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Effects of dietary copper, zinc, and ractopamine hydrochloride on finishing pig growth performance, carcass characteristics, and antimicrobial susceptibility of enteric bacteria[1,2]

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ABSTRACT: A total of 480 pigs (PIC 327 × 1050; initially 48.7 ± 2.3 kg) were used to determine the interactive effects of supplemental Cu, Zn, and ractopamine HCl (RAC) on finishing pig growth performance, carcass characteristics, and antimicrobial susceptibility of enteric bacteria. Treatments were arranged in a 2 × 2 × 2 factorial with the main effects of added Cu ($CuSO_4$; 0 vs. 125 mg/kg Cu), Zn (ZnO; 0 vs. 150 mg/kg Zn), and RAC (0 vs. 10 mg/kg during the last 28 d prior to marketing). All diets contained 11 mg/kg Cu and 73 mg/kg Zn from the trace mineral premix. Pens of pigs were balanced and blocked on initial BW and then randomly allotted to 1 of the 4 mineral treatment diets. At 28 d prior to marketing, pens within each block and mineral treatment were randomly assigned to receive either 0 or 10 mg/kg RAC in addition to the mineral treatment. Adding either Cu or Zn alone did not improve ADG or ADFI yet resulted in numerical improvements in overall G:F and caloric efficiencies, but improvements were not additive (Cu × Zn, $P = 0.057$, $P = 0.068$, and $P = 0.064$ for G:F and caloric efficiency on a ME and NE basis, respectively). Ractopamine improved ($P < 0.001$) overall ADG, G:F, and caloric efficiency, thereby increasing final BW by 3% with no change in ADFI. Ractopamine also increased ($P < 0.001$) HCW, percentage carcass yield, G:F, loin depth, and percent fat-free lean and decreased ($P = 0.014$) backfat. Adding Zn or Cu alone to diets containing RAC numerically improved percent yield and HCW G:F, but this effect was absent when the Cu or Zn was added to the control diet or when Cu and Zn were fed in combination in RAC diets (Cu × Zn × RAC, $P = 0.011$ and $P = 0.018$ for yield and HCW G:F, respectively). Fecal samples were collected on d 0 and at the conclusion of the finishing period (d 90) for bacterial isolation and antimicrobial susceptibility determinations according to Clinical and Laboratory Standards Institute minimal inhibitory concentrations breakpoints. *Enterococcus* spp. and *Escherichia coli* isolates displayed varying levels of resistance to certain antibiotics prior to initiation of treatments on d 0. Resistance to most antibiotics decreased ($P < 0.05$) over time or was stable for those that had a low baseline percentage of resistance. Neither Zn nor RAC adversely affected antimicrobial resistance. However, extended feeding of 125 mg/kg Cu throughout the finishing period seems to decrease enterococcal susceptability to tetracycline, tylosin, and quinupristin/dalfopristin.

Key words: antimicrobial resistance, enteric bacteria, finishing pig, growth performance

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INTRODUCTION

Ractopamine HCl (**RAC**), a β-adrenergic agonist, increases synthesis and accretion of skeletal muscle protein (Bergen et al., 1989) and has lypolytic ability (Mills et al., 2003). Ractopamine improves the rate and efficiency of gain and carcass leanness (Stoller et al., 2003; Kutzler et al., 2011). Previous studies have showed additional and mixed responses on addition of supplemen-

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tal Zn with ractopamine in the diet (Paulk et al., 2015). Copper also has been shown to improve growth and feed intake of finishing pigs (Coble et al., 2014).

Bacterial antibiotic resistance remains a paramount public health concern, yet limited research has been undertaken to determine the impact of lower doses of pharmacological mineral agents and β-agonists on the ecology of antimicrobial-resistant bacteria in finishing pigs. In enterococci, the plasmid-borne transferable copper gene (*tcrB*) that encodes resistance to Cu is associated with the prevalence of certain antibiotic resistance genes in enteric bacteria (Amachawadi et al., 2013), whereas a gene conferring zinc resistance (*czrC*) has been associated with decreased antibiotic susceptibility (Cavaco et al., 2010) among *Staphylococcus aureus* isolates of pigs. This suggests Cu and Zn feeding may co-select for both mineral and antibiotic resistance in livestock and may increase the propensity for dissemination of antibiotic resistance genes.

Endogenous catecholamines use the adrenergic receptors to induce physiological changes (Liang et al., 1985; Frishman, 2003). In addition, bacterial growth and plasmid transfer are upregulated by endogenous catecholamines such as adrenaline and epinephrine (Peterson et al., 2011). There is speculation adrenergic agonists like RAC could actually aid in the progression of antibacterial resistance. The objective of this study was to determine the interactive effects of Cu, Zn, and RAC on finishing pig performance and antimicrobial susceptibility of fecal enteric bacteria.

MATERIALS AND METHODS

Animal Experiment and Dietary Treatments

The protocol for 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. Pigs were housed in an environmentally controlled tunnel-ventilated barn and reared on completely slatted concrete flooring over deep pits for manure storage. Each pen was equipped with a 2-hole stainless steel dry self-feeder (Farmweld, Teutopolis, IL) and a cup waterer to provide pigs with ad libitum access to feed and water. Feed delivery to each individual pen was accomplished and recorded via a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN).

A total of 480 pigs (PIC 327 × 1050; initially 48.7 ± 2.3 kg) from 2 finishing groups were used for this study. Prior to placement on experimental finisher diets, the pigs did not receive any chlortetracycline in their feed or water to avoid potential confounding study impacts due to disturbances to the intestinal microbiome.

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

Item	Phase 1	Phase 2	Phase 3	Phase 4[2]
Ingredient, %				
Corn[3]	72.54	78.02	80.66	76.55
Soybean meal (47.7% CP)	25.00	19.70	17.20	21.20
Limestone	0.90	0.90	0.90	0.90
Monocalcium phosphate	0.60	0.50	0.40	0.40
Sodium chloride	0.35	0.35	0.35	0.35
L-Lys HCl	0.28	0.25	0.23	0.25
DL-Met	0.05	0.02	0.00	0.04
L-Thr	0.08	0.05	0.05	0.10
Trace mineral premix[4]	0.10	0.10	0.10	0.10
Vitamin premix[5]	0.10	0.10	0.10	0.10
Phytase[6]	0.02	0.02	0.02	0.02
$CuSO_4$, ZnO, and ractopamine HCl additives[7]	0–0.07	0–0.07	0–0.07	0–0.27
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lys	1.01	0.86	0.78	0.90
Ile:Lys	64	64	66	65
Leu:Lys	139	149	157	147
Met:Lys	30	29	29	31
Met + Cys:Lys	56	57	57	58
Thr:Lys	62	62	64	67
Trp:Lys	18.3	18.1	18.2	18.3
Val:Lys	70	73	75	72
Total Lys, %	1.14	0.97	0.89	1.02
CP, %	18.3	16.1	15.1	16.8
ME, kcal/kg	3,303	3,311	3,316	3,314
NE, kcal/kg	2,474	2,507	2,524	2,500
SID Lys:ME, g/Mcal	3.06	2.60	2.35	2.71
SID Lys:NE, g/Mcal	4.08	3.43	3.09	3.59
Ca, %	0.55	0.51	0.49	0.50
P, %	0.50	0.45	0.42	0.44
Available P, %	0.30	0.27	0.24	0.25

[1]All diets were fed in meal form and formulated to be fed in 4 phases from 36 to 57, 57 to 79, 79 to 100, and 100 to 132 kg BW.

[2]Phase 4 diets were fed for the final 28 d prior to slaughter.

[3]Corn levels represent control level prior to addition of treatment diet ingredients, which replaced an equivalent amount of corn in respective experimental diets.

[4]Provided per kilogram of diet: 27 mg Mn from manganese oxide, 110 mg Fe from iron sulfate, 110 mg Zn from zinc sulfate, 11 mg Cu from copper sulfate, 0.20 mg I from calcium iodate, and 0.20 mg Se from sodium selenite.

[5]Provided per kilogram of diet: 4,409 IU vitamin A, 661 IU vitamin D_3, 18 IU vitamin E, 1.8 mg vitamin K, 3.3 mg riboflavin, 11.0 mg pantothenic acid, 19.8 mg niacin, and 0.02 mg vitamin B_{12}.

[6]HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 540 phytase units/kg and an estimated release of 0.10% available P.

[7]Copper from $CuSO_4$ was added to treatment diets at either 0 or 125 mg/kg. All diets contained 11 mg/kg Cu from the trace mineral premix. Zinc from ZnO was added to treatment diets at either 0 or 150 mg/kg. All diets contained 73 mg/kg Zn from the trace mineral premix. Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to treatment diets at either 0 or 10 mg/kg during the final 28 d prior to marketing.

