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Differences in longissimus thoracis metabolites in feedlot steers with differing plasma Zn concentration and implant status

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This study examined the role of Zn status on muscle glucose and other metabolites. Angus steers (144; 525 $\pm$ 30 kg) with varying plasma Zn concentrations and implant status were used for this secondary experiment. Steers were assigned to implant (IMP) treatments: no implant (NO) or Component TE-200 (TE-200; Elanco, Greenfield, IN) on d 0. Zinc sulfate was supplemented at 0 (analyzed 54 mg Zn/kg DM), 30, or 100 mg Zn/kg DM starting d -60. Steers were fed in two blocks via GrowSafe bunks, and steer was the experimental unit. Jugular blood and longissimus thoracis biopsies were collected d 40 post-implant. Plasma Zn was quantified via ICP-OES and stratified into quintiles by concentration and IMP treatment. Samples (n = 48; 12 low and 12 high from each IMP group) were identified and designated to plasma Zn treatments (PLZN): low (LO, 1.1 mg Zn/L) or high (HI, 1.6 mg Zn/L). Corresponding muscle samples were analyzed via gas chromatography-mass spectrometry for non-targeted metabolomics. Data were analyzed using ProcMixed of SAS with fixed effects of PLZN, IMP, BLOCK, and PLZN×IMP. No interactions were noted. β-alanine, 3-hydroxybutyric acid, and glycine were greater in HI than LO ($P \leq 0.05$), while 3-hydroxybutyric acid, 2,3,4-trihydroxybutyric acid, and glycine were greater in TE200 than NO ($P \leq 0.03$). Lactic and malic acids tended to be greater in TE200 than NO ($P \leq 0.10$). Although both Zn groups were adequate, greater plasma Zn altered metabolites indicative of enhanced energy metabolism, potentially explaining benefits of Zn supplementation to feedlot cattle.

1 Introduction

Zinc is an essential trace mineral crucial to whole-body growth. It is a cofactor to over 300 enzymes, a component of many transcription factors, and is implicated in nearly every signaling pathway in higher organisms (Beyersmann and Haase, 2001; Cousins et al., 2006). The current requirement for Zn is 30 mg Zn/kg dry matter (DM) and has remained

unchanged for four decades (NASEM, 2016). However, consulting nutritionists often supplement at concentrations as high as 300% of the requirement (Samuelson et al., 2016). This may be to accommodate the 44% increase in average daily gain (ADG) of beef cattle between 1977 and 2007 (Capper, 2011). While the required Zn concentration prevents deficiency, modern feedlot cattle may need greater Zn to support growth.

In implanted cattle, we have observed improved growth when supplementing up to 150 mg Zn/kg DM (Messersmith et al., 2022; Messersmith and Hansen, 2024) but the mechanisms are not entirely understood. Several of our studies have noted decreased plasma Zn concentration in implanted cattle, which is often overcome by Zn supplementation, suggesting circulating Zn may be important in the Zn-induced growth response (Messersmith and Hansen, 2021; Messersmith et al., 2022; Smerchek et al., 2024; Messersmith and Hansen, 2024). In the prior study, growth performance was not influenced by supplemental Zn, potentially driven by high plasma Zn concentrations across dietary treatments and limited growth potential noted in the steers. However, Zn impacted circulating glucose and insulin, corresponding with increased d 20 skeletal muscle mRNA abundance of GLUT4 in implanted steers, implicating altered muscle energy demand (Smerchek et al., 2024).

In this study, like others who have examined extreme populations (Russell et al., 2016; Carlson et al., 2017), we analyzed selected highs and lows in plasma Zn within implanted and non-implanted steers to examine the muscle metabolome. We hypothesized that implanted steers and steers with greater plasma Zn concentration would have greater concentrations of muscle metabolites related to growth and energy metabolism.

2 Methods

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC-20-127).

