Effect of added zinc in diets with ractopamine hydrochloride on growth performance, carcass characteristics, and ileal mucosal inflammation mRNA expression of finishing pigs[1,2]

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ABSTRACT: Two experiments were conducted to determine the effects of increasing the dietary Zn content on growth performance, carcass characteristics, plasma Zn, and ileal mucosal inflammation mRNA expression of finishing pigs fed diets containing ractopamine HCl (RAC; Elanco Animal Health, Greenfield, IN). In Exp. 1, 312 pigs (327 × 1050; PIC, Hendersonville, TN; 94 kg BW) were used in a 27-d study. There were 2 pigs per pen and 26 pens per treatment. Treatments included a corn–soybean meal diet (control; 0.66% standardized ileal digestible [SID] Lys); a diet (0.92% SID Lys) with 10 mg/kg RAC; and the RAC diet plus 50, 100, or 150 mg Zn/kg from ZnO or 50 mg Zn/kg from a Zn AA complex (ZnAA; Availa-Zn; Zinpro, Eden Prairie, MN). All diets also contained 83 mg Zn/kg from $ZnSO_4$ in the trace mineral premix. Pigs fed the RAC diet without added Zn had increased ($P < 0.05$) ADG, G:F, HCW, carcass yield, and loin weight compared with pigs fed the control diet. Increasing Zn from ZnO in diets containing RAC tended to increase (linear, $P = 0.067$) G:F and loin weight (quadratic, $P = 0.064$). Pigs fed diets with 50 mg Zn/kg from ZnAA tended to have increased ($P = 0.057$) ADG compared with pigs fed the RAC diet. In Exp. 2, 320 pigs (327 × 1050; PIC; 98 kg BW) were used in a 35-d study. There were 2 pigs per pen and 20 pens per treatment. Treatments included a control diet (0.66% SID Lys); a diet (0.92% SID Lys) with 10 mg/kg RAC; or the RAC diet plus 75, 150, and 225 mg Zn/kg from ZnO or ZnAA. All diets also contained 55 mg Zn/kg from $ZnSO_4$ from the trace mineral premix. Pigs fed the RAC diet had increased ($P < 0.05$) ADG, G:F, HCW, loin depth, percentage lean, and liver weight compared with pigs fed the control diet. No Zn level or source effects or level × source interactions were observed for growth performance. A Zn level × source interaction (quadratic, $P = 0.007$) was observed in liver Zn concentrations. This resulted from liver Zn concentrations plateauing at 150 mg Zn/kg when ZnO was supplemented, while there was a linear increase when using ZnAA. Increasing Zn in diets containing RAC increased (linear, $P < 0.05$) plasma Zn on d 18 and 32. The expression of *IL-1*β was increased ($P = 0.014$) in mucosa of pigs fed the RAC diet compared with those fed the control diet. Expression of *IL-1*β decreased (linear, $P = 0.026$) in the mucosa of pigs fed increasing added Zn. In conclusion, adding Zn to diets containing RAC resulted in a trend for improved growth performance of pigs in 1 of 2 experiments. Also, additional Zn increased plasma Zn and reduced *IL-1*β.

Key words: beta agonist, inflammatory cytokines, liver, plasma, porcine, zinc amino acid complex, zinc oxide

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INTRODUCTION

Ractopamine HCl (**RAC**; Paylean; Elanco Animal Health, Greenfield, IN) is a feed additive used in late finishing pig diets to improve growth performance and carcass leanness (Apple et al., 2007). In addition, recent research demonstrates further improved ADG and G:F of pigs fed diets containing RAC with added

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Zn from a Zn AA complex (**ZnAA**) compared with those fed added Zn from an inorganic source (**ZnO or $ZnSO_4$**; Patience et al., 2011; Rambo et al., 2012). Fry et al. (2013) also observed increased G:F in pigs fed added Zn in diets containing RAC. Although previous research demonstrated improvements in performance of pigs fed the ZnAA or added Zn in diets containing RAC, the mechanism for these improvements is unknown.

Klasing (1992) suggested that the requirement for Zn may be greater for optimum immune response compared with growth as Zn plays an important role in multiple aspects of the immune system (Shankar and Prasad, 1998). Increases in relative protein expression of Interleukin-1 beta (***IL-1β***) or absolute number of tumor necrosis factor alpha (**TNF-α**) can increase intestinal injury leading to a decrease in intestinal barrier function (Ma et al., 2004; Al-Sadi and Ma, 2007). These increases also stimulate macrophages to produce Interleukin-8 (***IL-8)***, which is responsible for attracting neutrophils to the site of inflammation (Baggiolini and Clark-Lewis, 1992). Bao et al. (2003) determined in vitro that increasing Zn reduced gene expression of *IL-1*β, *TNF*-α, and *IL-8* in the monocyte-macrophage cell line. Therefore, we hypothesized that manipulation of dietary Zn above the nutritional requirement would modulate intestinal inflammation gene expression and result in improved performance. However, to the best of our knowledge, the response to RAC of gene expression of proinflammatory cytokines has not been studied. Therefore, our objective was to determine the effects of adding various concentrations of Zn from ZnO or a ZnAA (Availa-Zn; Zinpro, Eden Prairie, MN) on growth performance, carcass characteristics, plasma and tissue Zn concentrations, and ileal mucosal inflammation mRNA expression of finishing pigs fed diets containing RAC.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. Both experiments were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Pigs were housed in an environmentally controlled finishing building in 1.5 m^2 pens containing slatted flooring. Each pen was equipped with a single-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water.

Chemical Analysis

Samples of each diet were collected from feeders, blended, subsampled, and analyzed at Ward Laboratories, Inc. (Kearney, NE) for DM (method 934.01; AOAC, 2006), CP (method 990.03; AOAC, 2006), crude fat (method 920.39; AOAC, 2006), CF (method 978.10; AOAC, 2006), and ash (method 942.05; AOAC, 2006).