However, the pigs did receive dietary neomycin and oxytetracycline antibiotics immediately after weaning.

All finishing diets were a corn–soybean meal–based diet fed in meal form, which contained a trace mineral premix providing 73 mg/kg Zn and 11 mg/kg Cu to the diet (Table 1). The diets were formulated to be fed in 4 phases (36 to 57 kg, 57 to 79 kg, 79 to 100 kg, and 100 to 132 kg) during the finishing period and were prepared at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS).

Dietary treatments were arranged in a 2 × 2 × 2 factorial with the main effects of added copper sulfate ($CuSO_4$; 0 vs. 125 mg/kg Cu), added zinc oxide (ZnO; 0 vs. 150 mg/kg Zn), and RAC (0 vs. 10 mg/kg during the last 28 d prior to marketing; Paylean; Elanco Animal Health, Greenfield, IN). The dietary treatments were as follows: 1) a control, 2) the control + 125 mg/kg Cu, 3) the control + 150 mg/kg Zn, 4) the control + 125 mg/kg Cu + 150 mg/kg Zn, 5) the control + 10 mg/kg RAC during only the final 28 d, 6) the control + 125 mg/kg Cu + 10 mg/kg RAC during only the final 28 d, 7) the control + 150 mg/kg Zn + 10 mg/kg RAC during only the final 28 d, and 8) the control + 125 mg/kg Cu + 150 mg/kg Zn + 10 mg/kg RAC during only the final 28 d.

The finishing period of the first pig group spanned from January to April whereas pigs in the second finishing group were housed in a different room from March to June. Upon entry into the finisher, pigs were randomly allotted to pens of either 7 (group 1) or 8 (group 2) pigs per pen. Pens contained 4 gilts and either 3 (group 1) or 4 (group 2) barrows each. Pen space was maintained at 0.929 m^2/pig across both groups by adjusting the pen size according to the number of animals per pen.

The study design was structured as a randomized complete block design with a split plot and replicated over 2 finishing groups with 32 pens each. At the beginning of the study, 32 pens of pigs were arranged into 4 weight blocks per group based on similar pen initial average BW. Two pens per weight block were then randomly allotted to 1 of the 4 mineral treatment diets (negative control, +125 mg/kg Cu, +150 mg/kg Zn, or +125 mg/kg Cu with +150 mg/kg Zn) and balanced on initial pen average BW across blocks. At 28 d prior to marketing, pens within each block and mineral treatment diet were randomly assigned to receive either 0 or 10 mg/kg RAC in addition to their mineral treatment, and all diets were formulated to contain 0.90% standardized ileal digestible lysine. Ractopamine HCl treatment assignments were balanced across blocks on the current pen average BW at the time of allotment to RAC treatments. Hence, for the final 28 d of the finishing period, each of the 4 weight blocks in each group contained 1 pen per each of the 8 diet treatments.

Feed samples from each batch of feed were taken from feeders. Samples were pooled within each phase to form a composite diet sample that was subsequently analyzed for Ca, P, Cu, and Zn (method 985.01; AOAC, 2000) in duplicate with modifications of ashing a 0.35-g sample for 1 h at 535°C, digestion in an open crucible for 20 min in 15% nitric acid on a hot plate, and sample dilution to 50 mL and analysis on an inductively coupled plasma spectrometer (PerkinElmer 3300 XL and 5300 DV ICP; PerkinElmer Inc., Shelton, CT); DM (method 930.15; AOAC., 2000); CP (method 990.03; AOAC, 2000); crude fat (method 2003.05; AOAC, 2006); crude fiber (method 978.10; AOAC, 2000); and ash (method 942.05; AOAC, 2000) with modifications of a 1.5-g sample, 4 h ash time, and hot weight (Cumberland Valley Analytical Services, Hagerstown, MD; Table 2). Across both finishing groups, a composite sample of each of the 4 diets containing Paylean was assayed for RAC concentration (FDA, 2003; Covance Laboratories, Greenfield, IN; Table 2).

Pigs and feeders were weighed approximately every 3 wk to determine ADG, ADFI, feed:gain, and caloric efficiency on both ME and NE caloric efficiency ([(kcal energy/kg diet) × (total diet intake/pen)]/total pen weight gain) on a pen basis. Dietary ME and NE values were derived from feed ingredient energy values based on those published by the NRC (2012). At the conclusion of the 90- (group 1) or 83-d (group 2) experimental period, all pigs were individually weighed and tattooed with a unique identifier. Pigs were transported to a commercial harvesting facility (Triumph Foods LLC, St. Joseph, MO) and held in lairage overnight prior to processing and carcass data collection. Carcass characteristics measured at the plant included HCW immediately after evisceration and backfat and loin depths via an optical probe.

Percent carcass yield was calculated by dividing the individual HCW obtained at the packing plant by the corresponding individual final BW obtained at the farm. An average percentage carcass yield for each pen was then calculated by averaging the observed yields of pigs for each pen. Pen average HCW was calculated by multiplying the pen average percent yield by the pen average final live weight. Percentage lean was calculated (NPPC, 2000). Using the NPPC (2002) equations for standartized fat free lean Initial carcass weight on study d 0 was assumed to equal 75% of the initial pen average live BW; therefore, HCW gain on a pen basis was calculated using the following formula: final pen average HCW, kg − (0.75 × initial pen average BW on d 0). Subsequently, HCW ADG was calculated by dividing the average individual HCW gain of each pen by the number of study days. Similarly, HCW feed:gain was calculated for each pen by dividing the ADFI per pig (overall ADFI/pig) by average daily HCW gain.

Table 2. Analyzed dietary concentrations (as-fed basis)[1]

	Analyzed dietary composition[2]							Analyzed concentrations[3]		
Diets	DM, %	CP, %	CF, %	Ether extract, %	Ash, %	Ca, %	P, %	Cu, mg/kg [4]	Zn, mg/kg [5]	Ractopamine HCl, mg/kg [6]
Phase 1										
Control	87.70	20.6	2.9	2.85	5.26	0.74	0.58	25	117	–
Cu	87.70	20.4	2.6	2.97	5.20	0.74	0.57	196	116	–
Zn	87.65	20.3	2.6	2.77	5.15	0.76	0.60	26	298	–
Cu + Zn	87.70	20.2	2.8	3.00	5.44	0.77	0.57	187	299	–
Phase 2										
Control	87.75	18.0	2.5	3.22	5.10	0.80	0.53	24	140	–
Cu	87.65	18.3	2.6	3.26	5.43	0.85	0.55	179	126	–
Zn	87.45	18.2	2.7	3.28	5.38	0.71	0.52	44	279	–
Cu + Zn	87.70	17.7	2.5	3.63	5.07	0.71	0.52	182	307	–
Phase 3										
Control	87.40	16.9	2.6	3.23	4.36	0.67	0.48	25	138	–
Cu	87.55	17.0	2.6	3.37	4.88	0.72	0.50	181	133	–
Zn	86.35	17.1	2.3	3.28	4.75	0.75	0.52	49	276	–
Cu + Zn	81.85	16.8	2.6	3.26	5.13	0.77	0.50	175	309	–
Phase 4										
Control	75.30	19.1	3.0	3.71	5.14	0.71	0.52	39	129	–
Cu	88.15	18.8	2.8	3.45	4.95	0.74	0.51	182	106	–
Zn	87.75	18.8	2.5	3.57	5.19	0.78	0.52	31	320	–
Cu + Zn	87.65	18.7	2.7	3.97	4.86	0.74	0.53	191	297	–
Control + RAC[7]	87.55	18.7	2.5	3.57	5.05	0.75	0.51	29	127	9.3
Cu + RAC	87.50	18.6	2.7	3.67	5.12	0.77	0.52	197	138	9.7
Zn + RAC	88.55	19.1	2.8	3.48	5.29	0.76	0.54	33	250	8.0
Cu + Zn + RAC	87.85	18.8	2.7	4.00	5.20	0.74	0.52	200	340	9.3

[1]Phase 1, 2, 3, and 4 diets fed in meal form from approximately 48 to 68, 68 to 91, 91 to 109, and 109 to 136 kg BW, respectively.

[2]CF = crude fiber. Analysis performed by Cumberland Valley Analytical Services (Hagerstown, MD) on pooled diet samples within each dietary phase; results represent the average of both finishing groups.

[3]Mineral analysis was performed by Cumberland Valley Analytical Services using inductively coupled plasma spectrometry; means represent the average of 2 to 4 duplicate feed samples within each dietary phase and each finishing group, which were subsequently analyzed in duplicate.

