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Effects of supplemental zinc on growth, carcass characteristics, and liver abscess formation in steers with experimentally induced ruminal acidosis challenge

Erika L. Lundy-Woolfolk,[†] Olivia N. Genther-Schroeder,[‡,‖] Mark Branine,[$] and Stephanie L. Hansen[‡,1]

[†]Department of Animal Science, Iowa State University Extension and Outreach, Ames, IA 50011, USA
[‡]Department of Animal Science, Iowa State University, Ames, IA 50011, USA
[‖]Current address: Purina Animal Nutrition, Gray Summit, MO 63039, USA
[$]Zinpro Corporation, Eden Prairie, MN, USA

[1]Corresponding author: slhansen@iastate.edu

ABSTRACT

The study's aim was to evaluate the effect of dietary Zn supplementation on steer performance, biomarkers of inflammation and metabolism, and liver abscess formation in response to a mild acidosis challenge. Forty-two steers (417 ± 3.99 kg; *n* = 6/pen) were housed in pens with bunks designed to measure individual dry matter intake (**DMI**) and fed one of two diets containing either 0 (CON; *n* = 18) or 90 mg Zn/kg from a Zn-amino acid complex (**Zn-AA**; *n* = 18; AvailaZn; Zinpro) for 109 d. Six additional steers were fed the CON diet and did not undergo the acidosis challenge (**NON**; *n* = 6). The acidosis challenge included restricting steers to 50% of the previous 7 d daily DMI on days 46 and 47, steers were individually provided 10% of DMI as cracked corn (as-fed) at 0800 h followed by ad libitum feed access 2 h post-grain consumption. Steer was the experimental unit, and two contrasts were constructed: NON vs. CON and CON vs. Zn-AA. Blood samples were collected on days 40, 48, 53, 69, 80, and 108 and analyzed as repeated measures. Final body weight and overall average daily gain (2.29, 2.30, and 2.31 ± 0.920 kg/d for CON, Zn-AA, and NON, respectively) were not different ($P \geq 0.74$) between treatments. By design, DMI was greater ($P < 0.01$) for NON compared to CON on day 46 but was not different ($P \geq 0.41$) for the rest of the experiment. While hot carcass weight (423, 428, and 424 ± 7.9 kg for CON, Zn-AA, and NON, respectively) and ribeye area were not different ($P \geq 0.53$) due to treatment, marbling score tended ($P = 0.06$) to be greater in CON compared to Zn-AA. The 12th rib backfat thickness was greater ($P = 0.05$) in NON vs. CON steers. Liver abscess incidence tended to be greater ($P = 0.12$) in CON (24% abscesses) vs. Zn-AA (6% abscesses). NON had a greater incidence ($P = 0.05$; 50% abscesses) compared to CON. Overall, blood fibrinogen and leukocyte counts were not different between treatments ($P \geq 0.67$); however, neutrophil-to-lymphocyte ratio tended to be greater in NON vs. CON ($P = 0.08$). Serum aspartate aminotransferase and gamma-glutamyl transferase concentrations were greater in NON vs. CON ($P \leq 0.02$), and serum alkaline phosphatase concentration was lesser in CON vs. Zn-AA ($P < 0.01$). Overall, dietary Zn supplementation tended to lessen incidence of liver abscesses with limited impacts on overall cattle performance. Shifts in liver enzymes may represent opportunities to identify cattle with liver abscesses earlier in the feeding period.

Lay Summary

The study aimed to investigate the effects of dietary zinc supplementation on performance, inflammation, and liver abscess formation in steers in response to a mild acidosis challenge. Forty-two steers were divided into two groups: one fed a diet containing 0 mg Zn/kg dry matter (**DM**) (**CON**), and the other fed a diet containing 90 mg Zn/kg DM from Zn-amino acid chelate (**Zn-AA**). Six additional steers were fed the CON diet and did not undergo the acidosis challenge. Blood samples were collected and analyzed as repeated measures, and weights and carcass data were collected. The results showed that dietary zinc supplementation did not affect the overall average daily gain of the steers. However, liver abscess incidence tended to be greater in the CON group than in the Zn-AA group. The study suggests that dietary zinc supplementation can reduce the incidence of liver abscesses with limited impacts on overall cattle performance. The study also found that shifts in liver enzymes may represent opportunities to identify cattle with liver abscesses earlier in the feeding period.