Experiment 1

A total of 312 finishing pigs (327 × 1050; PIC, Hendersonville, TN), initially 94 kg BW, from 2 consecutive groups were used with treatments replicated equally in both groups. Pens of pigs were allotted to 1 of 6 dietary treatments, with either 2 barrows or 2 gilts per pen and 26 pens per treatment. Dietary treatments consisted of a corn–soybean meal–based control diet formulated to contain 0.66% standardized ileal digestible (**SID**) Lys, a RAC diet formulated to contain 0.92% SID Lys and 10 mg/kg RAC; the RAC diet plus 50, 100, or 150 mg Zn/kg from ZnO; or the RAC diet plus 50 mg Zn/kg from a ZnAA (Availa-Zn; Zinpro; Table 1). All diets also contained 83 mg Zn/kg from $ZnSO_4$ provided by the trace mineral premix. Experimental diets were fed in meal form, and ZnO or the ZnAA was added to the RAC diet at the expense of corn. A subsample of experimental diets was collected and analyzed for dietary Zn (Ward Laboratories, Inc.). Samples were prepared using the method outlined by the AOAC (2012) and analyzed using an iCAP 6000 series ICP Emission Spectrometer (Thermo Electron Corporation, Marietta, OH). Total Zn concentrations were analyzed in duplicate. Analyzed Zn values were 168 and 131 mg Zn/kg in the control and RAC diets, respectively; 163, 188, and 267 mg Zn/kg in the diets containing added ZnO; and 185 mg Zn/kg in the diet containing added ZnAA. Pigs and feeders were weighed on d 0, 14, and 27 to determine ADG, ADFI, and G:F.

On d 27, all pigs were weighed, individually tattooed, and shipped approximately 2 h to a commercial harvesting plant (Farmland Foods Inc., Crete, NE). Immediately after harvest, HCW was collected and percent carcass yield was calculated by dividing HCW by live weight obtained at the farm before transport to the packing plant. Additional carcass measurements were collected only on the second group of pigs. For the second group of pigs, last-rib backfat measurements and boneless loin weights were collected and percentage lean was calculated (NPPC, 2000).

All data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. In addition to treatment, the effects of gender and group were included as random effects. Hot carcass weight was used as a covariate for analyses of backfat thickness, percentage lean, and boneless loin weight.

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

Item	Control	RAC[2]	ZnO, mg Zn/kg			ZnAA,[3] mg Zn/kg
			50	100	150	50
Ingredient, %						
Corn	84.29	73.91	73.91	73.90	73.89	73.86
Soybean meal (46.5% CP)	13.65	24.00	24.00	24.00	24.00	24.00
Monocalcium P (21% P)	0.50	0.45	0.45	0.45	0.45	0.45
Limestone	0.90	0.90	0.90	0.90	0.90	0.90
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[4]	0.075	0.075	0.075	0.075	0.075	0.075
Trace mineral premix[5]	0.075	0.075	0.075	0.075	0.075	0.075
L-Lys HCl	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	—	0.015	0.015	0.015	0.015	0.015
L-Thr	—	0.025	0.025	0.025	0.025	0.025
RAC[6]	—	0.05	0.05	0.05	0.05	0.05
ZnO	—	—	0.007	0.014	0.021	—
ZnAA	—	—	—	—	—	0.05
Calculated analysis, %						
ME, kcal/kg	3,351	3,347	3,347	3,347	3,346	3,345
NE, kcal/kg	2,512	2,445	2,445	2,445	2,444	2,444
SID[7] Lys, %	0.66	0.92	0.92	0.92	0.92	0.92
Total Lys, %	0.75	1.03	1.03	1.03	1.03	1.03
SID Lys:ME/Mcal	1.97	2.75	2.75	2.75	2.75	2.75
Ca, %	0.51	0.53	0.53	0.53	0.53	0.53
Total P, %	0.44	0.47	0.47	0.47	0.47	0.47
Available P, %	0.16	0.16	0.16	0.16	0.16	0.16
Analyzed values						
DM, %	89.16	89.31	89.11	89.41	89.07	89.25
CP, %	13.44	17.34	17.64	17.76	17.52	18.02
Crude fiber, %	1.94	2.10	2.12	2.20	2.06	2.18
Fat,%	2.82	2.92	2.92	2.88	2.80	3.00
Ash, %	3.50	4.02	4.11	4.06	3.99	4.12
Zn, mg Zn/kg	168	131	163	188	267	185

[1]Diets were fed in meal form from d 0 to 27 of the experiment. Basal diets contained 83 mg Zn/kg from $ZnSO_4$ provided by the trace mineral premix.

[2]RAC = ractopamine HCl.

[3]ZnAA = Zn AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).

[4]Provided per kilogram of diet: 3,307 IU vitamin A, 413 IU vitamin D_3, 13 IU vitamin E, 1.3 mg vitamin K, 2.5 mg riboflavin, 8.3 mg pantothenic acid, 14.9 mg niacin, and 0.01 mg vitamin B_{12}.

[5]Provided per kilogram of diet: 20 mg Mn from manganese oxide, 83 mg Fe from iron sulfate, 83 mg Zn from zinc sulfate, 8 mg Cu from copper sulfate, 0.15 mg I from calcium iodate, and 0.15 mg Se from sodium selenite.

[6]Provided 10 mg/kg of RAC (Paylean; Elanco Animal Health, Greenfield, IN).

[7]SID = standardized ileal digestible.

Contrast statements consisted of 1) control vs. RAC diet, 2) increasing ZnO linear and quadratic polynomials, 3) RAC diet vs. the ZnAA diet, and 4) 50 mg Zn/kg from ZnO vs. from the ZnAA. Statistical significance was determined at $P < 0.05$ and P-values falling within $P > 0.05$ and $P < 0.10$ were considered a trend.