[4]Copper from $CuSO_4$ was added at 125 mg/kg to diets containing 11 mg/kg Cu from the trace mineral premix.

[5]Zinc from ZnO was added at 150 mg/kg to diets containing 73 mg/kg Zn from the trace mineral premix.

[6]Analysis was performed by Covance Laboratories (Greenfield, IN) on a composite sample of each of the 4 diets containing ractopamine HCl across both finishing groups.

[7]RAC = ractopamine HCl.

Fecal Sample Collection

Fecal samples from 5 randomly selected pigs per pen were collected into individual Whirl-Pak bags (Nasco, Ft. Atkinson, WI) on d 0 (baseline) and again on d 90 (before harvest) from the first group of pigs (32 pens). Samples were transported on ice to the Molecular Epidemiology and Microbial Ecology laboratory at Kansas State University (Manhattan, KS) for bacterial isolation and antimicrobial susceptibility analysis.

Bacterial Isolation and Identification

Fecal samples were stored at 4°C prior to processing. Approximately 1 g of feces from each of the 5 samples per pen was suspended in 9 mL PBS. Fifty microliters of the fecal suspension was then spread plated onto both M-*Enterococcus* agar and a MacConkey agar for the isolation of *Enterococcus* spp. and *Escherichia coli*, respectively, from each fecal sample. Unless otherwise specified, all the culture media was obtained from Difco (Becton, Dickinson and Company, Sparks, MD). M-*Enterococcus* plates were incubated at 42°C for 24 to 36 h and MacConkey plates were incubated at 37°C for 24 h.

Two putative colonies (pin-point red, pink, or metallic red) were selected from each M-*Enterococcus* agar and 2 distinct pink lactose fermenting colonies were picked from each MacConkey agar; each of these colonies was individually streaked onto a blood agar plate (Remel, Lenexa, KS) and incubated at 37°C for 24 h. Preliminary genus confirmation of each of the enterococcal isolates was done by esculin hydrolysis. An indole test was done to confirm each of the *E. coli* isolates. The 2 confirmed *E. coli* and 2 confirmed *Enterococcus* isolates per original fecal sample were preserved using cryo-protect beads (Cryocare; Key

Scientific Products, Round Rock, TX) and stored at −80°C for future use (Amachawadi et al., 2011).

Antimicrobial Susceptibility of Escherichia coli *and* Enterococcus *Isolates*

The microbroth dilution method as outlined by the Clinical and Laboratory Standards Institute (2013) was used on one of each *E. coli* and *Enterococcus* spp. bacterial isolate per original fecal sample to determine the minimal inhibitory concentrations using a Sensititre (TREK Diagnostic Systems, Oakwood Village, OH) micro-broth dilution procedure. For both *E. coli* and *Enterococcus* spp., bacterial isolate preserved in cryo-protect beads was streaked onto a blood agar plate and incubated at 37°C for 24 h. Individual colonies were selected and suspended in demineralized water (TREK Diagnostic Systems) and turbidity was adjusted to 0.5 McFarland turbidity standards. Then, 10 μL of the bacterial inoculum was added to Mueller–Hinton broth and vortexed to mix. A Sensititre automated inoculation delivery system (TREK Diagnostic Systems) was used to dispense 100 μL of the broth into National Antimicrobial Resistance Monitoring System panel plates designed for Gram-positive (CMV3AGPF; TREK Diagnostic Systems; Table 3) and Gram-negative (CMV3AGNF; TREK Diagnostic Systems; Table 4) bacteria. *Enterococcus faecalis* ATCC 29212 and *E. coli* ATCC 25922 (American Type Culture Collection, Manassas, VA) strains were included as quality controls for *Enterococcus* and *E. coli* susceptibility testing, respectively. Plates were incubated at 37°C for 18 h and then bacterial growth was assessed using Sensititre ARIS and Vizion systems (TREK Diagnostic Systems). Clinical and Laboratory Standards Institute guidelines were used to classify each bacterial isolate as resistant or nonresistant (intermediate and susceptible) according to the breakpoints established for each antimicrobial (CLSI, 2013).

Table 3. Resistance breakpoints and evaluated concentrations for antimicrobials of National Antimicrobial Resistance Monitoring System Gram-positive bacteria panel (CMV3AGPF; WHO, 2012)

Antimicrobial	WHO classification[1]	Concentration, μg/mL	Breakpoint, μg/mL[2]
Chloramphenicol	Highly important	2–32	≥32
Ciprofloxacin	Critically important	0.12–4	≥4
Daptomycin	Critically important	0.25–16	N/A[3]
Erythromycin	Critically important	0.25–8	≥8
Gentamicin	Critically important	128–1,024	>500
Kanamycin	Critically important	128–1,024	≥1,024
Lincomycin	Highly important	1–8	≥8
Linezolid	Critically important	0.5–8	≥8
Nitrofurantoin	Important	2–64	≥128
Penicillin	Critically important	0.25–16	≥16
Quinupristin/alfopristin	Highly important	0.5–32	≥4
Streptomycin	Critically important	512–2,048	>1,000
Tetracycline	Highly important	1–32	≥16
Tigecycline	Critically important	0.015–0.5	N/A[4]
Tylosin tartrate	Critically important	0.25–32	≥32
Vancomycin	Critically important	0.25–32	≥32

[1]World Health Organization (WHO) categorization of antimicrobials according to importance for human medicine (WHO, 2012).

[2]Breakpoints established by Clinical and Laboratory Standards Institute (2013).

[3]N/A = not applicable. A susceptibility breakpoint of ≤4 μg/mL for daptomycin exists but no resistant breakpoint has been established. In this study, isolates with a minimal inhibitory concentration ≥ 8 μg/mL were categorized as resistant.

[4]A susceptibility breakpoint of ≤0.25 μg/mL for tigecycline exists but no resistant breakpoint has been established. In this study, isolates with a minimal inhibitory concentration ≥ 0.5 μg/mL were categorized as resistant.

Statistical Analysis

Growth and carcass data were analyzed as a randomized complete block design with a 2 × 2 × 2 treatment structure and replicated over 2 groups. Pen was the experimental unit. The MIXED procedure in SAS (version 9.3; SAS Inst. Inc., Cary, NC) was used to model diet treatment as a fixed effect with random effects of group and initial weight block nested within group. The main effects of Cu, Zn, and RAC as well as their interactions were tested using a priori orthogonal CONTRAST statements. Hot carcass weight was used as a covariate in the analyses of backfat, loin depth, and percentage lean. The antibiotic susceptibility determination data was analyzed as a binary distribution using the SAS GLIMMIX procedure and a logit link function to model the data as a repeated measure with pen as the experimental unit and 5 sample observations per pen per day. Sample nested within pen was modeled as a random effect to account for clustering within pen. Treatment main effects of Cu, Zn, and RAC; day of sampling (baseline or d 90); and dietary main effect within day were evaluated for antibiotics having a nonzero resistance variance component for these fixed effects. For all data, the Kenward–Roger method was used to compute denominator degrees of freedom for tests of fixed effects. Results were considered statistically significant at $P \leq 0.05$; results with P-values >0.05 and ≤0.10 were considered marginally significant.

RESULTS

Analyzed CP levels were consistently greater than calculated levels for all diets, possibly as a reflection of higher protein levels in feedstuffs compared with nutrient book values used in the diet formulation (see Tables 1 and 2). Analyzed Cu and Zn levels reflected

Table 4. Resistance breakpoints and evaluated concentrations for antimicrobials of National Antimicrobial Resistance Monitoring System Gram-negative bacteria panel (CMV3AGNF; WHO, 2012)

Antimicrobial	WHO classification[1]	Concentration, µg/mL	Breakpoint, µg/mL[2]
Amoxicillin:clavulanic acid 2:1 ratio	Critically important	1/0.5–32/16	≥32/16
Ampicillin	Critically important	1–32	≥32
Azithromycin	Critically important	0.12–16	N/A[3]
Cefoxitin	Highly important	0.5–32	≥32
Ceftiofur	Critically important	0.12–8	≥8
Ceftriaxone	Critically important	0.25–64	≥4
Chloramphenicol	Highly important	2–32	≥32
Ciprofloxacin	Critically important	0.015–4	≥1
Gentamicin	Critically important	0.25–16	≥16
Nalidixic acid	Critically important	0.5–32	≥32
Streptomycin	Critically important	32–64	≥64
Sulfisoxazole	Highly important	16–256	≥512
Tetracycline	Highly important	4–32	≥16
Trimethoprim/ sulfamethoxazole	Highly important	0.12/2.4–4/76	≥4/76

[1]World Health Organization (WHO) categorization of antimicrobials according to importance for human medicine (WHO, 2012).

