Effects of supplemental zinc sulfate on growth performance, carcass characteristics, and antimicrobial resistance in feedlot heifers¹

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ABSTRACT: Effects of supplemental Zn as Zn sulfate on feedlot performance, carcass traits, and antimicrobial resistance were evaluated using 480 crossbred heifers (BW = 385 kg ± 13.08) in a randomized complete block design. Heifers were blocked by BW and randomly assigned within block to diets with 0, 30, 60, or 90 mg supplemental Zn/kg DM. Heifers were housed in dirt-surfaced pens (20 animals per pen; 6 pens per treatment) equipped with fence-line feed bunks and automatic water fountains. Heifers were fed once daily to ensure ad libitum intake. Plasma was collected on day 0 from five randomly selected heifers per pen and repeated on days 63 and 115 to determine plasma Zn concentrations. Random samples of freshly voided feces were collected from the surface of each pen the day of harvest to determine antibiotic resistance. Heifers were transported on day 144 to a commercial abattoir where hot carcass weight (HCW) and incidence of liver abscesses were recorded at harvest and HCW, dressed yield, ribeye area, 12th rib fat, quality and yield grades were recorded after 36 h of refrigeration. Plasma Zn concentration increased ($P = 0.02$) linearly in response to increasing concentrations of dietary Zn. Final BW

and ADG were unaffected by supplementation ($P \geq 0.29$). Quantified levels of resistance to ceftriaxone and tetracycline among fecal *Escherichia coli* were not impacted ($P > 0.05$) by dietary zinc concentrations. Increasing Zn concentrations tended to decrease (linear effect, $P = 0.07$) DMI, resulting in a linear ($P = 0.03$) and tendency for quadratic ($P = 0.12$) improvement in feed efficiency with increasing Zn concentration. No differences were detected for HCW, dressed yield, ribeye area, 12th rib fat, percentages of carcasses grading Select or Choice, or yield grade ($P > 0.53$), but added Zn tended to affect percentage of carcasses that graded Prime, peaking at 60 mg/kg added Zn (quadratic effect, $P = 0.07$). In vitro fermentations were performed using ruminal fluid cultures containing 0, 30, 60, 90, 120, or 150 mg Zn/kg substrate DM to determine impact of Zn on gas production, VFA concentrations, and in vitro DM disappearance (IVDMD). There were no effects of Zn on in vitro gas production, IVDMD, or most VFA ($P > 0.15$), but isovalerate decreased linearly in response to added Zn ($P = 0.05$). Supplementing finishing heifers up to 60 mg Zn/kg diet DM improved feed efficiency compared to other treatments.

Key words: feedlot cattle, feed efficiency, zinc

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INTRODUCTION

Zinc is a micro mineral that is an essential component to enzymes and protein gene expression that affect growth; hormone production, secretion,

and storage; skin and wound healing; immune function; reproduction; cell division; microbial growth; and macronutrient and nucleic acid metabolism (McDowell, 1992). Nutrient Requirements of Beef Cattle (NRBC; 2016) recommends a minimum of 30 mg Zn/kg diet to prevent growth retardation and skin abnormalities in beef cattle, but concentrations recommended by consulting nutritionists range from 34 to 130 mg added Zn per kg diet DM (Samuelson et al., 2016). Zinc supplementation has been observed to impact DMI, 12th rib fat thickness, and yield grade (Malcolm-Callis et al., 2000); however, in another study, no effects of Zn supplementation were observed (Nunnery et al., 2007). In addition, adding up to 20 mg/kg Zn in incubation fluid decreased in vitro DM disappearance (IVDMD; Arelovich et al., 2000).

Use of heavy metals provides potential for co-selection for antibiotic resistance, as genes encoding for metal resistance often are co-located with antibiotic resistance elements on the same integron, transposon, or plasmid, or when antibacterial activities are similar for metals and antibiotics (Chapman, 2003). Zinc concentrations elevated above dietary requirements previously were found to coincide with increases in methicillin resistance in *Staphylococcus aureus* in swine (Amachawadi et al., 2015), along with increased levels of multi-drug resistant *Escherichia coli* in piglets (Bednorz et al., 2013), but there is a literature gap concerning impact of dietary concentrations of Zn on enteric bacterial populations of fed cattle.

The objectives of this study were to evaluate feedlot performance, carcass traits, and antimicrobial resistance patterns in finishing heifers supplemented four concentrations of Zn as Zn sulfate and to assess changes in fermentative activity with in vitro cultures of mixed ruminal content. We hypothesized supplementing increased concentrations for Zn would have deleterious effects on performance, microbial fermentation, and increased antibiotic resistance.

MATERIALS AND METHODS

Protocols and procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Beef Cattle Research Center in Manhattan, KS.

Experimental Design

The study was conducted using 480 crossbred yearling heifers (initial BW = 385 kg $\pm$ 13.08) in

a randomized complete block design with four treatments. Heifers were blocked by initial BW and randomly assigned within block (three blocks) to experimental pens, and pens were randomly assigned to treatment. Heifers were housed in 24 pens containing 20 animals per pen (6 pens per treatment). To determine if block had an effect on responses to treatment, each block contained two treatment replications per block. Treatments consisted of heifers supplemented with 0, 30, 60, or 90 mg Zn/kg diet DM (0, 30, 60, or 90Zn). The basal ration contained 51.4 mg Zn/kg diet DM, thus providing an estimated 5.14, 81.4, 111.4, or 141.4 mg Zn/kg diet DM for the respective treatments. Previous research at the Kansas State University Beef Cattle Research Center indicated supplementing finishing cattle with 60 mg Zn/kg diet DM as Zn sulfate plus 3 g/animal daily (~96 mg total supplemental Zn/kg diet) of Zn methionine (Zinpro 120, Zinpro Corporation, Eden Prairie, MN) may reduce feed intake (L. Miranda and J. S. Drouillard, Kansas State University, Manhattan, KS, personal communication); therefore, 90 mg Zn/kg diet DM was used for the high concentration. Final finishing diet compositions are presented in Table 1.

