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Effects of increasing supplemental zinc to non-implanted and implanted finishing steers

Elizabeth M. Messersmith and Stephanie L. Hansen[1]

Department of Animal Science, College of Agriculture and Life Sciences, Iowa State University, Ames, IA 50011, USA
[1]Corresponding author: slhansen@iastate.edu

Abstract

The effects of supplemental Zn within steroidal implant strategy on performance, carcass characteristics, trace mineral status, and muscle gene expression were tested in a 59-d study using 128 Angus-crossbred steers (492 ± 29 kg) in a 2 × 4 complete randomized design. Implant strategies included no implant (**NoIMP**) or Component TE-200 (**TE200**; Elanco, Greenfield, IN) administered on day 0. Zinc was supplemented at 0, 30, 100, or 150 mg Zn/kg dry matter (**Zn0**, **Zn30**, **Zn100**, **Zn150**, respectively) from $ZnSO_4$. Steers were stratified by body weight (**BW**) to pens (n = 5 or 6 steers/pen) equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) and assigned treatments (n = 15, 16, or 17 steers/treatment). Cattle were weighed on days −1, 0, 18, and 59 with blood collected on days −1, 18, 40, and 59. Muscle samples were collected from the *longissimus thoracis* on day 11 and liver samples were collected on day 55 or 56. Data were analyzed using the Mixed Procedure of SAS via contrast statements testing the linear and quadratic response to Zn supplementation within implant treatment and NoIMP vs. TE200 for performance, carcass, blood, and liver parameters. Specific contrast statements were formed for the analysis of gene expression in muscle including: Zn0 vs. Zn150 within NoIMP and TE200, NoIMP vs. TE200 (Zn0 and Zn150 only), and the linear effect of supplementing Zn0, Zn100, and Zn150 within TE200. Steer was the experimental unit. Day 18 BW and days 0 to 18 average daily gain (**ADG**) were linearly increased due to Zn supplementation within TE200 ($P \leq 0.002$) in conjunction with a linear increase from Zn in day 11 muscle epidermal growth factor receptor, matrix metalloproteinase 2, and phosphodiesterase 4B gene expression of TE200 steers ($P \leq 0.05$). Plasma Zn on days 18 and 40 linearly increased with increasing Zn supplementation regardless of implant treatment ($P \leq 0.03$) and was lesser for TE200 than NoIMP steers on day 18 ($P = 0.001$). Day 59 BW and hot carcass weight (**HCW**) were greater for TE200 vs. NoIMP ($P \leq 0.002$) and HCW of implanted steers tended to linearly increase with increasing Zn supplementation ($P = 0.09$). No effects of Zn supplementation were observed in NoIMP for HCW, BW, or ADG ($P \geq 0.17$). Yield grade and 12th rib fat tended to quadratically decrease within NoIMP ($P \leq 0.09$), with Zn100 being the most lean. These data indicate increasing supplemental Zn influences steroidal implant signaling machinery while increasing the Zn status and implant-induced growth of feedlot cattle.

Lay Summary

This 59-d study explored the effects of zinc supplementation and an anabolic implant on the performance and carcass quality of Angus-crossbred steers. Researchers provided different levels of zinc to the steers, some of which received a combination growth implant. They found that zinc, especially when used with the implant, improved weight gain and hot carcass weight. Zinc also influences muscle genes related to growth. These findings suggest that zinc supplementation can enhance the benefits of growth implants in beef cattle, leading to better performance.

Key words: cattle, steroidal implant, zinc

Abbreviations: ADG, Average daily gain; AKT1, protein kinase B; BF, 12th rib fat thickness; BW, Body weight; DM, Dry matter; DMI, Dry matter intake; EEF1A2, eukaryotic translation elongation factor 1 alpha 2; EEF2K, eukaryotic elongation factor 2 kinase; EGFR, epidermal growth factor receptor; ESR1, estrogen receptor 1; FOXO3, forkhead box O-3; G:F,Gain to feed ratio; GPER1, G protein-coupled estrogen receptor 1; HCW, Hot carcass weight;IGF1R, insulin-like growth factor 1 receptor; MAPK1, mitogen-activated protein kinase 1; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9; MTOR, mammal target of rapamycin kinase; MYF5, myogenic factor 5; MYOD, myogenic differentiation 1; MYOG, myogenin; NoIMP, No Implant treatment; PAX7, paired box protein 7; PDE4B, phosphodiesterase 4B; PUN, Plasma urea nitrogen; REA, Ribeye area; RPS6KB1, ribosomal protein S6 kinase B1; RPS9, ribosomal protein S9; SLC30A10, solute carrier family 30 member 10; SLC30A7, solute carrier family 30 member 7; SLC39A14, solute carrier family 39 member 14; SLC39A7, solute carrier family 39 member 7; STA, Specific target amplification; TE200, Component TE-200 implant treatment; YG, Yield grade; Zn0, Zinc at 0 mg/kg DM; Zn30, Zinc at 30 mg/kg DM; Zn100, Zinc at 100 mg/kg DM; Zn150, Zinc at 150 mg/kg DM

Introduction

Steroidal implants have been commonly used in US beef production since the 1950s (Preston, 1999), resulting in improvements in the average daily gain (**ADG**) of cattle by 16% to 20% (Bartle et al., 1992; Duckett and Pratt, 2014). This steroidal implant-induced growth response is elicited through genomic and nongenomic modes of action that regulate gene transcription (Yen, 2015) and stimulate growth processes through secondary messengers (Heinlein and Chang, 2002; Filardo and Thomas, 2012). Zinc may augment both genomic and nongenomic steroidal implant signaling. For example, Zn acts on the nongenomic G protein-coupled estrogen receptor (**GPER1**) to phosphorylate epidermal growth factor receptor (**EGFR**) and insulin-like growth factor-1 receptor (**IGF1R**) in a dose-dependent manner (Pisano et al., 2017), leading to the

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activation of growth processes. Zinc supports transcription factors, deoxyribonucleic acid, and protein synthesis (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976; Cousins et al., 2006), all vital components of growth. Zinc's role in DNA synthesis was specifically demonstrated when thymidine kinase activity, a key enzyme for the incorporation of thymidine into DNA, was decreased in the liver of Zn-deficient rats (Duncan and Dreosti, 1976). The steroidal implant, zeranol, increased Zn absorption and retention in lambs (Hufstedler and Greene, 1995) and the supplementation of 200 mg Zn/kg dry matter (**DM**) basis from $ZnSO_4$ to steers resulted in a greater ADG response to a potent steroidal implant than steers receiving a control diet that exceeded NASEM (30 mg Zn/kg DM; 2016) Zn recommendations (Huerta et al., 2002). These data suggest administration of steroidal implants may increase the Zn requirements of the animal to accommodate increased growth rates and greater supplementation of Zn may promote steroidal implant-induced growth. Therefore, the objective of this study was to determine the effects of increasing dietary Zn supplementation within steroidal implanted and non-steroidal implanted steers on performance, carcass characteristics, liver and plasma Zn concentrations, and the expression of genes associated with growth and Zn metabolism. It was hypothesized that optimal Zn supplementation to improve growth and subsequent effects on relative gene expression measures would differ between steroidal implanted and non-steroidal implanted steers.

Materials and Methods

All procedures and protocols utilized in this study were approved by the Iowa State University Institutional Animal Care and Use Committee (log number: IACUC-20-053).