Experiment 2

A total of 320 finishing pigs (327 × 1050; PIC), initially 98 kg BW, from 4 consecutive groups were used with treatments replicated equally in all groups. Pens of pigs were randomly allotted to 1 of 8 dietary treatments with either 2 barrows or 2 gilts per pen and 20 replicate pens per treatment. Dietary treatments included a corn–soybean meal–based control diet formulated to 0.66% SID Lys; a RAC diet formulated to 0.92% SID Lys and 10 mg/kg of RAC; and the RAC diet plus 75, 150, or 225 mg Zn/kg from either ZnO or the ZnAA (Table 2). All diets also contained 55 mg Zn/kg from $ZnSO_4$ provided by the trace mineral premix. Experimental diets were fed in meal form, and ZnO or the ZnAA was added to the RAC diet at the expense of corn. Diets were fed for the last 41 d before slaughter for group 1 and the last 35 d for group 2, 3, and 4. Analyzed total Zn concentrations were 66 and

Table 2. Diet composition, Exp. 2 (as-fed basis)[1]

			ZnO, mg Zn/kg			ZnAA,[3] mg Zn/kg		
Item	Control	RAC[2]	75	150	225	75	150	225
Ingredient, %								
Corn	83.06	74.24	74.23	74.22	74.20	74.17	74.10	74.03
Soybean meal (46.5% CP)	15.22	23.97	23.97	23.97	23.97	23.97	23.96	23.95
Monocalcium P (21% P)	0.25	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Limestone	0.75	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[4]	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Trace mineral premix[5]	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
L-Lys HCl	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	–	0.015	0.015	0.015	0.015	0.015	0.015	0.015
L-Thr	–	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Phytase[6]	–	–	–	–	–	–	–	–
RAC[7]	–	0.05	0.05	0.05	0.05	0.05	0.05	0.05
ZnO	–	–	0.010	0.021	0.031	–	–	–
ZnAA	–	–	–	–	–	0.075	0.150	0.225
Calculated analysis, %								
ME, kcal/kg	3,361	3,357	3,357	3,357	3,356	3,355	3,352	3,350
NE, kcal/kg	2,302	2,269	2,268	2,268	2,268	2,267	2,265	2,263
SID[8] Lys, %	0.70	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Total Lys, %	0.79	1.03	1.03	1.03	1.03	1.03	1.03	1.03
SID Lys:ME/Mcal	2.08	2.74	2.74	2.74	2.74	2.74	2.74	2.74
Ca, %	0.41	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Total P, %	0.39	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Available P, %	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Analyzed values								
DM, %	10.60	10.60	10.34	10.39	10.52	10.24	10.12	10.19
CP, %	89.41	89.41	89.66	89.62	89.48	89.77	89.88	89.82
Crude fiber, %	14.45	18.15	18.15	18.75	18.00	18.05	17.65	18.10
Fat,%	1.90	2.00	2.00	2.10	2.10	2.35	2.15	1.95
Ash, %	3.50	3.40	3.15	2.85	3.10	2.80	3.10	3.10
Zn, mg Zn/kg	66	77	134	241	308	154	256	318

[1]Diets were fed in meal form from d 0 to 27 of the experiment. Basal diets contained 83 mg Zn/kg from $ZnSO_4$ provided by the trace mineral premix.

[2]RAC = ractopamine HCl.

[3]ZnAA = Zn AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).

[4]Provided per kilogram of diet: 3,307 IU vitamin A, 413 IU vitamin D_3, 13 IU vitamin E, 1.3 mg vitamin K, 2.5 mg riboflavin, 8.3 mg pantothenic acid, 14.9 mg niacin, and 0.01 mg vitamin B_{12}.

[5]Provided per kilogram of diet: 17 mg Mn from manganese oxide, 55 mg Fe from iron sulfate, 55 mg Zn from zinc sulfate, 8 mg Cu from copper sulfate, 0.15 mg I from calcium iodate, and 0.15 mg Se from sodium selenite.

[6]Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 408 phytase units/kg of feed, with a release of 0.1% available P.

[7]Provided 10 mg/kg of RAC (Paylean; Elanco Animal Health, Greenfield, IN).

[8]SID = standardized ileal digestible.

77 mg Zn/kg in the control and RAC diets, respectively; 134, 241, and 308 mg Zn/kg in the diets containing added ZnO; and 154, 256, and 318 mg Zn/kg in the diets containing the added ZnAA.

One pig was randomly selected from 16 pens per treatment (balanced across sex and group) for blood collection on d 0, 8, 18, and 32 of the experiment and ileal mucosal swabs at harvest. On the final day of the experiment, pigs were harvested at 1 of 2 locations. The pigs that were selected for bleeding and 1 randomly selected pig from the remaining pens were weighed, individually tattooed, and shipped to the Kansas State University Meats Laboratory (Manhattan, KS) for harvest. The remaining pigs were weighed, tattooed, and shipped approximately 2.5 h to a commercial harvesting plant (Triumph Foods LLC, St. Joseph, MO).

Pigs harvested at the commercial packing plant were tattooed to allow individual identification for carcass data collection. Hot carcass weight was collected immediately following evisceration, and each carcass was evaluated for percent yield, backfat and loin depth, and percent lean. Fat and loin depth were

collected using an optical probe (Fat-O-Meater; SFK Technology A/S, Henlev, Denmark) inserted between the 10th and 11th rib approximately 7 cm from the dorsal midline. Percent lean was calculated using equations from the National Pork Producers Council (2000).

Pigs harvested at the Kansas State University Meats Laboratory were tattooed to allow for individual carcass identification during data collection. Immediately following evisceration, HCW and liver weights were collected. In addition, liver samples were taken from the top left lobe for Zn analysis and mucosal swabs of the distal ileum were collected for mRNA expression analysis (Jones et al., 2014). After the visceral organ mass was removed, a 15-cm segment of the ileum was collected 1 m proximal to the ileal–cecal junction. The ileum was cut along the mesenteric border, placed on a cold metal tray, and flushed with cold saline. A glass microscope slide was then used to collect scrapings of the mucosa's luminal surface. Mucosa scrapings were flash-frozen in liquid nitrogen and stored at –80°C for later analyses. Carcasses were chilled (–18°C) for 24 h and then the left side of each carcass was ribbed between the 10th and 11th rib interface. At this time, 10th rib backfat and loin depth were measured by trained university personnel using a ruler commonly used in non-computer-assisted carcass data collection. Fat thickness was measured three-fourths of the way up the loin muscle with the ruler perpendicular to the skin (National Pork Producers Council, 2000). The ruler was held perpendicular to the skin in the same location to measure the loin depth. Percent lean was calculated as previously described. A 30-cm portion of the longissimus lumborum muscle (beginning at the 10th rib) from the left side of each pig was collected for immunohistochemical and fresh pork quality analysis. The results for the fresh pork quality analysis are reported in Paulk et al. (2014).

