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Effects of increasing supplemental zinc in beef feedlot steers administered a steroidal implant and beta agonist

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ABSTRACT

Ninety-two Angus-crossbred steers (424 ± 28.3 kg initial body weight) were used in a 98-d study to assess the effects of increasing Zn supplementation on cattle performance, liver and plasma trace mineral concentrations, blood metabolites, and carcass characteristics. All steers were implanted with a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0 and fed 300 mg·steer^{-1}·d^{-1} of ractopamine hydrochloride (Zoetis, Parsippany, NJ) from d 70 to 98. Cattle were fed via GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada), and steer served as the experimental unit (n = 22 or 23 steers/treatment). Supplemental Zn was administered through the diet at 0, 100, 150, or 180 mg Zn/kg on a dry matter basis from $ZnSO_4$ (Zn0, Zn100, Zn150, or Zn180, respectively). Cattle were weighed on d −1, 0, 9/10, 20, 41, 59, 69, 70, 78/79, 97, and 98. Blood was collected on d 0, 9/10, 69, 78/79, and 97, and liver biopsies on d 9/10 and 78/79 (n = 12 steers/treatment). Data were analyzed as a complete randomized design. Contrast statements were formed to test the linear, quadratic, and cubic effects of Zn supplementation and test Zn0 vs. Zn supplementation. Day 10 and 70 body weight (BW) and d 0 to 10 and 0 to 70 average daily gain were linearly increased with Zn supplementation ($P \leq 0.05$), and greater for Zn supplemented steers ($P \leq 0.03$). No effects of Zn supplementation were observed on final BW, dressing percentage, ribeye area, 12th rib fat, or marbling ($P \geq 0.11$). Hot carcass weight tended to be 7 kg greater for Zn supplemented steers than Zn0 ($P = 0.07$), and yield grade linearly increased with increasing Zn supplementation ($P = 0.02$). Day 10 liver Mn concentrations tended to quadratically decrease ($P = 0.08$) with increasing Zn supplementation, though d 79 liver Mn concentrations and arginase activity were not influenced by Zn ($P \geq 0.28$). Day 10 liver arginase activity tended to be ($r = 0.27$; $P = 0.07$) and d 10 serum urea nitrogen was correlated with d 10 liver Mn ($r = 0.55$; $P < 0.0001$). Zinc supplementation linearly increased d 10 liver Zn and d 10, 69, 79, and 97 plasma Zn concentrations ($P \leq 0.05$). A cubic effect of Zn was observed on d 79 liver Zn ($P = 0.01$) with lesser liver Zn in Zn0 and Zn150 steers. These data suggest increasing dietary Zn improves growth directly following the administration of a steroidal implant and that steroidal implants and beta agonists differ in their effects on protein metabolism.

Key words: arginase, beef steers, manganese, protein metabolism, zinc sulfate

INTRODUCTION

Steroidal implants and beta agonists are technologies commonly used in the U.S. feedlot industry (NAHMS, 2013; Samuelson et al., 2016). The economic importance of these technologies is demonstrated by the 16%–20% improvement in average daily gain (**ADG**) of implanted cattle (Duckett and Pratt, 2014) and improvements in hot carcass weight (**HCW**) and ribeye area (**REA**) of cattle fed a beta agonist (Lean et al., 2014). However, just as energy has been shown to be a potentially limiting factor in implant-induced growth of cattle (Guiroy et al., 2002), so too might other nutrients be preventing optimal growth responses to these technologies. Zinc may benefit growth of cattle administered both steroidal implants and beta agonists because of its function in over 2000 transcription factors (Cousins et al., 2006) and its role in both deoxyribonucleic acid (**DNA**) and protein synthesis (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976). Messersmith and Hansen (2021) reported increasing supplemental Zn up to 150 mg Zn/kg dry matter (**DM**) linearly increased ADG of implanted cattle, but not nonimplanted cattle, within 18 d of implant administration. Similarly, Genther-Schroeder et al. (2016a, 2016b) observed a linear improvement in final body weight (**BW**) of cattle fed a beta agonist when increasing Zn supplementation up to 150 mg Zn/kg DM, but no effects of Zn were observed in cattle fed no beta agonist. These data suggest Zn supplementation above NASEM (2016) recommendations may be crucial to optimizing the growth of cattle utilizing growth promoting technologies. However, growth rates of cattle utilizing these technologies appear to be greatest early after administration (Johnson et al., 1996; Maxwell et al., 2015), indicating Zn may have the greatest potential to improve growth during this time. The objective of this study was to examine the effects of increasing Zn supplementation from inorganic Zn on growth and mineral status of feedlot cattle with particular emphasis on periods of peak growth following administration of a steroidal implant and the initiation of beta agonist feeding. It was hypothesized cattle growth would linearly increase with increasing Zn supplementation during both periods of rapid growth.

MATERIALS AND METHODS

Care and Use of Animals

The Iowa State University Institutional Animal Care and Use Committee (log number: IACUC-20-182) approved all procedures and protocols used in this study.