[2]Breakpoints established by Clinical and Laboratory Standards Institute (2013).

[3]N/A = not applicable. The National Antimicrobial Resistance Monitoring System has not established breakpoints for azithromycin interpretation; therefore, there is no Clinical and Laboratory Standards Institute resistant breakpoint.

the addition of 125 mg/kg Cu and 150 mg/kg Zn to respective treatment diets. Analyzed concentrations were at the upper end of the Association of American Feed Control Officials acceptable analytical variation range for the minerals (AOAC, 2000). Analyzed levels of all other nutrients were similar to calculated levels.

Growth Performance

No significant Cu × Zn × RAC interactions were observed in the growth performance responses measured in this study. There was no difference among pig BW on d 0 except that the pigs that would eventually receive RAC later in the finishing period were initially slightly heavier ($P = 0.012$) than those pigs that would not receive RAC (Table 5). Prior to the final 28 d of the finishing period, no effects of Cu or Zn on ADG or ADFI were observed and there were no differences in BW on d 62. However, a significant interaction (Cu × Zn, $P < 0.05$) between Cu and Zn was observed for feed and caloric efficiency, where pigs fed diets with added Zn alone had numeric improvement in efficiency but no improvement when both minerals were added together.

On the final day of the study (d 90 and 83 for groups 1 and 2, respectively), pigs receiving RAC for the previous 28 d had heavier ($P < 0.001$) BW than pigs that had not received RAC (Table 5). The heavier final BW of pigs receiving RAC was the result of greater ($P < 0.001$) ADG in the final 28 d before harvest, which consequently improved ($P < 0.001$) their G:F and caloric efficiency on both a ME and NE basis compared with that of pigs not fed RAC. In contrast, no effect of added Cu and/or Zn was observed on the ADG or G:F of pigs during the final 28 d of the finishing period. There were no effects of the minerals or RAC on ADFI in the final 28 d of the finishing period aside from a marginally significant effect of pigs fed Zn and RAC in combination having less (Zn × RAC, $P = 0.084$) ADFI than would have been expected considering added Zn numerically increased ADFI and RAC numerically decreased ADFI when each was fed not in combination with the other.

No effects of added Cu, Zn, and/or RAC were observed on overall ADFI (Table 5). Overall ADG, G:F, and caloric efficiency on both a ME and NE basis were improved ($P < 0.001$) due to feeding RAC in the last 28 d of the finishing period. Conversely, there were no main effects of added Cu or Zn on overall growth performance. Feeding Cu or Zn alone numerically improved overall G:F and overall caloric efficiency but had no influence on overall efficiencies when both were added to the diet (Cu × Zn, $P = 0.057$, $P = 0.068$, and $P = 0.064$ for G:F, ME, and NE, respectively).

Carcass Characteristics

The added Cu and Zn had minimal effects on growth performance and, congruently, had minimal effects on carcass characteristics. No differences in pen average HCW or HCW ADG due to the added minerals were observed. However, the finishing growth performance improvements induced by feeding RAC during the last 28 d prior to marketing resulted in increased ($P < 0.001$) HCW and HCW ADG (Table 6).

Feeding RAC also increased ($P < 0.001$) percentage carcass yield, and the magnitude of the increase was numerically greater when either Cu or Zn was added to the diet containing RAC; however, the minerals did not provide any carcass yield benefit when both were fed together with RAC (Cu × Zn × RAC, $P = 0.011$; Table 6). No main effects of Cu or Zn on percentage carcass yield were observed. Efficiency of carcass gain followed a pattern similar to percent carcass yield, with no main effect due to added Cu or Zn and with a Cu × Zn × RAC interaction ($P = 0.018$). Again, RAC improved ($P < 0.001$) HCW G:F, with further numeric improvement when either Cu or Zn was added with RAC, but no improvement due to the minerals was observed when both were included in diets together without RAC. When compared on a common HCW basis, pigs fed RAC had less ($P =$

Table 5. Effects of added Cu, Zn, and ractopamine HCl on growth performance of finishing pigs[1]

	Added Cu,[2] 125 mg/kg								
	–	+	–	+	–	+	–	+	
	Added Zn,[3] 150 mg/kg								
	–	–	+	+	–	–	+	+	
	Ractopamine HCl,[4] 10 mg/kg								
Item	–	–	–	–	+	+	+	+	SEM
BW, kg									
d 0	48.2	48.6	48.5	48.6	49.4	48.8	48.8	48.9	0.57
d 62[5]	108.5	108.8	109.2	108.9	108.7	108.7	109.0	109.0	2.59
d 90[a6]	134.5	133.8	135.0	134.1	138.4	138.1	138.6	138.3	4.29
d 0 to 62[5]									
ADG, kg	1.03	1.03	1.03	1.03	1.02	1.03	1.03	1.03	0.024
ADFI, kg	2.62	2.61	2.61	2.63	2.63	2.61	2.60	2.65	0.038
G:F[b]	0.39	0.39	0.40	0.39	0.39	0.39	0.40	0.39	0.013
ME caloric efficiency[b7]	8,428	8,395	8,376	8,432	8,590	8,442	8,370	8,537	269.2
NE caloric efficiency[b7]	6,375	6,346	6,334	6,376	6,498	6,381	6,329	6,456	200.9
d 62 to 90[5,6]									
ADG,[a] kg	0.88	0.90	0.92	0.90	1.06	1.06	1.07	1.05	0.056
ADFI, kg	2.78	2.78	2.88	2.80	2.86	2.79	2.80	2.76	0.101
G:F[a]	0.32	0.32	0.32	0.32	0.37	0.38	0.38	0.38	0.008
ME caloric efficiency[a7]	10,483	10,325	10,403	10,309	8,939	8,739	8,689	8,733	229.4
NE caloric efficiency[a7]	7,909	7,795	7,849	7,776	6,746	6,594	6,557	6,589	173.1
Overall (d 0 to 90)[6]									
ADG,[a] kg	0.98	0.99	0.99	0.99	1.03	1.03	1.04	1.03	0.011
ADFI, kg	2.67	2.67	2.70	2.68	2.70	2.67	2.67	2.68	0.053
G:F[ac]	0.37	0.37	0.37	0.37	0.38	0.39	0.39	0.38	0.006
ME caloric efficiency[ac7]	8,996	8,957	8,973	8,986	8,697	8,535	8,472	8,595	134.5
NE caloric efficiency[ac7]	6,799	6,768	6,780	6,790	6,573	6,448	6,402	6,495	100.1

[a]Ractopamine effect ($P < 0.001$).

[b]Zinc × Cu interaction ($P < 0.05$).

[c]Zinc × Cu interaction ($P < 0.10$).

[1]A total of 480 pigs (PIC 327 × 1050; initially 48.7 ± 2.3 kg) were used in a 90- (group 1) or 83-d (group 2) study with 7 (group 1) or 8 (group 2) pigs per pen and 8 replications per treatment.

[2]Copper from $CuSO_4$ was added to treatment diets at either 0 or 125 mg/kg . All diets contained 11 mg/kg Cu from the trace mineral premix.

[3]Zinc from ZnO was added to treatment diets at either 0 or 150 mg/kg . All diets contained 73 mg/kg Zn from the trace mineral premix.

[4]Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to treatment diets at either 0 or 10 mg/kg during the final 28 d prior to marketing.

[5]d 62 (group 1) corresponds to d 55 of group 2 and marks the beginning of the 28-d period of ractopamine HCl treatments in addition to the mineral combination treatments.

[6]d 90 (group 1) corresponds to d 83 of group 2 and is the final day of the study.

[7]Caloric efficiency is expressed as kilocalories per kilogram of live weight gain.

0.014) backfat and greater ($P < 0.001$) loin depth and percentage of fat-free lean compared with pigs not fed RAC. In contrast, no differences in backfat, loin depth, or fat-free lean were observed due to added Cu or Zn.

Antimicrobial Resistance

Antibiotic susceptibility results are shown in Tables 7 through 10. For each antibiotic, the percentage resistant for each of the 8 dietary treatments is listed in Tables 7 and 9. As shown in these tables, we were unable to calculate variance components and related test statistics on some of the antibiotics as they were associated with either 0 or 100% resistance within each sampling day. However, evaluation of the treatment main effects rather than interactive effects resulted in model convergence for some of these antibiotics as displayed in Tables 8 and 10.

