0.017$) ADG, ADFI, and BW. Feeding CTC also resulted in a marginally significant improvement (quadratic, $P = 0.083$) in caloric efficiency with pigs fed CTC at 55 ppm having the best G:F. Except for a small decrease ($P = 0.025$) in the G:F of pigs previously fed pharmacological Zn, no differences were observed in the posttreatment period (d 26 to 47) growth rates or feed intakes of pigs that had previously received ZnO or CTC in their diets. Nevertheless, the improvements in ADG and ADFI from feeding pharmacological Zn from ZnO during the treatment period were maintained over the posttreatment period as evidenced by greater ($P < 0.05$) overall ADG and ADFI from d 5 to 47. The respective improvements in ADG and ADFI due to feeding CTC during the treatment

Table 3. Effects of ZnO and CTC on nursery pig growth performance (exp. 1)[a,b]

								Probability, $P <$				
ZnO, ppm:	0			2,500				ZnO × CTC			CTC	
CTC, ppm:	0	55	441	0	55	441	SEM	Linear	Quadratic	ZnO	Linear	Quadratic
BW, kg												
d 5	6.46	6.46	6.43	6.45	6.46	6.44	0.077	0.923	0.974	0.981	0.751	0.912
d 26	13.91	14.45	14.57	14.78	14.80	15.24	0.203	0.986	0.175	<0.001	0.011	0.251
d 47	28.90	29.81	29.61	29.66	29.86	30.04	0.425	0.952	0.409	0.240	0.387	0.239
d 5 to 26												
ADG, kg	0.35	0.38	0.39	0.40	0.40	0.42	0.008	0.894	0.121	<0.001	0.002	0.208
ADFI, kg	0.50	0.51	0.53	0.55	0.54	0.57	0.012	0.826	0.399	<0.001	0.017	0.914
G:F	0.71	0.74	0.73	0.73	0.73	0.73	0.013	0.646	0.362	0.567	0.436	0.119
ME caloric efficiency[c]	4,684	4,466	4,482	4,534	4,472	4,460	78.2	0.611	0.315	0.359	0.207	0.083
NE caloric efficiency[c]	3,456	3,295	3,306	3,345	3,299	3,289	57.7	0.613	0.315	0.350	0.200	0.083
d 26 to 47												
ADG, kg	0.71	0.73	0.72	0.71	0.72	0.74	0.015	0.262	0.648	0.978	0.449	0.405
ADFI, kg	1.15	1.16	1.17	1.16	1.17	1.21	0.024	0.500	0.845	0.273	0.155	0.757
G:F	0.62	0.63	0.61	0.61	0.61	0.61	0.007	0.424	0.635	0.025	0.155	0.289
d 5 to 47												
ADG, kg	0.53	0.55	0.55	0.55	0.56	0.58	0.009	0.416	0.322	0.045	0.062	0.244
ADFI, kg	0.83	0.84	0.85	0.86	0.86	0.89	0.016	0.555	0.665	0.022	0.058	0.830
G:F	0.65	0.66	0.65	0.65	0.65	0.65	0.006	0.743	0.329	0.235	0.639	0.070
ME caloric efficiency[c]	5,066	4,947	5,044	5,072	5,033	5,055	50.5	0.732	0.360	0.364	0.754	0.083
NE caloric efficiency[c]	3,732	3,644	3,716	3,736	3,707	3,723	37.2	0.730	0.361	0.368	0.762	0.083

[a] A total of 240 nursery pigs (PIC 1050, initially 21 d of age and 6.08 kg BW) were used in a 47-d study with 5 pigs per pen and 8 pens per treatment.

[b] Experimental treatment diets were fed from d 5 to d 26, and a common diet was fed to all pigs from d 26 to 47.

[c] Caloric efficiency is expressed as kcal per kg of live weight gain.

period remained marginally significant (linear, $P = 0.062$ and 0.058, respectively) overall from d 5 to 47. Also, the overall feed and caloric efficiency of pigs fed CTC had marginally significant quadratic improvements (quadratic, $P < 0.10$) as 55 ppm of CTC was added to the diet with no further improvement at the 441 ppm level.