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Animals and Experimental Design

Ninety-two Angus-crossbred steers (424 ± 28 kg) were used in a complete randomized design to determine the effect of increasing Zn supplementation on cattle receiving both a steroidal implant and fed a beta agonist. Cattle were stratified by BW into pens (n = 5 or 6 steers/pen), so that pen weights were similar at the start of the study. All cattle were implanted with a high potency implant (Component TE-200; 200 mg trenbolone acetate + 20 mg estradiol + 29 mg tylosin tartrate; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist for the last 28-d of the trial starting on d 70 (300 mg·steer^{-1}·d^{-1} of ractopamine hydrochloride; donated by Zoetis, Parsippany, NJ). Steers were fed a dry-rolled corn-based finishing diet (Table 1) ad libitum throughout the 98-d trial. Feed was delivered once daily (0800 hours) to GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada). Radio frequency tags on each steer relayed individual steer feed disappearance data from the bunk to GrowSafe software. Therefore, individual intake data were recorded for each animal in each pen. Cattle were fed 1 of 4 Zn treatments (n = 22 or 23 steers per treatment): 0, 100, 150, or 180 mg supplemental Zn/kg on a DM basis (Zn0, Zn100, Zn150, or Zn180, respectively) from $ZnSO_4$ administered daily through a dried distillers grains plus solubles-based premix in the total mixed ration (**TMR**). Zinc treatments were chosen to represent no supplemental Zn, industry supplementation of Zn (Samuelson et al., 2016), and supranutritional concentrations of Zn previously supplemented in the literature (Genther-Schroeder et al., 2018).

Following the completion of the 98-d study, cattle were harvested at a commercial abattoir (National Beef, Tama, IA) via industry accepted practices. Trained National Beef personnel collected HCW on the day of harvest while REA, 12th rib fat (**RF**), and marbling data were collected following a 48-h chill. Yield grade data represents the abattoir assigned number and was not calculated via the USDA yield grade calculation due to a lack of kidney, pelvic, and heart fat data.

Table 1. Diet composition

Ingredient	% DM basis
Dry-rolled corn	45.0
Sweet Bran[1]	20.0
Corn silage	15.0
DDGS[2]	18.06
Limestone	1.5
Salt	0.31
Vitamin and mineral premix[3,4]	0.1165
Rumensin	0.0135
Analyzed composition	
Crude protein[5]	15.4
Neutral detergent fiber[5]	19.8
Ether extract[5]	4.8
Cu, mg/kg DM[6]	12
Fe, mg/kg DM[6]	118
Mn, mg/kg DM[6]	30
Zn, mg/kg DM[6]	39
Calculated composition[7], Mcal/kg	
NEm	2.05
NEg	1.39

[1]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).
[2]Dried distillers grains with solubles.
[3]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet.
[4]Wtih the exception of Zn, trace minerals were supplemented at NASEM (2016) recommendations for Co, Cu, I, Mn, and Se, from inorganic sources. Diets were supplemented with 0, 100, 150, or 180 mg Zn/kg dry matter (**DM**) from $ZnSO_4$.
[5]Analysis of Zn0 total mixed ration (**TMR**) conducted by Dairyland Laboratories (Arcadia, WI).
[6]Analyzed values for trace minerals represent the Zn0 TMR measured by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA). Dietary Zn was analyzed as 148, 167, and 205 mg Zn/kg DM for Zn100, Zn150, and Zn180, respectively.
[7]Calculations for net energy of maintenance (**NEm**) and net energy of gain (**NEg**) utilized NASEM (2016) nutrient values of diet ingredients.

Sample Collection and Analysis

Consecutive day BW were taken at the beginning of the trial (d −1 and 0), the start of the beta agonist period (d 69 and 70), and at the end of the trial (d 97 and 98) with interim BW recorded on d 9/10, 20, 41, 59, and 78/79 (n = 22 or 23 steers/treatment) to monitor the effects of Zn treatment relative to implant administration and beta agonist use. Weights recorded on d 9/10 and 78/79 were taken in accordance with the liver biopsy pen schedule. Liver biopsies were collected from the same steers on d 9 or 10, and again on d 78 or 79 with 2 of the 4 pens per treatment sampled on each day (n = 12 steers/treatment). Liver samples were collected via biopsy procedures outlined by Engle and Spears (2000) and stored at 20 °C before analysis for trace mineral concentration.

Liver arginase activity was analyzed in triplicate utilizing procedures adapted from Lin et al. (2017). Briefly, liver samples were homogenized with a phosphate buffered saline, protease, and phosphatase buffer cocktail followed by centrifugation of the sample at 14,000 × g for 15 min. The resulting supernatant was diluted with 30× phosphate buffered saline solution. The diluted supernatant (50 µl) was added to test tubes in triplicate and incubated with 220 µl glycine–NaOH buffer (pH 9.6) and 100 µl arginine (68 mM, pH 9.6) at 37 °C for 10 min. Following incubation the reaction was stopped with the addition of 900 µl $H_2SO_4/H_3PO_4/H_2O$ (1:3:7). Next, 40 µl of α-isonitrosopropiophenone (9%) dissolved in ethanol was added before samples were heated at 95 °C for 30 min to develop color. Samples were cooled in the dark for 15 min before being transferred to a 96-well plate and read at 540 nm. Sample urea production was normalized to protein content of the sample analyzed using a commercial Coomassie Bradford Protein Assay kit (Thermo Fisher Scientific, Waltham, MA) and arginase activity was determined by the amount of urea produced per µg protein in 1 min (nM urea/µg protein/min). Intra-assay and inter-assay CV were 10.4% and 10.9%, respectively for the arginase assay.

Samples of each TMR were collected weekly and dried in a forced air oven at 70 °C for 48 h to calculate diet dry matter. Dried TMR samples were ground through a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and composited by month within each treatment. Nutrient analysis of the composited Zn0 TMR was conducted by Dairyland Laboratories (Arcadia, WI) in accordance with procedures outlined by Heiderscheit and Hansen (2020). Blood was collected from the same steers on d 0, 9/10, 69, 78/79, and 97 (n = 12 steers/treatment) to assess how implants and beta agonists affect blood metabolites. Vacuum capped tubes (Becton Dickerson, Rutherford, NJ) containing either trace mineral grade K_2EDTA for plasma trace mineral analysis or

no additives for serum collection were used for jugular venipuncture collection of blood. Blood samples were stored at –20 °C until analysis. Serum urea nitrogen (**SUN**) concentrations were measured at all timepoints from serum samples using a commercial kit (Teco Diagnostics, Anaheim, CA). Intra-assay and inter-assay CV were 6.3% and 5.9%, respectively for SUN analysis. Non-esterified fatty acid (**NEFA**) concentrations were also measured in serum for d 69, 78/79, and 97 using a commercial kit (Wako Pure Chemical Industries Ltd., Chuo-Ku Osaka, Japan). Intra-assay and inter-assay CV for NEFA data were 5.9 and 5.5%, respectively.