Zinc Analysis

Samples were collected via jugular venipuncture into heparinized (143 units of sodium heparin) vacutainer tubes (Tyco Health Care Group LP, Mansfield, MA), inverted, and immediately placed on ice until samples were processed. Whole blood was centrifuged (2,000 × *g* for 15 min at 4°C) and the plasma was removed and frozen at –20°C. Plasma was deproteinized by diluting 1:4 in 12.5% trichloroacetic acid followed by centrifugation at 2,000 × *g* for 15 min at 4°C (GS-6KR; Beckman-Coulter, Brea, CA), with the resulting supernatant collected for analysis. Zinc analysis was determined by flame atomic absorption spectrophotometry according to the methods of Shaw et al. (2002; UNICAM 989 Solar AA Spectrometer; Thermo Elemental Corp., Franklin, MA).

Liver, loin, and feed samples were microwave digested (MARS 5; CEM Corp., Matthews, NC) in 10 mL of HNO_3 followed by addition of 2 mL of H_2O_2 (Shaw et al., 2002). Samples were brought to constant volume and diluted appropriately for Zn analysis described previously.

Ileal Mucosal Gene Expression

Approximately 100 mg of ileal mucosa was homogenized in Trizol (Life Technologies, Grand Island, NY) for the isolation of nucleic acids (Gonzalez et al., 2013). Extracted nucleic acids were purified using the Purelink RNA Mini kit (Life Technologies, Carlsbad, CA). Total RNA was collected with the addition of 90 µL of ribonuclease (**RNase**)-free water on the membrane for 1 min followed by centrifuging the column at 12,000 × *g* for 2 min at room temperature. Total RNA concentration and quality (absorbance [**A**] ratio at 260 and 280 nm) was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). All extractions yielded RNA with A260:280 nm ratios greater than 1.9 and all samples possessed A260:230 nm ratios greater than 1.8. Extracted RNA was stored at –80°C until PCR analysis.

Fifty nanograms of total RNA was treated to RNase-free deoxyribonuclease and then reverse transcribed to cDNA (High Capacity cDNA Archive kit; Life Technologies, Carlsbad, CA) in a 20-µL reaction, according to the manufacturers' recommendations. Five nanogram equivalents of total RNA was amplified with gene-specific primers (Table 3), DNA polymerase, and SYBRGreen chemistry (PerfeCta Sybr fast mix; Quanta Biosciences, Gaithersburg, MD) in a Realplex^2 S PCR System (Eppendorf North America, Hauppauge, NY). Thermal cycling parameters included an initial heating step of 50°C for 2 min and an initial denaturing step of 95°C for 10 min followed by 50 cycles of 15 s at 95.0°C, an annealing step for 30 s at the appropriate temperature for each primer, and an extension step of 20 s at 68.0°C. A final dissociation step was included at 95°C for 15 s followed by annealing at 60°C for 15 s. Melting temperature analysis was then conducted between 60 and 95°C using a 20-min ramp time and continuous fluorescence detection to determine primer specificity for each reaction. Primer efficiencies were determined from the slope of varying input concentrations, with an acceptable range for amplification being –3.0 to –3.8. All sequences and efficiencies can be found in Table 3. Sequencing of the amplicon products ensured that all primers amplified the gene of interest (University of California at

Table 3. Sequences, annealing temperatures, amplicon length, and efficiency of primers used for real-time PCR quantification of gene expression

Small intestine genes	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	T_m,[1] °C	Amplicon length	Efficiency
Interleukin-1 β	CCTCCTCCCAGGCCTTCTGT	GGGCCAGCCAGCACTAGAGA	62.0	178	1.01
Interleukin-8	TCCTGCTTTCTGCAGCTCTC	GGGTGGAAAGGTGTGGAATG	60.5	100	1.09
Tumor necrosis factor α	GCAGGAGCCACCACGCTCTT	CGTGGGCGACGGGCTTATCT	62.0	147	0.90
Normalizing gene					
Ribosomal protein L4	AGGAGGCTGTTCTGCTTCTG	TCCAGGGATGTTTCTGAAGG	60.5	184	1.06

[1]T_m = melting temperature.

Davis, DNA Sequencing Facility, Davis, CA). Due to sample numbers and the need to conduct the PCR analysis for each gene over multiple plates, plates were balanced so that an equal number of treatments represented on each plate. Additionally, a pooled control sample representative of all treatment groups was run on each plate as an internal standard. Normalized expression (ΔCt) for each sample was determined using *Ribosomal protein L4* (***RPL4***) as an endogenous control gene. The average normalized expression of the pooled control sample was used as the calibrator to calculate relative gene expression (Livak and Schmittgen, 2001; McNeill et al., 2007). For each sample, relative expression was calculated as $2^{-\Delta\Delta Ct}$, in which ΔΔCt represents ΔCt sample – ΔCt calibrator (Livak and Schmittgen, 2001).

Statistical Analysis

All growth performance, carcass characteristic, and relative gene expression data was analyzed as a generalized randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc.) with pen as the experimental unit for the growth performance and carcass characteristic data and selected pig within pen for the relative gene expression data. Dietary treatment served as the fixed effect while gender, group, and barn were included as random blocking factors. For plasma Zn concentration analysis, the statistical structure was the same except day of bleeding served as a fixed effect in addition to dietary treatment. Day of bleeding also served as the repeated measure with animal as the subject. Contrast statements consisted of 1) negative control vs. positive control RAC diet, 2) the 3-way and all possible 2-way interactions between increasing added Zn and source and day, 3) increasing Zn linear and quadratic polynomials, 4) added Zn from ZnO vs. from the ZnAA, and 5) increasing day linear and quadratic polynomials. Statistical significance was determined at $P < 0.05$ and *P*-values falling within $P > 0.05$ and $P < 0.10$ were considered a trend.