Key words: Acidosis, cattle, liver abscess, ruminal pH, zinc

INTRODUCTION

The liver plays a crucial role in a variety of metabolic functions in the body, and severe liver abscesses result in decreased growth performance, carcass weight, and ultimate animal value (Reinhardt and Hubbert, 2015). An estimated 10% to 20% of cattle on feed develop liver abscesses (Elanco, 2014; Rezac et al., 2014). While the pathogenesis of liver abscess development is not well understood, it is believed that diets rich in rapidly fermentable starch increase risk of ruminal acidosis (Owens et al., 1998) and lead to rumen epithelial damage and pathogen entrance to the hepatic portal system (Jensen et al., 1954; Kleen et al., 2003). The ruminal epithelium is vital for absorption of short-chain fatty acids, while providing a barrier between ruminal contents and the bloodstream. Persistent exposure to high-concentrate diets in the finishing phase could maximize subacute acidosis potential, assaulting the

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rumen wall until it eventually reaches its threshold, creating rumenitis and thus, liver abscess development. Minimal work has investigated Zn influence on ruminal barrier function. However, zinc amino-acid complex (Zn-AA) improves lower gut morphology in heat-stressed ruminants (Opgenorth et al., 2021). In addition, Zn supplementation protects the intestinal barrier and prevents excessive permeability in other species (Rodriguez et al., 1996; Sturniolo et al., 2002; Zhang and Guo, 2009; Sanz-Fernandez et al., 2014) and decreases anaerobic bacterial translocation to the mesenteric lymph nodes in weaned piglets (Broom et al., 2006). Commercial work has demonstrated that supplementing 360 mg Zn/steer daily from Availa-Zn can decrease the incidence and severity of liver abscesses in feedlot steers (Larson and Branine, 2015). Collectively, these data suggest that increasing dietary Zn may improve ruminal integrity and decrease abscess formation. The aim of this study was to determine the effect of Zn-amino acid supplementation on liver abscess formation in steers receiving a mild grain challenge.

MATERIALS AND METHODS

Animals, Experimental Design, and Sample Analysis

All procedures and protocols were approved by the Iowa State University (ISU) Institutional Animal Care and Use Committee (log number 7-16-8317-B).

Forty-two steers (417 ± 3.99 kg; n = 6/pen) were stratified by weight and housed under roof in concrete pens equipped with individual intake recording bunks (Dahlke et al., 2008) at the ISU Beef Nutrition Research Unit (Ames, IA) and fed one of two diets containing either 0 (CON, n = 18; NON, n = 6) or 90 mg supplemental Zn/kg from a Zn-amino acid complex (Zn-AA; n = 18; Availa-Zn).

Steers received a growing diet for 32 d (25% roughage, DM basis), followed by a 7-d transition diet (15% roughage, DM), prior to the final diet containing 5% roughage (Table 1). Steers were fed their respective finishing diet for 7 d to ensure steers were approaching ad libitum intake before steers were exposed to a challenge model stimulating mild acidosis similar to a method described by Dohme et al. (2008). The acidosis challenge included restricting steers to 50% of the previous 7 d daily dry matter intake (**DMI**) on days 46 and 47, steers were offered 10% of DMI of cracked corn (as-fed) at 0800 h, two steers in each challenge pen at a time to ensure all animals consumed the allotted corn, followed by ad libitum feed access 2 h post-grain consumption. One pen of the non-supplemental Zn-fed steers did not undergo the challenge to serve as the negative control for the acidosis challenge (NON, n = 6 steers). Throughout the rest of the trial, steers received the respective finishing diet at ad libitum intake. Diets did not contain Tylosin. Total mixed rations were sampled weekly and dried in a forced air oven at 70 °C for 48 h to determine weekly DM, which was used to calculate individual steer DMI. Feed efficiency (gain:feed; **G:F**) was calculated for each period by dividing the total amount of body weight (**BW**) gain by the total amount of DMI for that period. Dried diet samples were ground through a 2-mm screen (Retsch Zm100 grinder; Glen Mills Inc., Clifton, NJ) and samples were composited by diet for nutrient analyses.