Liver samples and composited TMR samples were acid digested via procedures outlined by Pogge and Hansen (2013) and Richter et al. (2012), respectively. Trace mineral concentrations of liver, TMR, and plasma samples were analyzed via inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA) following the procedures described by Richter et al. (2012) and Pogge and Hansen (2013). Instrument accuracy was ensured by using a standard on each run (Trace Elements Serum Control #66816; UTAK Laboratories Inc., Valencia, CA; Bovine Liver #1577c; National Institute of Standards and Technology, Gaithersburg, MD).

Statistical Analysis

Data were analyzed using the Mixed Procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Zinc treatment was utilized as the fixed effect. Contrast statements were formed using PROC IML to determine contrast coefficients to test for linear, quadratic, and cubic effects of Zn supplementation. Additionally, a contrast statement was formed to test for differences between non-Zn supplemented and Zn supplemented treatments. Steer was the experimental unit for all analysis ($n = 22$ or 23 steers/treatment for performance parameters or $n = 12$ steers/treatment for blood and liver data). Initial BW was used as a covariate in performance and carcass data analysis, except for d 0 BW, while initial blood values were used as a covariate for the respective analysis of plasma trace mineral concentrations, SUN, and NEFA, excluding the analysis of d 0 plasma trace mineral and SUN concentrations and d 69 NEFA. The Correlation Procedure of SAS was utilized to assess correlations between liver arginase activity, SUN concentrations, and liver Mn concentrations. All data are reported as the least squares means ± the standard error of the mean. Cook's *D* statistical test was used to test for outliers. Cook's *D* values above 0.20 were removed from analysis. Two steers were removed from all performance and carcass analysis due to chronic health events ($n = 1$ each for Zn100 and Zn180). Statistical significance was determined as $P \leq 0.05$ and tendencies between $0.06 \leq P \leq 0.10$.

RESULTS

Performance and Carcass Characteristics

Body weights from throughout the trial are included in Table 2. A positive linear response to Zn supplementation was observed for d 10 and 70 BW ($P \leq 0.05$) that can be explained by the 3.3 kg advantage in d 10 BW and 9.7 kg advantage in d 70 BW of all Zn supplemented steers vs. non-Zn supplemented steers ($P \leq 0.03$). Furthermore, a tendency for a cubic response to Zn supplementation on d 20 BW was observed ($P = 0.10$) in which Zn0 and Zn150 had lesser BW than Zn100 and Zn180. No other polynomial effects were observed for BW throughout the trial ($P \geq 0.11$). However, d 79 BW tended to be 8.3 kg greater for Zn supplemented steers than non-Zn supplemented steers ($P = 0.09$).

Both d 0 to 10 and d 0 to 70 ADG linearly increased with Zn supplementation (Table 3; $P \leq 0.05$) and were greater for Zn supplemented steers than Zn0 ($P \leq 0.03$). However, neither dry matter intake (**DMI**) or feed efficiency (**G:F**) for d 0 to 70 were affected ($P \geq 0.15$). Although final BW was not influenced by Zn supplementation ($P \geq 0.15$), a cubic response to Zn supplementation was observed for d 70 to 98 ADG and G:F ($P \leq 0.02$) driven by lesser ADG and G:F of Zn150 during the beta agonist feeding period. Furthermore, Zn supplementation tended to decrease d 70 to 98 G:F ($P = 0.08$), although no effects of Zn supplementation were observed on d 70 to 98 DMI ($P \geq 0.16$). Likewise, overall (d 0 to 98) G:F tended to cubically respond to Zn supplementation ($P = 0.06$) with Zn100 having the greatest G:F and Zn150 the least G:F. However, no effects of Zn supplementation were observed for d 0 to 98 ADG or DMI ($P \geq 0.16$).

Carcass-adjusted G:F tended to quadratically decrease (Table 4; $P = 0.10$) with Zn150 having the lowest G:F. However, Zn supplementation did not affect carcass-adjusted final BW or ADG ($P \geq 0.15$). Hot carcass weight, dressing percentage, REA, **RF**, and marbling were not affected by polynomial effects of Zn supplementation ($P \geq 0.11$). However, HCW tended to be 7 kg greater for Zn supplemented steers than Zn0 ($P = 0.07$). Yield grade linearly increased with increasing Zn supplementation ($P = 0.02$), corresponding to Zn supplemented cattle having greater yield grade than Zn0 steers ($P = 0.04$). This is in accordance with the numerical ($P = 0.11$) linear increase in RF with increasing Zn supplementation.

Blood Metabolites

Although dietary treatments had not yet begun, d 0 SUN linearly decreased with increasing Zn concentrations (Table 5; $P = 0.02$) and were lesser for Zn supplemented steers than non-Zn supplemented steers ($P = 0.01$) before the administration of the implant. Zinc supplementation did not influence SUN concentrations ($P \geq 0.47$) 10 d-post administration of the implant but tended to quadratically increase SUN on d 69 ($P = 0.08$). No further effects of Zn supplementation were observed for d 0, 69, 79 or 97 SUN ($P \geq 0.19$). Day 0 to 10 percent change in SUN was not affected by Zn supplementation ($P \geq 0.24$). The percent change in SUN from d 69 to 79 linearly ($P = 0.05$) and quadratically ($P = 0.001$) decreased with Zn100 having the greatest decrease in SUN.