RESULTS

Experiment 1

Analyzed Zn concentrations were greater than the calculated levels for the control and RAC diets (Table 1; calculated concentrations equaled 103 and 107 mg Zn/kg and analyzed levels equaled 168 and 131 mg Zn/kg, respectively). Analyzed Zn concentrations were within the acceptable limits for analytical variation according to the Association of American Feed Control Officials (2010) for the added Zn diets. There is no explanation for increased levels of analyzed Zn for the control and RAC diets; however, there were still increases in analyzed Zn content with increasing added Zn treatments.

From d 0 to 14, pigs fed the RAC diet had improved ($P < 0.05$) ADG, G:F, and d-14 BW compared with pigs fed the control diet (Table 4). Increasing dietary Zn from ZnO tended to improve (linear, $P = 0.072$) G:F. Pigs fed diets with RAC plus 50 mg Zn/kg from the ZnAA had increased ($P < 0.05$) ADG and G:F compared with pigs solely fed RAC. No differences in growth performance were observed among pigs fed diets containing 50 mg Zn/kg from ZnO or the ZnAA.

From d 14 to 27, pigs fed the RAC diet had a tendency for increased ($P = 0.060$) ADG and reduced ($P = 0.016$) ADFI, resulting in improved ($P = 0.001$) G:F compared with those fed the control diet. No differences were observed in performance when Zn from ZnO or the ZnAA was added to the RAC diet. Performance did not differ among pigs fed diets with 50 mg Zn/kg from either source.

Overall (d 0 to 27), pigs fed the RAC diet had improved ($P < 0.05$) ADG, G:F, final BW, HCW, and boneless loin weight. Pigs fed RAC also had a tendency for increased ($P = 0.053$) carcass yield and a tendency for reduced ($P = 0.075$) ADFI compared with those fed the control diet without RAC. Increasing Zn from ZnO tended to increase (linear, $P = 0.067$) G:F and boneless loin weights (quadratic, $P = 0.064$). Pigs fed the diet with 50 mg Zn/kg from the ZnAA tended to have increased ($P = 0.057$) ADG compared with

Table 4. Effects of added Zn and ractopamine HCl (RAC) on growth performance and carcass characteristics of finishing pigs, Exp. 1[1]

								Probability, $P <$				
			ZnO,[2] mg Zn/kg			ZnAA,[2,3] mg Zn/kg		Control vs.	ZnO		ZnAA vs.	
Item	Control	RAC	50	100	150	50	SEM	RAC	Linear	Quadratic	RAC	50 mg Zn/kg (ZnO)
d 0 to 14												
ADG, kg	1.08	1.33	1.35	1.35	1.39	1.42	0.03	0.001	0.242	0.810	0.041	0.122
ADFI, kg	3.35	3.29	3.26	3.23	3.26	3.33	0.06	0.443	0.712	0.670	0.618	0.466
G:F	0.324	0.406	0.415	0.418	0.425	0.430	0.008	0.001	0.072	0.929	0.029	0.153
d 14 to 27												
ADG, kg	0.99	1.07	1.11	1.10	1.07	1.10	0.03	0.060	0.988	0.256	0.572	0.658
ADFI, kg	3.27	3.05	3.12	3.06	2.98	3.10	0.07	0.016	0.394	0.239	0.548	0.847
G:F	0.302	0.354	0.359	0.359	0.362	0.355	0.008	0.001	0.516	0.913	0.997	0.686
d 0 to 27												
ADG, kg	1.04	1.20	1.24	1.23	1.24	1.27	0.02	0.001	0.385	0.568	0.057	0.382
ADFI, kg	3.31	3.17	3.19	3.15	3.13	3.22	0.06	0.075	0.492	0.675	0.535	0.759
G:F	0.314	0.383	0.389	0.391	0.397	0.395	0.005	0.001	0.067	0.930	0.116	0.437
Carcass characteristics												
HCW, kg	89.5	93.8	94.9	94.0	94.2	95.3	0.70	0.001	0.876	0.494	0.120	0.686
Carcass yield,[4] %	73.9	74.4	74.8	74.5	74.4	74.65	0.179	0.053	0.758	0.184	0.365	0.501
Backfat depth, mm[5,6]	24.62	23.63	23.70	23.36	22.34	22.81	0.922	0.430	0.264	0.517	0.502	0.465
Loin wt, kg[5,6]	3.86	4.05	3.97	4.03	4.13	3.99	0.054	0.015	0.165	0.064	0.461	0.678
Lean, %[5,6,7]	51.74	52.15	52.12	52.25	52.63	52.48	0.37	0.426	0.300	0.546	0.495	0.460

[1]A total of 312 pigs (PIC 327 × 1050; PIC, Hendersonville, TN; 2 consecutive groups of 156 pigs) were used in a 27-d study with 2 pigs per pen and 26 pens per treatment.

[2]Diets contained 10 mg/kg of RAC (Paylean; Elanco Animal Health, Greenfield, IN).

[3]ZnAA = Zn AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).

[4]Percentage carcass yield was calculated by dividing HCW by live weight obtained at the farm before transport to the packing plant.

[5]Data was collected on the second group of pigs (13 pens per treatment).

[6]Adjusted using HCW as a covariate.

[7]Percentage lean was calculated using equations from the National Pork Producers Council (2000).

pigs fed the RAC diet. No differences were observed in performance between pigs fed diets with 50 mg Zn/kg from either source.

Experiment 2

Diet Analysis. Analyzed Zn levels for experimental diets are reported in Table 1. Analyzed Zn concentrations were within the acceptable limits for analytical variation according to the Association of American Feed Control Officials (2010)

Growth Performance and Carcass Characteristics. From d 0 to 14, pigs fed the RAC diet had improved ($P < 0.05$) ADG and G:F compared with pigs fed the control diet (Table 5). There were no interactions between Zn source and level or a Zn level or source main effect. There was a trend for increased ($P < 0.10$) ADG and ADFI in pigs fed diets with added Zn from ZnO compared with pigs fed diets with added Zn from the ZnAA.