Animals and experimental design

One hundred and twenty-eight Angus-crossbred steers (492 ± 29 kg) were utilized in a complete randomized design arranged as a 2 × 4 factorial resulting in 8 treatments to test the effect of Zn supplementation within steroidal implant treatment. Steroidal implant treatments included no steroidal implant (**NoIMP**) or a Component TE-200 (**TE200**; 200 mg trenbolone acetate + 20 mg estradiol; donated by Elanco Animal Health, Greenfield, IN) administered on day 0. Cattle were fed 0, 30, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively) from $ZnSO_4$ starting on day 0. Zinc treatments were added to the diet through dried distiller grains with solubles-based premix. Prior to the start of the experiment, all steers received a corn silage-based growing diet supplemented with 30 mg Zn/kg DM from $ZnSO_4$. Steers were fed once daily (0800 h) and transitioned to a dry-rolled corn-based diet during the first 14 d of the experiment and remained on the finishing diet through the remainder of the 59-d study (Table 1). The Zn0 diet analyzed 48, 40, and 42 mg Zn/kg DM for transition 1, transition 2, and finishing, respectively. Cattle were stratified by body weight (**BW**) into pens (n = 5 or 6 steers per pen) to evenly disperse BW across treatments. Pens were equipped with a single GrowSafe (GrowSafe Systems Ltd., Airdrie, AB, Canada) bunk and cattle were provided ad libitum access to feed. Radio-frequency tags on each steer relayed individual steer feed disappearance data from the bunk to GrowSafe software. Cattle were randomly assigned to steroidal implant and supplemental Zn treatments within pen (n = 3 pens/treatment). All cattle within a pen received the same steroidal implant and supplemental Zn treatment, and individual intake and performance data were collected for each steer. Therefore, the steer was the experimental unit. Cattle were harvested on day 61 at a commercial abattoir (Greater Omaha Beef, Omaha, NE) via industry-accepted practices. Trained Greater Omaha Beef personnel collected hot carcass weight (**HCW**) while ribeye area (**REA**), 12th rib fat (**BF**), marbling score, and yield grade (**YG**) were obtained through a camera grading system following a 48-h chill.

Table 1. Compositional analysis of non-zinc supplemented diet

Ingredient, % of diet DM	Transition 1 days 0 to 6	Transition 2 days 7 to 13	Finisher days 14 to 59
Dry rolled corn	30	45	57
Sweet Bran[1]	25	20	20
DDGS[2]	18.06	13.06	13.06
Bromegrass hay	5	8	8
Corn silage	20	12	-
Limestone	1.5	1.5	1.5
Salt	0.31	0.31	0.31
Vitamin & mineral premix[3]	0.1159	0.1159	0.1159
Rumensin	0.0135	0.0135	0.0135
Analyzed composition, %			
Crude protein[4]	16.6	13.1	13.9
NDF[4]	28.0	31.2	20.9
Ether extract[4]	5.5	5.0	4.4
Zn[5], mg/kg DM	48	40	42
Cu[5], mg/kg DM	17	13	12
Calculated composition[6], Mcal/kg			
NEm	1.97	1.98	2.05
NEg	1.32	1.33	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. All diets included NASEM (2016) recommendations for Co, Cu, I, Mn, and Se. Diets were supplemented with Zn at 0, 30, 100, or 150 mg Zn/kg DM. All trace minerals were from inorganic sources.
[4]Analysis of Zn0 TMR was conducted by Dairyland Laboratories (Arcadia, WI).
[5]Trace minerals were analyzed by inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA). Analyses were of Zn0 TMR. Analysis of Zn30, Zn100, and Zn150 were 68, 122, and 148 mg Zn/kg DM, 77, 121, and 107 mg Zn/kg DM, and 48, 101, and 137 mg Zn/kg DM for transition 1, transition 2, and finishing diets, respectively.
[6]Net energy of maintenance (NEm) and gain (NEg) were calculated using ingredient nutrient values from NASEM (2016).

Sample collection and analysis

Total mixed ration (**TMR**) samples of each treatment were collected weekly to calculate DM by drying in a forced air oven for 48 h at 70 °C. Dried TMR samples were ground through a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and composited by month within each dietary Zn treatment. The composited Zn0 TMR was sent to Dairyland Laboratories (Arcadia, WI) for nutrient analysis (methods denoted in Heiderscheit and Hansen, 2020). BWs were taken on days −1, 0, 18, 40, 55/56, and 59 (n = 15, 16, or 17 steers/treatment). Blood was collected on days −1, 18, and 40 (n = 8, 9, or 10 steers/treatment) via jugular venipuncture in vacuum-capped tubes (Becton Dickerson, Rutherford,

NJ) containing trace mineral grade K_2EDTA for plasma trace mineral analysis or sodium heparin for plasma urea nitrogen (**PUN**) analysis. Blood tubes were spun in a temperature-controlled centrifuge (4 °C) at 1,000 × *g* for 10 min for K_2EDTA tubes and 20 min for sodium heparin tubes before storing at −20 °C until analysis was completed. A commercial kit (Teco Diagnostics, Anaheim, CA) was utilized to determine PUN concentrations with an intra-assay and inter-assay CV of 6.33% and 7.15%, respectively. PUN data are not discussed herein but are shown in **Supplementary Table 1**.

Muscle biopsies were conducted on 9 randomly selected steers per treatment (n = 3 steers/pen) on d 11 utilizing procedures adapted from Pampusch et al. (2008). At time points split across 2 d, half of the cattle were biopsied on each day. Biopsies were extracted from the *longissimus thoracis* between the 10th and 13th rib spaces. Muscle samples were flash-frozen in liquid nitrogen and stored at −80 °C prior to analysis.

Due to limitations in sample analysis, muscle samples from only 5 of the 8 treatments (NoIMP: Zn0 and Zn150 and TE200: Zn0, Zn100, and Zn150) were analyzed for quantitative gene expression using the 48.48 Dynamic Array Integrated Fluidic Circuit (Fluidigm, San Francisco, CA) as described by Suasnavas et al. (2015). Prior to quantitative gene expression analysis, RNA was isolated from muscle samples using Trizol Reagent (Life Technologies, Carlsbad, CA, USA) and cDNA was synthesized using random primers and Superscript III Reverse Transcriptase (Invitrogen, Waltham, MA, USA) in accordance with manufacturer's instructions (McGill et al., 2016). Twenty-four genes specific to the steroidal implant growth mechanism and the biological functions of Zn were targeted for analysis (**Supplementary Table 2**) and primer sets for each gene were designed and validated by Fluidigm. In brief, a Specific Target Amplification (**STA**) was performed to enrich each sample for target-specific cDNA prior to quantitative gene expression analysis, in accordance with Fluidigm protocol. For STA thermal cycling, each reaction consisted of 1.25 µL of the primer mix, 2.5 µL of the TaqMan PreAmp Master Mix (Applied Biosystems, Foster City, CA), and 1.25 µL of cDNA diluted 1:20 in nuclease-free water. Enzyme activation took place at 95 °C for 10 min before amplification was conducted for 14 cycles (95 °C for 15 s then 60 °C for 4 min). The Fluidigm IFC chip was run on the Biomark thermocycler/detection module. The average expression of housekeeping genes eukaryotic translation elongation factor 1 alpha 2 (**EEF1A2**) and ribosomal protein S9 (**RPS9**) was utilized as a reference to determine the relative gene expression of each parameter using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Relative gene expression was calculated relative to the NoIMP-Zn0 treatment.