Escherichia coli *Resistance*

All the tested fecal *E. coli* isolates were susceptible to ciprofloxacin, sulfisoxazole, and trimethoprim/sulfamethoxazole at either d 0 (baseline) or 90 (Table 7). None of the isolates grown in the presence of nalidixic acid were resistant except for 1 isolate (5% resistant) on d 0 in 1 pen subsequently fed the Cu and RAC treat-

Table 6. Effects of added Cu, Zn, and ractopamine HCl on finishing pig carcass characteristics[1]

	Added Cu,[2] 125 mg/kg								
	–	+	–	+	–	+	–	+	
	Added Zn,[3] 150 mg/kg								
	–	–	+	+	–	–	+	+	
	Ractopamine HCl,[4] 10 mg/kg								
Item	–	–	–	–	+	+	+	+	SEM
Carcass characteristics									
HCW,[a] kg	99.3	98.4	99.4	99.0	103.3	103.9	103.9	103.1	4.32
Carcass yield,[a] %	73.8	73.5	73.6	73.8	74.6	75.2	74.9	74.5	0.86
Backfat,[a5] mm	19.2	19.3	18.7	19.3	18.2	18.0	18.1	17.8	0.89
Loin depth,[5] cm	6.55	6.48	6.48	6.55	6.77	6.92	6.85	6.90	0.22
Fat-free lean,[a5,6] %	51.5	51.7	51.6	51.5	52.6	52.8	52.6	53.1	0.30
Carcass performance									
HCW ADG,[a] kg	0.73	0.72	0.73	0.72	0.77	0.78	0.78	0.77	0.020
HCW G:F[ab]	0.27	0.27	0.27	0.27	0.28	0.29	0.29	0.29	0.003

[a]Ractopamine effect ($P < 0.001$).

[b]Ractopamine × Zn × Cu interaction ($P = 0.018$).

[1]A total of 480 pigs (PIC 327 × 1050; initially 48.7 ± 2.3 kg) were used in a 90- (group 1) or 83-d (group 2) study with 7 (group 1) or 8 (group 2) pigs per pen and 8 replications per treatment.

[2]Copper from $CuSO_4$ was added to treatment diets at either 0 or 125 mg/kg . All diets contained 11 mg/kg Cu from the trace mineral premix.

[3]Zinc from ZnO was added to treatment diets at either 0 or 150 mg/kg . All diets contained 73 mg/kg Zn from the trace mineral premix.

[4]Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to treatment diets at either 0 or 10 mg/kg during the final 28 d prior to marketing.

[5]Adjusted for individual HCW using HCW as a covariate.

[6]Standardized fat-free lean (NPPC, 2000)/HCW.

ment diet; all other baseline isolates as well as d-90 isolates were categorized as susceptible. All baseline *E. coli* isolates were resistant to ampicillin, but by d 90, the percentage decreased to 85% of isolates from pigs that had received the control diet, 90% of isolates from pigs that had received the Cu and Zn diet, and 95% of isolates from pigs that had received the Zn and RAC diet whereas 100% resistance was still observed for isolates from pigs fed all other treatments.

For *E. coli* isolates, the percentage resistant to amoxicillin:clavulanic acid 2:1 ratio, cefoxitin, ceftiofur, ceftriaxone, gentamicin, streptomycin, and tetracycline decreased ($P < 0.05$) from d 0 to 90 (Table 8). Ractopamine HCl did not affect the percentage of antibiotic-resistant *E. coli* isolates. Zinc did not adversely affect antibiotic resistance among fecal *E. coli* isolates. Pigs fed Zn had a greater rate (Zn within day, $P < 0.05$) of decreasing percent resistant to streptomycin compared with pigs fed the diets without added Zn. This resulted in a significant decrease ($P < 0.05$) in resistant *E. coli* isolates from baseline to d 90 for pigs fed Zn but only a numerical decrease for those fed diets without Zn. Because *E. coli* isolates from all pigs fed diet treatments containing Zn were 100% susceptible to gentamicin on d 90, the corresponding variance components were zero so no statistics were computed (Table 7).

Copper treatment did not affect antimicrobial resistance over time aside from a marginally significant effect ($P = 0.069$) on resistance to ceftiofur and ceftriaxone. *Escherichia coli* isolates had a similar pattern of resistance to both ceftiofur and ceftriaxone, where isolates from pigs subsequently fed Cu had less antibiotic resistance on d 0 than isolates from pigs not fed Cu. However, the resistance level decreased by d 90 among pigs not fed Cu but did not decrease among pigs fed Cu so that by d 90, the resistance was not different between pigs that had been fed Cu versus those that had not been fed Cu.

Enterococcus *Resistance*

All the tested fecal *Enterococcus* spp. isolates were susceptible to both nitrofurantoin and vancomycin antibiotics (Table 9). On d 0, prior to initiation of diet treatments, enterococcal isolates displayed a low level of resistance (≤10%) to chloramphenicol, gentamicin, linezolid, penicillin, and tigecycline. However, by d 90, before harvesting, no isolates that were resistant to these antibiotics were observed across all dietary treatments. Across all treatments, the estimated percentage of enterococcal isolates that were resistance to erythromycin, lincomycin, and quinupristin/dalfopristin decreased ($P < 0.05$) from d 0 to 90 whereas resistance to tetracycline increased ($P < 0.05$) throughout the finishing period (Table 10). Neither Zn nor RAC treatments affected antibiotic susceptibility of the enterococcal isolates.

Table 7. Effects of added Cu, Zn, and ractopamine HCl on percentage fecal *Escherichia coli* antimicrobial resistance according to National Antimicrobial Resistance Monitoring System (CLSI, 2013) established breakpoints[1]

	Added Cu,[2] 125 mg/kg							
	–	+	–	+	–	+	–	+
	Added Zn,[3] 150 mg/kg							
	–	–	+	+	–	–	+	+
	Ractopamine HCl,[4] 10 mg/kg							
Item	–	–	–	–	+	+	+	+
Amoxicillin:clavulanic acid 2:1 ratio								
Baseline	35	10	30	15	15	20	35	10
Day 90	5	15	5	10	5	0	15	5
Ampicillin								
Baseline	100	100	100	100	100	100	100	100
Day 90	85	100	100	90	100	100	95	100
Cefoxitin								
Baseline	35	10	30	15	15	20	35	10
Day 90	5	15	5	10	5	0	15	5
Ceftiofur								
Baseline	35	10	30	15	15	20	35	10
Day 90	5	25	10	10	5	5	15	10
Ceftriaxone								
Baseline	35	10	30	15	15	20	35	10
Day 90	5	25	10	10	5	5	15	10
Chloramphenicol								
Baseline	25	0	20	10	15	10	5	15
Day 90	5	5	10	10	15	10	0	10
Ciprofloxacin								
Baseline	0	0	0	0	0	0	0	0
Day 90	0	0	0	0	0	0	0	0
Gentamicin								
Baseline	25	15	30	25	15	10	25	20
Day 90	5	5	0	0	10	0	0	0
Nalidixic acid								
Baseline	0	0	0	0	0	5	0	0
Day 90	0	0	0	0	0	0	0	0
Streptomycin								
Baseline	35	20	35	25	15	20	30	30
Day 90	10	20	5	0	15	5	5	0
Sulfisoxazole								
Baseline	0	0	0	0	0	0	0	0
Day 90	0	0	0	0	0	0	0	0
Tetracycline								
Baseline	100	80	70	90	80	80	95	90
Day 90	60	80	70	80	85	75	70	75
Trimethoprim/sulfamethoxazole								
Baseline	0	0	0	0	0	0	0	0
Day 90	0	0	0	0	0	0	0	0

[1]Values represent the percentage resistant of 20 *E. coli* isolates per sampling day (d 0 baseline or d 90); 5 random fecal samples were collected per pen per day and 1 *E. coli* isolate per fecal sample was assessed. There were a total of 224 pigs (PIC 327 × 1050; initially 49 ± 1.9 kg) housed with 7 (group 1) pigs per pen and 4 replicate pens per treatment.

[2]Copper from $CuSO_4$ was added to treatment diets at either 0 or 125 mg/kg . All diets contained 11 mg/kg Cu from the trace mineral premix.

[3]Zinc from ZnO was added to treatment diets at either 0 or 150 mg/kg . All diets contained 73 mg/kg Zn from the trace mineral premix.

[4]Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to treatment diets at either 0 or 10 mg/kg during the final 28 d prior to the end of the study (d 62 to 90).