Nonesterified fatty acid concentrations were measured beginning on d 69, at the start of the beta agonist feeding period. After 10 d of beta agonist supplementation, d 79 NEFA concentrations linearly decreased ($P = 0.05$) with increasing Zn concentrations. A tendency for a cubic response to Zn supplementation ($P = 0.08$) was observed for d 97 NEFA concentrations with Zn0 and Zn150 having greater NEFA concentrations than Zn100 and Zn180. No effects of Zn supplementation were observed for d 69 NEFA concentrations ($P \geq 0.27$) or d 69 to 79 percent change in NEFA ($P \geq 0.19$).

Liver Trace Mineral Concentrations and Arginase Activity

Liver Cu tended to quadratically increase with increasing Zn supplementation on d 10 (Table 6; $P = 0.07$) while d 10

Table 2. Effect of zinc supplementation on body weights of implanted and beta agonist-fed finishing beef steers

	Treatments[1]				SEM	Contrasts[2]			
	Zn0	Zn100	Zn150	Zn180		Lin	Quad	Cub	No Zn vs. Zn
Steers (*n*)[3]	23	22	23	22					
Weight, kg									
d 0 (start implant)	422	424	425	424	5.6	0.81	0.84	0.96	0.76
d 10	440	444	443	444	1.3	0.03	0.33	0.61	0.02
d 20	464	468	465	468	1.6	0.21	0.67	0.10	0.15
d 41	494	500	494	497	2.6	0.59	0.20	0.11	0.26
d 59	522	528	525	526	3.7	0.48	0.43	0.65	0.32
d 70 (start beta agonist)	535	546	543	545	3.9	0.05	0.34	0.51	0.03
d 79	555	566	560	564	4.2	0.18	0.32	0.26	0.09
d 98	589	599	591	599	5.0	0.28	0.62	0.15	0.19

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 mg·steer^{-1}·d^{-1} from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).
[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).
[3]Initial body weight and pre-trial average daily gain (**ADG**; d –22 through d 0) served as covariates in performance analysis. Initial body weight was not used as a covariate in d 0 body weight.

Table 3. Effects of increasing zinc supplementation on average daily gain, dry matter intake, and feed efficiency

	Treatments[1]				SEM	Contrasts[2]			
	Zn0	Zn100	Zn150	Zn180		Lin	Quad	Cub	No Zn vs. Zn
Steers (*n*)[3]	23	22	23	22					
ADG, kg									
d 0 to 10	1.62	2.00	1.93	1.98	0.126	0.03	0.29	0.58	0.02
d 0 to 70	1.58	1.74	1.71	1.74	0.056	0.05	0.35	0.51	0.03
d 70 to 79	2.28	2.28	1.88	2.06	0.160	0.13	0.74	0.16	0.25
d 70 to 98	1.93	1.91	1.71	1.91	0.070	0.25	0.58	0.02	0.27
d 0 to 98	1.68	1.79	1.71	1.78	0.051	0.27	0.57	0.16	0.18
DMI, kg									
d 0 to 70	10.1	10.0	10.6	10.5	0.27	0.15	0.40	0.40	0.37
d 70 to 98	10.1	9.9	10.7	10.5	0.30	0.21	0.42	0.16	0.49
d 0 to 98	10.1	10.0	10.6	10.5	0.27	0.16	0.40	0.30	0.40
G:F									
d 0 to 70	0.160	0.177	0.162	0.167	0.0065	0.56	0.16	0.19	0.23
d 70 to 98	0.192	0.195	0.160	0.177	0.0072	0.01	0.47	0.004	0.08
d 0 to 98	0.169	0.182	0.162	0.166	0.0057	0.46	0.08	0.06	0.83

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 mg·steer^{-1}·d^{-1} from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).
[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).
[3]Initial body weight and pre-trial average daily gain (**ADG**; d -22 through d 0) served as covariates in performance analysis of ADG, dry matter intake (**DMI**), and feed efficiency (**G:F**).

liver Mn concentrations tended to quadratically decrease ($P = 0.08$) with increasing Zn supplementation. Liver Zn concentrations linearly increased with increasing Zn supplementation ($P = 0.04$) on d 10. However, no effects of Zn were observed for d 10 liver Fe concentrations ($P \geq 0.20$).

After 10 d of beta agonist supplementation, d 79 liver Cu and Fe concentrations quadratically decreased with increasing Zn supplementation ($P \leq 0.04$) and d 79 liver Cu concentrations were lesser for Zn supplemented steers than Zn0 ($P < 0.01$). Liver Cu concentrations on d 79 also linearly

Table 4. Effects of increasing zinc supplementation on carcass-adjusted performance and carcass characteristics

	Treatments[1]				SEM	Contrasts[2]			
	Zn0	Zn100	Zn150	Zn180		Lin	Quad	Cub	No Zn vs. Zn
Steers (*n*)[3]	23	22	23	22					
Carcass-adjusted[4]									
Final weight, kg	588	599	591	599	4.9	0.23	0.61	0.15	0.16
ADG, kg	1.66	1.77	1.69	1.77	0.049	0.22	0.59	0.15	0.15
G:F	0.168	0.180	0.161	0.165	0.0059	0.45	0.10	0.11	0.90
Carcass characteristics									
Hot carcass weight, kg	384	393	389	391	3.2	0.17	0.25	0.36	0.07
Dress, %	65.3	65.6	65.8	65.2	0.33	0.79	0.29	0.41	0.53
Ribeye area, cm^2	88.3	90.3	87.6	88.8	1.09	0.95	0.33	0.13	0.65
12th rib fat, cm	1.18	1.18	1.27	1.38	0.079	0.11	0.22	0.89	0.33
Marbling[5]	442	456	452	457	18.6	0.57	0.84	0.81	0.54
Yield grade[6]	2.1	2.3	2.5	2.6	0.16	0.02	0.74	0.86	0.04