Table 1. Ingredient and nutrient composition of the diets (% dry matter)

	Growing diet[1]	Transition diet[2]	Final diet[3]
Ingredient	% of diet DM		
Dry rolled corn	45.0	55.0	65.0
Grass hay	25.0	15.0	5.0
Modified distillers grains with solubles	25.0	25.0	25.0
Dried distillers grains with solubles	3.0	3.0	3.0
Limestone	1.5	1.5	1.5
Salt	0.31	0.31	0.31
Rumensin	0.0135	0.0135	0.0135
Vitamins and trace minerals	0.1765	0.1765	0.1765
Analyzed composition	DM basis		
CP, %	16.8		14.7
Fat, %	5.0		5.4
NDF, %	25.2		16.4
S, %	0.36		0.33
Cu, mg/kg[4]	14.5		11.44
Fe, mg/kg[4]	134		84
Zn, mg/kg[4,5]	32.0		33.6
Calculated composition[6]			
NEg, Mcal/kg	1.28	1.39	1.48

[1]Fed from days 0 to 32.
[2]Fed from days 33 to 39.
[3]Fed from days 40 to 109.
[4]Trace mineral analysis was completed using inductively coupled plasma optical emission spectroscopy as described by Pogge et al. (2014).
[5]Analyzed Zn concentrations for Zn-AA treatment diets were 125 mg/kg for the Growing diet, and 113 mg/kg for the final diet.
[6]Composition was calculated using values from NRC (2000) in BRANDS (ISU).

All steers were weighed on days −1, 0, 28, 53, 69, 80, 81, 108, and 109. Steers were implanted on day −1 with Component TE-IS (Elanco Animal Health, Indianapolis, IN) and on day 53 with Component TE-S. All live BW were subject to a 4% pencil shrink prior to calculating average daily gain (**ADG**) and G:F. Ractopamine hydrochloride (Actogain; Zoetis, Parsippany, NJ) was fed at a rate of 300 mg/steer/d for 28 d prior to harvest.

Blood samples were collected on days 48 and 53 (days 1 and 6 post-acidosis challenge) to evaluate plasma trace mineral concentrations. Samples were also collected for complete blood count and the acute phase protein fibrinogen, white blood cell differentiation, as well as a large animal routine chemistry profile, including measurements of several hepatic enzymes including gamma-glutamyltransferase, alkaline phosphatase, and aspartate transaminase (Iowa State University Veterinary Diagnostic Laboratory, Ames, IA).

Individual steers were equipped with a wirelessly accessible ruminal bolus (eBolus eCow Ltd, Exeter, Devon, UK) to continuously monitor ruminal pH throughout the study, including the time of the acidosis challenge. Fecal grab samples via rectal palpation were also collected on days 41, 48, 53, and 69 to monitor fecal pH as an indicator of starch by-pass and fermentation in the large intestine (Depenbusch et al., 2008). Briefly, 1 g of feces was added to a clean test tube containing 9 mL of deionized water, vortexed, and fecal pH was determined with a calibrated pH meter.

At the end of the finishing period, steers were harvested at a commercial packing plant (National Beef, Tama, IA). At the abattoir, gross pathology of the liver was assessed and scored (Rezac et al., 2014) for abscesses and other lesions. Liver abscess scores (**LAS**) were scored using the Elanco Liver Check System (Greenfield, IN) on a 0, A, and A+ scale. Livers free of abscesses were classified as a LAS of 0. Mild abscesses, up to four small abscesses less than 2.54 cm in diameter, were classified as LAS of A. Larger or more severe abscesses, with active areas of inflammation on the liver or when portions of the liver were attached to the diaphragm were classified as a LAS of A+. A section of the liver was collected for trace mineral analysis. Hot carcass weight was collected, and then carcasses were chilled for 48 h, after which ribeye area (**REA**), backfat thickness (**BF**), percent kidney, pelvic, heart fat (**KPH**), marbling score were collected, and yield grade (**YG**) was calculated. Dressing percentage was calculated by dividing hot carcass weight (**HCW**) by the shrunk live final BW and multiplying by 100.

Liver samples collected at slaughter were acid digested in trace mineral grade nitric acid (Fisher Scientific, Fair Lawn, NJ) as described by Pogge and Hansen (2013). Plasma sample preparation, and liver and plasma samples analysis for Cu, Fe, and Zn concentrations via inductively coupled plasma optical emission spectrometry (Optima 7000 DV, Perkin Elmer, Waltham, MA) were conducted as previously described by Pogge and Hansen (2013).