Liver biopsies using procedures described by Engle and Spears (2000) were conducted on day 55/56 with half of the steers sampled on each day (n = 8, 9, or 10 steers/treatment). Liver samples were stored at −20 °C before analysis for common trace minerals involved in growth including Cu, Fe, Mn, and Zn. Liver and composited TMR samples were acid-digested following the procedures of Pogge and Hansen (2013) and Richter et al. (2012), respectively. Trace mineral concentrations of liver, TMR, and plasma were measured via inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA) as described by Pogge and Hansen (2013) and Richter et al. (2012). A standard was utilized on each run to verify instrument accuracy (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 via the Mixed Procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with the fixed effect of treatment. Contrast statements were formed using the IML Procedure of SAS 9.4 (SAS Inst. Inc.) to specifically test for linear and quadratic effects of Zn supplementation within NoIMP and TE200 treatments as well as to test NoIMP vs. TE200, regardless of Zn treatment. Steer served as the experimental unit for all analyses (n = 15, 16, or 17 steers/treatment for performance and carcass parameters or n = 8, 9, or 10 steers/treatment for blood and liver data). Initial BW was utilized as a covariate in all performance and carcass data analysis and initial plasma Zn concentrations served as covariates for subsequent time-point analysis. Because not all treatments were included in the assessment of relative gene expression in day 11 muscle samples additional contrast statements were formed. These statements included testing the effect of Zn0 vs. Zn150 within NoIMP and TE200 treatments, NoIMP vs. TE200 (Zn0 and Zn150 only), and the linear effect of Zn0, Zn100, and Zn150 within TE200. Outliers were assessed using the Cook's D statistical test with values above 0.20 removed from analysis. One steer was removed from performance and carcass parameters (NoIMP-Zn30) due to poor performance related to health concerns. Data are reported as the least squares mean with the standard error of the mean, and statistical significance was determined as $P \leq 0.05$ with tendencies between $0.05 < P \leq 0.10$.

Results

Performance

No differences in BW were observed on day 0 (**Table 2**; $P \geq 0.67$) between Zn treatments. However, day 18 BW, days 0 to 18 ADG, and days 0 to 18 feed efficiency (**G:F**) linearly increased as Zn supplementation increased within TE200 ($P \leq 0.02$) and were greater for steroidal implanted steers than non-steroidal implanted steers ($P \leq 0.01$). Steroidal implanted steers also tended to have greater days 0 to 18 dry matter intake (**DMI**) than NoIMP ($P = 0.09$). Zinc supplementation tended to quadratically decrease days 0 to 18 DMI ($P = 0.09$) and quadratically increase days 0 to 18 G:F ($P = 0.05$) of NoIMP.

By the end of the trial, TE200 were 8.25 and 13.25 kg heavier than NoIMP for day 59 BW and carcass-adjusted final BW, respectively ($P \leq 0.002$). Furthermore, TE200 steers had greater days 0 to 59 and carcass-adjusted ADG and G:F ($P \leq 0.02$). However, days 0 to 59 DMI was not influenced by Zn supplementation or steroidal implant treatment ($P \geq 0.15$). A tendency for a linear increase due to Zn supplementation in carcass-adjusted final BW and ADG was observed in steroidal implanted steers ($P \leq 0.10$), while carcass-adjusted G:F quadratically increased within NoIMP ($P = 0.04$) with Zn100 having the greatest G:F.

Carcass characteristics

HCW tended to linearly increase (**Table 3**; $P = 0.09$) while dressing percentage linearly increased ($P = 0.01$) with increasing Zn supplementation within TE200. Steroidal implanted

Table 2. Effects of zinc[1] supplementation on performance parameters of non-steroidal implanted and steroidal implanted[2] beef feedlot steers

	NoIMP				TE200				Contrasts[3]					
	Zn0	Zn30	Zn100	Zn150	Zn0	Zn30	Zn100	Zn150	SEM	L-NoIMP	Q-NoIMP	L-TE200	Q-TE200	NoIMP vs. TE200
Steer (*n*)[4]	17	15	16	16	15	16	16	16						
BW, kg														
day 0	490	495	491	492	495	492	492	489	7.6	0.99	0.91	0.67	0.95	0.98
day 18	528	532	532	531	531	534	534	540	1.8	0.34	0.18	0.002	0.34	0.005
day 59	595	596	603	596	605	607	608	603	3.8	0.50	0.17	0.81	0.26	0.002
days 0 to 18														
ADG, kg	2.01	2.24	2.22	2.18	2.17	2.31	2.32	2.65	0.101	0.34	0.18	0.002	0.34	0.005
DMI, kg	11.4	11.2	11.2	12.1	11.5	11.4	11.7	12.0	0.24	0.11	0.09	0.09	0.48	0.52
G:F	0.177	0.192	0.200	0.181	0.188	0.203	0.199	0.222	0.0094	0.71	0.05	0.02	0.61	0.01
Carcass-adjusted overall[5]														
Final BW	594	593	600	593	604	606	610	613	4.0	0.81	0.35	0.10	0.99	<0.0001
ADG	1.70	1.69	1.79	1.68	1.87	1.89	1.96	2.01	0.066	0.81	0.35	0.10	0.99	<0.0001
G:F	0.151	0.152	0.163	0.145	0.162	0.168	0.170	0.174	0.0053	0.74	0.04	0.12	0.86	<0.0001

[1]Cattle were supplemented 0, 30, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively) from $ZnSO_4$.
[2]Steroidal implant strategies included no steroidal implant (**NoIMP**) or a Component TE-200 (**TE200**; 200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on day 0.
[3]Contrast statements were formed to test for linear (L) and quadratic (Q) effects of Zn supplementation within non-steroidal implanted (**L-NoIMP** and **Q-NoIMP**) or steroidal implanted (**L-TE200** or **Q-TE200**) steers. A separate contrast statement was formed to test for differences between non-steroidal implanted steers and all steroidal implanted steers (**NoIMP vs. TE200**).
[4]Initial body weight (BW) was utilized as a covariate in all performance analyses including body weight (**BW**), average daily gain (**ADG**), dry matter intake (**DMI**), and gain:feed (**G:F**).
[5]Carcass adjusted performance was calculated using the average dressing percentage for all treatments: 62.25%.

steers had greater HCW, dressing percentage, and REA ($P \leq 0.02$) than NoIMP. Both BF and YG tended to quadratically decrease with increasing Zn supplementation within NoIMP ($P \leq 0.09$) with Zn100 having lesser BF and YG than other Zn treatments. However, marbling was not influenced by Zn supplementation or steroidal implant treatment ($P \geq 0.17$).

Trace mineral and PUN concentrations

As expected, day −1 plasma Zn concentrations were not influenced by Zn or steroidal implant treatments (Table 4; $P \geq 0.16$). Day 18 and 40 plasma Zn concentrations linearly increased with increasing Zn supplementation within both NoIMP and TE200 ($P \leq 0.03$). Furthermore, plasma Zn was lesser for steroidal implanted steers on day 18 than NoIMP ($P = 0.001$), but no effect of steroidal implant was observed for day 40 plasma Zn concentrations ($P = 0.49$).

Liver Zn, Cu, and Fe concentrations measured on days 55 or 56 were not affected by increasing Zn supplementation ($P \geq 0.15$) or steroidal implant treatment ($P \geq 0.20$). Liver Mn tended to be lesser for TE200 steers than NoIMP steers ($P = 0.10$) and was not affected by Zn supplementation ($P \geq 0.15$).

Gene expression

The relative gene expression of steroidal implant hormone receptors and signaling machinery are displayed in Figure 1. Neither androgen receptor (Figure 1A), estrogen receptor 1 (**ESR1**; Figure 1B), or IGF1R (Figure 1C) were influenced by Zn0 or Zn150 supplementation within NoIMP or TE200 treatments ($P \geq 0.17$), steroidal implant treatment ($P \geq 0.14$), or a linear response to 0, 100, or 150 mg Zn/kg DM supplementation within TE200 steers ($P \geq 0.26$). However, EGFR relative gene expression was greater for Zn150 than Zn0 within TE200 (Figure 1D; $P = 0.03$). Furthermore, EGFR gene expression was greatest for steroidal implanted cattle ($P = 0.02$) and linearly increased with supplementation of 0, 100, and 150 mg Zn/kg DM within TE200 ($P = 0.05$).