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 mg·steer^{-1}·d^{-1} from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).
[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).
[3]Initial body weight and pre-trial average daily gain (**ADG**; d -22 through d 0) served as covariates in carcass-adjusted final weight, ADG, and feed efficiency (**G:F**), as well as carcass characteristics analysis.
[4]Carcass-adjusted data were calculated using treatment dressing percentage averages: 65.30, 65.60, 65.77, and 65.21% for Zn0, Zn100, Zn150, and Zn180, respectively.
[5]Marbling scores: slight = 300, small = 400, modest = 500, moderate = 600.
[6]Yield grade was assigned by the personnel at the commercial abattoir.

Table 5. Effects of zinc supplementation on serum urea nitrogen and non-esterified fatty acids.

	Treatments[1]				SEM	Contrasts[2]			
	Zn0	Zn100	Zn150	Zn180		Lin	Quad	Cub	No Zn vs. Zn
Steers (*n*)	12	12	12	12					
SUN[3], mg/dL									
d 0	13.6	11.4	11.7	11.3	0.61	0.02	0.27	0.53	0.01
d 10	9.7	9.9	10.3	9.7	0.59	0.78	0.61	0.47	0.69
d 69	12.2	13.3	12.4	11.5	0.70	0.61	0.08	0.99	0.82
d 79	11.2	11.5	10.8	11.5	0.77	0.95	0.99	0.47	0.92
d 97	11.1	13.0	11.7	12.2	0.75	0.39	0.22	0.27	0.19
Δ SUN[4], %									
d 0 to 10	–21.53	–12.86	–11.84	–17.29	5.470	0.39	0.31	0.71	0.24
d 69 to 79	–11.58	–18.39	–12.68	1.80	3.525	0.05	0.001	0.33	0.65
NEFA[3], mEq/L									
d 69	137	156	130	122	18.0	0.55	0.27	0.66	0.96
d 79	177	166	162	128	13.8	0.05	0.26	0.35	0.14
d 97	142	119	141	108	11.9	0.20	0.92	0.08	0.21
Δ NEFA[4], %									
d 69 to 79	31.75	17.91	42.08	7.14	17.643	0.61	0.78	0.19	0.65

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed ractopamine hydrochloride from d 70 to 98 (300 mg·steer^{-1}·d^{-1}, donated by Zoetis, Parsippany, NJ).
[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).
[3]Serum urea nitrogen (**SUN**) data were analyzed with d 0 values as a covariate, except for d 0 analysis. Nonesterified fatty acid (**NEFA**) data were analyzed with d 69 values as a covariate, expect for d 69 analysis.
[4]Percent change (Δ) was calculated between timepoints for SUN and NEFA data using individual steer data. No covariates were utilized in Δ analysis.

Table 6. Effect of increasing zinc supplementation on liver trace mineral concentrations and liver arginase after implant and beta agonist administration.

	Treatments[1]				SEM	Contrasts[2]			
	Zn0	Zn100	Zn150	Zn180		Lin	Quad	Cub	No Zn vs. Zn
Steers (*n*)	12	12	12	12					
Liver[3], mg/kg DM									
d 10									
Cu	205	210	204	163	14.6	0.13	0.07	0.33	0.47
Fe	132	139	139	136	4.2	0.31	0.36	0.76	0.20
Mn	7.7	6.9	7.3	7.6	0.34	0.74	0.08	0.77	0.30
Zn	108	111	111	119	3.0	0.04	0.31	0.26	0.13
d 79									
Cu	265	248	214	166	14.6	<0.01	0.02	0.59	<0.01
Fe	170	152	151	187	12.3	0.75	0.04	0.26	0.62
Mn	8.1	7.7	7.7	7.4	0.46	0.28	0.95	0.76	0.33
Zn	116	132	120	143	5.8	0.02	0.65	0.01	0.03
Liver arginase activity, nmol/μg/min									
d 10	0.97	0.78	0.99	1.20	0.114	0.19	0.02	0.94	0.86
d 79	0.87	0.90	0.86	1.00	0.099	0.51	0.59	0.41	0.65

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (**Zn0**, **Zn100**, **Zn150**, and **Zn180**, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 mg·steer^{-1}·d^{-1} from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).
[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).
[3]Liver biopsies were collected on d 9/10 and 78/79, representing 9 or 10 d-post administration of either growth promoting technology.

Table 7. Correlations between liver arginase activity, liver manganese, serum urea nitrogen, and average daily gain.