Animal Processing, Housing, and Handling

On arrival at Kansas State University Beef Cattle Research Center, heifers were allowed ad libitum access to ground brome hay and water. Approximately 1 wk after arrival, heifers were identified with uniquely numbered ear tags, vaccinated with Bovi-Shield Gold 5 (Zoetis, Florham Park, NJ) and Ultra-Bac 7 Somubac (Zoetis), and treated with a topical parasiticide (Permethrin; Bayer Animal Health, Shawnee, KS) and metaphylaxis (Micotil; Elanco, Greenfield, IN). Heifers received a grower ration consisting of 50% roughage and 50% concentrate for 4 mo prior to study initiation. Before beginning the trial and 2 mo thereafter, heifers were implanted with Component TE-200 with Tylan (20 mg estradiol and 200 mg trenbolone acetate implant; Elanco Animal Health). Heifers were housed in dirt surfaced pens that contained fence-line feed bunks and automatic waterers that were shared between adjacent pens and were provided ad libitum access to feed and water for 144 d. BWs for each pen were measured at the beginning and end of feedlot finishing, and used to compute ADG and G:F.

Blood Collection and Analysis

Five heifers per pen were randomly selected at the beginning of the study and were tagged with colored tags for identification for blood collections.

Table 1. Diet composition of finishing heifers supplemented 0, 30, 60, or 90 mg Zn/kg diet DM

Item	Treatment, mg Zn/kg diet DM			
	0	30	60	90
Ingredient, % DM				
Steam flaked corn	58.90	58.90	58.90	58.90
Wet corn gluten feed	30.00	30.00	30.00	30.00
Alfalfa hay	7.00	7.00	7.00	7.00
Feed additive premix ¹	2.16	2.16	2.16	2.16
0 mg/kg Zn vitamin–mineral premix ²	1.94	1.29	0.65	—
90 mg/kg Zn vitamin–mineral premix ²	—	0.65	1.29	1.94
Calculated nutrient composition ³ , %				
Neutral detergent fiber	19.03	19.03	19.03	19.03
Crude protein	14.06	14.06	14.06	14.06
Ca	0.70	0.70	0.70	0.70
P	0.48	0.48	0.48	0.48
Analyzed nutrient composition ⁴ , mg/kg				
Zn	51.35	81.35	111.35	141.35

¹Formulated to provide 300 mg/d monensin, 90 mg/d tylan (Elanco Animal Health, Greenfield, IN), and 0.4 mg/d melengesterol acetate (Elanco Animal Health, Greenfield, IN) in a ground corn carrier. Melengesterol acetate was removed from the diet the final 18 d on feed due to a shortage of the product. Ractopamine hydrochloride (**RH**; Elanco Animal Health, Greenfield, IN) was mixed in a ground corn carrier and fed at a rate of 0.09 kg/d to supply 300 mg RH/animal daily the final 28 d prior to harvest.

²Formulated to provide the following nutrient levels: added 2,200 IU/kg vitamin A; added 22 IU/kg vitamin E (α tocopherol acetate); 0.10 mg/kg added Co as cobalt carbonate; 10 mg/kg added Cu as copper sulfate; 0.5 mg/kg added I as ethylenediamine dihydriodide; 20 mg/kg added Mn as manganous sulfate; 0.1 mg/kg added Se as sodium selenite; 0.3% of diet DM as sodium chloride, and 0.7% total dietary Ca using limestone. Vitamin–mineral premixes were formulated to contain 0 or 90 mg supplemental Zn/kg diet DM (Zn sulfate) and were blended for rations formulated to contain 30 or 60 mg/kg supplemental Zn.

³Calculated using values from [NRC \(2000\)](#).

⁴Zn was analyzed for individual feed ingredients according to the procedures adapted by [Murthy et al. \(1971\)](#) and Analytical Methods for Atomic Absorption Spectrophotometry ([Perkin-Elmer, 1996](#)), and reported dietary concentrations were computed as weighted means of ingredient additions to each diet.

Blood was collected ~2 h prior to feeding by jugular venipuncture days 0, 63, and 115 from the same five heifers per pen for determination of plasma Zn concentration. Heifers were sorted from pen and away from feed ~15 to 20 min for blood collection. Trace mineral-free blood collection tubes containing K₂ EDTA as anticoagulant (BD Vacutainer, Franklin Lakes, NJ) were used for blood collections. Samples were immediately placed on ice and were centrifuged within 20 min of sampling at 2,550 $\times$ g for 10 min at room temperature. Plasma was transferred by pipette into 5-mL plastic tubes, capped, and frozen at –20 °C for subsequent analysis. Plasma samples for Zn analysis were diluted 1 mL plasma:3 mL deionized water (v/v), then diluted samples were analyzed using an atomic absorption spectrometer (Perkin Elmer Atomic Absorption Spectrometer 3110, PerkinElmer, Waltham, MA) set at a wavelength of 213.9 nm with a 0.2-nm slit width. Standards were prepared from a 500 mg/L Zn stock solution that contained Zn metal and 1% (v/v) HCl.

Diet Preparation, Feeding, and Feed Analyses

Heifers were transitioned to finishing diets through three graduated step-up diets with an 11%

increase in steam-flaked corn and proportional decrease in roughage over a period of 7 d/step to allow gradual adaptation to the final high-grain diet. During the step up period, inclusion level of wet corn gluten feed, vitamin–mineral premix, and feed additive premix remained constant. Vitamin–mineral premixes were formulated to provide either 0 or 90 mg supplemental Zn/kg diet DM as zinc sulfate (0Zn or 90Zn premix). To obtain vitamin–mineral mixes containing 30 or 60 mg supplemental Zn/kg diet DM, 0Zn premix:90Zn premix ratios of 66:34 or 34:66 were combined, respectively. Ractopamine hydrochloride (**RH**; Elanco Animal Health, Greenfield, IN) was mixed in a ground corn carrier and fed at a rate of 90 g/d to supply 300 mg RH/heifer daily during the final 28 d prior to harvest. Melengesterol acetate (Elanco Animal Health) was removed from the feed additive premix the final 18 d on feed due to a shortage of product.