Copper did not affect the percentage of enterococcal isolates resistant to daptomycin, erythromycin, kanamycin, lincomycin, or streptomycin over time (Table 10). However, the percentage of resistance to quinupristin/dalfopristin and tylosin tartrate was affected ($P = 0.023$) by Cu treatment over time. Resistance to quinupristin/dalfopristin and tylosin decreased ($P < 0.05$) from d 0 to 90 among isolates from pigs not fed added Cu whereas the percent of resistant isolates from pigs fed added Cu did not differ from d 0 to 90. Consequently, the percentage of quinupristin/dalfopristin-resistant isolates from pigs fed Cu was greater ($P < 0.05$) on d 90 than that of isolates from pigs that were not fed Cu. Copper treatment also affected ($P = 0.003$) the percentage of enterococcal isolates resistant to tetracycline over time, as baseline isolates from pigs subsequently fed added Cu were initially more ($P < 0.05$) susceptible to tetracycline than isolates from pigs that would not be fed added Cu. However, resistance to tetracycline among enterococcal isolates from pigs fed added Cu increased so that by d 90, there was no difference in *Enterococcus* spp. susceptibility to tetracycline. There were no ciprofloxacin-resistant enterococcal isolates detected on d 90 from pigs fed diets without added Cu, so the corresponding variance components were zero and no statistics were computed (Table 9). However, baseline resistance across all treatments initially ranged from 0 to 15% and over time, isolates from pigs fed diets containing added Cu had a smaller numerical decrease in resistance to ciprofloxacin compared with isolates from pigs fed diets without added Cu.

DISCUSSION

The combined or lone supplementation of 125 mg/kg Cu and 150 mg/kg Zn above trace mineral premix levels failed to improve gain, feed intake, efficiency, or carcass characteristics. The growth benefits of feeding added Cu and Zn to weaned pigs in the nursery phase are well established and are seemingly driven largely

Table 8. Main effects of added Cu, Zn, and ractopamine HCl on percentage fecal *Escherichia coli* antimicrobial resistance according to National Antimicrobial Resistance Monitoring System (CLSI, 2013) established breakpoints[1]

	Cu[2]			Cu, d,	Zn[3]			Zn, d,	Ractopamine HCl[4]			RAC,[5] d,
Item	–	+	SEM	*P*-value <	–	+	SEM	*P*-value <	–	+	SEM	*P*-value <
Amoxicillin:clavulanic acid 2:1 ratio[d]												
Baseline	28.8	13.8	5.61	0.253	20.0	22.5	5.19	0.791	22.5	20.0	5.13	0.788
Day 90	7.5	7.5			6.3	8.8			8.8	6.3		
Cefoxitin[d]												
Baseline	28.8	13.8	5.61	0.253	20.0	22.5	5.19	0.791	22.5	20.0	5.13	0.788
Day 90	7.5	7.5			6.3	8.8			8.8	6.3		
Ceftiofur[d]												
Baseline	28.8	13.8	5.48	0.069	20.0	22.5	5.08	0.980	22.5	20.0	5.08	0.726
Day 90	8.8	12.5			10.0	11.3			12.5	8.8		
Ceftriaxone[d]												
Baseline	28.8	13.8	5.48	0.069	20.0	22.5	5.08	0.980	22.5	20.0	5.08	0.726
Day 90	8.8	12.5			10.0	11.3			12.5	8.8		
Chloramphenicol												
Baseline	16.3	8.8	3.77	0.216	12.5	12.5	3.41	0.810	13.8	11.3	3.52	0.566
Day 90	7.5	8.8			8.8	7.5			7.5	8.8		
Gentamicin[d]												
Baseline	23.8	17.5	5.09	0.576	16.3	25.0	–	–	23.8	17.5	5.07	0.741
Day 90	3.8	1.3			5.0	0			2.5	2.5		
Streptomycin[d]												
Baseline	28.8	23.8	5.94	0.900	22.5[ab]	30.0[a]	5.58	0.030	28.8	23.8	5.81	0.898
Day 90	8.8	6.3			12.5[b]	2.5[c]			8.8	6.3		
Tetracycline[d]												
Baseline	86.3	85.0	6.38	0.558	85.0	86.3	6.19	0.819	85.0	86.3	6.27	0.896
Day 90	71.3	77.5			75.0	73.8			72.5	76.3		

[a–c]Means within main effect and antibiotic lacking common superscripts differ, $P < 0.05$.

[d]Percentage of resistant isolates decreased (day, $P < 0.05$) between d 0 (baseline) and 90.

[1]Values represent the percentage resistant among 80 *E. coli* isolates for determination of treatment main effects within day. Five random fecal samples were collected per pen per day (d 0 baseline or d 90) and 1 *E. coli* isolate per fecal sample was assessed. There were a total of 224 pigs (PIC 327 × 1050; initially 49 ± 1.9 kg) housed with 7 (group 1) pigs per pen and 4 replicate pens per treatment.

[2]Copper from $CuSO_4$ was added to treatment diets at either 0 or 125 mg/kg. All diets contained 11 mg/kg Cu from the trace mineral premix.

[3]Zinc from ZnO was added to treatment diets at either 0 or 150 mg/kg . All diets contained 73 mg/kg Zn from the trace mineral premix.

[4]Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to treatment diets at either 0 or 10 mg/kg during the final 28 d prior to the end of the study (d 62 to 90).

[5]RAC = ractopamine HCl.

by increased feed intake although efficiency of gain can also be improved (Sales, 2013; Ma et al., 2015). In contrast, the response to supplementation later in the growth period, such as the finishing period of our study, is less consistent.

In a series of experiments conducted by the NCR-42 Committee on Swine Nutrition (1974), the response to Cu was highly variable but, overall, failed to improve gain or efficiency over the growing–finishing period. In contrast, Davis et al. (2002) reported improved gain and efficiency with 175 mg/kg Cu. Several studies have demonstrated that a decrease in or complete elimination of any trace mineral supplementation including Cu and Zn to a typical corn–soybean meal–based diet during all or part of the finishing period did not significantly worsen growth performance or carcass characteristics (Shelton et al., 2004; Ma et al., 2012; Gowanlock et al., 2013). It should be noted that some of these studies included antibiotics in the diets, which may have bolstered performance in the absence of trace mineral supplementation. The pigs in the current study were enrolled in this finishing period study at a relatively heavy weight (49 kg) and had high levels of feed intake across all treatments. Moreover, this study was conducted in a research setting conducive to high growth performance. Together, these factors possibly precluded any potential for mineral supplementation to improve the growth responses of these pigs.

Prior to the final 28 d of the finishing period, a significant interaction between Cu and Zn was observed for feed and caloric efficiency, where pigs fed diets with either added Cu or Zn alone had numeric improvement in efficiency but no improvement when both minerals were added together. In the final 28 d of finishing period, no evidence for interactive effects

Table 9. Effects of added Cu, Zn, and ractopamine HCl on percentage fecal *Enterococcus* spp. antimicrobial resistance according to National Antimicrobial Resistance Monitoring System (CLSI, 2013) established breakpoints[1]

	Added Cu,[2] 125 mg/kg							
	–	+	–	+	–	+	–	+
	Added Zn,[3] 150 mg/kg							
	–	–	+	+	–	–	+	+
	Ractopamine HCl,[4] 10 mg/kg							
Item	–	–	–	–	+	+	+	+
Chloramphenicol								
Baseline	0	10	0	5	0	0	10	0
Day 90	0	0	0	0	0	0	0	0
Ciprofloxacin								
Baseline	10	15	5	5	10	15	0	0
Day 90	0	5	0	10	0	5	0	0
Daptomycin[5]								
Baseline	5	0	5	15	5	5	10	10
Day 90	10	0	15	10	5	10	15	5
Erythromycin								
Baseline	80	80	80	85	75	80	65	80
Day 90	15	30	15	25	5	30	20	5
Gentamicin								
Baseline	0	5	5	0	0	0	0	0
Day 90	0	0	0	0	0	0	0	0
Kanamycin								
Baseline	10	5	20	20	5	0	5	15
Day 90	0	0	10	10	0	10	0	0
Lincomycin								
Baseline	100	100	95	95	100	95	95	95
Day 90	95	70	85	60	90	80	90	70
Linezolid								
Baseline	0	0	0	5	0	0	5	0
Day 90	0	0	0	0	0	0	0	0
Nitrofurantoin								
Baseline	0	0	0	0	0	0	0	0
Day 90	0	0	0	0	0	0	0	0
Penicillin								
Baseline	0	5	0	5	10	5	5	0
Day 90	0	0	0	0	0	0	0	0
Quinupristin/dalfopristin								
Baseline	40	45	55	40	65	40	50	50
Day 90	5	20	10	35	10	25	25	40
Streptomycin								
Baseline	10	5	5	15	0	0	5	15
Day 90	5	5	10	5	0	15	0	0
Tetracycline								
Baseline	75	70	80	40	75	55	70	40
Day 90	60	90	65	80	70	65	85	80
Tigecycline[6]								
Baseline	0	0	5	0	0	0	0	0
Day 90	0	0	0	0	0	0	0	0
Tylosin tartrate								
Baseline	30	20	45	20	15	5	25	25
Day 90	15	30	10	30	5	30	20	5
Vancomycin								
Baseline	0	0	0	0	0	0	0	0
Day 90	0	0	0	0	0	0	0	0

[1]Values represent the percentage resistant of 20 *Enterococcus* spp. isolates per sampling day (d 0 baseline or d 90); 5 random fecal samples were collected per pen per day and 1 enterococcal isolate per fecal sample was assessed. There were a total of 224 pigs (PIC 327 × 1050; initially 49 ± 1.9 kg) housed with 7 (group 1) pigs per pen and 4 replicate pens per treatment.