Matrix metalloproteinase 2 (**MMP2**) tended to be greater for Zn150 than Zn0 within TE200 (Figure 1E; $P = 0.10$) and linearly increased ($P = 0.04$) with increasing supplemental Zn at 0, 100, and 150 mg Zn/kg DM within TE200. Similarly, phosphodiesterase 4B (**PDE4B**) relative gene expression tended to be greater for Zn150 than Zn0 within TE200 steers (Figure 1F; $P = 0.10$) but was lesser for steroidal implanted steers than NoIMP supplemented Zn0 or Zn150 ($P = 0.02$). However, PDE4B gene expression linearly increased with the supplementation of 0, 100, and 150 mg Zn/kg DM within TE200 ($P = 0.03$). The linear responses observed in both MMP2 and PDE4B gene expression were largely driven by high relative gene expression of Zn100-TE200 steers.

The relative gene expression of select genes involved in growth signaling are found in Figure 2. No effects of Zn or IMP were observed through contrast statements formed for gene expression for protein kinase B (**AKT1**; Figure 2A), eukaryotic elongation factor 2 kinase (**EEF2K**; Figure 2B), ribosomal protein S6 kinase B1 (**RPS6KB1**; Figure 2C), mitogen-activated protein kinase 1 (**MAPK1**; Figure 2D), or forkhead box O-3 (**FOXO3**; Fig 2E; $P \geq 0.17$). However, some markers of satellite cell development were influenced by steroidal implant strategy (Figure 3). Both myogenin (**MYOG**; Figure 3B) and myogenic factor 5 (**MYF5**; Figure 3C) were greater for Zn0 and Zn150 steroidal implanted steers than NoIMP counterparts ($P \leq 0.04$). No further

Table 3. Effects of zinc[1] supplementation within non-steroidal implanted and steroidal implanted[2] beef steers on carcass characteristics

	NoIMP				TE200					Contrasts[3]				
	Zn0	Zn30	Zn100	Zn150	Zn0	Zn30	Zn100	Zn150	SEM	L-NoIMP	Q-NoIMP	L-TE200	Q-TE200	NoIMP vs. TE200
Steer (*n*)[4]	17	15	16	16	15	16	16	16						
Hot carcass weight, kg	370	369	373	369	376	377	380	381	2.5	0.80	0.39	0.09	0.98	<0.0001
Dress, %	62.2	62.0	61.9	62.0	62.3	62.1	62.4	63.2	0.30	0.62	0.65	0.01	0.13	0.02
Ribeye area, cm^2	84.4	81.7	86.5	83.1	89.4	88.8	87.9	87.6	1.96	0.76	0.56	0.48	0.91	0.001
12thrib fat, cm	1.61	1.67	1.43	1.69	1.50	1.44	1.52	1.56	0.086	0.87	0.09	0.43	0.67	0.12
Marbling[5]	491	470	483	495	472	460	452	472	22.0	0.70	0.52	0.96	0.43	0.17
Yield grade[6]	3.6	3.6	3.4	3.7	3.5	3.4	3.5	3.5	0.08	0.87	0.09	0.43	0.67	0.12

[1]Zinc was supplemented to cattle at 0, 30, 100, or 150 mg Zn/kg DM from $ZnSO_4$ (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively).
[2]Steroidal implant strategies included no steroidal implant (**NoIMP**) or a Component TE-200 (**TE-200**; 200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on day 0.
[3]Contrast statements were formed to test for linear (L) and quadratic (Q) effects of Zn supplementation within non-steroidal implanted (**L-NoIMP** and **Q-NoIMP**) or steroidal implanted (**L-TE200** or **Q-TE200**) steers. A separate contrast statement was formed to test for differences between non-steroidal implanted steers and all steroidal implanted steers (**NoIMP vs. TE200**).
[4]Intial body weight was utilized as a covariate for carcass characteristics.
[5]Marbling scores: slight = 300, small = 400, modest = 500, moderate = 600, slightly abundant = 700, moderately abundant = 800.
[6]Yield grade was assigned by personnel of the commercial abattoir.

Table 4. Effects of zinc[1] supplementation within non-steroidal implanted and steroidal implanted[2] beef steers on trace mineral and plasma urea nitrogen concentrations

	NoIMP				TE200					Contrasts[3]				
	Zn0	Zn30	Zn100	Zn150	Zn0	Zn30	Zn100	Zn150	SEM	L-NoIMP	Q-NoIMP	L-TE200	Q-TE200	NoIMP vs. TE200
Steer (*n*)	10	9	9	9	8	9	9	9						
Plasma Zn[4], mg/L														
day −1	1.22	1.31	1.32	1.32	1.28	1.28	1.37	1.35	0.053	0.17	0.41	0.16	0.67	0.42
day 18	1.31	1.34	1.43	1.44	1.25	1.21	1.33	1.39	0.037	0.003	0.53	0.001	0.36	0.001
day 40	1.33	1.25	1.36	1.42	1.29	1.27	1.35	1.38	0.038	0.01	0.12	0.03	0.82	0.49
day 55 liver[5], mg/kg DM														
Zn	115	113	122	115	118	118	125	127	6.1	0.69	0.59	0.20	0.97	0.20
Mn	8.7	7.9	8.6	8.6	8.2	7.9	7.6	8.4	0.46	0.65	0.55	0.82	0.15	0.10
Cu	310	274	277	252	278	268	268	293	29.9	0.15	0.86	0.67	0.49	0.94
Fe	181	181	173	184	178	170	171	184	11.2	0.99	0.47	0.59	0.28	0.59

[1]Zinc was supplemented to cattle at 0, 30, 100, or 150 mg Zn/kg DM from $ZnSO_4$ (**Zn0**, **Zn30**, **Zn100**, or **Zn150**, respectively).
[2]Steroidal implant strategies included no steroidal implant (**NoIMP**) or a Component TE-200 (**TE-200**; 200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on day 0.
[3]Contrast statements were formed to test for linear (L) and quadratic (Q) effects of Zn supplementation within non-steroidal implanted (**L-NoIMP** and **Q-NoIMP**) or steroidal implanted (**L-TE200** or **Q-TE200**) steers. A separate contrast statement was formed to test for differences between non-steroidal implanted steers and all steroidal implanted steers (**NoIMP vs. TE200**).
[4]Plasma Zn from day −1 was utilized as a covariate in days 18 and 40 plasma Zn analysis.
[5]Liver samples were collected on days 55/56 with half of the samples collected on each d.

differences in relative gene expression were observed for markers of satellite cell development ($P \geq 0.11$).

Within TE200, Zn150 tended to have greater relative gene expression of the Zn transporter solute carrier family 30 member 7 (**SLC30A7**) than Zn0 (**Figure 4A**; $P = 0.07$), and SLC30A7 gene expression tended to linearly increase with supplementation of 0, 100, and 150 mg Zn/kg DM within TE200 steers ($P = 0.06$). Furthermore, TE200 steers had lesser SLC30A7 relative gene expression than NoIMP ($P = 0.05$). Neither solute carrier family 39 member 7 (**SLC39A7**; **Figure 4B**) or solute carrier family 39 member 14 (**SLC39A14**; **Figure 4C**) relative gene expression were influenced by the tested contrast statements ($P \geq 0.11$).