	Liver Mn[1]		SUN[1]		ADG[2]	
	Corr[3]	*P*-value	Corr[3]	*P*-value	Corr[3]	*P*-value
d 10						
Liver arginase	0.27	0.07	–0.04	0.77	–0.01	0.95
Liver Mn	–	–	0.55	< 0.0001	–0.25	0.09
SUN	–	–	–	–	–0.10	0.49
d 79						
Liver arginase	0.19	0.21	0.07	0.64	–0.15	0.33
Liver Mn	–	–	0.29	0.05	0.003	0.98
SUN	–	–	–	–	–0.18	0.23

[1]Liver biopsies and serum urea nitrogen (**SUN**) were collected on d 9/10 and 78/79, representing 9 or 10 d-post administration of either growth promoting technology.
[2]Average daily gain (**ADG**) represents the average daily gain of steers from d 0 to 10 for correlations within d 10 values and d 70 to79 for correlations within d 79 values.
[3]Corr: *r*, Pearson's correlation coefficient.

decreased ($P < 0.01$). A linear ($P = 0.02$) and cubic response ($P = 0.01$) for d 79 liver Zn concentrations was observed with Zn0 and Zn150 having lesser liver Zn concentrations than Zn100 and Zn180. However, grouped together, Zn supplemented steers had greater liver Zn concentrations than Zn0 ($P = 0.03$) on d 79. Liver Mn concentrations on d 79 were not influenced by Zn supplementation ($P \geq 0.28$).

Liver arginase activity measured 10 d post-implant administration (d 10) quadratically decreased ($P = 0.02$) with Zn100 having the lowest arginase activity. However Zn supplementation did not further influence d 10 or d 79 liver arginase activity ($P \geq 0.41$).

Correlations Between Markers of Growth and Protein Degradation

Correlations between d 10 or 79 liver arginase activity, liver Mn, SUN, and d 0 to 10 or d 70 to 79 ADG are presented in Table 7. Day 10 liver arginase activity tended to be positively correlated ($r = 0.27$; $P = 0.07$) with d 10 liver Mn concentrations but not d 10 SUN concentrations ($r = –0.04$; $P = 0.77$) or d 0 to 10 ADG ($r = –0.01$; $P = 0.95$). However, d 10 liver Mn concentrations were correlated with d 10 SUN concentrations ($r = 0.55$; $P < 0.0001$) and tended to be correlated with d 0 to 10 ADG ($r = 0.25$; $P = 0.09$). Day 10 SUN were not correlated with d 0 to 10 ADG ($r = 0.10$; $P = 0.49$).

Following 10 d of beta agonist supplementation, d 79 liver arginase activity was not correlated with either d 79 liver Mn ($r = 0.19$), SUN concentrations ($r = 0.07$), or d 70 to 79 ADG ($r = –0.15$; $P \geq 0.21$). However d 79 liver Mn concentrations were positively correlated with d 79 SUN concentrations ($r = 0.29$; $P = 0.05$). Neither d 79 liver Mn ($r = 0.003$) or SUN ($r = -0.18$) were correlated with d 70 to 79 ADG ($P \geq 0.23$).

Plasma Trace Mineral Concentrations

Although treatments had not yet been assigned, plasma Zn concentrations quadratically decreased (Table 8; $P = 0.01$) and were lesser ($P = 0.05$) within Zn supplemented treatments on d 0. Plasma Cu and Fe on d 0 and plasma Cu on d 10 were not affected by Zn supplementation ($P \geq 0.23$). Plasma Fe on d 10 quadratically increased ($P = 0.05$) with Zn100 and Zn150 having the greatest plasma Fe concentrations. Furthermore, d 10 plasma Zn concentrations linearly increased ($P = 0.02$) with increasing Zn supplementation and Zn supplemented steers had greater plasma Zn concentrations than Zn0 on d 10 ($P = 0.02$).

By the start of the beta agonist period (d 69), plasma Cu concentrations tended ($P = 0.10$) to quadratically decrease

Table 8. Effects of increasing zinc supplementation on plasma trace mineral concentrations after implant and beta agonist administration.

	Treatments[1]				SEM	Contrasts[2]			
	Zn0	Zn100	Zn150	Zn180		Lin	Quad	Cub	No Zn vs. Zn
Steers (*n*)	12	12	12	12					
Plasma[3], mg/L									
d 0									
Cu	0.90	0.87	0.87	0.86	0.045	0.51	0.92	0.79	0.50
Fe	2.13	1.90	2.06	1.79	0.152	0.24	0.99	0.23	0.25
Zn	1.32	1.21	1.18	1.31	0.040	0.24	0.01	0.14	0.05
d 10									
Cu	0.98	0.96	0.99	1.01	0.032	0.65	0.38	0.97	0.96
Fe	2.04	2.23	2.23	2.05	0.081	0.50	0.05	0.47	0.17
Zn	1.07	1.17	1.15	1.19	0.034	0.02	0.54	0.33	0.02
d 69									
Cu	0.97	0.91	0.90	0.96	0.034	0.52	0.10	0.51	0.23
Fe	1.86	2.26	2.24	2.17	0.157	0.10	0.23	0.99	0.06
Zn	1.17	1.33	1.28	1.33	0.048	0.03	0.32	0.32	0.02
d 79									
Cu	1.00	0.98	0.99	1.00	0.047	0.95	0.70	0.98	0.92
Fe	1.96	2.20	2.03	1.91	0.144	0.89	0.15	0.83	0.61
Zn	1.28	1.39	1.39	1.39	0.042	0.04	0.35	0.82	0.03
d 97									
Cu	0.97	0.95	0.96	1.01	0.031	0.52	0.24	0.68	0.89
Fe	2.17	2.18	2.26	2.20	0.111	0.69	0.94	0.66	0.72
Zn	1.20	1.31	1.29	1.35	0.047	0.05	0.71	0.39	0.05

[1]Steers were fed 0, 100, 150, or 180 mg Zn/kg DM from $ZnSO_4$ throughout the 98-d trial (Zn0, Zn100, Zn150, and Zn180, respectively). All cattle were implanted with a Component TE-200 (TE-200; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) on d 0 and fed a beta agonist at 300 mg·steer^{-1}·d^{-1} from d 70 to 98 (ractopamine hydrochloride, donated by Zoetis, Parsippany, NJ).
[2]Contrast statements were formed to test linear (**Lin**), quadratic (**Quad**), and cubic (**Cub**) effects of Zn supplementation and to test for a difference between Zn0 and all other Zn treatments (**No Zn vs. Zn**; Zn100, Zn150, and Zn180).
[3]Blood was collected on d 0, 9/10, 69, 78/79, and 97. Trace mineral concentrations from d 0 were utilized as a covariate in analysis of that respective mineral on subsequent days.