Feed additive premixes and vitamin–mineral mixes were weighed daily and subsequently mixed into total mixed rations. Diets were mixed once daily in a truck mounted mixer and delivered to cattle at ~0800 hours. Feed was monitored and adjusted daily prior to feeding so <0.23 kg/heifer of residual feed remained the following day. Weights

of fresh feed were recorded daily, and unconsumed feed was removed from the bunk and weighed every 28 d or as needed to determine DMI. Subsamples of unconsumed feed were dried at 55 °C for 48 h to determine DM content. DMI was calculated as: $[(\text{total feed offered} \times \% \text{ DM}) - (\text{total feed refused} \times \% \text{ DM})]/(\text{number of animal} \times \text{day})$.

Feed ingredient samples were collected weekly or with each load of feed and subsamples were dried at 55 °C for 48 h to determine DM content. Dried samples were composited into monthly samples, ground to pass a 2-mm screen in a Wiley mill, and analyzed for Zn (Murthy et al., 1971). Zinc analysis was conducted using atomic absorption spectroscopy (Perkin Elmer Atomic Absorption Spectrometer 3110, PerkinElmer, Waltham, MA) set at a wavelength of 213.9 nm with a 0.2-nm slit width. Nutrient composition of diets was then computed as a weighted mean of the individual ingredient concentrations for each nutrient.

Harvest

On day 144, final BW (gross BW $\times$ 0.96) was obtained, then heifers were transported 322 km to a commercial abattoir in Lexington, NE. On the day of harvest, hot carcass weight (HCW), and incidence and severity of liver abscesses were recorded. Liver abscesses were scored according to the procedure described by Brown et al. (1975): 0 = no abscesses, A⁻ = 1 or 2 small abscesses or abscess scars, A⁰ = 2 to 4 small, well-organized abscesses, and A⁺ = 1 or more large or active abscesses with or without adhesions. After ~36 h of refrigeration, USDA yield grades, marbling score, 12th rib fat thickness, and ribeye area were collected by camera images (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany) provided by the abattoir, and quality grade and incidence of dark cutting beef were determined by a certified USDA grader.

Microbial Isolation Methods

Samples from 10 freshly voided fecal pats were collected directly from the surface of each pen immediately before cattle were weighed, loaded onto trucks, and transported to the abattoir. Samples were homogenized, subsampled, and stored in 5-mL culture tubes containing 2 g of feces and 2 mL of glycerol, then stored at -80 °C. Later, samples were removed from the freezer; then, 1 g of sample was added to 9 mL of sterile PBS to create a final 1:20 dilution in a 15-mL glass culture tube and

vortexed thoroughly. Fifty microliters of solution were spiral plated using the Eddy Jet 2 instrument (Neutec Group Inc., NY) onto plain MacConkey agar (BD Difco, NJ), MacConkey agar containing ceftriaxone (Sigma-Aldrich, MO) at 4 µg/mL and MacConkey agar containing tetracycline (Sigma-Aldrich, MO) added at 16 µg/mL. Antibiotic plates contained only one antibiotic per plate. All samples were plated to each of the three plate types. Plates were incubated for 18 h at 37 °C. Each plate was counted for presumptive *E. coli* cfus (based on morphology and pink coloration; Aperce et al., 2016) using the Flash & Go instrument (Neutec Group Inc., NY).

In Vitro Fermentation

Ruminal fluid was collected from a ruminally fistulated Holstein heifer consuming a forage-based diet ~10 h after feeding. Ruminal fluid was strained through four layers of cheese cloth, placed directly into pre-warmed insulated water jugs, and transported 2 km to the Preharvest Food Safety Laboratory on the Kansas State University campus (Manhattan, KS). Upon arrival, ruminal fluid was strained through eight layers of cheese cloth, placed into a large separatory funnel, gassed with nitrogen, and incubated at 39 °C for ~1 h to allow stratification into layers. Layers consisted of a top mat layer, a bottom sediment layer consisting of feed particles, and an intermediate layer consisting of microbe-rich fluid. The bottom sediment layer was discarded, and the intermediate layer was collected taking care to minimize mixing with the mat layer for in vitro fermentation use.

The study was conducted as a randomized complete block (replicate) design with treatments consisting of 0, 30, 60, 90, 120, and 150 mg added Zn/kg substrate DM (five replicates) as Zn sulfate heptahydrate. Zinc sulfate heptahydrate was solubilized in 10 mL deionized water equal to treatment concentrations. Three grams of substrate (90% ground corn, 10% soybean meal) were added to each fermentation flask and pre-wetted with 140 mL of McDougall's buffer (McDougall, 1948). Blank bottles (one per replicate) received no substrate and served for baseline measurements of VFA profiles and substrate contributions of ruminal fluid. One milliliter of the Zn solution was added to the appropriate flask or an equal volume of deionized water was added to flasks serving as blanks and flasks receiving no supplemental Zn. Strained ruminal fluid (10 mL/bottle) was added and initial pH was recorded using Thermo Orion

benchtop pH meter (model 230 A, Thermo Fisher Scientific Inc., Waltham, MA) while bottles were gassed briefly with nitrogen gas. Fermentation flasks were then capped with an Ankom gas pressure monitor (Ankom Gas Production System, Ankom Technology), placed into a shaking incubator set at 39 °C (New Brunswick Scientific Inc., New Brunswick, NJ), and were gently agitated continuously for 24 h. Gas pressure within each vessel was monitored and cumulative pressure was recorded in 15-min intervals.