Discussion

Steroidal implants improve cattle ADG (Bartle et al., 1992; Duckett and Pratt, 2014) with the greatest potential for gain occurring in the first 40 d post-steroidal implant during peak hormonal payout (Johnson et al., 1996). It was hypothesized

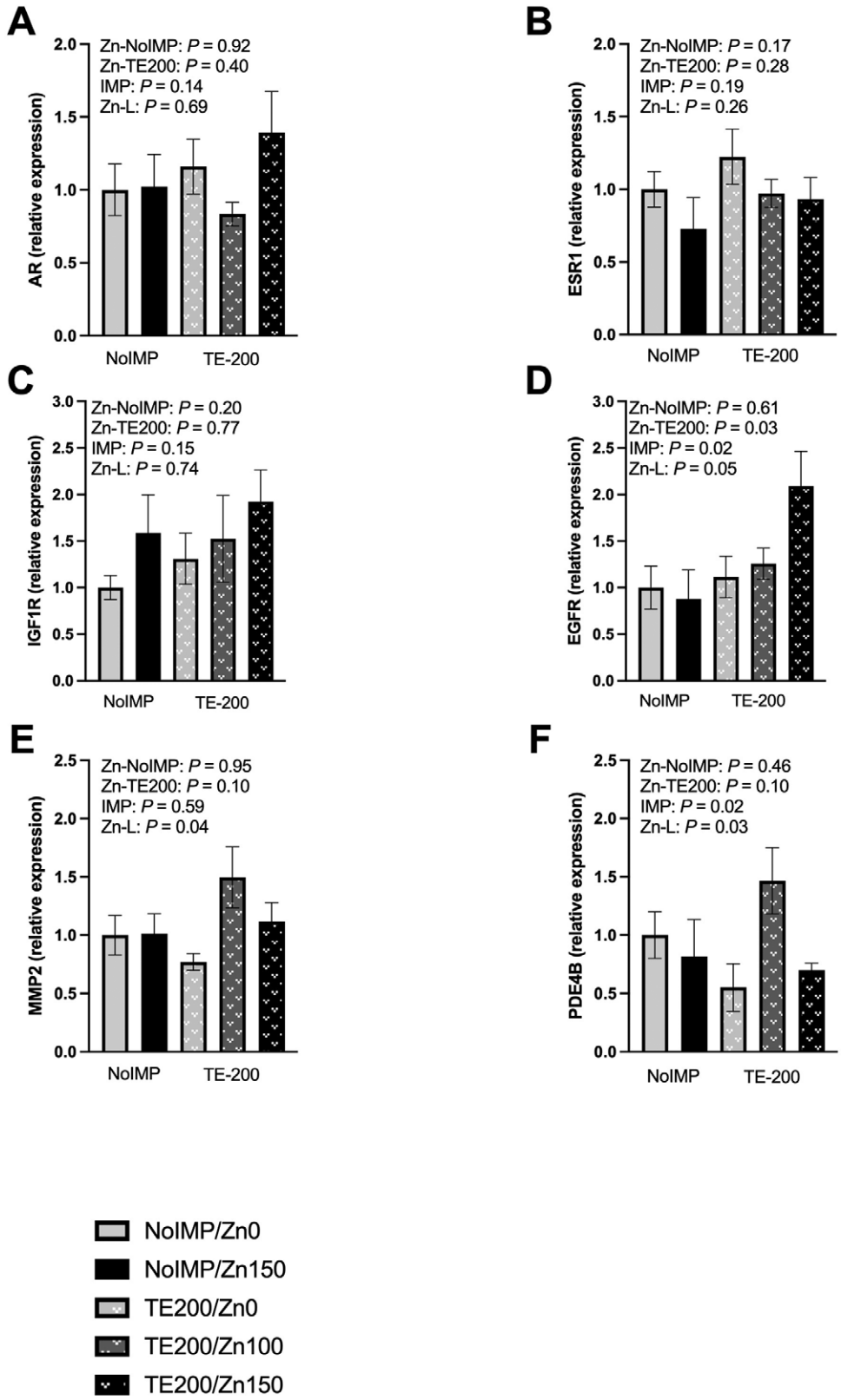

Figure 1. Relative gene expression of steroidal implant receptors and growth signaling machinery from the muscle of steers receiving no steroidal implant (**NoIMP**) or a steroidal implant (**TE200**) and fed 0, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn100**, **Zn150**, respectively). Samples were collected from the *longissimus thoracis* on day 11 and relative gene expression was calculated relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of steroidal implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Androgen receptor (AR), **B)** estrogen receptor (ESR1), and **C)** insulin-like growth factor-1 receptor (IGF1R) gene expression were not influenced by Zn or steroidal implant contrast statements ($P \geq 0.14$). **D)** Epidermal growth factor receptor (EGFR) gene expression was greater for Zn150 than Zn0 steers administered TE200 ($P = 0.03$) and linearly increased with 0, 100, and 150 mg/kg DM of supplemental Zn within TE200 steers ($P = 0.05$). Steroidal implanted cattle (Zn0 and Zn150) had greater EGFR gene expression than NoIMP ($P = 0.02$). **E)** Matrix metalloproteinase 2 (MMP2) gene expression tended to be greater

for Zn150 than Zn0 within TE200 ($P = 0.10$) and linearly increased with 0, 100, and 150 mg/kg DM of supplemental Zn for TE200 steers ($P = 0.04$). **F)** Phosphodiesterase 4B (PDE4B) gene expression tended to be greater for Zn150 than Zn0 within TE200 ($P = 0.10$) and linearly increased in TE200 steers with 0, 100, and 150 mg/kg DM of supplemental Zn ($P = 0.03$). Steroidal implanted cattle had lesser muscle PDE4B gene expression than NoIMP for Zn0 and Zn150 cattle ($P = 0.02$).

increasing Zn concentrations up to 5 times NASEM (2016) recommendations (30 mg Zn/kg DM) may be needed to optimize steroidal implant-induced growth of cattle. Zinc is involved in DNA and protein synthesis through a multitude of biological pathways including phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (MTOR; Jung et al., 2009). Indeed, increasing supplemental Zn linearly increased ADG of steroidal implanted cattle within 18 d of steroidal implant administration, though no effects of Zn were observed for non-steroidal implanted steers at this time. Similarly, Huerta et al. (2002) found steers supplemented with 200 mg Zn/kg DM from $ZnSO_4$ had a 9.8% greater growth response to a high potency steroidal implant than steers fed a control diet containing 84 mg Zn/kg DM. Niedermayer et al. (2018) observed improved gain and HCW in steers supplemented with trace minerals (Cu, Co, Fe, Mn, Se, and Zn) at 2 to 3 times NASEM (2016) recommendations, regardless of receiving a steroidal implant or not. In contrast to the current study, Niedermayer et al. (2018) supplemented cattle with a high concentration of trace minerals for twice as long (124 d). The short feeding period (59 d) of the current study may have limited potential Zn responses within non-steroidal implanted steers. However, the proximity of cattle to a mature BW may have also contributed to the lack of response to Zn supplementation within non-steroidal implanted cattle. Spears and Kegley, (2002) found Zn supplemented steers (33 mg Zn/kg DM) had improved performance during the growing period followed by a diminished Zn growth response during the finishing period. The current study was conducted during the Spring of 2020 during the COVID-19 pandemic explaining the heavy initial BW (492 kg) of cattle enrolled in the study. Perhaps, cattle maturity hindered the potential growth benefits of supplemental Zn in non-steroidal implanted steers. As cattle mature on the growth curve, lean tissue accretion lessens (Owens et al., 1995).