Statistical Analysis

Steer growth performance, carcass data, blood analyses, and harvest liver mineral concentrations were analyzed by ANOVA as a complete randomized design using the Mixed procedure of SAS (SAS Institute, Inc., Cary, NC) where steer was the experimental unit. The model included the fixed effect of diet and random effect of pen. Treatment distributions of LAS data were determined using PROC Glimmix of SAS. Area under the curve for ruminal pH data was calculated using R (The R Foundation, Vienna, Austria) and resulting means were analyzed using repeated measures. The model included the fixed effects of treatment, day of study and the interaction, with the repeated effect of day. Covariance structures were selected based on the best model fit. Outliers were determined using Cook's D and removed if Cook's D ≥ 0.5. Two single df contrast statements were developed to assess the effects of grain challenge (CON vs. NON) and effects of Zn supplementation within grain-challenged steers (CON vs. Zn-AA). Blood data were analyzed using the fixed effects of LAS score. Significance was declared at $P \leq 0.05$, and tendencies were declared from $P = 0.06$ to 0.15. Means reported are least square means (LSMEANS) ± SEM. One steer from CON was removed from trial during the ractopamine (**RAC**) period. While data collected from the pre-RAC period were utilized, data from the steer were eliminated from RAC and harvest analyses.

RESULTS AND DISCUSSION

The liver is a vital organ required for essential life functions, including nutrient metabolism, immunoregulation, hormonal control, and detoxification of blood. Damages to the liver, including liver abscesses, are thought to result in decreased functionality and ultimately, decreased animal growth and performance. Liver abscess development is often attributed to high starch feeding programs during the finishing phase, though mechanisms that contribute to liver abscess formation are poorly understood. Among other routes, liver abscesses occur when bacteria from the gastrointestinal tract translocate into the hepatic portal system and eventually enter the liver (Nagaraja and Lechtenberg, 2007). Once in the liver, the body attempts to detoxify the foreign pathogens and eventually abscesses may develop (Nagaraja and Lechtenberg, 2007).

No differences were noted between treatments for pre-challenge BW, DMI, ADG, or G:F ($P \geq 0.24$; Table 2). Liver abscess incidence tended to be greater ($P = 0.12$) in CON when compared with Zn-AA (Table 3), though the study was insufficiently powered to identify differences in severity of liver abscesses (A vs. A+). However, CON had a lesser incidence ($P \leq 0.05$) compared to NON. It is interesting to note that when NON and CON steers are considered together (collectively receiving the same non-Zn supplemented diet), numerical incidence of liver abscess was 30% in comparison with 6% incidence in the Zn-supplemented treatment. While the present study is with small pens and individual animal as the experimental unit, two large experiments at commercial research feedlots showed similar depressive effects of Zn on liver abscess. As summarized in Larson and Branine (2015), cattle fed steam-flaked corn diets containing Tylosin had 19% fewer liver abscesses ($P < 0.05$) when diets were supplemented with up to 90 mg Zn/kg DM (as Zn methionine) and received ractopamine hydrochloride. Combined, these data suggest that ~90 mg Zn/kg DM may provide some protection against liver abscess formation. In addition, research data suggests that Availa-Zn may reduce liver abscesses either in the absence (current study) or presence of antibiotics (Larson and Branine, 2015). Zinc has been demonstrated to prevent excessive intestinal permeability (Rodriguez et al., 1996; Sturniolo et al., 2002; Sanz-Fernandez et al., 2014) and increase the protein expression of gastrointestinal tight

Table 2. Effect of dietary Availa-Zn supplementation on live animal performance of feedlot cattle pre-acidosis challenge[1]

Item	Treatment[2]		SEM[3]	Contrast *P*-value
	CON	Zn-AA		CON vs. Zn-AA[5]
	n = 18	*n* = 18		
Initial BW[6], kg	416	417	4.0	0.83
Day 41 BW, kg	515	517	4.2	0.83
Growing period DMI, kg (days 1 to 33)	25.9	25.3	0.70	0.50
Transition period DMI, kg (days 34 to 40)	26.5	26.6	0.88	0.94
Pre-challenge period DMI, kg (days 41 to 45)	25.6	24.1	0.95	0.24
Pre-challenge DMI, kg (days 1 to 45)	26.0	25.3	0.70	0.47
ADG (days 1 to 41)	2.41	2.43	0.079	0.83
Pre-challenge G:F (days 1 to 41)	0.211	0.219	0.0075	0.43

[1]Acidosis challenge = CON and Zn-AA steers were restricted to 50% of the previous 7 d DMI on day 46, then provided 10% (as-fed) cracked corn at 0800 h, and 2 h later provided TMR ad libitum on day 47.
[2]Treatments: NON – non-challenged with no supplemental zinc; CON – no supplemental zinc; Zn-AA – supplemental Availa-Zn at 90 mg/kg.
[3]Highest treatment SEM reported.
[4]Contrast comparing NON and CON.
[5]Contrast comparing CON and Zn-AA.
[6]A 4% pencil shrink was applied to all live body weights.