From d 14 to 35 and compared with pigs fed the control diet, pigs fed the RAC diet had improved ($P = 0.006$) G:F, which resulted from decreased ($P = 0.011$) ADFI. There were no Zn level × source interactions or a Zn level or source main effect for performance.

Overall (d 0 to 35), pigs fed the RAC diet had improved ($P < 0.05$) ADG, G:F, d 35 BW, HCW, loin depth, and percentage lean and reduced ($P < 0.05$) ADFI and backfat depth compared with those fed the control diet. There was no Zn level × source interaction or Zn level or source main effect for performance and carcass characteristics.

Plasma and Tissue Zn Levels and Liver Weights. Plasma Zn concentrations were not affected by the treatment × day interaction (Table 6). As day increased, there was an increase (quadratic, $P < 0.001$) in plasma Zn. On each of the plasma collection days, Zn concentration was not different between the RAC and control pigs. Also, there was no Zn level × source interaction or Zn source effect. However, pigs fed RAC diets with added Zn had increased (linear, $P < 0.05$) plasma Zn concentrations on d 18 and 32.

There was no difference in liver Zn concentrations between pigs fed either the RAC or control diet (Table 5). A Zn level × source interaction (quadratic, $P = 0.007$) was observed and this resulted from con-

Table 5. Effects of level and source of added Zn on growth performance and carcass characteristics of finishing pigs fed ractopamine HCl (RAC), Exp. 2[1]

			ZnO,[2] mg Zn/kg			ZnAA,[2,3] mg Zn/kg				Probability,[4] $P <$			
Item	Control	RAC	75	150	225	75	150	225	SEM	Control vs. RAC	Zn linear	Zn quadratic	Source
d 0 to 14													
ADG, kg	1.10	1.33	1.31	1.31	1.32	1.26	1.31	1.23	0.04	0.001	0.242	0.826	0.100
ADFI, kg	3.28	3.16	3.07	3.15	3.11	3.00	3.08	2.95	0.14	0.178	0.223	0.714	0.074
G:F	0.338	0.426	0.427	0.418	0.425	0.422	0.425	0.417	0.013	0.001	0.604	0.998	0.774
d 14 to 35													
ADG, kg	1.00	1.03	1.06	1.07	1.06	1.08	1.03	1.05	0.04	0.419	0.683	0.586	0.619
ADFI, kg	3.46	3.22	3.16	3.18	3.23	3.17	3.19	3.14	0.09	0.011	0.777	0.587	0.636
G:F	0.293	0.324	0.336	0.339	0.332	0.343	0.324	0.335	0.017	0.006	0.497	0.272	0.762
d 0 to 35													
ADG, kg	1.04	1.15	1.16	1.17	1.17	1.15	1.14	1.12	0.03	0.001	0.737	0.740	0.173
ADFI, kg	3.39	3.19	3.12	3.17	3.18	3.10	3.14	3.07	0.10	0.014	0.494	0.612	0.245
G:F	0.311	0.365	0.373	0.371	0.369	0.373	0.365	0.367	0.014	0.001	0.885	0.419	0.599
Carcass characteristics													
HCW, kg	99.0	101.7	102.5	101.7	102.8	101.9	101.7	101.0	1.34	0.047	0.966	0.814	0.288
Carcass yield,[5] %	72.62	73.17	73.39	72.90	73.56	72.75	73.53	73.03	0.271	0.120	0.575	0.647	0.381
Loin depth,[6] mm	65.03	68.88	69.53	71.62	69.65	70.30	69.76	69.26	1.271	0.033	0.602	0.256	0.629
Backfat depth,[6] mm	22.82	19.28	18.72	19.01	20.09	19.11	19.18	18.81	0.888	0.005	0.837	0.607	0.738
Lean,[6,7] %	51.37	53.65	54.53	54.22	53.75	54.14	54.10	53.95	0.640	0.012	0.858	0.326	0.836

[1]A total of 320 pigs (PIC 327 × 1050; PIC, Hendersonville, TN) were used with 2 pigs per pen and 20 pens per treatment.

[2]Diets contained 10 mg/kg of RAC (Paylean; Elanco Animal Health, Greenfield, IN).

[3]ZnAA = Zn AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).

[4]No interactive effects ($P > 0.12$) of Zn level × source.

[5]Percentage yield was calculated by dividing HCW by live weight obtained at the farm before transport to the packing plant.

[6]Adjusted using HCW as a covariate.

[7]Percentage lean was calculated using equations from the National Pork Producers Council (2000).

centrations plateauing at 150 mg Zn/kg for ZnO supplemented pigs and concentrations increasing linearly when the ZnAA was added. There were no treatment effects on loin Zn concentration.

Pigs fed the RAC diets without added Zn had increased ($P = 0.028$) liver weights compared with those fed the control diet (Table 6). There was no Zn level × source interaction or a Zn level effect for liver weight. Pigs fed the RAC diets with added Zn from ZnO tended to have heavier ($P = 0.091$) liver weights compared with pigs fed the RAC diet with added Zn from the ZnAA.

Illeal Mucosal mRNA Expression. There was no Zn level × source interaction or a Zn source effect for *IL-1*β mRNA expression (Table 7). The expression of *IL-1*β was increased ($P = 0.014$) in mucosa of pigs fed the RAC diet compared with those fed the control diet. However, the relative mRNA expression of *IL-1*β decreased (linear; $P = 0.026$) in the mucosa of pigs fed added Zn. There were no treatment differences in *IL-8* or *TNF*-α relative mRNA expression.