Biopsies of the *longissimus thoracis* collected on day 11 post-steroidal implant administration allow for a closer examination of growth processes affected by Zn. Considering no effects of steroidal implant or Zn supplementation were observed for androgen and E_2 receptor relative gene expression, the current study was unable to assess the direct effects of Zn on the genomic steroidal implant mode of action. However, upon estrogen stimulation from a steroidal implant, GPER initiates non-genomic signaling through the cleaving action of MMP2 or 9 on heparin-binding epidermal growth factor-like growth factor and the subsequent activation of EGFR to trigger growth processes including cell proliferation (Thornton et al., 2015). Pisano et al. (2017) found Zn acts through GPER to activate signaling through IGF1R and EGFR as Zn depletion hindered IGF1R and EGFR signaling. The linear response of EGFR relative gene expression to increasing Zn supplementation within steroidal implanted steers aligns with the findings of Pisano et al. (2017) and the linear increase in days 0 to 18 ADG of the current study. As a component of the non-genomic steroidal implant signaling mechanism, EGFR leads to the activation of downstream proteins such as extracellular-signal-regulated-kinase 1 and 2 (**ERK-1/-2**) that induce cell proliferation and migration (Wells, 1999; Filardo et al., 2000). Both ERK and AKT activation in human breast cancer cells have been observed with increasing Zn exposure (Pisano et al., 2017), indicating the effects of Zn supplementation on growth pathways are observed downstream of cell surface receptors. However, MAPK1 (representative of ERK-2) gene expression and AKT1 gene expression were not influenced by steroidal implant or Zn supplementation within steroidal implanted steers of the current study. Measurements at the protein level may be more reflective of Zn's effects on steroidal implant-induced signaling as Zn is associated with increased protein phosphorylation (Wu et al., 1999; Samet et al., 2003; Pisano et al., 2017). Although the small sample size in the present study may have limited relative gene expression results, maintaining an α level of $P \leq 0.05$ for statistical significance and $0.05 < P \leq 0.10$ for tendencies protected relative gene expression data from both Type I and Type II if the α level was too high or not high enough, respectively.

As components of non-genomic steroidal implant-induced signaling (Shakur et al., 2001; Thornton et al., 2015), MMP2 and PDE4B showed interesting responses to Zn supplementation. The linear response to Zn for MMP2 and EGF4 gene expression corresponds with increased days 0 to 18 growth of steroidal implanted steers. As a regulator of G protein-coupled receptor signaling, PDE4B degrades intracellular cyclic adenosine monophosphate (**cAMP**) to inhibit downstream non-genomic signaling (Shakur et al., 2001). Gene expression of PDE4B is inhibited by Zn in cell culture (von Bulow et al., 2005). Therefore, it was hypothesized increasing Zn supplementation would decrease the relative gene expression of PDE4B within steroidal implanted steers in a dose-dependent manner, leading to sustained cAMP signaling and increased growth rates. Indeed, steroidal implanted steers had lesser PDE4B gene expression than non-steroidal implanted steers. However, a linear increase in PDE4B relative gene expression with increasing Zn supplementation of steroidal implanted steers was observed, largely attributed to high Zn100 expression. It is uncertain why PDE4B gene expression would increase with Zn supplementation, though the small sampling size may have exaggerated this response.

Interestingly, non-steroidal implanted steers supplemented with 100 mg Zn/kg DM seemed to perform comparably to steroidal implanted steers throughout the trial and had numerically greater HCW than NoIMP counterparts. Therefore, supplementing 100 mg Zn/kg DM to non-steroidal implanted cattle may make up for the lost growth potential of not utilizing a steroidal implant. Although Zn supplementation did not affect day 59 BW, the early effects of Zn on steroidal implant-induced growth performance were apparent in HCW, and Zn linearly increased the dressing percentage of steroidal implanted steers. It is worth considering that as carcass transfer increases late in the feeding period (Macdonald et al., 2007), Zn may be particularly important to help transfer live gain to carcass gain (Genther-Schroeder et al., 2016).

Within the limited literature focused on Zn supplementation to cattle utilizing growth-promoting technologies, the growth response to supplemental Zn is inconsistently transferred to HCW. For example, the 7 kg BW advantage

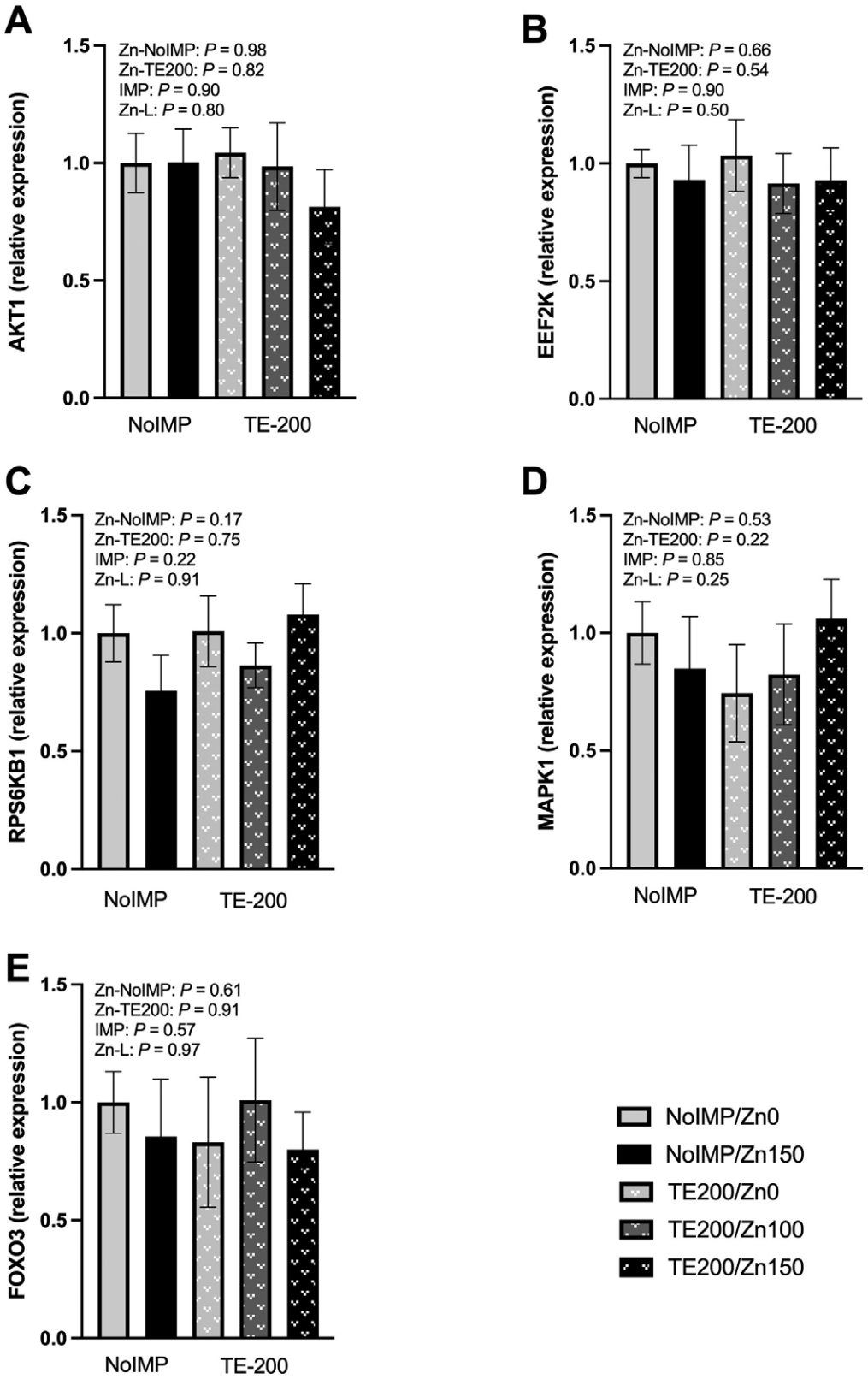

Figure 2. Relative gene expression of growth signaling proteins from the muscle of steers not steroidal implanted (**NoIMP**) or steroidal implanted (**TE200**) and supplemented 0, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn100**, **Zn150**, respectively). Samples were collected on day 11 from the *longissimus thoracis*. Relative gene expression was calculated relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of steroidal implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Protein kinase B (AKT1), **B)** eukaryotic elongation factor 2 kinase (EEF2K), **C)** ribosomal protein S6 kinase B1 (RPS6KB1), **D)** mitogen-activated protein kinase 1 (MAPK1), and **E)** forkhead box O-3 (FOXO3) gene expression were not affected by Zn or steroidal implant contrast statements ($P \geq 0.17$).