Experiment 2

During the d 5 to 33 treatment period, higher CTC levels linearly increased ($P = 0.028$) ADG and induced marginally significant increases (linear, $P = 0.079$) in ADFI which resulted in marginally heavier (linear, $P = 0.074$) BW on d 33 (Tables 4 and 5). When the pigs ceased consuming CTC and were fed the common diet from d 33 to 47, pigs previously fed CTC had a numerical reduction ($P = 0.101$) in ADG compared to pigs which had not previously been fed CTC. Consequently, higher levels of CTC did not linearly affect overall ADG or ADFI from d 5 to 47 and although CTC had failed to affect G:F during either the treatment period or the succeeding common period, CTC induced marginally significant improvements (quadratic, $P = 0.093$) in overall G:F and caloric efficiency with pigs fed 55 ppm CTC having the best feed efficiency.

During the treatment period, there was a marginally significant 3-way interaction between EO, Cu, and Zn (Cu × EO × Zn, $P = 0.098$). Adding EO to the control diet numerically reduced ADG, whereas it had no impact on growth rate when added to diets containing pharmacological levels of Zn and Cu. Pharmacologic Cu and Zn each increased ($P < 0.01$) ADG, resulting in greater ($P < 0.05$) BW on d 33 at the end of the treatment period. No interactions between treatment ingredients were observed for feed intake; pharmacologic Zn increased ($P = 0.006$) ADFI, pharmacologic Cu induced a marginally significant improvement ($P = 0.055$) in ADFI, and EO did not affect feed intake during the treatment period. Despite the concomitant increases in both ADG and ADFI, a marginally significant improvement in caloric efficiency ($P = 0.089$ for ME and 0.084 for NE) was observed due to pharmacologic Zn. The main effect

Table 4. Effects of added dietary Cu, Zn, EO, and CTC on nursery pig growth performance (exp. 2)[a,b]

Added Cu[c]:	–	+	–	–	+	+	–	+	–	–	
Added Zn[d]:	–	–	+	–	+	–	+	+	–	–	
EO[e]:	–	–	–	+	–	+	+	+	–	–	
CTC, ppm:	0	–	–	–	–	–	–	–	55	441	SEM
BW, kg											
d 5	6.57	6.56	6.56	6.53	6.61	6.69	6.56	6.62	6.53	6.55	0.084
d 33	18.94	19.61	19.82	19.08	20.47	19.91	19.85	19.96	18.88	19.64	0.361
d 47	29.02	29.32	29.68	28.83	30.56	29.50	29.47	29.89	28.90	29.28	0.503
d 5 to 33											
ADG, kg	0.44	0.46	0.47	0.42	0.49	0.47	0.47	0.48	0.43	0.46	0.012
ADFI, kg	0.56	0.58	0.61	0.55	0.61	0.62	0.61	0.62	0.55	0.59	0.018
G:F	0.77	0.79	0.77	0.76	0.81	0.76	0.78	0.76	0.79	0.78	0.012
ME caloric efficiency[f]	4,262	4,163	4,253	4,338	4,049	4,304	4,196	4,293	4,165	4,193	65.2
NE caloric efficiency[f]	3,145	3,071	3,137	3,200	2,986	3,175	3,095	3,166	3,073	3,092	48.1
d 33 to 47											
ADG, kg	0.72	0.69	0.70	0.70	0.72	0.68	0.69	0.71	0.72	0.69	0.016
ADFI, kg	1.17	1.13	1.15	1.13	1.16	1.12	1.13	1.14	1.12	1.12	0.028
G:F	0.62	0.62	0.61	0.62	0.62	0.61	0.61	0.62	0.64	0.61	0.113
d 5 to 47											
ADG, kg	0.53	0.54	0.55	0.51	0.57	0.54	0.55	0.55	0.53	0.54	0.011
ADFI, kg	0.76	0.76	0.79	0.73	0.79	0.79	0.78	0.79	0.74	0.77	0.019
G:F	0.70	0.71	0.69	0.69	0.72	0.69	0.70	0.70	0.72	0.70	0.009
ME caloric efficiency[f]	4,726	4,652	4,721	4,754	4,553	4,752	4,704	4,702	4,590	4,666	61.5
NE caloric efficiency[f]	3,483	3,429	3,479	3,504	3,355	3,502	3,467	3,465	3,383	3,439	45.3