DISCUSSION

Both experiments consisted of a control diet without RAC and a RAC diet without added Zn. This allowed us to confirm the effects of the RAC diet on finishing pig performance and carcass characteristics independent of added Zn. Apple et al. (2007) summarized 23 publications determining the effects of RAC on performance and carcass characteristics of finishing pigs. They concluded that adding 10 mg/kg RAC to finishing pig diets resulted in a 11.7%, 13.3%, 2.4 kg, and 3.5 cm^2 average increase in ADG, G:F, HCW, and LM area, respectively, and a 1.4 mm reduction in 10th rib fat depth. In Exp. 1 conducted herein, improvements in ADG, G:F, and HCW were greater than the average percentage improvement determined in the meta-analysis. However, values still fell within the range of differences observed. Similarly, RAC diets increased loin weight in Exp. 1. Although Apple et al. (2007) determined that RAC reduces 10th rib backfat thickness, it is not as consistent a response, with the change ranging from –16.1 to 6.6%. Data from Exp. 1 did not show reductions in backfat thickness. In Exp. 2 conducted herein, improvements in ADG and HCW

Table 6. Effects of level and source of added Zn on plasma, liver, and loin Zn concentrations and liver weights of finishing pigs fed ractopamine HCl (RAC), Exp. 2[1]

			ZnO, mg Zn/kg			ZnAA,[2] mg Zn/kg				Probability, $P <$			
Item	Control	RAC	75	150	225	75	150	225	SEM	Control vs. RAC	Zn linear	Zn quadratic	Source
Plasma,[3,4,5] μg/mL													
d 0	1.06	1.01	1.04	1.05	1.04	1.01	1.06	1.06	0.038	0.328	0.248	0.743	0.845
d 8	1.08	1.07	1.09	1.06	1.15	1.08	1.11	1.16	0.046	0.920	0.114	0.395	0.512
d 18	1.13	1.06	1.12	1.16	1.11	1.10	1.18	1.17	0.039	0.159	0.023	0.157	0.441
d 32	1.08	1.01	1.07	1.07	1.13	1.07	1.09	1.13	0.039	0.187	0.005	0.981	0.769
DM basis,[6] μg/g													
Liver[7]	306.24	292.84	314.12	345.38	329.26	289.59	326.44	394.80	17.44	0.551	0.001	0.500	0.570
Loin[8]	61.47	59.15	62.43	58.05	56.41	60.15	55.79	58.68	2.80	0.501	0.326	0.695	0.702
Liver wt,[8,9] kg	1.90	2.05	2.06	2.02	2.00	1.96	1.95	1.97	0.058	0.028	0.203	0.555	0.091

[1]Diets contained 10 mg/kg of RAC (Paylean; Elanco Animal Health, Greenfield, IN).

[2]ZnAA = Zn AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).

[3]Values represent 128 pigs, 1 pig randomly selected from 16 pens per treatment.

[4]No interactive effects ($P > 0.212$) of Zn level × source or treatment × day.

[5]There was an increase (quadratic, $P < 0.001$) in plasma Zn from Day 0 to 18.

[6]Values represent 160 pigs, 1 pig randomly selected from 20 pens per treatment.

[7]There was a Zn level × source interaction (quadratic, $P = 0.007$).

[8]No interactive effects ($P > 0.234$) of Zn level × source.

[9]Liver weights were measured with the gallbladder still intact.

were similar to the average improvement determined in the meta-analysis. Improvements in G:F and reductions in backfat depth were greater than the average. However, values still fell within the range of differences observed. Similarly, RAC diets increased loin depth in Exp. 2.

The NRC (2012) has a Zn requirement estimate of 50 mg Zn/kg for growing pigs from 100 to 135 kg BW. Although the NRC (2012) estimates increased requirements of AA for 115 to135 kg pigs when RAC is added to the diet, there is not a similar increase in the Zn requirement estimate. However, recent data might suggest otherwise, as Fry et al. (2013) observed a tendency for improved G:F in finishing pigs fed diets containing 5 mg/kg RAC (with 79 mg Zn/kg from the trace mineral premix) with 40 mg Zn/kg from either $ZnSO_4$ or the ZnAA. Our results from Exp. 1 agree with Fry et al. (2013) in that the addition of up to 150 mg Zn/kg from ZnO tended to improve G:F in finishing pigs fed diets containing RAC. However, data from Exp. 2 did not support this observation as performance was not influenced by increasing level of added Zn. Although Fry et al. (2013) determined that added Zn may enhance the response to RAC, they also reported 2 additional experiments in which they did not observe improved performance when Zn was added to RAC diets (with 79 mg Zn/kg from the trace mineral premix). Other studies have also failed to demonstrate improvements in the response to RAC when supplemental Zn was added at levels above that contributed in the trace mineral premix (Rambo, 2013; Gowanlock et al., 2013).

Previous research demonstrated improved ADG in pigs fed RAC diets with 50 mg Zn/kg from the ZnAA compared with supplementing different inorganic Zn sources (Patience et al., 2011; Rambo et al., 2012). However, these studies did not include a RAC treatment without added Zn. Therefore, it is not possible to determine if the supplemental Zn elicited additional benefits over that observed from a diet containing only RAC. Both of the experiments herein did not indicate a difference in performance and carcass characteristics among finishing pigs supplemented Zn from ZnO vs. from the ZnAA. This is similar to observations of Fry et al. (2013) and Rambo (2013). In attempt to explain the variability in the response to added Zn from the ZnAA vs. inorganic Zn, Patience et al. (2013) conducted an experiment to determine if the Lys:calorie ratio of a finishing pig diet with RAC could affect the response to different added Zn sources. They observed no difference in performance of pigs fed added Zn independent of the Lys:calorie ratio.