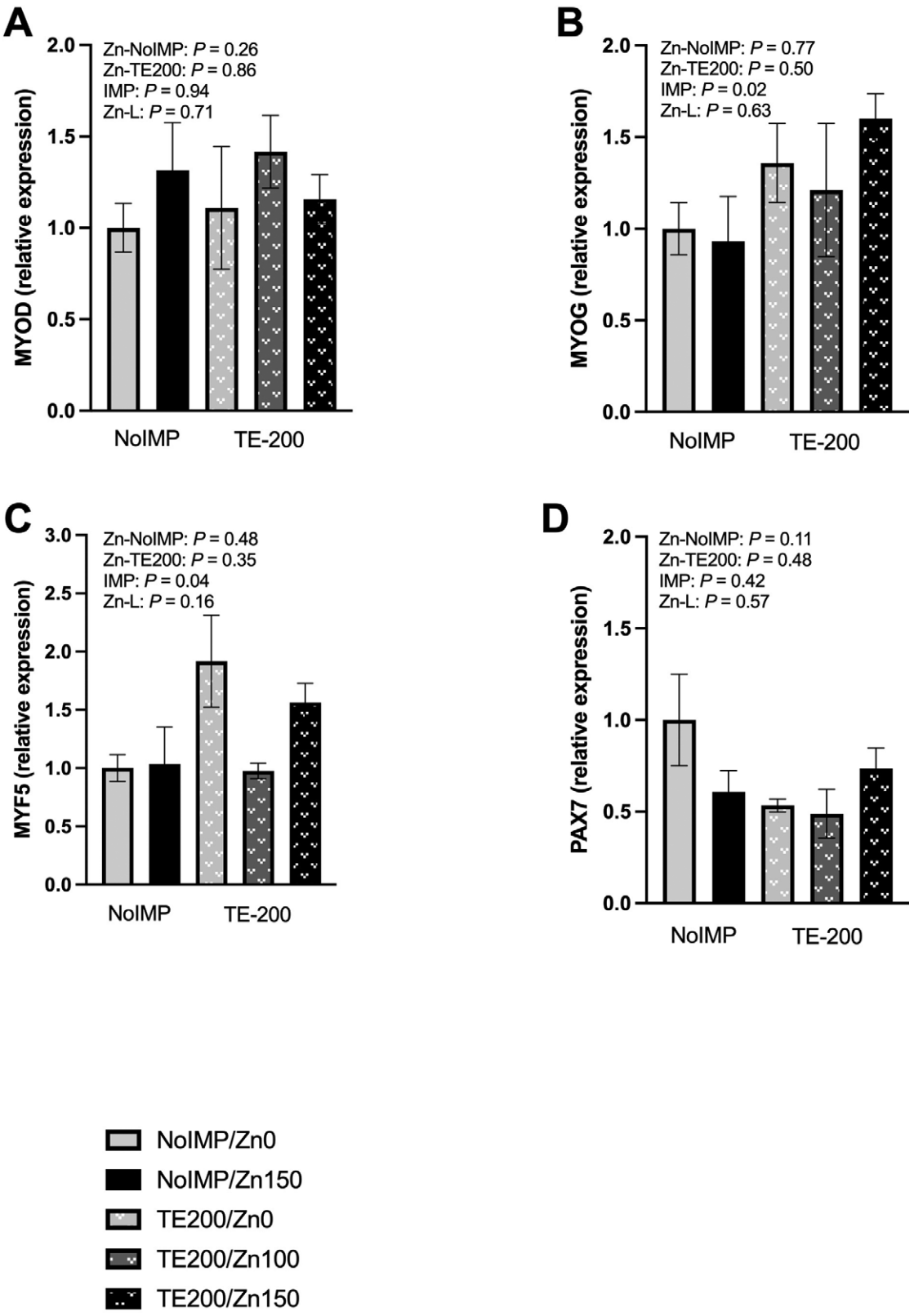

Figure 3. The relative gene expression of markers of satellite cell development was analyzed in non-steroidal implanted (**NoIMP**) and steroidal implanted (**TE200**) steers that were supplemented 0, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn100**, **Zn150**, respectively). Samples from the *longissimus thoracis* were collected on day 11 and relative gene expression was calculated relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of steroidal implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Myogenic differentiation 1 (MYOD) gene expression was not influenced by the tested contrast statements ($P \geq 0.26$). **B)** Myogenin (MYOG) and **C)** myogenic factor 5 (MYF5) gene expression were greater for TE200 than NoIMP for Zn0 and Zn150 treatments ($P \leq 0.04$). **D)** Paired box protein 7 (PAX7) gene expression was not influenced by the tested contrast statements ($P \geq 0.11$).