with Zn150 having the lowest plasma Cu concentration. Plasma Fe concentrations tended to increase ($P = 0.10$) and plasma Zn concentrations did increase ($P = 0.03$) with increasing Zn supplementation on d 69. Furthermore, d 69 plasma Fe concentrations tended to be greater ($P = 0.06$) and d 69 plasma Zn concentrations were greater ($P = 0.02$) for Zn supplemented steers. During beta agonist supplementation, d 79 and 97 plasma Zn concentrations linearly increased with increasing Zn supplementation ($P \leq 0.05$) and were greater for Zn supplemented steers than Zn0 ($P \leq 0.05$). No effects of Zn supplementation were observed for d 79 or 97 plasma Cu or Fe ($P \geq 0.15$).

DISCUSSION

Steroidal implants increase ADG of cattle 16%–20% (Bartle et al., 1992; Johnson et al., 1996; Duckett and Pratt, 2014), but it is thought the greatest growth stimulation occurs during the first 40 d after implant administration, corresponding to peak hormonal payout of the implant (Johnson et al., 1996). Similarly, beta agonists induce rapid growth (Johnson et al., 2014; Lean et al., 2014), albeit across a much shorter timeframe. This growth appears to be greatest early in the beta agonist feeding period (Maxwell et al., 2015; Genther-Schroeder et al., 2018). Adequate Zn supplementation is vital to DNA synthesis (Williams and Chesters, 1970) and results in increased phosphorylation of mTOR and FOXO (Gao et al., 2014) indicative of Zns importance to protein synthesis and degradation. Because of the need for Zn to support protein synthesis, it was hypothesized that increasing Zn supplementation would be most beneficial during these rapid growth periods occurring shortly after administration of these widely used growth promoting technologies.

Indeed, Zn supplementation linearly increased d 10 BW and d 0 to 10 ADG, with all three concentrations of supplemental Zn similarly improving implanted cattle growth over un-supplemented controls. Messersmith and Hansen (2021) found supplementing up to 150 mg Zn/kg DM from $ZnSO_4$ (0, 30, 100, or 150 mg Zn/kg DM) linearly improved ADG during the first 18-d after administration of the same high potency implant used in this study. In heifers, supplementation of 100 vs. 30 mg Zn/kg DM from $ZnSO_4$ resulted in a 6 kg advantage in BW 29 d after administering a high potency implant (Messersmith et al., 2021b). Collectively, it appears dietary Zn may be limiting implant-induced growth, and Zn supplementation at greater rates may be necessary to capture the full growth potential of this technology. In addition to supplemental Zn, basal dietary Zn concentrations in each of these studies were well above NASEM (30 mg Zn/kg DM; 2016) recommendations at 42 or 68 mg Zn/kg DM

(Messersmith et al., 2021b; Messersmith and Hansen, 2021) with the current study diet analyzing at 39 mg Zn/kg DM.

The current trial's early benefits of Zn supplementation on growth performance of steers persisted until the start of beta agonist supplementation on d 70. Though BW tended to be greater for Zn supplemented steers on d 79, Zn supplementation did not affect d 98 live or carcass-adjusted final BW. While controls lagged in performance early in the trial, once beta agonist supplementation began on d 70, these steers matched or exceeded rates of daily gain of Zn supplemented steers. In combination with the large increase in NEFA concentrations from d 69 to 79 for Zn0 steers, this growth response may suggest Zn0 steers were experiencing greater rates of lipolysis in response to beta agonist supplementation. Perhaps, the greater liver Cu concentrations of Zn0 steers on d 79 supported greater lipolytic rates, as Cu status has been shown to influence in vitro lipolysis (Johnson and Engle, 2003; Messersmith et al., 2021a). More research is needed to clarify the potential interaction between tissue Zn and Cu concentrations and the beta agonist growth response. Genther-Schroeder et al. (2016a, 2016b) found a linear increase in final BW, ADG, and G:F in beta agonist-fed steers when Zn was supplemented up to 150 mg Zn/kg DM total. Likewise, supplementation of 120 or 160 mg Zn/kg DM improved the performance of steers receiving a beta agonist only (Genther-Schroeder et al., 2016b; Wellmann et al., 2020). However, these studies used blended organic and inorganic Zn sources (Genther-Schroeder et al., 2016a, 2016b; Wellmann et al., 2020) compared to only $ZnSO_4$ used in the current study. It is unclear why Zn source may affect performance response during the beta agonist feeding period. More work is needed to clarify the optimum concentrations and sources of Zn to utilize in feedlot cattle diets during this time.

Hot carcass weight tended to be greater in Zn supplemented steers, corresponding to an 8 and 6 kg advantage in final BW for Zn100 and Zn180, respectively, over Zn0 and Zn150 steers. Zinc impacts on carcass accretion appear to be subtle but relatively consistent across published works in modern cattle utilizing growth promoting technologies (Genther-Schroeder et al., 2016a, 2016b; Wellmann et al., 2020; Messersmith et al., 2021b; Messersmith and Hansen, 2021). Although 12th rib fat was not statistically affected by Zn supplementation, numerically 12th rib fat linearly increased with increasing Zn supplementation in agreement with Zn effects observed by Spears and Kegley (2002) and Greene et al. (1988). This numerical trend in 12th rib fat is likely why YG linearly increased with increasing Zn supplementation.