Table 3. Effect of dietary Availa-Zn supplementation on carcass characteristics and distribution of quality grade, yield grade, and liver abscess scores of steers that experienced an acidosis challenge[1]

	Treatment[2]			SEM[3]	Contrast *P*-value	
	NON	CON	Zn-AA		NON vs. CON[4]	CON vs. Zn-AA[5]
	n = 6	*n* = 17	*n* = 18			
Hot carcass weight, kg	424	423	428	7.85	0.96	0.53
Ribeye area, cm^2	92.7	92.6	92.5	3.18	0.97	0.98
Backfat, cm	2.29	1.85	1.91	0.184	0.05	0.73
KPH[6], %	2.2	2.1	2.1	0.130	0.75	0.95
Marbling score[7]	488	506	448	35.7	0.67	0.06
Yield grade	4.1	3.7	3.8	0.246	0.15	0.67
Select, %	0	12	22		0.16	0.41
Low choice, %	50	44	61		0.90	0.32
Ave. choice and higher, %	50	44	17		0.28	0.11
Yield grade 2, %	0	12	11		0.25	0.95
Yield grade 3, %	50	59	56		0.74	0.85
Yield grade 4, %	50	29	33		0.38	0.80
Liver abscess score[8] 0, %	50	76	94		0.24	0.12
Liver abscess score A, %	17	24	6		0.72	0.12
Liver abscess score A+, %	33	0	0		0.02	1.00

[1]Acidosis challenge = CON and Zn-AA steers were restricted to 50% of the previous 7 d DMI on day 46, then provided 10% (as-fed) cracked corn at 0800 h, and 2 h later provided TMR ad libitum on day 47.
[2]Treatments: NON – non-challenged with no supplemental zinc; CON – no supplemental zinc; Zn-AA – supplemental Availa-Zn at 90 mg/kg.
[3]Highest treatment SEM reported.
[4]Contrast comparing NON and CON.
[5]Contrast comparing CON and Zn-AA.
[6]Kidney, pelvic, and heart fat.
[7]Marbling score: 300 = slight, 400 = small, and 500 = modest.
[8]Liver abscess score: 0 = no abscesses; A = one or two small abscesses or abscess scars; A+ = one or more large, active abscesses.

junction proteins (e.g. rumen, colon) in other species (Zhang and Guo, 2009), and although the link between zinc supplementation and rumen health is yet uncharacterized, many of the tight junction proteins affected by Zn in other species can also be found within the rumen epithelium (Sturniolo et al., 2002; Graham and Simmons, 2005). Additionally, Zn is critical in host defense, and through roles in macrophages, and/or wound healing (Sandstead et al., 1970), may help the liver resolve abscesses and regrow liver tissue, though these factors have yet to be examined in cattle feeding experiments.

In this study, 20% of the steers were affected with liver abscesses, which is greater than normal incidence in mid-western states where finishing diets often do not contain Tylosin (13% average liver abscesses; Reinhardt and

Table 4. Effect of dietary Availa-Zn supplementation on live animal performance of feedlot cattle pre- and post-acidosis challenge[1]

Item	Treatment[2]			SEM[3]	Contrast *P*-value	
	NON	CON	Zn-AA		NON vs CON[4]	CON vs Zn-AA[5]
	n = 6	*n* = 18	*n* = 18			
Final BW, kg	667	662	665	11.9	0.74	0.78
50% DMI restriction, kg (day 46)	10.4	7.0	6.2	0.72	0.01	0.20
Corn DMI, kg (day 47)	0	1.23	1.17	0.015	0.01	0.01
Post-challenge period DMI, kg (days 48 to 81)	11.6	12.0	11.7	0.48	0.42	0.32
Ractopamine period DMI, kg (days 82 to 109)	12.6	13.1	12.9	0.52	0.42	0.63
Trial DMI, kg	11.7	12.4	11.9	0.48	0.18	0.17
Post-challenge ADG, kg/d (days 48 to 81)	2.21	2.31	2.31	0.920	0.35	0.94
Ractopamine period ADG, kg/d (days 82 to 109)	2.52	2.12	2.21	0.199	0.10	0.61
Trial ADG, kg/d	2.31	2.29	2.30	0.095	0.89	0.88
Post-challenge G:F (days 48 to 81)	0.226	0.214	0.226	0.0127	0.41	0.22
Ractopamine period G:F (days 82 to 109)	0.196	0.159	0.169	0.1400	0.17	0.25
Trial G:F	0.198	0.186	0.195	0.0088	0.20	0.21

[1]Acidosis challenge = CON and Zn-AA steers were restricted to 50% of the previous 7 d DMI on day 46, then provided 10% (as-fed) cracked corn at 0800 h, and 2 h later provided TMR ad libitum on day 47.
[2]Treatments: NON – non-challenged with no supplemental zinc; CON – no supplemental zinc; Zn-AA – supplemental Availa-Zn at 90 mg/kg.
[3]Highest treatment SEM reported.
[4]Contrast comparing NON and CON.
[5]Contrast comparing CON and Zn-AA.
[6]A 4% pencil shrink was applied to all live body weights.