2.1 Animals and experimental design

This study utilized samples from a subset of a larger study (Smerchek et al., 2024). Briefly, 144 single-source Angus-cross steers (525 ± 30 kg) were used in a 2 × 3 randomized design with steers blocked by body weight (BW) to one of two blocks to accommodate sampling logistics and assigned to one of two implant treatments: no implant or Component TE-200 (200 mg trenbolone acetate + 20 mg estradiol; Elanco Animal Health, Greenfield, IN) on d 0. Zinc was supplemented as $ZnSO_4$ at 0 mg Zn/kg DM (analyzed 53 mg Zn/kg DM), 30 mg Zn/kg DM (CON + 30 mg Zn/kg DM), or 100 mg Zn/kg DM (CON + 100 mg Zn/kg DM), starting 60 d prior to implant. Steers were stratified by BW

into pens (n = 6 steers/pen) equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to determine individual feed disappearance and steer was the experimental unit. Steers were fed a dry-rolled corn-based diet ad libitum delivered daily at 0800 h (45% dry-rolled corn, 20% Sweet Bran, 10% DDGS, 15% corn silage, and 5% basal premix on a dry matter basis), and Zn treatments were delivered via premix utilizing dry distillers grains plus solubles as a carrier at 5% diet DM.

2.2 Sample collection and analysis

Blood and longissimus thoracis (LT) muscle samples for this experiment were collected 40 days after terminal implant (Smerchek et al., 2024), approximately aligned with peak hormone payout from uncoated implant pellets (Parr et al., 2014).

Trace mineral concentration of plasma samples was measured using inductively coupled plasma optical emission microscopy (Optima 7000 DV, Perkin Elmer, Waltham, MA; Pogge et al., 2012). Standards were used to verify instrument accuracy (UTAK Laboratories INC., Valencia, CA). Plasma Zn samples (n = 144) were stratified into quintiles by plasma Zn concentration to identify the 12 highest (HI) and 12 lowest (LO) samples within non implanted (NO) or implanted (TE-200) groups (n = 48 total; Table 1). Samples were selected from both the early and late BW blocks.

For these 48 steers, snap-frozen LT samples from the same day were pulverized and 100 mg of sample was weighed while frozen prior to metabolomics analysis. Muscle metabolites were extracted, dried, and analyzed in accordance with methods adapted from Heiderscheit and Hansen (2022). Metabolites were identified by the W.M. Keck Metabolomics Research Laboratory at Iowa State University (Ames, IA) using an Agilent Technologies Model 6890 GC coupled to Model 5975 controlled by the Agilent ChemStation software. The reference library was based on metabolites observed in Heiderscheit and Hansen (2022) which utilized the 2017 mass spectral library from the National Institutes of Standards and Technology. Metabolites that could not be assigned to a metabolite reference were excluded from analysis.

2.3 Statistical analysis

Data were analyzed as a complete randomized design using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with fixed effects of plasma zinc grouping (PLZN), implant status (IMP), PLZN × IMP, and block. Metabolites with >30% of values missing were removed from the dataset. Data were logarithmically transformed to achieve normality and presented means were back-transformed. Outliers were characterized as greater than three standard deviations from the treatment mean and were excluded from analysis. No interactions were observed for any muscle metabolite and thus main effects are presented (n = 12 per PLZN × IMP treatment).

TABLE 1 Treatment plasma Zn concentration[1].

| | LO[2] × NO[3] | LO[2] × TE200[3] | HI[2] × NO[3] | HI[2] × TE200[3] | SEM | P - value | | |
						PLZN	IMP	PLZN × IMP
Item								
Steers (n)	12	12	12	12				
Plasma Zn (mg/L)[4]	1.18[y]	1.09[z]	1.66[w]	1.46[x]	0.2	0.01	0.01	0.10

[1]Plasma samples obtained d 40 via jugular venipuncture.
[2]Plasma Zn treatments (PLZN; 24 steers/treatment; collected d 40) included LO and HI determined by plasma Zn concentration.
[3]Implant treatments (IMP; 24 steers/treatment) included NO (no implant) and TE200 (200 mg trenbolone acetate + 20 mg estradiol; administered d 0; Elanco Animal Health, Greenfield, IN).
[4]Means with different superscripts (w,x,y,z) tend to differ ($P < 0.10$).

3 Results

Plasma Zn concentrations are displayed in Table 1. There was a tendency for a PLZN×IMP interaction ($P = 0.09$) where, by design, HI was greater than LO, but implanted steers had lesser plasma Zn in HI and LO. In longissimus thoracis, there was a PLZN effect for beta-alanine, 3-Hydroxybutyric acid (BHB), and glycine, where HI was greater than LO ($P \leq 0.05$; Figure 1). An IMP effect was noted for BHB, 2,3,4-trihydroxybutyric acid, and glycine in which TE200 was greater than NO ($P = 0.03$; Figure 2). Additionally, lactic acid and malic acid tended to be greater in TE200 than NO ($P \leq 0.10$; Figure 2). Several other metabolites related to amino acid and energy metabolism were identified but not affected by treatment ($P > 0.10$; Table 2).