[a] A total of 350 nursery pigs (PIC 1050, initially 6.05 kg BW) were used in a 47-d study with 5 pigs per pen and 7 replicate pens per treatment except for 4 treatments (EO, Cu + Zn, Cu + EO, Cu + Zn + EO), which had 6 replicate pens each.

[b] Experimental treatment diets were fed from d 5 to d 33. All diets contained 16.5 ppm Cu and 110 ppm of Zn from the trace mineral premix.

[c] Cu from $CuSO_4$ was added to treatment diets at either 0 or 125 ppm.

[d] 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.

[e] Regano EX (Ralco Animal Nutrition, Marshall, MN) was added to treatment diets at either 0 or 0.1%.

[f] Caloric efficiency is expressed as kcal per kg of live weight gain.

of EO was a negative impact on ($P = 0.009$) G:F during the treatment period and a Cu (regardless of Zn inclusion) × EO interaction ($P = 0.024$) was observed due to the numeric improvements in G:F induced by Cu being eliminated when EO was also fed in combination. These interactive (Cu × EO, $P = 0.025$) and main effects ($P = 0.015$) of EO were also observed when considering efficiency on a caloric basis for both ME and NE during the treatment period.

During the 14-d common period from d 33 to 47, no differences were observed in growth performance due to previous diet treatment except for a marginally significant interaction between previous Cu and Zn treatments whereby ADG of pigs previously fed either Cu or Zn were numerically decreased compared with those previously fed the control when the minerals were fed individually, but not when previously fed in combination (Cu × Zn, $P = 0.095$). No interactions between treatment ingredients were observed on overall growth performance from d 5 to 47. Feeding pharmacological Zn for 28 d increased ($P < 0.05$) overall ADG and ADFI over the entire 42-d experiment which resulted in greater ending BW on d 47 compared to that of pigs not fed added Zn. Similarly, pigs that received pharmacologic Cu for 28 d had greater ($P = 0.018$) overall ADG and marginally significant improvements ($P = 0.099$) in ending d 47 BW compared to that of pigs not receiving added Cu. In contrast, dietary inclusion of EO for 28 d did not affect pig gain rate or feed intake but resulted in a marginally significant decrease ($P = 0.086$) in overall G:F from d 5 to 47 compared to that of pigs not receiving the EO.

Table 5. Probability ($P <$) for effects of added Cu, Zn, EO, and CTC on nursery pig growth performance (exp. 2)[a,b]

	Probability, $P <$								
								CTC	
	Cu	Zn	EO	Cu × Zn	Cu × EO	Zn × EO	Cu × Zn × EO	Linear	Quadratic
BW, kg									
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 5 to 33									
ADG, g	0.003	<0.001	0.605	0.120	0.822	0.707	0.098	0.028	0.755
ADFI, g	0.055	0.006	0.444	0.173	0.182	0.798	0.444	0.079	0.392
G:F	0.150	0.186	0.009	0.958	0.024	0.886	0.137	0.833	0.265
ME caloric efficiency[c]	0.138	0.089	0.015	0.870	0.025	0.858	0.144	0.645	0.226
NE caloric efficiency[c]	0.137	0.084	0.015	0.870	0.025	0.858	0.144	0.631	0.226
d 33 to 47									
ADG, g	0.928	0.608	0.136	0.095	0.692	0.965	0.782	0.101	0.987
ADFI, g	0.675	0.696	0.355	0.347	0.668	0.978	0.675	0.377	0.222
G:F	0.659	0.972	0.488	0.321	0.930	0.958	0.845	0.340	0.135
d 5 to 47									
ADG, g	0.018	0.001	0.207	0.573	0.621	0.825	0.111	0.422	0.771
ADFI, g	0.225	0.025	0.818	0.425	0.225	0.942	0.304	0.499	0.240
G:F	0.141	0.293	0.086	0.549	0.145	0.972	0.549	0.973	0.093
ME caloric efficiency[c]	0.131	0.207	0.111	0.560	0.147	0.976	0.561	0.937	0.084
NE caloric efficiency[c]	0.131	0.202	0.111	0.560	0.147	0.977	0.560	0.930	0.084