[2]Copper from $CuSO_4$ was added to treatment diets at either 0 or 125 mg/kg . All diets contained 11 mg/kg Cu from the trace mineral premix.

[3]Zinc from ZnO was added to treatment diets at either 0 or 150 mg/kg . All diets contained 73 mg/kg Zn from the trace mineral premix.

[4]Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to treatment diets at either 0 or 10 mg/kg during the final 28 d prior to the end of the study (d 62 to 90).

[5]A susceptibility breakpoint of ≤4 μg/mL for daptomycin exists but no resistant breakpoint has been established. In this study, isolates with a minimal inhibitory concentration ≥ 8 μg/mL were categorized as resistant.

[6]A susceptibility breakpoint of ≤0.25 μg/mL for tigecycline exists but no resistant breakpoint has been established. In this study, isolates with a minimal inhibitory concentration ≥ 0.5 μg/mL were categorized as resistant.

between the minerals was observed, possibly indicating the combined level of pharmacological minerals in the lower BW pigs was detrimental to efficient gain. Accordingly, although supplementation of both Cu and Zn at levels well above the pig's physiological requirements improve weaned pig performance (Pérez et al., 2011; Shelton et al., 2011), extended supplementation of minerals may lead to poor performance. Yet Kline et al. (1972) reported no main or interactive effects of up to 500 mg/kg Cu and up to 300 mg/kg Zn on feed efficiency when fed from 17.3 to 90.8 kg.

In the present study, RAC fed at 10 mg/kg during the final 28 d of the finishing period improved late finishing growth rate, final BW, HCW, and percentage carcass yield without increasing feed intake, thereby causing greater caloric and feed efficiency. Apple et al. (2007) conducted a meta-analysis across a wide range of genotypes and environments and reported that ractopamine minimally affects feed intake when fed at 5 to 10 mg/kg but can slightly decrease feed intake when considered over a large number of studies. In addition, pigs fed 10 mg/kg exhibited respective improvements over controls in daily gain, feed efficiency, and HCW of 11.8, 13.3, and 3.1%, respectively, and percentage improved with a range from 0.7 to 2.2% improvement. Accordingly, pigs of the present study demonstrated no difference in feed intake and similar magnitudes of improvement in daily

Table 10. Main effects of added Cu, Zn, and ractopamine HCl on percentage fecal *Enterococcus* spp. antimicrobial resistance according to National Antimicrobial Resistance Monitoring System (CLSI, 2013) established breakpoints[1]

	Cu[2]			Cu, d,	Zn[3]			Zn, d,	Ractopamine HCl[4]			RAC,[5] d,
Item	–	+	SEM	*P*-value <	–	+	SEM	*P*-value <	–	+	SEM	*P*-value <
Ciprofloxacin												
Baseline	6.3	8.8	–	–	12.5	2.5	4.39	0.265	8.8	6.3	3.95	0.645
Day 90	0	5.0			2.5	2.5			3.8	1.3		
Daptomycin[6]												
Baseline	6.3	7.5	3.58	0.338	3.75	10.0	3.50	0.654	6.3	7.5	3.29	0.824
Day 90	11.3	6.3			6.25	11.3			8.8	8.8		
Erythromycin[d]												
Baseline	75.0	81.3	5.44	0.719	78.8	77.5	5.47	0.785	81.3	75.0	5.50	0.930
Day 90	13.8	22.5			20.0	16.3			21.3	15.0		
Kanamycin												
Baseline	10.0	10.0	3.95	0.555	5.0	15.0	4.26	0.668	13.8	6.3	4.25	0.895
Day 90	2.5	5.0			2.5	5.0			5.0	2.5		
Lincomycin[d]												
Baseline	97.5	96.3	5.11	0.367	98.8	95.0	4.81	0.435	97.5	96.3	4.77	0.480
Day 90	90.0	70.0			83.8	76.3			77.5	82.5		
Quinupristin/dalfopristin[d]												
Baseline	52.5[a]	43.8[ab]	6.61	0.023	47.5	48.8	6.64	0.243	45.0	51.3	6.73	0.741
Day 90	12.5[c]	30.0[b]			15.0	27.5			17.5	25.0		
Streptomycin												
Baseline	5.0	8.8	3.55	0.955	3.8	10.0	3.74	0.169	8.8	5.0	3.63	0.956
Day 90	3.8	6.3			6.3	3.8			6.3	3.8		
Tetracycline[d]												
Baseline	75.0[a]	51.3[b]	5.43	0.003	68.8	57.5	5.69	0.113	66.3	60.0	5.76	0.518
Day 90	70.0[a]	78.8[a]			71.3	77.5			73.8	75.0		
Tylosin tartrate												
Baseline	28.8[a]	17.5[ab]	5.34	0.023	17.5	28.8	5.61	0.157	28.8	17.5	5.52	0.725
Day 90	12.5[bc]	23.8[ab]			20.0	16.3			21.3	15.0		

[a–c]Means within main effect and antibiotic lacking common superscripts differ, $P < 0.05$.

[d]Percentage of resistant isolates differed (day, $P < 0.05$) between d 0 (baseline) and 90.

[1]Values represent the percentage resistant among 80 *Enterococcus* spp. isolates for determination of treatment main effects within day. Five random fecal samples were collected per pen per day (d 0 baseline or d 90) and 1 enterococcal isolate per fecal sample was assessed. There were a total of 224 pigs (PIC 327 × 1050; initially 49 ± 1.9 kg) housed with 7 (group 1) pigs per pen and 4 replicate pens per treatment.

[2]Copper from $CuSO_4$ was added to treatment diets at either 0 or 125 mg/kg. All diets contained 11 mg/kg Cu from the trace mineral premix.

[3]Zinc from ZnO was added to treatment diets at either 0 or 150 mg/kg . All diets contained 73 mg/kg Zn from the trace mineral premix.

[4]Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN) was added to treatment diets at either 0 or 10 mg/kg during the final 28 d prior to the end of the study (d 62 to 90).

[5]RAC = ractopamine HCl.

[6]A susceptibility breakpoint of ≤4 μg/mL for daptomycin exists but no resistant breakpoint has been established. In this study, isolates with a minimal inhibitory concentration ≥ 8 μg/mL were categorized as resistant.

gain, feed efficiency, HCW, and dressing percent of 17.5, 15.3, 4.5, and 1.5%, respectively.

When compared on a common HCW basis, pigs of the study herein that were fed RAC had less backfat and greater loin depth and percentage of fat-free lean compared with pigs not fed RAC. In the current study, the magnitude of the increase in percent yield due to ractopamine was numerically greater for pigs fed either added Cu or Zn throughout the entire finishing period; however, the minerals did not provide any carcass yield benefit when both were fed together and RAC was also fed. No other significant interactions between ractopamine and the minerals were observed in the growth and carcass responses. These observations indicate that 150 mg/kg Zn supplemented throughout the entire finishing period did not improve growth and carcass responses when fed with or without ractopamine.

In contrast, several studies have suggested that additional improvements to the increased feed efficiency realized through ractopamine feeding can be achieved with concurrent Zn supplementation. This supposition is biologically based on the fact that Zn mediates insulin-like growth factor-1 (IGF-1) induced cell proliferation and growth (MacDonald, 2000; Salgueiro et al., 2002)

and may be a key nutrient to support the lean gain of pigs fed ractopamine. Patience et al. (2011), Rambo et al. (2012), and Fry et al. (2013) reported that an organic source of Zn improved performance over that of Zn from an inorganic source. Most recently, Paulk et al. (2015) reported where Zn from ZnO linearly improved feed efficiency in one study but Zn from inorganic and organic sources had no response in the presence of ractopamine in a second study. Conversely, when Gowanlock et al. (2013) fed diets without any trace mineral supplementation for the entire finishing period or supplemented just Zn from an organic source, no significant differences in growth performance (gain, feed intake, or feed efficiency) or in carcass characteristics (HCW, back fat depth, or loin muscle area) were observed due to a lack of Zn supplementation despite the inclusion of 10 mg/kg ractopamine for 21 d prior to marketing. To summarize, the response to Zn supplementation is highly variable irrespective of whether the Zn is supplemented from an organic or an inorganic source.