4 Discussion

Trace minerals support many biological pathways that profoundly impact functions related to cattle growth (Suttle, 2010). This study investigated the effects of plasma Zn concentration on muscle metabolites. As supplementing Zn increased plasma Zn concentration and hot carcass weight of steers (Messersmith and Hansen, 2021; Messersmith et al., 2022) and post-mortem rate of tenderness and LT metabolites related to energy metabolism (Schulte et al., 2023) we hypothesized steers with greater plasma Zn concentration would have more favorable energetic metabolites to support growth.

β-alanine is the rate-limiting metabolite of carnosine synthesis (Artioli et al., 2010), and was increased in HI PLZN. Carnosine helps buffer cellular H^{+} ions, making it an important antioxidant and transition metal chelator. It is also present at higher concentrations in Type II muscle fibers (Dunnett and Harris, 1997). β-alanine is used to improve stamina in athletes (Artioli et al., 2010). Cônsolo et al. (2020) found Nellore steers with greater genetic potential for growth had greater muscle carnosine compared to low-growth potential counterparts. Similarly, cattle with more tender steaks had greater β-alanine and β-alanine was negatively correlated with Warner-Bratzler Shear Force (WBSF; r = -0.45) (Antonelo et al., 2020), aligning with Schulte et al. (2023) who observed improved post-mortem LT tenderness in Zn-supplemented steers. Heiderscheit and Hansen (2022) examined the effects of three concentrations of Zn supplementation (0, 70, and 120 mg Zn/kg DM) on LT metabolites before and after an 18 h transit event. Pre-transit, steers supplemented 70 mg Zn/kg DM had lesser L-alanine than steers supplemented 120 mg Zn/kg DM. Porcine satellite cells treated with carnosine had greater proliferation and Akt/mTOR activity (Liu et al., 2022; Kalbe et al., 2023), potentially offering a mechanism by which Zn improves the response to steroidal implants in previous live-animal studies (Messersmith and Hansen, 2021).