[a] A total of 350 nursery pigs (PIC 1050; initially 6.05 kg BW) were used in a 47-d study with 5 pigs per pen and 7 replicate pens per treatment except for 4 treatments (EO, Cu + Zn, Cu + EO, Cu + Zn + EO), which had 6 replicate pens each.

[b] Experimental treatment diets were fed from d 5 to d 33. All diets contained 16.5 ppm Cu and 110 ppm of Zn from the trace mineral premix.

[c] Caloric efficiency is expressed as kcal per kg of live weight gain.

DISCUSSION

In the present study, feeding pharmacological levels of 2,500 ppm Zn from ZnO for 21 d or at 3,000 ppm for 7 d then 2,000 ppm for another 21 d increased rates of gain with coinciding increases in feed intake but did not affect feed efficiency in either experiment. This is in agreement with recent meta-analysis by Sales (2013) which provides a summation of evidence that feeding pharmacological concentrations of Zn from ZnO to weaned pigs improves rate of gain, feed intake, and efficiency of gain. While pharmacologic Zn from ZnO is capable of inducing efficiency improvements, this efficiency response is more variable than the consistently observed improvements in gain and feed intake. In contrast to the meta-analysis of Sales (2013), no evidence for improved efficiency of gain due to Zn supplied above the pigs' physiological requirement was observed in the present study. Similar findings were reported by Woodworth et al. (2005) when 3,000 ppm Zn was fed for the first 10 d postweaning then 2,000 ppm Zn was fed from d 10 to 20. They found efficiency was initially improved from d 0 to 10 but no improvement in efficiency due to pharmacological Zn could be detected from d 0 to 20.

In the present study, increased feed intake resulted in a linear improvement in BW gain as dietary inclusion of CTC increased from 0 to 55 ppm to 441 ppm for 21 or 28 d. In modern multisite production systems, dietary antibiotics remain efficacious in improving weaned pig growth but appear to have limited application for improving efficiency of gain (Dritz et al., 2002). Marginal evidence for CTC improving overall feed and caloric efficiencies in exp. 1 indicated the greatest improvements were obtained when 55 ppm of CTC was added to the diet, with no further improvement at 441 ppm. This observation is not unexpected when considering that CTC levels greater than 55 ppm are indicated for the control or prevention of clinical disease while the lower dosage is indicated for production benefit. CTC appeared to elicit a greater improvement in growth rate and feed intake in exp. 1 than in exp. 2 in which there was no difference in overall growth performance of pigs due to feeding increasing levels of CTC. In exp. 1, pigs fed 441 ppm CTC had an 8% improvement in ADG compared to that of control pigs while pigs in exp. 2 had a 5% improvement. Summarizing a large number of studies, Dritz et al. (2002) reported a 5% improvement in ADG due to feeding antibiotic among nursery pigs in commercial environments yet cautioned that antibiotic growth responses are smaller when baseline growth performance is high. Several factors could have contributed to the variation in CTC response in the present experiments, such as a greater number of replicate pens fed CTC in exp. 1 than in exp. 2, or initial BW of pigs (6.45 kg in exp. 1 and 6.55 kg in exp. 2).

No interactions between pharmacologic Zn from ZnO and CTC were observed, indicating that the effects of each are additive in nature. Similar effects of pharmacological Zn and other broad-spectrum antibiotics fed in combination have been reported. Hill et al. (2001) observed additive improvements in growth rate, feed intake, and efficiency of gain when a subtherapeutic level of carbadox was fed with up to 3,000 ppm Zn from ZnO in weaned pig diets. Furthermore, Woodworth et al. (2005) reported additive improvements in rate of gain due to feeding a combination of neomycin and tetracycline with a pharmacological level of Zn for 20 d. In addition, they reported improved feed intake due to Zn but no interactive effect of Zn and concomitant feeding of the antibiotics on feed intake.