After a 24-h fermentation period, gas pressures were converted into moles of gas (n) using the following 'ideal' gas law (Ankom Technology Corp., 2014):

$$n = p(V / RT)$$

In this formula, n is gas produced (mol), p is pressure (kPa), V is head-space volume in the flasks (L), T is temperature (K), and R is gas constant (8.314472 L kPa K⁻¹ mol⁻¹). Moles of gas were subsequently converted into volume of gas (mL) using Avogadro's law (Ankom Technology Corp.).

$$\text{gas produced (mL)} = n \times 22.4 \times 1,000$$

where 22.4 is the volume occupied by 1 mol of gas at 39 °C.

Final pH was recorded for each bottle using Thermo Orion benchtop pH meter (model 230 A, Thermo Fisher Scientific Inc.) after 24 h of incubation. Four milliliters of fluid contents from each bottle were combined with 1 mL of 25% (w/v) meta-phosphoric acid solution and frozen at -20 °C. Remaining samples in fermentation flasks were placed into 19.0 × 12.7-cm aluminum pans and dried for 24 h at 105 °C to determine IVDMD. Calculations of percent IVDMD were as follows: [substrate weight - (dry pan weight - initial pan weight - average residue weight of blanks)]/substrate weight.

Volatile Fatty Acid Analyses

Samples of culture fluid were removed from the freezer, thawed at room temperature, and 2 mL of each mixture were placed into micro centrifuge tubes and centrifuged at 17,000 × g for 10 min. Supernatant was transferred by pipette into 12 × 75 gas chromatography vials and sealed. A volatile fatty acid mixture (Supelco, Inc., Bellefonte, PA) was used as the external; standard. The analyses were carried out using an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and a 15 m × 0.53 mm

× 0.5 μm Supelco 25326 Nukol column (Supelco, Inc.) using hydrogen gas as the carrier. The initial oven temperature was 100 °C and increased 10 °C/min to a final temperature of 220 °C held for 2 min and flow rate was 5.1 mL/min with an average velocity of 45 cm/s.

Statistical Analyses

All growth performance and carcass characteristics were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC). There were no block × treatment effects when block was included as a fixed effect, therefore block was removed from the fixed effects statement and included in the random statement for analyses. Supplemental Zn concentration was the fixed effect and pen was the experimental unit. For plasma Zn concentration analysis, the statistical model was the same except day of plasma collection and the interaction between treatment and day were included as fixed effects. In vitro VFA production, final pH, and IVDMD were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.) using replicate as the random effect, fermentation flask as the experimental unit, and supplemental Zn concentration as the fixed effect. Comparative and descriptive statistics were employed concerning microbial growth on plain versus antibiotic-impregnated plates. Zero-inflated Poisson regression models were run (STATA ver. 14 IC, STATA Corp., College Station, TX) accounting for the increased frequencies of no-growth on those MacConkey plates with antibiotics added. These outcome variables were regressed on a rounded (integer) log₁₀ scale. Gas production for in vitro fermentation was analyzed as a repeated measure using the MIXED procedure of SAS. The model included fixed effects of supplemental Zn concentration, time and the interaction between supplemental Zn and time, and replicate (block) as the random effect. The repeated statement included time, bottle within treatment as the subject, and compound symmetry as the covariance structure. The Kenward-Rogers correction was applied for degrees of freedom estimation for all analyses. All variables were tested for the polynomial contrast 0Zn vs. the average of supplemental Zn concentrations, and supplemental Zn concentrations were tested for linear and quadratic responses. Least-squares means were calculated for each treatment. Separations of means were determined to be significant at α level ≤ 0.05 and P -values falling between 0.05 and 0.15 were considered tendencies.

RESULTS AND DISCUSSION

One animal supplemented with 60Zn was removed from the study due to severe lameness. One heifer from the 30Zn treatment died before completion of the study but cause of death was not apparent at necropsy. Two heifers from the 0Zn group died during the study, one of which was diagnosed with septicemia originating from a shoulder abscess, and the other from severe interstitial emphysema in the lungs.

Plasma Zn

A linear increase in plasma Zn concentration was observed with increasing supplemental Zn ($P = 0.02$; [Figure 1](#)). In addition, an effect of day was detected, in which plasma Zn concentration increased as the number of days on feed increased, regardless of supplemental Zn concentration ($P < 0.01$), but no Zn $\times$ day interaction was observed ($P = 0.99$). [NRC \(2001\)](#) indicated serum Zn concentrations within the range of 0.7 to 1.3 $\mu\text{g/mL}$ is considered normal; however, stress, infection, or disease can cause a redistribution of Zn out of extracellular fluids resulting in decreased serum Zn concentrations causing plasma or serum Zn concentrations to be an unreliable indicator of Zn status ([Hambidge et al., 1986](#)).

Microbial Growth

Mixed effect linear regression, controlling for the clustering of pen, indicated that dietary zinc concentration did not impact total bacterial growth on plain MacConkey agar ($P = 0.898$). We found no evidence to suggest that zinc concentration was

associated with ceftriaxone ($P = 0.178$) or tetracycline ($P = 0.61$) resistance plate counts for zero-inflated Poisson regression models. [Figure 2](#) shows the frequency of growth in cfus per gram of fecal sample on MacConkey agar with 4 $\mu\text{g/mL}$ of ceftriaxone on a rounded $\log_{10}$ scale as zinc concentration increases in milligram per kilogram above standard nutrient supplementation. Likewise, [Figure 3](#) shows the frequency of growth in cfus per gram of fecal sample on MacConkey agar infused with tetracycline at 16 $\mu\text{g/mL}$. These results suggest that there is no co-selection of third generation cephalosporin or tetracycline resistance among *E. coli* associated with increasing levels of zinc (to 90 mg/kg) in cattle feed. These results are consistent with those of [Jacob et al. \(2010\)](#), who found no difference in antibiotic susceptibility of *E. coli* isolates based upon zinc or copper supplementation during a 32-d trial where feedlot cattle were fed 1 $\times$ or 10 $\times$ the National Research Council recommended dose of those metals. Little exists in the literature regarding how increasing dietary zinc concentrations in cattle feed may impact antimicrobial resistance among enteric bacteria. There is some debate concerning associations between increased dietary heavy metals and antimicrobial resistance within swine models, however ([Feldpausch et al., 2016](#)).