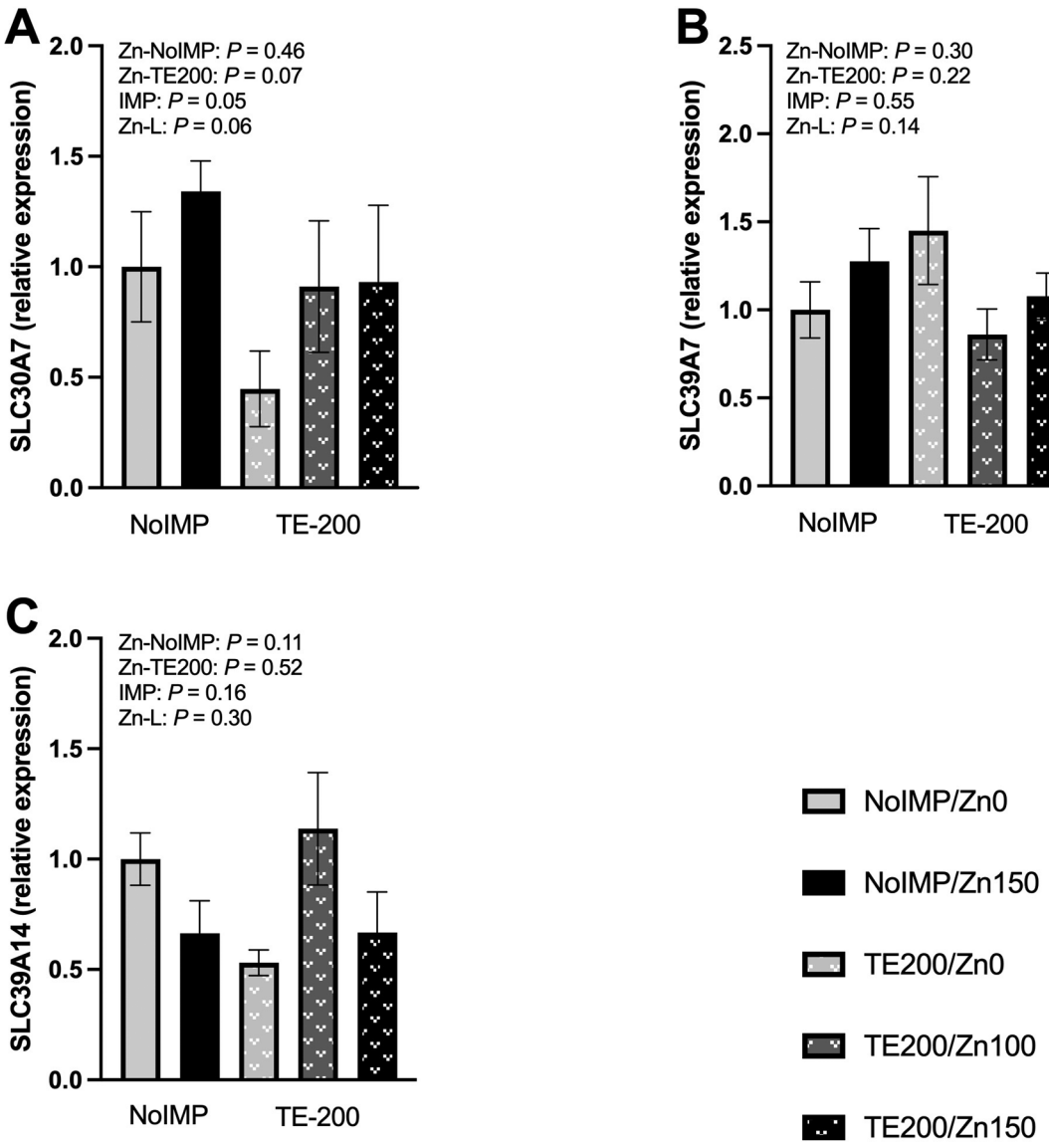

Figure 4. Relative gene expression of Zn transporters in the muscle of non-steroidal implanted (**NoIMP**) and steroidal implanted (**TE200**) steers supplemented 0, 100, or 150 mg Zn/kg DM (**Zn0**, **Zn100**, **Zn150**, respectively). Samples were collected on day 11 from the *longissimus thoracis* and relative gene expression was determined relative to NoIMP-Zn0 treatment. Contrast statements were formed to test the effect of increasing Zn from Zn0 to Zn150 within NoIMP (**Zn-NoIMP**) and TE200 (**Zn-TE200**), in addition to the main effect of steroidal implant (**IMP**; Zn0 and Zn150) and the linear effect of increasing Zn as Zn0, Zn100, and Zn150 within TE200 (**Zn-L**). **A)** Solute carrier family 30 member 7 (SLC30A7) gene expression tended to be greater for Zn150 than Zn0 within TE200 steers ($P = 0.07$) and tended to linearly increase with increasing Zn supplementation (Zn0, Zn100, and Zn150) within TE200 ($P = 0.06$). Gene expression of SLC30A7 was lesser for TE200 than NoIMP ($P = 0.05$). **B)** Solute carrier family 39 member 7 (SLC39A7) and **C)** solute carrier family 39 member 14 (SLC39A14) were not affected by the tested contrast statements ($P \geq 0.11$).

Messersmith et al. (2021) observed in steroidal implanted heifers supplemented 100 vs. 30 mg Zn/kg DM 48 d before harvest was not observed in HCW. Similarly, Huerta et al. (2002) found no differences in HCW due to Zn supplementation of 0 or 200 mg Zn/kg DM in steers and heifers, despite ADG differences throughout the trial. More statistical power appears necessary to pick up these subtle, yet impactful effects of Zn on HCW. Indeed, a post hoc power analysis revealed a *n* of 64 to detect an HCW response between Zn0 and Zn150 of steroidal implanted steers. However, power was sufficient to detect differences in days 0 to 8 ADG ($n = 13$).

Plasma Zn concentrations linearly increased with increasing Zn supplementation regardless of steroidal implant treatment on days 18 and 40. Interestingly, Zn100 and Zn150 plasma Zn concentrations were relatively similar and did not exceed 1.44 mg Zn/L, supporting the tightness of plasma Zn regulation when fed these physiological (not pharmacological) Zn diets. Considering the adequacy range of 0.8 to 1.4 mg Zn/L for plasma Zn concentrations proposed by Kincaid (2000), cattle Zn status was adequate to high.

Plasma Zn concentrations were lesser on day 18 in steroidal implanted steers. These data agree with the steroidal implant-induced decrease in plasma Zn concentrations observed by Messersmith (2018) 13 d post-steroidal implant administration. However, the depression in plasma Zn concentrations observed by Messersmith (2018) persisted through day 73, unlike the present study where day 40 plasma Zn was not affected by steroidal implant administration. Perhaps the depression of plasma Zn concentrations did not persist through day 40 due to the lesser growth response after the first 18 d post

steroidal implant administration. The lesser plasma Zn concentrations of steroidal implanted vs. non-steroidal implanted steers may be due to increased Zn demand in the muscle for growth processes (Oberleas and Prasad, 1969; Duncan and Dreosti, 1976) corresponding with the linear increase in days 0 to 18 performance and gene expression of steroidal implant signaling machinery such as EGFR and MMP2 of TE200 steers due to Zn supplementation. Furthermore, this steroidal implant-induced growth response appears to influence the Zn transporter SLC30A7 (ZnT7) that is important in the uptake of cytosolic Zn by both the Golgi apparatus and sarcoplasmic reticulum (Kirschke and Huang, 2002; Tuncay et al., 2017).

No effects of Zn supplementation or steroidal implant administration were observed on d 55/56 liver Cu, Fe, or Zn concentrations. Interestingly, Reichhardt et al. (2021) observed a decrease in liver Zn concentrations 2 d post-steroidal implant administration, but not on day 10 suggesting mobilization of liver Zn occurs during a limited window following steroidal implant administration. Furthermore, changes in liver Cu and Fe due to steroidal implants have been inconsistent when measured (Niedermayer et al., 2018; Reichhardt et al., 2021). The inconsistent response to steroidal implant in liver Cu and Fe concentrations may be due to the high concentrations of these minerals stored in the liver offsetting the mobilization of these minerals into the bloodstream. In contrast, on days 55/56 liver Mn concentrations were decreased due to steroidal implant administration, consistent with Smerchek et al. (2024) who reported on the relationship between liver Mn concentrations and decreasing PUN in steroidal implanted cattle, especially early in the steroidal implant period.

In summary, this study demonstrates that Zn supplementation can enhance the growth response to steroidal implants in beef cattle, particularly during the initial weeks following steroidal implant administration. The observed effects on performance parameters, coupled with changes in gene expression related to growth factor signaling and Zn homeostasis, suggest Zn plays a crucial role in supporting steroidal implant-induced growth. The benefits of Zn supplementation were most pronounced in steroidal implanted cattle, with minimal effects observed in non-steroidal implanted animals. These findings have important implications for beef cattle management, indicating that current Zn recommendations may be insufficient for maximizing the growth potential of steroidal implanted cattle. However, the complex interactions between Zn status, steroidal implant response, and time underscore the need for further research to refine Zn supplementation strategies in beef cattle production.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

Conflict of interest statement

The authors declare no conflict of interest.

Author Contributions

Elizabeth Messersmith (Conceptualization, Data curation, Project administration, Writing—original draft, Writing—review & editing), and Stephanie Hansen (Conceptualization, Data curation, Funding acquisition, Project administration, Writing—original draft, Writing—review & editing)

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