Early research established that supplementing basal diets with graded levels of 0 to 500 ppm Cu from $CuSO_4$ induced nonlinear responses in weaned pig growth performance with maximum growth rates, feed intake, and efficiency of gain achieved when feeding approximately 250 ppm Cu (Roof and Mahan, 1982; Cromwell et al., 1989). However, 75% to 80% of this maximum response was realized by supplementation with a more moderate level of 125 ppm Cu Cromwell et al. (1989) and Stahly et al. (1980) reported that rate of gain and feed intake were maximized at 125 ppm Cu when diets included antibiotics. In the present study, feeding Cu above the nursery pigs' physiological requirement at level 125 ppm for 28 d improved rate of gain with a marginally significant increase in feed intake but no significant improvement in G:F. In our previous study, supplementation of Cu at 125 ppm elicited positive response in efficiency of gain in addition to increased rate of gain and voluntary feed intake compared to those of pigs not fed additional Cu (Shelton et al., 2011). It is possible that a greater response to Cu may have occurred in the present study if a greater concentration of Cu had been fed.

Including 0.1% *Origanum* EO supplement containing approximately 5% oil into weaned pig diets for 28 d failed to affect daily gain or feed intake but resulted in poorer feed and caloric efficiencies in the present study. The interactive effect of pharmacologic Cu and *Origanum* EO on feed efficiency suggests that *Origanum* EO had an antagonistic effect on the numeric improvement in gain efficiency induced by Cu. Previous research in weaned pigs has shown that oregano EO supplied at equivalent concentrations to that fed in the present study, or at 2× greater or lesser concentrations, for 28 d did not elicit any improvements in growth rate, feed efficiency, or feed intake (Neill et al., 2006). Moreover, no improvements in any growth performance parameters were observed due to the feeding of the combined oil extracts of oregano (5% carvacrol), cinnamon (3% cinnamaldehyde), and Mexican pepper (2% capsicum oleoresin) when included in the diet at up to 300 ppm (Manzanilla et al., 2004). Little research has demonstrated detrimental effects of oregano EO although Jugl-Chizzola et al. (2006) did report that weaned pigs had a lesser preference for feed containing oregano herbs (supplying 0.002 or 0.02% EO) than for unsupplemented feed and Zhang et al. (2012) reported decreased G:F during the initial 2-wk period of feeding oregano EO plant extract to weaned pigs.

Origanum vulgaris successfully improved rate of gain over that of pigs receiving no EO nor antibiotic therapy, although no differences were observed in feed intake (Papatsiros et al., 2009) among pigs infected with proliferative enteropathy. Also, oregano EO has been shown to be as effective as a feed-grade antibiotic in inducing improved rate of gain and feed conversion ratios in poultry (Mathlouthi et al., 2010). However, compared to pharmacologic Zn or Cu, there is a scarcity of literature supporting the supposition that oregano plant EO improves growth performance in non-disease-challenged weaned pigs. Li et al. (2012) did report that feeding an EO blend containing thymol and cinnamaldehyde improved ADG and decreased *Escherichia coli* in the cecum, colon, and rectum of weaned pigs. However, as summarized by Zeng et al. (2015), there are currently few studies which would support positive effects of carvacrol and thymol fed in combination on nursery pig growth. Further additional research is warranted to determine more specific in vivo applications for the active compounds of oregano EO and appropriate dose and feeding strategies, the role of *Origanum* EO in stimulating weaned pig growth performance appears limited.

In the present study, lack of interaction among pharmacological Zn, Cu, and *Origanum* EO for feed intake indicates any individual improvements due to Zn and Cu are additive. Few studies have evaluated any interactive effects of EO with other growth-promoting agents although no interactions were observed between oregano EO and feed-grade antibiotics when both were fed together (Neill et al., 2006).