We observed greater glycine concentration in the LT of both HI PLZN and TE200 steers. Glycine is acquired from dietary sources or *de novo* synthesis, resulting mainly from serine and its precursors (Alves et al., 2019). Glycine is readily catabolized, donating nitrogen to the greater pool for transamination (Matthews et al., 1981) and is integral to collagen and the extracellular matrix (Parry, 1988). Steroidal implants increase circulating insulin-like growth factor-1, promoting Type II collagen synthesis (Fortier et al., 1999; Preston, 1999). Genther-Schroeder et al. (2018) found no effects of increasing dietary Zn on meat collagen content when all steers were fed ractopamine hydrochloride (Genther-Schroeder et al., 2018). However, Schulte et al. (2023) found that ractopamine-fed steers had greater glycine concentrations in post-mortem LT than in control. While steroidal implants and β-agonists have different mechanisms of action, growth induced by these technologies may influence glycine metabolism. Matrix metalloproteinase 9 is implicated in the biological response to steroidal implants (Kamanga-Sollo et al., 2014; Thornton et al., 2015) and is involved in extracellular matrix remodeling (Koulicoff et al., 2023). In postmortem LT, steers supplemented 60 mg Zn/kg DM as $ZnSO_4$ + 60 mg Zn/kg DM as Zn-AA had increased matrix metalloproteinase 9 activity compared to unsupplemented steers, contributing to altered extracellular matrix degradation (Koulicoff et al., 2023). In contemporaries to steers from the present study, increasing supplemental Zn increased mRNA abundance of matrix metalloproteinase 2 in muscle 20 days post implant (Smerchek et al., 2024). Further, Zn treatment of HTR-8/SVneo cells has been shown to influence expression of STAT3 and matrix metalloproteinase 2/9 (Zong et al., 2017), known to impact satellite cell proliferation (Thornton et al., 2015). Increased glycine in both HI PLZN and TE200 groups may be related to extracellular matrix remodeling associated with increased protein synthesis and satellite cell fusion to muscle fibers, both key modes of
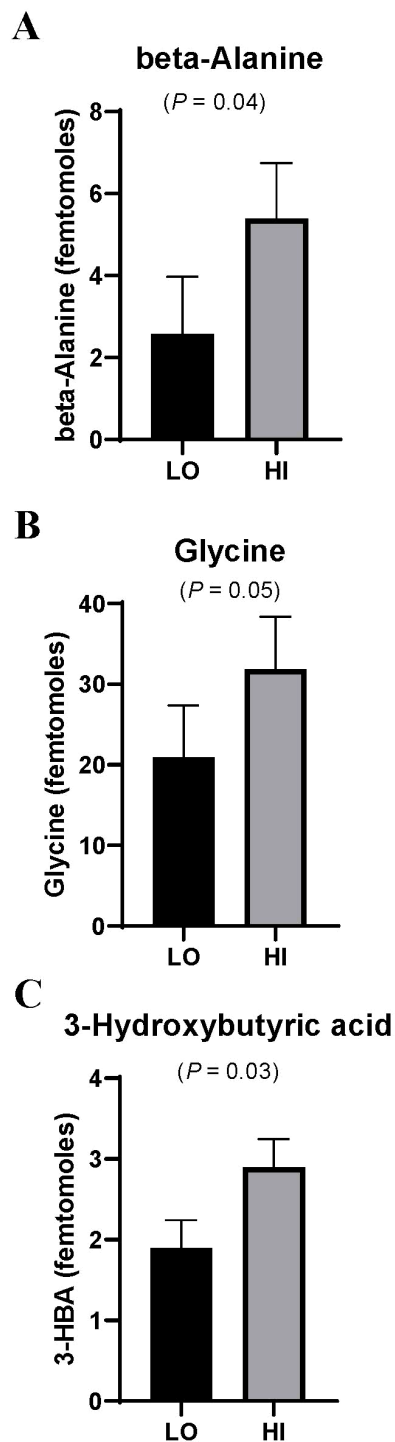
FIGURE 1
(A-C) Effects of LO or HI plasma Zn (PLZN) on muscle metabolites of beef steers ($P \leq 0.05$). Steers were selected for PLZN treatment groups from the lowest and highest quintile of plasma Zn concentration (PLZN; LO = 1.1 mg Zn/L; HI = 1.6 mg Zn/L; 24 steers/treatment).

implant-induced growth (Johnson et al., 1998; Reichhardt et al., 2021) and influenced by Zn (Ninh et al., 1998; Tang and Shay, 2001; Haase and Maret, 2003; Plum et al., 2014).