Table 5. Effect of dietary Availa-Zn supplementation on rumen pH of steers that experienced an acidosis challenge[1]

	Treatment[2]			SEM[3]	Contrast *P*-value			
	NON	CON	Zn-AA		NON vs. CON[4]	CON vs. Zn-AA[5]	Day	Trt × day
		n = 24	*n* = 18					
Pre-challenge[6]								
AUC 5.8, pH*hr/d	—	48.06	62.39	6.909	—	0.12	0.04	0.05
Minimum pH	—	5.57	4.56	0.057	—	0.17	0.99	0.90
Maximum pH	—	6.28	6.26	0.051	—	0.79	<0.01	0.04
	n = 6	*n* = 18	*n* = 18					
Restriction day, day 46								
Minimum pH	5.59	5.57	5.43	0.138	0.92	0.21		
Maximum pH	6.31	6.21	6.19	0.159	0.58	0.88		
Challenge day, day 47								
Minimum pH	5.40	5.30	5.24	0.110	0.45	0.46		
Maximum pH	6.31	6.70	6.61	0.147	0.03	0.44		
Post-challenge[7]								
AUC 5.8, pH*h/d	67.30	65.22	68.43	12.243	0.88	0.75	0.03	0.36
Minimum pH	5.44	5.49	5.45	0.068	0.47	0.72	0.09	0.31
Maximum pH	6.24	6.26	6.18	0.090	0.81	0.26	0.53	0.22

[1]Acidosis challenge = CON and Zn-AA steers were restricted to 50% of the previous 7 d DMI on day 46 then provided 10% (as-fed) cracked corn at 0800 h, and 2 h later provided TMR ad libitum on day 47. Blood samples were taken on days –6, 1, 6, 22, 33, and 61 relative to the acidosis challenge. Day –6 samples prior to the challenge were used as covariates in analysis.
[2]Treatments: NON – non-challenged with no supplemental zinc; CON – no supplemental zinc; ZINC – supplemental Availa-Zn at 90 mg/kg.
[3]Highest treatment SEM reported.
[4]Contrast comparing NON and CON.
[5]Contrast comparing CON and ZINC.
[6]Days 40 to 45 of study.
[7]Days 48 to 53 of study.

Hubbert, 2015). Liver abscesses not only impact packers through condemnation of livers but also producer economics as adhered abscesses often result in excessive trim of the carcass, decreasing hot carcass weights. In a large pen study, Lundy et al. (2015) noted approximately 20% lesser ADG during the reimplant period of steers with A+ LAS at harvest. While only two steers in the present study had liver abscesses of this severity, HCW was decreased by 11.8 kg relative to

Table 6. Effect of acidosis challenge[1] and dietary Availa-Zn supplementation on blood parameters of steers that experienced an acidosis challenge[1]