If growth is the primary response criterion used to establish Zn requirements in finishing pigs fed RAC diets, then the Zn provided by the premix (83 mg Zn/kg from $ZnSO_4$) and endogenous Zn from the ingredients may not have been sufficient for pigs in Exp. 1. However, Zn provided by the premix (55 mg Zn/kg from $ZnSO_4$) and endogenous Zn from the ingredients was sufficient to support maximum performance in Exp. 2. The results from the 2 experiments were inconsistent and do not provide a clear conclusion for Zn concentrations in RAC-containing diets fed to fin-

Table 7. Effects of level and source of added Zn on mRNA expression of inflammatory cytokine genes in the distal ileum of finishing pigs fed ractopamine HCl (RAC), Exp. 2[1,2]

			ZnO,[4] mg Zn/kg			ZnAA,[4,5] mg Zn/kg				Probability,[6] $P <$			
Item[3]	Control	RAC	75	150	225	75	150	225	SEM	Control vs. RAC	Zn linear	Zn quadratic	Source
IL-1β	4.62	10.12	5.63	7.62	5.54	5.74	4.91	5.61	1.776	0.014	0.026	0.122	0.498
IL-8	3.84	3.75	4.82	4.53	3.90	3.94	3.10	3.65	1.867	0.942	0.881	0.621	0.233
TNF-α	7.22	6.65	7.20	5.95	6.00	6.15	5.87	4.82	1.305	0.727	0.302	0.769	0.414

[1]Values represent 128 pigs, 1 pig randomly selected from 16 pens per treatment.

[2]All values indicate relative expression of genes. Normalized expression (**Δ**Ct) for each sample was determined using *ribosomal protein* L4 as an endogenous control gene. The average normalized expression of the pooled control sample was used as the calibrator to calculate relative gene expression. For each sample relative expression was calculated as $2^{-\Delta\Delta Ct}$, in which **ΔΔ**Ct represents **Δ**Ct sample – **Δ**Ct calibrator (Livak and Schmittgen, 2001).

[3]*IL-1β = Interleukin-1 β*; *IL-8 = Interleukin-8*; *TNF-α = Tumor necrosis factor α.*

[4]Diets contained 10 mg/kg of RAC (Paylean; Elanco Animal Health, Greenfield, IN).

[5]ZnAA = Zn AA complex (Availa-Zn; Zinpro, Eden Prairie, MN).

[6]No interactive effects ($P > 0.333$) of Zn level × source.

ishing pigs. Published data has also provided inconsistent results and has not explained the variability of this response through the factorial arrangement of treatments. However, research has suggested that the requirement for Zn may be greater for immune response when animals are stressed (Klasing, 1992). In addition, Rambo (2013) attributed the inconsistency of obtained results to health status of the pigs used.

Ractopamine is a member of the phenylethanolamine class of β-adrenergic agonists, which are structurally and functionally similar to the endogenous catecholamines, epinephrine and norepinephrine (Barnes, 1995; Beermann, 2002). These compounds act as repartitioning agents directing nutrients toward skeletal muscle accretion and away from adipose tissue deposition (Beermann, 2002; Mersmann, 1998). This was demonstrated in Exp. 2 through increased loin depth and reduced backfat thickness. In addition, the data indicated that the RAC diet increased the relative expression of proinflammatory cytokine *IL-1*β in distal ileum mucosa of pigs compared with those fed the control diet. Increasing interleukin-1β is correlated with elevated intestinal inflammation (Reinecker et al., 1991) and is also associated with increases in intestinal tight junction permeability (Al-Sadi and Ma, 2007). Therefore, our data would suggest that feeding RAC diets results in elevated inflammation of the pig's small intestine based on increased relative expression of *IL-1*β. To the best of our knowledge, there has been no previous data that demonstrates the effects of RAC diets on the relative expression of proinflammatory cytokines in the small intestines of pigs. However, the response to other β-agonist and endogenous catecholamines on proinflammatory cytokine expression in various tissues has been studied. Research in mice also determined that catecholamines released from the sympathetic nerve terminals and adrenal gland resulted in increased *IL-1*β expression in the liver and spleen (Jung et al., 2000).

Although there were increases in *IL-1*β in our study, there were no differences in relative expression of proinflammatory cytokines *TNF*-α or *IL-8*. Verghese et al. (1994) evaluated different cyclic adenosine monophosphate (**cAMP**)-phosphodiesterase isoforms that inhibited degradation of cAMP to determine if these isoforms could regulate cytokine release from lipopolysaccharide (**LPS**)-challenged human monocytes. The authors concluded that increasing cellular levels of cAMP reduced the accumulation of *TNF*-α gene expression. However, they increased accumulation of *IL-1*β mRNA. Additional research has also determined that increasing cAMP can inhibit LPS-induced production of *TNF*-α and *IL-8* in human promonocytic THP-1 cells (Farmer and Pugin, 2000). When feeding the β-adrenergic agonist RAC, it binds to a β-adrenergic receptor, which activates intracellular adenylyl cyclase, leading to increases in intracellular levels of cAMP (Mersmann, 1998). Therefore, increasing intracellular cAMP by feeding RAC may explain the observed differences in *IL-1*β but not *TNF*-α and *IL-8* in the current experiment.

Although pigs fed RAC had increased relative expression of the proinflammatory cytokine *IL-1*β in the distal ileum mucosa, increasing Zn in RAC diets reduced the relative expression of *IL-1*β. Previous data conducted in vitro has determined that Zn deficiency increased gene expression of *IL-1*β, *TNF*-α, and *IL-8* in the monocyte-macrophage cell line (Bao et al., 2003). The authors reported that the reduction in these proinflammatory cytokines due to added Zn is not clearly defined. They speculated that the mechanism revolves around the increase in a Zn finger protein, A20, when increasing levels of Zn are added to the cell media. Jaattela et al. (1996) determined that

A20 protein prevents an increase in the expression of *IL-1* and *TNF*-α by inhibiting the activation of nuclear factor-κβ-like transcription factors.

Conclusion

Adding Zn to diets containing RAC resulted in a trend for improved performance of pigs in 1 of 2 experiments. Therefore, adding Zn to diets containing RAC may improve finishing pig performance. However, the results are inconsistent. Due to the decrease in relative expression of *IL-1*β, we speculate that the variability of the response in pigs fed RAC diets with added Zn is mediated at the intestinal level and variability in the response may possibly be due to levels of stressors present in the environment. Therefore, more research is warranted to better define the relationship between RAC, Zn, and intestinal inflammation.

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