Feedlot Performance

Effects of supplemental Zn on feedlot performance are presented in [Table 2](#). There were no effects of treatment, linear or quadratic effects of Zn, or difference between cattle receiving 0Zn and the average of Zn supplemented cattle for final BW ($P \geq 0.37$) or ADG ($P \geq 0.29$). Zinc supplementation

Figure 1. Effects of supplementing 0, 30, 60, and 90 mg Zn/kg diet DM (0, 30, 60, or 90Zn) as Zn sulfate on plasma Zn concentration of finishing heifers with six blocks per treatment. Basal ration contained 51.35 mg/kg Zn. Plasma was collected from the same five heifers per pen days 0, 63, and 115. There was no quadratic effect of Zn ($P = 0.77$), but there was a tendency for a difference between heifers supplemented 0Zn vs. the average of heifers receiving added Zn ($P = 0.10$).

Figure 2. Frequency of rounded log₁₀ cfu count per gram of fecal sample by zinc concentration (mg/kg) above standard nutrient supplementation on MacConkey agar with 4 µg/mL of ceftriaxone. Differences among diets were not significant ($P = 0.178$) using Zero-inflated Poisson regression models.

Figure 3. Frequency of rounded log₁₀ cfu count per gram of fecal sample by zinc concentration (mg/kg) above standard nutrient supplementation on MacConkey agar with 16 µg/mL of tetracycline. Differences among diets were not significant ($P = 0.609$) using Zero-inflated Poisson regression models.

has commonly been observed to have no effect on final BW or ADG when supplemented in increased concentrations, regardless of source (Malcolm-Callis et al., 2000; Huerta et al., 2002; Nunnery et al., 2007).

Supplementing increased concentrations of Zn tended to linearly decrease DMI ($P = 0.07$), but there was no quadratic effect of Zn ($P = 0.87$). Feed efficiency improved linearly with increasing supplemental Zn ($P = 0.03$) and a tendency was observed

for a quadratic effect of Zn ($P = 0.12$) in G:F. Feed efficiency tended to be optimized with addition of 60Zn followed by a 3% reduction in G:F when 90Zn was supplemented. Heifers supplemented Zn, regardless of concentration, had improved feed efficiencies compared to heifers supplemented 0Zn ($P = 0.04$), suggesting finishing cattle requires increased concentrations of Zn than the 30 mg/kg Zn recommended by NRC (2000) for beef cattle. Malcolm-Callis et al. (2000) supplemented 20, 100,

Table 2. Feedlot performance of finishing heifers supplemented 0, 30, 60, or 90 mg Zn/kg diet DM¹

Item	Treatment, mg Zn/kg diet DM				P-value ²				
	0	30	60	90	SEM	Trt	C1	C2	C3
Pens	6	6	6	6	—	—	—	—	—
Initial BW, kg	386	384	385	386	13.08	0.64	0.93	0.33	0.40
Final BW ³ , kg	603	603	610	602	19.69	0.53	0.79	0.37	0.67
ADG, kg	1.51	1.52	1.56	1.51	0.05	0.52	0.77	0.29	0.57
DMI, kg	10.25	10.12	9.95	9.88	0.35	0.33	0.07	0.87	0.15
G:F	0.1470	0.1497	0.1573	0.1522	0.0024	0.03	0.04	0.12	0.04

^{a,b}Within a row, means without a common superscript letter are different ($P \leq 0.03$).

¹A steam-flaked corn-based diet that contained a basal concentration 51.4 mg/kg Zn. Vitamin–mineral premixes were formulated to contain 0 or 90 mg supplemental Zn/kg diet DM (Zn sulfate) and were blended for rations formulated to contain 30 or 60 mg/kg supplemental Zn.

²Effect of treatment (Trt), linear effect of Zn (C1), quadratic effect of Zn (C2), and 0 mg Zn/kg diet DM vs. average treatments with added zinc (C3).

³Gross BW $\times$ 0.96.

and 200 mg Zn/kg DM as zinc sulfate to finishing steers fed a diet containing ~70 mg/kg Zn. Similar to current results, the authors observed increased supplemental Zn tended to reduce DMI in a linear manner; however, decreased DMI did not result in improved feed efficiency. [Genther-Schroeder et al. \(2018\)](#) supplemented steers 0, 60, 90, 120, and 150 mg Zn/kg as Zn-amino acid complex to diets that contained 60 mg Zn/kg as Zn sulfate and a basal concentration of 30 mg Zn/kg DM. The authors observed no differences in ADG, DMI, of G:F. Similarly, when comparing a basal ration containing 52.5 mg Zn/kg diet DM to diets supplementing 75 mg Zn/kg DM as Zn sulfate, Zn methionine, or Zn proteinate fed to heifers, Zn supplementation did not affect ADG, but feed efficiency tended to increase with added Zn, regardless of source ([Nunnery et al., 2007](#)). In contrast, supplementing 25 mg Zn/kg DM (as Zn oxide or two forms of Zn proteinate) to steers had no effect on DMI or feed efficiency compared to cattle consuming a basal diet that contained 26 mg/kg Zn ([Spears and Kegley, 2002](#)). [Kessler et al. \(2003\)](#) supplemented finishing bulls with 10 mg Zn/kg DM in the form of Zn oxide, Zn proteinate, or Zn polysaccharide and observed no effect of Zn supplementation for final BW, growth rate, or feed efficiency compared to bulls fed a basal ration consisting of 35 mg/kg Zn. Differences among studies could be due Zn source, sex of the animals, feeding duration, or level of Zn in basal diets. Zinc requirements for finishing cattle are not clearly defined and little is known regarding factors that may influence Zn requirements. Increased supplemental Zn has been observed to interfere with absorption of other minerals, such as copper ([Ivan and Grieve, 1975](#)); however, there is minimal research indicating effects of Zn source on absorption of other minerals. In the current trial, it is conceivable that increasing supplemental Zn as