	Treatment[2]			SEM[3]	Contrast *P*-values	
	NON	CON	Zn-AA		NON vs. CON[4]	CON vs. Zn-AA[5]
	n = 6	*n* = 18	*n* = 18			
Plasma						
Cu, mg/L						
Day 48	1.11	1.15	1.136	0.10	0.66	0.81
Day 53	0.92	0.93	1.051	0.07	0.98	0.02
Fe, mg/L						
Day 48	2.07	2.29	2.251	0.35	0.58	0.89
Day 53	2.31	1.99	1.874	0.23	0.23	0.54
Zn, mg/L						
Day 48	1.22	1.34	1.347	0.17	0.56	0.94
Day 53	1.10	1.06	1.179	0.16	0.85	0.40
Whole blood						
White blood cell, cell number × 10^3/μL						
Day 48	9.55	9.61	9.34	0.908	0.96	0.73
Day 53	9.29	9.97	9.63	1.008	0.56	0.68
Fibrinogen, mg/dL						
Day 48	283	306	328	82.2	0.82	0.74
Day 53	383	394	439	35.8	0.79	0.14
Neutrophil, cell number × 10^3/μL						
Day 48	2.37	2.77	2.72	0.389	0.38	0.88
Day 53	2.42	2.95	3.09	0.380	0.23	0.65
Lymphocyte, cell number × 10^3/μL						
Day 48	6.24	5.86	5.64	0.563	0.56	0.64
Day 53	6.02	6.06	5.61	0.655	0.96	0.41
Monocyte, cell number × 10^3/μL						
Day 48	0.49	0.55	0.51	0.083	0.54	0.59
Day 53	0.47	0.55	0.54	0.091	0.43	0.85
Eosinophil, cell number × 10^3/μL						
Day 48	0.21	0.29	0.32	0.064	0.24	0.53
Day 53	0.18	0.26	0.25	0.046	0.12	0.75
Basophil, cell number × 10^3/μL						
Day 48	0.09	0.10	0.09	0.010	0.40	0.24
Day 53	0.07	0.09	0.09	0.013	0.14	0.46
Neutrophil:Lymphocyte						
Day 48	0.39	0.47	0.50	0.063	0.29	0.57
Day 53	0.41	0.49	0.57	0.053	0.21	0.07
Serum						
Albumin, g/dL						
Day 48	3.2	3.1	3.2	0.10	0.60	0.46
Day 53	3.1	3.1	3.1	0.12	0.91	0.43
Alkaline phosphatase, IU/L						
Day 48	148.0	140.2	164.0	14.45	0.64	0.05
Day 53	148.3	131.6	143.0	15.91	0.37	0.39
Aspartate aminotransferase, IU/L						
Day 48	96.0	95.7	93.3	6.12	0.97	0.64
Day 53	102.6	99.1	98.9	8.42	0.72	0.98
Bilirubin, mg/dL						
Day 48	0.18	0.20	0.22	0.03	0.51	0.49
Day 53	0.20	0.20	0.18	0.03	0.91	0.42
Creatine, mg/dL						
Day 48	1.1	1.1	1.2	0.06	0.62	0.17

Table 6. Continued

	Treatment[2]			SEM[3]	Contrast *P*-values	
	NON	CON	Zn-AA		NON vs. CON[4]	CON vs. Zn-AA[5]
Day 53	1.3	1.2	1.3	0.10	0.37	0.12
Creatine kinase, IU/L						
Day 48	127.2	110.4	120.1	18.71	0.45	0.54
Day 53	124.0	126.2	210.0	88.16	0.98	0.26
Gamma-glutamyl transferase, IU/L						
Day 48	47.7	44.2	47.4	6.21	0.63	0.53
Day 53	55.8	44.9	51.8	7.29	0.20	0.27
Glucose, mg/dL						
Day 48	97.0	98.3	97.9	3.11	0.71	0.88
Day 53	98.0	97.2	92.8	4.15	0.87	0.17
Urea nitrogen mg/dL						
Day 48	7.8	8.9	8.9	0.66	0.16	0.92
Day 53	10.0	9.7	11.0	2.55	0.93	0.54

[1]Acidosis challenge = CON and Zn-AA steers were restricted to 50% of the previous 7 d DMI on day 46, then provided 10% (as-fed) cracked corn at 0800 h, and 2 h later provided TMR ad libitum on day 47. Blood samples were taken on days 1 (day 48) and 6 (day 53) relative to the acidosis challenge.
[2]Treatments: NON – non-challenged with no supplemental zinc; CON – no supplemental zinc; ZINC – supplemental Availa-Zn at 90 mg/kg.
[3]Highest treatment SEM reported.
[4]Contrast comparing NON and CON.
[5]Contrast comparing CON and ZINC.
[6]Cell number × 10^3/μL.
[7]Neutrophil to lymphocyte ratio, cell number × 10^3/μL.

Table 7. Effect of acidosis challenge[1] and dietary Availa-Zn on harvest liver trace mineral concentrations of steers

	Treatment[2]			SEM[3]	Contrast *P*-value	
	NON	CON	Zn-AA		NON vs. CON[4]	CON vs. Zn-AA[5]
	n = 6	*n* = 16	*n* = 18			
Liver mineral, mg/kg DM						
Copper	348	365	327	34.1	0.67	0.20
Iron	186	148	167	14.7	0.04	0.15
Zinc	180	161	163	20.8	0.32	0.88

[1]Acidosis challenge = CON and Zn-AA steers were restricted to 50% of the previous 7 d DMI on day 46, then provided 10% (as-fed) cracked corn at 0800 h, and 2 h later provided TMR ad libitum on day 47. Blood samples were taken on days −6, 1, 6, 22, 33, and 61 relative to the acidosis challenge. Day −6 samples prior to the challenge were used as covariates in analysis.
[2]Treatments: NON – non-challenged with no supplemental zinc; CON – no supplemental zinc; Zn-AA – supplemental Availa-Zn at 90 mg/kg.
[3]Highest treatment SEM reported.
[4]Contrast comparing NON and CON.
[5]Contrast comparing CON and Zn-AA.

steers with an A or 0 LAS, similar to the 9 kg decrease reported by Lundy et al. (2015). Interestingly, Lundy et al. (2015) supplemented a similar rate of Zn, 80 to 90 mg Zn/kg DM, but from $ZnSO_4$.