Considering steers across all treatments were implanted on d 0 and started a beta agonist on d 70, the well-defined decrease in SUN concentrations observed from d 0 to 10 and d 69 to 79 agree with data from Parr et al. (2014) and Harris et al. (2020) and indicate these technologies result in decreased protein degradation and/or increased protein anabolism. Although Zn did not influence SUN concentrations during beta agonist supplementation, the quadratic decrease in the percent change in SUN concentrations from d 69 to 79 suggests supplemental Zn influences protein degradation. However, this response may instead be directly related to the quadratic tendency observed for d 69 SUN. Interestingly, this percent change in SUN concentrations from d 69 to 79 was greatest in Zn100 steers. These steers had numerically greater ADG during this period than Zn150 and Zn180, suggesting growth rates did influence protein degradation even though d 79 SUN were not correlated with d 70 to 79 ADG.

We have previously observed steroidal implants decrease liver Mn concentrations (Messersmith, 2018; Niedermayer et al., 2018; Messersmith et al., 2021b; Reichhardt et al., 2021). Interestingly, cytosolic Mn in the liver is associated with arginase (Rosebrough et al., 1987), the terminal enzyme of the urea cycle (Bond et al., 1983; Watts, 1990). Given that steroidal implants decrease the demand for the urea cycle (Galbraith, 1980), we suspected the decrease in liver Mn of implanted cattle was associated with decreasing liver arginase. We found d 10 liver arginase activity was positively correlated with d 10 liver Mn concentrations and tended to be correlated with d 0 to 10 ADG. These data support the implant-induced decreases in liver Mn previously observed and suggest changes in protein degradation during the implant response are impacting liver Mn concentrations. Liver Mn concentrations in the ruminant may be more directly influenced by N metabolism than previously thought.

In contrast to the first 10 days following implant administration, there was no correlation between d 79 liver arginase activity and d 79 liver Mn concentrations, corresponding to the first 10 d of beta agonist feeding. Perhaps, steroidal implants and beta agonists affect N metabolism differently. Bryant et al. (2010) found steroidal implants improved ADG by 20.7% over nonimplanted heifers during the beta agonist supplementation period while ractopamine hydrochloride supplementation increased ADG by 60.7% over the 28-d beta agonist feeding period. Although beta agonist supplementation induces high growth rates, Ji and Orcutt (1991) indicate beta agonists stimulate protein synthesis more than they decrease protein degradation. Therefore, liver arginase activity and liver Mn concentrations would not be expected to change during beta agonist supplementation due to their link to protein degradation, consistent with the results observed in this study.

By analyzing the liver at time points coordinated with projected peak growth responses of steroidal implants and beta agonists, liver trace minerals were expected to shift with growth pressure. Liver Zn has a debatable value as a marker of Zn status (Suttle, 2010); however, the linear response to Zn supplementation on d 10 and 79 suggests high concentrations of supplemental Zn can influence liver Zn stores. Plasma Zn concentrations provide a measurement of Zn that more readily changes with Zn needs. For example, Messersmith (2018) observed implanted steers had lesser plasma Zn 13 and 73 d post-implant administration, suggesting increased demand for Zn to support implant-induced growth. In the current study, plasma Zn concentrations were linearly increased with increasing Zn supplementation at all timepoints. Similar to Messersmith's (2018) findings, plasma Zn concentrations decreased for all Zn treatments from d 0 to 10; however, this decrease in plasma Zn differs across treatments. Steers receiving no supplemental Zn decreased plasma Zn from d 0 to 10 by 19%, while Zn100, Zn150, and Zn180 dropped by 3, 3, and 9%, respectively. Perhaps the increased growth rates observed in Zn supplemented treatments during this period were because of increased availability of circulating Zn to support protein accretion. If so, these data suggest a threshold for plasma Zn concentrations may be necessary to accommodate implant-induced growth.

Interestingly, plasma Zn concentrations increased in all treatments in the first 10 d of beta agonist supplementation

even though cattle had been on Zn treatments for 69 d before the start of the beta agonist. Both Zn0 and Zn150 increased 9%, while Zn100 and Zn180 increased plasma Zn concentrations by 5%. On an average, these changes in plasma Zn concentrations were not as strong as the changes observed within the first 10 d after implant administration and had an opposite effect. Even as growth rates increased across treatments due to ractopamine feeding, it is possible plasma Zn concentrations were sufficiently high in all treatments at start of beta agonist feeding on d 69 to prevent any drop in circulating Zn. More research is warranted to determine how each technology impacts circulating Zn stores and if protein synthesis and degradation rates influence plasma Zn concentrations.

Together these data indicate increasing dietary Zn supplementation well above NASEM (2016) recommendations increased growth which was most dramatic very early after implant administration. Therefore, Zn may be needed to prevent the decline in circulating Zn observed in implanted cattle (Huerta et al., 2002; Messersmith, 2018) and support the demands of growth. It is unclear why steers supplemented 150 mg Zn/kg DM experienced lesser performance than steers supplemented with 100 or 180 mg Zn/kg DM throughout the trial. These data suggest 100 mg Zn/kg DM is optimal for improving performance, and no further benefits were noted in treatments receiving more than 100 mg supplemental Zn/kg DM. Furthermore, these data provide a link between growth rates, liver Mn concentrations, and protein degradation, indicating implants influence liver Mn concentrations through regulation of the urea cycle and its terminal enzyme, arginase. Further research to determine strategic Zn supplementation programs to accommodate the growth of cattle administered steroidal implants and fed a beta agonist and to understand differences in N metabolism of cattle utilizing these technologies is warranted.

Conflict of Interest Statement

None declared.

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