Zn sulfate may have caused unknown interactions with other trace minerals, resulting in reduced DMI. It also is conceivable that increased concentrations of Zn sulfate may adversely affect ruminal microbiota. Our results suggest supplementing increased concentrations of Zn may depress DMI; however, ADG was not affected, resulting in improved feed efficiency with a tendency for no further improvement when zinc was supplemented above 60 mg/kg diet DM (111.4 mg total Zn/kg diet DM).

Carcass Characteristics

Effects of Zn supplementation on carcass characteristics are presented in [Table 3](#). A tendency for a treatment effect was observed for marbling score ($P = 0.08$) with carcasses from heifers supplemented 60Zn tending to have greater marbling scores compared to carcasses from heifers supplemented 90 or 30Zn ($P \leq 0.07$), but not when compared to carcasses from heifers supplemented with 0Zn ($P = 0.17$). No effects of treatment, linear or quadratic effects of Zn, or differences between 0Zn and the average of Zn treatments were observed for HCW, dressing percentage, incidence of liver abscesses, ribeye area, 12th rib fat thickness, or overall yield grade, ($P \geq 0.18$). Effects of Zn supplementation on yield and quality grades are presented in [Table 4](#). Tendencies for quadratic effects of Zn were observed for carcasses grading USDA Prime ($P = 0.07$) and USDA yield grades 1 and 3 ($P = 0.07$ and $P = 0.15$, respectively), with carcass from heifers fed 60Zn tending to have more carcasses grading Prime and fewer yield grade 1 carcasses compared to the other treatments. Zinc supplementation did not affect other yield or quality grades ($P \geq 0.22$).

Zinc finger proteins have been observed to have major regulatory roles in the process of adipogenesis ([Wei et al., 2013](#)). At least one Zn finger protein has

Table 3. Effects of supplementing 0, 30, 60, and 90 mg Zn/kg diet DM on carcass characteristics of finishing heifers¹

Item	Treatment, mg Zn/kg diet DM				SEM	P-value ²			
	0	30	60	90		Trt	C1	C2	C3
Pens	6	6	6	6	—	—	—	—	—
HCW ³ , kg	380	379	383	379	12.53	0.60	0.82	0.54	0.82
Dressed yield, %	63.01	62.86	62.81	62.99	0.26	0.80	0.89	0.34	0.54
Liver abscesses, %	11.1	6.0	7.6	10.0	3.12	0.55	0.89	0.18	0.32
Ribeye area, cm ²	94.7	93.7	93.6	93.9	2.84	0.88	0.60	0.55	0.44
12th-rib fat thickness, cm	1.45	1.40	1.47	1.40	0.08	0.53	0.68	0.87	0.55
Marbling score ⁴	444	434	454	440	11.28	0.08	0.72	0.66	0.87

¹A steam-flaked corn-based diet that contained a basal concentration 51.4 mg/kg Zn. Vitamin–mineral premixes were formulated to contain 0 or 90 mg supplemental Zn/kg diet DM (Zn sulfate) and were blended for rations formulated to contain 30 or 60 mg/kg supplemental Zn.

²Effect of treatment (Trt), linear effect of Zn (C1), quadratic effect of Zn (C2), and 0 mg Zn/kg diet DM versus average treatments with added zinc (C3).

³HCW = hot carcass weight.

⁴Marbling scores were determined by camera imaging (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany); small = 400 to 499; modest = 500 to 599.

Table 4. Effects of supplementing 0, 30, 60, and 90 mg Zn/kg diet DM on USDA quality and yield grades of carcasses from heifers¹

Item, %	Treatment, mg Zn/kg diet DM				SEM	P-value ²			
	0	30	60	90		Trt	C1	C2	C3
Pens	6	6	6	6	—	—	—	—	—
USDA Prime	1.7	2.5	5.1	0.0	1.53	0.16	0.71	0.07	0.64
USDA premium choice	18.8	15.2	17.8	20.8	4.57	0.76	0.61	0.39	0.85
USDA low choice	46.1	49.0	51.7	48.3	4.90	0.86	0.66	0.51	0.52
USDA select	24.8	24.8	21.2	26.7	4.77	0.80	0.91	0.51	0.90
Sub-select ³	6.9	6.0	2.5	4.2	2.22	0.46	0.22	0.55	0.28
Dark cutter	1.7	2.5	1.7	0.0	1.25	0.43	0.23	0.26	0.81
Overall yield grade	2.5	2.4	2.6	2.3	0.15	0.55	0.64	0.38	0.90
Yield grade 1	16.4	12.9	10.3	21.7	5.41	0.21	0.45	0.07	0.75
Yield grade 2	36.0	42.3	35.6	35.0	3.37	0.26	0.46	0.24	0.62
Yield grade 3	33.2	34.7	42.4	31.7	4.86	0.28	0.87	0.15	0.52
Yield grade 4	14.4	9.3	11.7	10.8	3.19	0.70	0.56	0.51	0.32
Yield grade 5	0.0	0.8	0.0	0.8	0.64	0.54	0.51	1.00	0.40

¹A steam-flaked corn-based diet that contained a basal concentration 51.4 mg/kg Zn. Vitamin–mineral premixes were formulated to contain 0 or 90 mg supplemental Zn/kg diet DM (Zn sulfate) and were blended for rations formulated to contain 30 or 60 mg/kg supplemental Zn.