The effects of feed antibiotic levels and pharmacologic Zn or Cu in weaned pig diets are reported as additive in several studies. Additive effects of up to 3,000 ppm Zn from ZnO and subtherapeutic doses of a feed-grade antibiotic on weaned pig growth performance have been reported (Hill et al., 2001; Woodworth et al., 2005). The effects of 250 ppm Cu from $CuSO_4$ were nonadditive when fed together with feed-grade antibiotics in the diet of grow-finish pigs (Ribeiro de Lima et al., 1981) but in weaned pigs, additive effects have been observed (Stahly et al., 1980; Edmonds et al., 1985) even with 125 ppm Cu (Roof and Mahan, 1982).

Additivity of pharmacological levels of Cu and Zn has been demonstrated less consistently, possibly due to interactions between the minerals competing for absorption at the gut level. Some studies have demonstrated nonadditive effects between pharmacological levels of 250 ppm Cu from $CuSO_4$ and 3,000 ppm Zn from ZnO (Smith et al., 1997; Hill et al., 2000) while others have demonstrated additive growth responses when feeding 125 ppm Cu in combination with Zn (3,000 ppm for 14 d postweaning and 2,000 ppm from d 14 to 42 postweaning; Shelton et al., 2011) or when feeding Cu at either 250 ppm from $CuSO_4$ or 100 ppm from an organic Cu source in combination with 3,000 ppm Zn (Pérez et al., 2011).

Few studies monitor for potential treatment carryover effects into the immediate posttreatment period. Minimal carryover effects from any of the dietary treatments on subsequent nursery pig growth performance were observed in this study. The observed overall gain response of pigs previously fed both pharmacologic Cu and Zn for 28 d provides marginal evidence that previous feeding of Cu plus Zn may not be as detrimental to rate of gain after supplementation of the pharmacologic levels of Cu and Zn has ceased compared to gain of pigs which were previously fed each mineral without the other. Bunch et al. (1963) observed 250 ppm CuSO4 improved ADG from weaning to 57 kg BW but when no $CuSO_4$ was subsequently fed from 57 to 91 kg BW the pigs which had previously received the added Cu had a better rate of gain compared to pigs which had not previously received $CuSO_4$.

In exp. 1 only, a small depression in feed efficiency was observed in pigs following cessation of pharmacologic Zn supplementation in the previous 21 d but no carryover effect of the Zn on efficiency was observed in exp. 2. Woodworth et al. (2005) have showed that the feeding antibiotic combination of neomycin and oxytetracycline for 21 d and reported that pigs previously receiving the antimicrobial exhibited decreased feed efficiency in the 7 d following cessation of the antimicrobial feeding. Numerically, but nonsignificantly, depressed feed efficiency was also observed in pigs previously fed pharmacologic Zn compared to pigs which had not received previous Zn treatment. Together, these observations suggest that modulated or depressed rates of gain or efficiency of gain following a period of stimulated growth are not unprecedented. Moreover, no further improvements in the growth performance of the pigs in these studies were observed after the antibiotics and added minerals were removed from the diets. Similar results were reported by Bosi et al. (2011) when considering the later growth performance of weaned pigs which had experienced improved ADG, ADFI, and G:F due to antibiotic feeding compared to that of pigs which had not been fed antibiotics in the preceding 21-d period; no differences were observed in the pigs' growth performance during the 7-d common period following cessation of antibiotic feeding.

CONCLUSION

This study illustrates the value of feeding pharmacological concentrations of Cu, Zn, and CTC to weaned pigs to promote growth but no improvement due to *Origanum* EO was observed. Although there were no improvements in feed efficiency due to Cu or Zn, the inclusion of an EO had a negative effect on feed and caloric efficiency. There were minimal carryover effects from the dietary treatments on subsequent nursery pig growth performance. Also, this study agrees with previous research findings with the data collectively suggesting that the benefits of feeding CTC and pharmacological levels of Zn are additive for nursery pigs. Furthermore, most effects of Cu, Zn, and EO on piglet growth performance appear to occur independently. In conclusion, pharmacological levels of Cu, Zn, or CTC improved weanling pig performance while *Origanum* EO elicited no growth performance benefits.

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