Both HI PLZN and TE200 steers had greater concentrations of BHB in LT. Similarly, 2,3,4-trihydroxybutyric acid was greater in TE200 and is an oxidized derivative of BHB. Butyric acid is preferentially absorbed by the rumen epithelium for its energy requirements. In cattle fed energy-dense diets, epithelial monocarboxylate transporters provide the animal with ketone bodies produced by intraepithelial breakdown of volatile fatty acids (Gäbel et al., 2002). Additionally, β-Hydroxy-β-methylbutyrate (HMB), a metabolite of leucine metabolism, leads
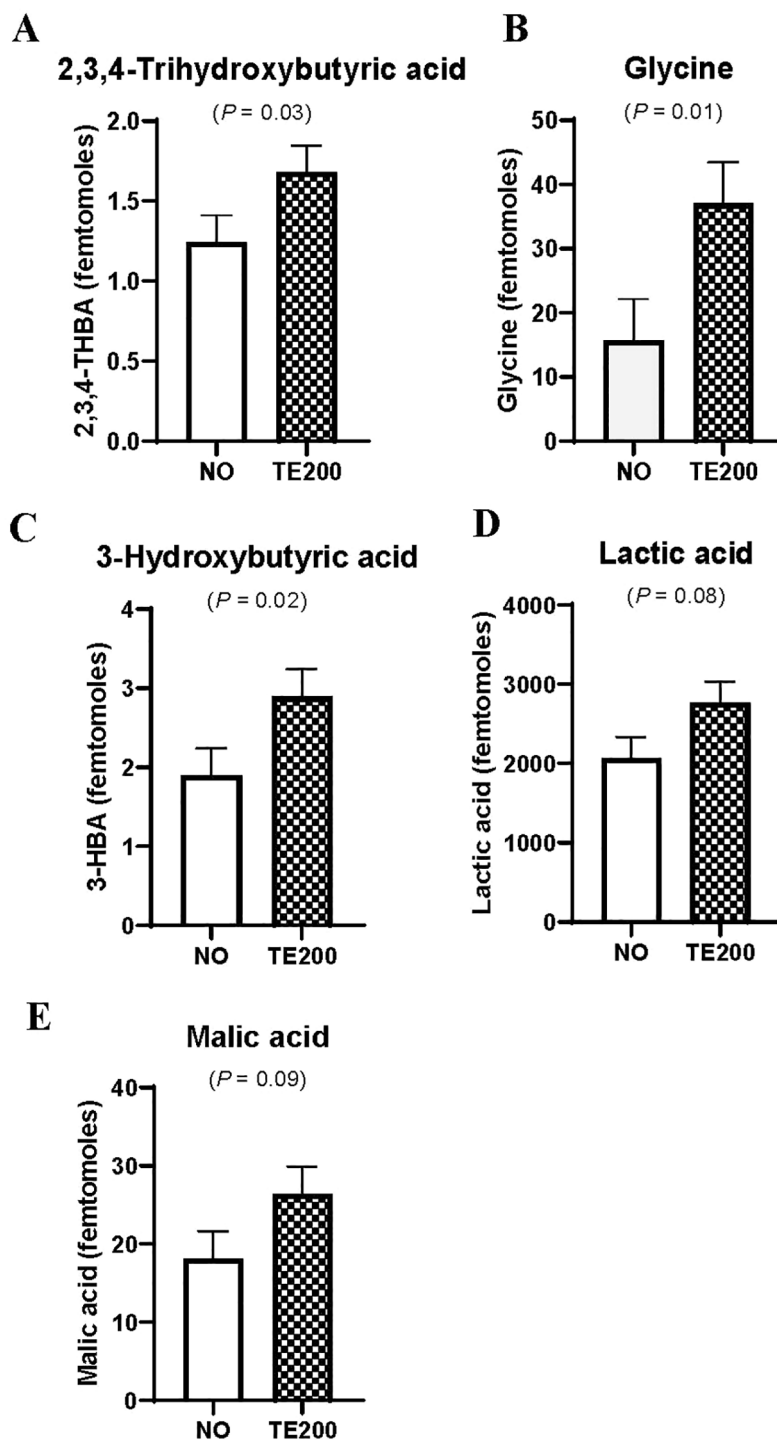
FIGURE 2
(A−E) Effects of NO or TE200 on muscle metabolites of beef steers collected 40 d after implant ($P \leq 0.10$). Steers received a steroidal implant treatment (IMP) on d 0: 1) no implant; NO; or 2) high potency combination implant; TE200 (200 mg trenbolone acetate + 20 mg estradiol; administered d 0; Elanco Animal Health, Greenfield, IN; 24 steers/treatment).

to increased BHB levels. Rats injected with HMB showed higher BHB concentrations in plasma and gastrocnemius (Ikeda et al., 2021). In aged rats with limb disuse for 14 days, HMB treatment led to a higher proportion of paired box 7 and myogenic differentiation-1 positive stem cells in the plantaris compared to controls (Alway et al., 2013). In the live-animal study (Smerchek et al., 2024), myogenic regulatory factor 5 expression increased with increasing $ZnSO_4$, but not due to implant status. While not assessed in the present study, it is intriguing to consider differences in LT BHB may reflect satellite cell differentiation differences.

TABLE 2 Longissimus thoracis metabolites detected that were not different reported as concentrations[1].