²Effect of treatment (Trt), linear effect of Zn (C1), quadratic effect of Zn (C2), 0 mg Zn/kg diet DM vs. average treatments with added zinc (C3).

³Includes carcasses that graded standard and commercial.

been isolated in bovine stromal vascular cells, and it has been implicated as a key initiator in early adipose determination (Huang et al., 2012), providing a possible explanation for the tendencies of Zn supplementation to affect marbling score, and percentage of carcasses grading Prime and yield grade one. Supplementing steers 20, 100, or 200 mg Zn/kg DM as Zn sulfate with a basal dietary Zn concentration of 70 mg/kg DM resulted in quadratic responses of Zn for 12th rib fat thickness and yield grade with carcasses from steers supplemented 100 mg Zn/kg DM having increased 12th rib fat thickness and increased yield grade compared to carcasses from steers supplemented 20 or 200 mg Zn/kg DM, but

supplementation did not affect HCW, dressing percentage, ribeye area, or marbling score (Malcom-Callis et al., 2000). Huerta et al. (2002) observed a tendency for decreased 12th rib fat thickness and dressing percentage, but no effects on marbling score, yield grade, or quality grade when heifers were supplemented 200 mg Zn/kg DM from Zn methionine or Zn sulfate compared to heifers fed a diet containing 64 mg/kg Zn regardless of source of Zn. In the same study, steers were supplemented the same concentration of Zn compared to steers fed a control ration containing 84 mg/kg Zn. Regardless of Zn source, no differences were observed between supplemented and un-supplemented steers for any

carcass trait. Likewise, [Genther-Schroeder et al. \(2018\)](#) observed no differences in carcass performance when steers were supplemented 0, 60, 90, 120, and 150 mg Zn/kg as Zn-amino acid complex to diets that contained 60 mg Zn/kg as Zn sulfate and a basal concentration of 30 mg Zn/kg DM. Steers supplemented 25 mg Zn/kg DM from Zn oxide or two forms of Zn proteinate had increased quality grades and increased marbling scores compared to un-supplemented steers, regardless of source ([Spears and Kegley, 2002](#)), when the basal ration contained 26 mg Zn/kg DM. The authors speculated increasing supplementary Zn concentrations in studies that fed basal diets above [NRC \(2000\)](#) recommendations had minimal impact on carcass traits, whereas a greater response to Zn supplementation was observed in studies that compared increased supplemental Zn to basal diets at or below [NRC \(2000\)](#) recommendations for dietary Zn. In addition, differences in study design, Zn source, Zn content in the basal diet, and supplemental Zn content make it difficult to compare effects of Zn supplementation across studies. Our study indicated Zn supplementation up to 90 mg/kg DM (141.35 mg/kg total dietary Zn) minimally impacted carcass traits; however, [Van Bibber-Krueger et al. \(2017\)](#) observed an interaction between Zn and ractopamine hydrochloride (RH), where supplementing 100 mg Zn/kg DM decreased ribeye area and increased yield grade, but these effects were no longer observed after supplementing heifers with RH ([Van Bibber-Krueger et al., 2017](#)). These same results were not observed in steers fed 85 or 150 mg/kg (60 mg Zn/kg DM from Zn amino acid complex) total dietary Zn ([Genther-Schroeder et al., 2016](#)). The lack of congruence among studies with steers

and heifers may suggest different Zn requirements for males vs. females. Heifers in the current study were supplemented with ractopamine hydrochloride during the final 28 d on feed, which may have obscured effects for carcass traits that occurred due to feeding increased concentrations of supplemental Zn. In addition, heifers in the current study received adequate Zn with the basal ration containing 51.4 mg Zn/kg diet DM, which may also contribute to limited responses to Zn supplementation.

In vitro Fermentation

Increased concentrations of supplemental Zn have previously been observed to affect cellulose digestion ([Martinez and Church, 1970](#)), but the effects of Zn supplementation on in vitro fermentation containing high concentrate substrates has not been evaluated. In the current study, no treatment $\times$ time interaction ($P = 0.67$; [Figure 4](#)), or effect Zn supplementation ($P = 0.59$) was observed for in vitro gas production. Gas production was affected by time ($P < 0.01$) with gas production increasing with increasing fermentation time. Very little literature is available to compare the current results with gas production from other research trials. Our study suggests supplementing up to 150 mg Zn/kg substrate DM does not affect in vitro gas production with addition of high concentrate substrate.

Effects of supplemental Zn on pH, IVDMD, and VFA concentrations are presented in [Table 5](#). Including supplemental Zn in the in vitro fermentation flasks did not affect terminal pH or IVDMD ($P \geq 0.20$). [Wang et al. \(2013\)](#) and [Kathirvelan and Balakrishnan \(2008\)](#) observed decreased IVDMD when 20 $\mu\text{g/mL}$ Zn from Zn sulfate and 10 mg/kg

Figure 4. Effects of adding 0, 30, 60, 90, 120, or 150 mg Zn/kg substrate DM (0, 30, 60, 90, 120, or 150) as zinc sulfate to fermentation flasks containing 10 mL ruminal fluid, 140 mL McDougal's buffer, and 3 g substrate (90% ground corn, and 10% soybean meal) on in vitro gas production over 24 h fermentation period using the Ankom Gas Production System (Ankom Technology, Macedon, NY) with five replicates per treatment. There were no linear or quadratic effects Zn ($P = 0.69$; $P = 0.82$, respectively) or a difference between 0 mg Zn/kg substrate vs. the average of Zn treatments ($P = 0.80$).