Several chromosomal or plasmid-borne genes have been implicated to carry antibiotic resistance determinants among bacteria, whereas phenotypic resistance is conferred through multiple and often complex mechanisms. Although antibiotic-resistant genes are ubiquitously present among both humans and animals (Agga et al., 2015), it is important to understand what dietary factors, if any, may contribute to increased antibiotic resistance among fecal bacteria of finishing swine. Supplementing diets with 150 mg/kg Zn above the basal premix trace mineral level throughout the entire finishing period did not adversely affect *E. coli* or *Enterococcus* susceptibility to antibiotics. However, Zn actually decreased *E. coli* resistance to streptomycin and possibly to gentamicin. Curiously, both streptomycin and gentamicin belong to the aminoglycoside drug class, which primarily targets the 16S rRNA, thereby interfering with ribosomal function (Davies, 1971). This aminoglycosidic action is predominantly thwarted through bacterial production of enzymes that chemically modify the antibiotic or protect 16S rRNA, and these mechanisms are encoded by transferable plasmid-borne genes (Yamane et al., 2005), which are not restricted to within-species transfer (Shaw et al., 1993). The observations of the current study indicate Zn may downregulate aminoglycoside resistance among *E. coli* through some mechanistic role in these processes.

Although methicillin-resistant *S. aureus* (**MRSA**) has a higher phenotypic resistance to Zn and a greater prevalence of the Zn-resistant gene (*czrC*) compared with methicillin-susceptible *S. aureus* (**MSSA**; Aarestrup et al., 2010; Cavaco et al., 2011), it is unclear whether Zn and methicillin experience co-selection (Yazdankhah et al., 2014). Both MRSA and MSSA isolates showed similar resistance to erythromycin, penicillin, and tetracycline despite the difference in Zn susceptibility (Aarestrup et al., 2010). In contrast to the results of the current study, Bednorz et al. (2013) reported pharmacological Zn from ZnO increased multiple drug resistance among Gram-negative bacteria possibly through co-selection or through increased rate of plasmid assimilation. Vahjen et al. (2015) reported that pharmacological levels of Zn from ZnO increased tetracycline (*tet*A) and sulfonamide (*sul*1) resistance genotypes. However, Jacob et al. (2010) observed no effect of feeding cattle 300 mg/kg Zn on expression of *erm*(B) and *tet*(M) genes, which are associated with macrolide and tetracycline resistance, respectively. Furthermore, feeding Zn at this level did not affect resistance pattern among *E. coli* isolates to erythromycin, penicillin, or tylosin or affect enterococcal isolate resistance to chloramphenicol, ciprofloxacin, gentamicin, linezolid, penicillin, streptomycin, or vancomycin in cattle. Therefore, extended feeding of a concentration of 150 mg/kg Zn in finishing pig diets may not elicit the antimicrobial resistance responses that higher concentrations might appear to stimulate.

Supplementation of 125 mg/kg Cu above basal premix trace mineral level throughout the finishing period increased *Enterococcus* resistance to tetracycline and antagonized the decrease in *Enterococcus* resistance to the streptogramin antibiotics quinupristin/dalfopristin and a macrolide drug, tylosin tartrate, over time. All 3 of these drug classes are protein synthesis inhibitors and interfere with the normal ribosomal function in bacteria. Transferable Cu resistance gene (*tcrB*) carrying plasmids have been observed to also carry genes resistant to macrolides and tetracyclines on the same plasmid (Hasman and Aarestrup, 2002; Hasman et al., 2006; Amachawadi et al., 2011, 2013) indicating that Cu may co-select for greater Cu tolerance and multiple drug resistance. Along with tetracycline, phenotypic erythromycin resistance has been associated with Cu-resistant enterococci (Amachawadi et al., 2013; Silveira et al., 2014). As such, Cu would have been expected to also increase resistance to erythromycin, another macrolide drug, in addition to tylosin tartrate, but this effect was not observed in the present study. However, 100 mg/kg Cu in cattle did not affect genotypic resistance to macrolides and tetracycline (Jacob et al., 2010). Hence, the phenotypic expression of bacterial resistance to all macrolide drugs may not be observable at a dose of 125 mg/kg in finishing pigs.

In the case of *E. coli*, Cu supplementation decreases susceptibility to 2 important antibiotics, ceftiofur and ceftriaxone, third-generation cephalosporins. This is in agreement with another study with pigs that reported an association between ceftiofur and tetracycline resistance of *E. coli* in young pigs mediated by $bla_{\text{CMY-2}}$ and *tetA*

genes, respectively (Agga et al., 2014). This strong association between cephalosporins and tetracyclines among *E. coli* isolates from a swine production system is a clear indication of maintenance of resistance determinants meant for cephalosporin resistance via a tetracycline genetic element. In the current study, Cu significantly increased tetracycline resistance among *Enterococcus* but not among *E. coli* isolates, possibly because Gram-negative bacteria are less sensitive to high concentrations of Cu than Gram-positive bacteria (Aarestrup and Hasman, 2004). Therefore, Cu may increase Gram-negative bacterial resistance to cephalosporins apart from co-selection with tetracycline.

Feeding pigs 10 mg/kg RAC for 28 d prior to marketing did not affect the susceptibility of fecal *E. coli* or *Enterococcus* to antibiotics. This novel observation suggests that feeding ractopamine to pigs according to the typical production practice does not pose a significant risk of increasing bacterial resistance to a wide range of antibiotics. Limited research has investigated the roles of α- and β-adrenergic receptors in mediating the bacterial response to catecholamines. Although both the α- and β-receptors appear to be involved, the α-adrenergic receptor may have a dominant role in facilitating bacterial growth, conjugative gene transfer, and increased bacterial virulence in response to catecholamines (Peterson et al., 2011). As such, β-adrenergic agonists such as RAC may have a limited ability to influence antimicrobial resistance.

In the present study, both *E. coli* and *Enterococcus* isolates, which were resistant to a number of antibiotics on d 0, gradually decreased throughout the finishing period regardless of dietary treatments. Similar observations have led to the postulation that the gastrointestinal microbiota of older animals is less vulnerable to a population with multiple antibiotic-resistant bacteria compared with younger animals (Langlois et al., 1986; Dewulf et al., 2007; Berge et al., 2010). This phenomenon could also be an artifact of greater antimicrobial use among younger livestock inducing microbial drug resistance early in life and then decreasing selection pressure over time causing these resistant microbes to have poorer relative competitiveness and survival (Dewulf et al., 2007).

In the present study, resistance to tetracycline was high among both *E. coli* and *Enterococcus* isolates and *E. coli* isolates also showed a high level of resistance to ampicillin. This low bacterial susceptibility to tetracycline and ampicillin may be reflective of these drugs' use in upstream production within the sow herd. Tetracycline resistance is conferred through *tet* and *otr* genes (Roberts, 2011), and subtherapeutic use of tetracycline has been shown to induce tetracycline-resistant genotypes as well as phenotypes (Funk et al., 2006; Dewulf et al., 2007; Agga et al., 2014). In addition, tetracycline resistance is associated with ampicillin drug resistance among Gram-negative bacteria (Funk et al., 2006; Dewulf et al., 2007). In the present study, the ampicillin resistance remained high over time but tetracycline resistance decreased throughout the finishing period among *E. coli* isolates whereas tetracycline resistance concomitantly increased among *Enterococcus* isolates. However, tetracycline resistance was initially greater in baseline *E. coli* isolates than in baseline *Enterococcus* isolates and both exhibited a similar, intermediate level of resistance by d 90. Genetic exchange between Gram-negative and Gram-positive bacteria can occur so that plasmid-mediated transfer of a common tetracycline resistance gene or genes from *E. coli* to *Enterococcus* would not be impossible (Courvalin, 1994; Roberts, 2011).

Conclusion

The results from the present study showed that the supplementation of 125 mg/kg Cu or 150 mg/kg Zn above basal premix trace mineral levels in diets containing RAC did not improve finishing pig growth performance of pigs with high feed intake levels as observed in this study. Inclusion of 10 mg/kg RAC in the diet for 28 d prior to marketing dramatically improved carcass leanness as well as the feed and caloric efficiencies of pigs. Ractopamine HCl did not adversely affect antimicrobial resistance among fecal bacterial isolates. Extended feeding of 125 mg/kg Cu throughout the finishing period resulted in less bacterial susceptibility to some antibiotics whereas there were no adverse effects of feeding 150 mg/kg of added Zn noted on antimicrobial resistance. In general, with the exception of tetracycline, resistance to most antibiotics decreased over time or was stable for antibiotics that had a low percentage of resistance at baseline.

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