Metabolite name	Treatment mean (femtomoles)[3]				SEM	P - value		
	LO[2]	HI[2]	NO[2]	TE200[2]		PLZN	IMP	PLZN × IMP
Amino acid[4]								
L-5-Oxoproline	10.8	11.6	9.5	12.9	0.15	0.84	0.21	0.87
L-Alanine	35.0	39.0	31.3	42.7	0.15	0.84	0.51	0.89
Isoleucine	1.4	1.7	1.4	1.7	0.16	0.55	0.56	0.22
Leucine	6.4	6.7	6.1	7.0	0.15	0.64	0.45	0.77
Serine	4.6	5.5	4.1	6.1	0.15	0.86	0.79	0.95
Threonine	3.2	3.7	2.9	4.0	0.16	0.97	0.73	0.62
Metabolic intermediate[4]								
Imidazol-2-amine	321	281	282	319	0.15	0.21	0.11	0.35
5-Aminovaleric acid	1.8	2.2	2.0	2.0	0.15	0.74	0.64	0.73
2-Aminomalonic acid	1.6	2.2	1.2	2.5	0.17	0.46	0.32	0.36
Phosphoric acid	2.7	2.1	2.3	2.5	0.15	0.28	0.59	0.75
Urea	10.4	9.6	9.0	11.0	0.16	0.68	0.71	0.36
Glyceric acid	1.2	1.1	1.0	1.3	0.15	0.97	0.72	0.23
Butanedioic acid	4.2	5.0	4.7	4.4	0.15	0.44	0.39	0.81
Silanol	1358	1475	1316	1517	0.15	0.22	0.15	0.13
Sugar[4]								
beta-D-Mannopyranose	75.6	79.6	75.5	79.7	0.16	0.45	0.92	0.66
Glucose	115.2	115.7	101.5	129.3	0.15	0.66	0.21	0.97
Myoinositol	17.9	15.2	17.8	15.4	0.15	0.60	0.91	0.19

[1]d 40 longissimus thoracis sample collection.
[2]Plasma Zn treatments (PLZN; 24 steers/treatment) included LO and HI determined by plasma Zn concentration.
Implant treatments (IMP; 24 steers/treatment) included NO (no implant) and TE200 (200 mg trenbolone acetate + 20 mg estradiol; administered d 0; Elanco Animal Health, Greenfield, IN).
[3]Metabolites reported as femtomoles.
[4]Metabolites listed by molecule class.

Prior to transit, Zn-supplemented steers had greater BHB in LT relative to control, and BHB decreased from pre-transit to post-transit to support energy demands (Heiderscheit and Hansen, 2022). Schulte et al. (2023) found that post-mortem LT of steers fed greater Zn concentrations or fed ractopamine hydrochloride had greater BHB concentrations than their respective control treatments. In support of these findings, we found that steers with greater plasma Zn concentration had greater BHB concentrations, indicating HI PLZN improved energy availability to muscle. Steroidal implants impact both satellite cell fusion into myofibers and fiber type-specific differences in energy metabolism, and differences to ketones in the present study suggest differential energy metabolism and satellite cell activity. Fiber type was not assessed in the present study.

Both lactic acid and malic acid tended to be greater in TE200 steers. In the forward reaction, Zn-dependent lactate dehydrogenase converts pyruvate to lactate while oxidizing nicotinamide adenine dinucleotide (Price, 1962). Similarly, malic acid is an energy metabolism intermediate of the TCA cycle, contributing to energy production through NADPH generation. Differential lactic and malic acid levels suggest altered energy metabolism in implanted cattle, potentially due to a shift towards glycolytic metabolism. Increased malic acid may result from decreased conversion to oxaloacetate via malate dehydrogenase, indicating a bottleneck or regulatory change in the TCA cycle in which malate is spared for fatty acid synthesis. A study examining the effects of Nellore cattle fed to achieve high and low growth rates in feedlot and pasture systems noted decreased malonate, a key compound in fatty acid synthesis, in low ADG feedlot steers compared to high ADG pastured steers, indicating greater energy demand to support *de novo* fatty acid synthesis in the feedlot steers (Gómez et al., 2022). Relatedly, feedlot steers with high ADG had greater circulating BHB (Gómez et al., 2022), which is rapidly sequestered from blood in high performance cattle to support lipid metabolism (Imaz et al., 2022), matching our findings. Ractopamine-fed steers supplemented 120 mg Zn/kg DM compared to non-ractopamine-fed steers with equal Zn supplementation had lesser abundance of malate dehydrogenase in post-mortem LT (Schulte et al., 2023).

5 Conclusions

Plasma Zn concentration influenced several metabolites related to satellite cell proliferation and energy metabolism that may explain why Zn supplementation enhances cattle growth. However, unlike previous studies, energy metabolites affected by PLZN or IMP indicate modification of TCA cycle intermediates. While there are limitations with the use of metabolites for inferences of muscle metabolism, data obtained from this study, specifically metabolites affecting intracellular pH and satellite cell proliferation, highlight areas affected by Zn status to be validated in further research to substantiate these exploratory findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Iowa State University Institutional Animal Care and Use Committee (IACUC-20-127). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

BO: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. DS: Conceptualization, Investigation, Methodology, Writing – review & editing. SH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1640542/full#supplementary-material

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