Table 5. Effects of adding 0, 30, 60, 90, 120, or 150 mg Zn/kg substrate DM as zinc sulfate to fermentation flasks containing 10 mL ruminal fluid, 140 mL McDougal's buffer, and 3 g substrate¹ on pH, IVDMD, and VFA production following 24 h fermentation using Ankom Gas Production System (Ankom Technology, Macedon, NY)

Item	Treatment, mg Zn/kg substrate						P-value ²				
	0	30	60	90	120	150	SEM	Trt	C1	C2	C3
Replicates	5	5	5	5	5	5	—	—	—	—	—
pH	6.01	6.06	6.04	6.02	6.01	6.00	0.02	0.50	0.67	0.26	0.41
IVDMD, %	52.40	49.67	51.07	51.47	52.07	51.80	1.17	0.60	0.75	0.20	0.30
Volatile fatty acid, mM											
Acetate	36.04	34.89	33.28	33.54	35.91	34.65	1.25	0.53	0.69	0.07	0.25
Propionate	42.90	41.70	41.56	42.10	41.92	43.08	0.68	0.52	0.48	0.30	0.17
Butyrate	8.68	8.68	8.43	8.99	8.53	8.74	0.31	0.91	0.99	0.40	0.80
Isobutyrate	1.02	1.39	1.12	0.76	1.00	1.05	0.36	0.89	0.56	0.80	0.91
Isovalerate	0.093	0.068	0.054	0.048	0.025	0.081	0.032	0.43	0.05	0.90	0.12
Valerate	0.405	0.360	0.377	0.372	0.342	0.370	0.043	0.81	0.26	0.95	0.24
Isocaproic	0.027	0.010	0.009	0.003	0.012	0.056	0.025	0.52	0.58	0.56	0.44
Caproic	0.023	0.000	0.000	0.000	0.003	0.040	0.019	0.44	0.46	0.44	0.26
Heptanoic	0.035	0.013	0.010	0.007	0.022	0.070	0.031	0.48	0.68	0.43	0.43
A:P ratio	0.839	0.835	0.800	0.797	0.856	0.803	0.020	0.21	0.93	0.04	0.44

¹Substrate used for each fermentation flask consisted of 90% corn and 10% soybean meal.

²Effect of treatment (Trt), linear effect of Zn (C1), quadratic effect of Zn (C2), and 0 mg Zn/kg diet DM vs. average treatments with added zinc (C3).

Zn from Zn chloride, respectively, was added to in vitro cultures containing primarily forage as the substrate. [Arelovich et al. \(2000\)](#) observed a linear decrease in IVDMD with addition of 0, 5, 10, 15, or 20 mg/kg added Zn as ZnCl was incubated with prairie hay as the substrate. [Eryavuz and Dehority \(2009\)](#) suggested decreased cellulose digestion with increased supplemental Zn may be due to inhibitory effects of cellulolytic enzymes produced by microorganisms rather than a direct inhibitory effect on the bacteria. This may explain the decreased IVDMD as Zn concentration increased in in vitro cultures containing high forage substrates. Our results suggest increased supplemental Zn to in vitro cultures containing 90% ground corn and 10% soybean meal did not affect IVDMD or pH.

A tendency for a quadratic decrease was observed for acetate ($P = 0.07$) concentration with addition of 60 and 90 mg Zn/kg substrate DM tending to have the lowest acetate concentrations compared to other treatments. There was no effect of added Zn on propionate concentration ($P \geq 0.17$), thus a quadratic effect of Zn was observed for A:P ratio ($P = 0.04$). A linear effect of Zn was observed for isovalerate ($P = 0.05$), in which concentration of isovalerate decreased with increasing supplemental Zn. Concentrations of butyrate, isobutyrate, valerate, isocaproic acid, caproic acid, and heptanoic acid were not affected by supplemental Zn ($P \geq 0.23$). [Bateman et al. \(2002\)](#) performed an in vitro fermentation study using 50:50 forage to concentrate

ratio and added 0 or 1,350 mg Zn/kg DM as Zn sulfate to continuous culture fermenters. The authors observed increased acetate, and decreased butyrate concentrations with addition of Zn; however, when ruminal fluid from cows fed diets containing similar supplemental Zn concentrations from Zn sulfate were analyzed, no differences were observed for any VFA concentrations. These data suggest there could be differences in fermentation between in vitro and in vivo VFA production, or that in vivo production of VFA is masked by absorption. Similarly, Zn supplementation had no effect on VFA concentrations of ruminal fluid from steers supplemented 430 mg Zn/kg DM as Zn chloride, and fed a diet consisting of 39% chopped alfalfa hay and 61% concentrate ([Arelovich et al., 2008](#)). [Spears et al. \(2004\)](#) supplemented steers 20 mg Zn/kg DM from Zn sulfate, Zn methionine, or Zn glycine complex (basal diet consisted of 46.4% dried citrus pulp, 39.3% cottonseed hulls, 5% corn, and 7% soybean meal 2.3% mineral mix) and observed decreased valerate with addition of Zn, regardless of source; however, Zn methionine supplementation resulted in increased propionate, and decreased butyrate compared to the control and other Zn treatments. Effects of Zn supplementation on VFA concentrations appear to vary depending on diet of animals tested, substrate used for in vitro cultures, or when comparing differences between in vitro and in vivo fermentation. Our results suggest adding up to 150 mg Zn/kg substrate DM minimally impacted in vitro fermentation.

Overall, Zn supplementation up to 150 mg/kg substrate weight minimally affected in vitro fermentation and supplementing up to 60 mg Zn/kg diet DM (111.4 mg/kg total dietary Zn) had the greatest improvement in feed efficiency with minimal effects on carcass characteristics; however, differences in carcass characteristics may have been masked with the addition of ractopamine hydrochloride to the diet the final 28 d on feed.

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