Treatment had no effect ($P \geq 0.15$; Table 3) on HCW, REA, KPH, or YG. However, BF was lesser ($P = 0.05$) for CON-fed steers compared to NON steers, whereas CON and Zn-AA steers were not different ($P = 0.73$). Marbling scores were not different between CON and NON steers ($P = 0.78$). Treatment did not affect the distribution of most quality and yield grades ($P \geq 0.16$). However, similar to the work of Genther-Schroeder et al. (2018), there was a tendency for marbling scores to be greater ($P = 0.06$) for CON compared to Zn-AA and a tendency for distribution of avg. choice and higher to be greater for CON ($P = 0.11$). This may be explained by the numerical trend for Zn-AA steers to have better ADG during the RAC feeding period at the end of the trial, as previous work by Genther-Schroeder et al. (2016a,b) has suggested that Zn-AA in combination with $ZnSO_4$ improves cattle growth response to ractopamine hydrochloride.

The grain challenge conducted in the current study was designed to induce mild acidosis in steers, since liver abscesses have been largely correlated to ruminal damage following bouts of acidosis. By design, DMI was lesser ($P < 0.01$) for CON compared to NON on day 46 (restriction day) but was not different ($P \geq 0.42$) for the rest of the experiment (Table 4). Corn intake as part of the acidosis model on day 47 was greater ($P < 0.01$) in CON challenged steers compared to NON. Interestingly, CON steers consumed more corn than ZN-AA fed steers ($P = 0.01$). However, in the present study, non-challenged steers actually had greater incidence of liver abscesses than challenged control steers, and had the only recorded A+ abscesses in the study. Because of the close nature of the pens used in this experiment, it is possible that NON steers experienced psychological stress from watching cohorts in other treatments receive grain, but the potential difference in stress between a gastrointestinal insult and psychological stress is unclear. DMI was not different between CON and Zn-AA on the restriction day and throughout the finishing period ($P \geq 0.32$).

During days 40 to 45 (pre-acidosis challenge) of the study, minimum and maximum rumen pH were not different between Zn-AA and CON-fed steers ($P \geq 0.17$; Table 5). However, for pre-challenge area under the curve (AUC), set at a threshold of a rumen pH below 5.8 (pH threshold indicating subacute ruminal acidosis; Dohme et al., 2008), there was a tendency for a treatment by day ($P = 0.05$) effect for AUC during the pre-challenge period, caused by a

Table 8. Effect of liver abscess score (LAS)[1] at harvest on blood parameters

	LAS Score			SEM[2]	*P*-value
	0	A	A+		
	n = 33	*n* = 6	*n* = 2		
Serum					
Albumin, g/dL					
Day 48	3.2	3.1	3.1	0.13	0.46
Day 53	3.1	3.0	3.0	0.12	0.21
Alkaline phosphatase, IU/L					
Day 48	151.5	153.9	143.9	26.97	0.95
Day 53	138.4	138.9	145.9	20.41	0.94
Aspartate aminotransferase, IU/L					
Day 48	94.2	92.4	110.2	9.32	0.24
Day 53	97.6[b]	106.8[b]	155.5[a]	14.14	0.001
Bilirubin, mg/dL					
Day 48	0.21	0.19	0.18	0.051	0.73
Day 53	0.19	0.18	0.22	0.047	0.79
Creatine, mg/dL					
Day 48	1.1	1.1	1.2	0.08	0.40
Day 53	1.2	1.2	1.2	0.13	0.87
Creatine kinase, IU/L					
Day 48	112.6[b]	256.0[a]	112.5[ab]	72.94	0.01
Day 53	167.3	136.7	137.6	112.35	0.88
Gamma-glutamyl transferase, IU/L					
Day 48	46.0	41.8	60.0	10.52	0.34
Day 53	48.6	44.5	77.0	12.10	0.07
Glucose, mg/dL					
Day 48	98.0	96.8	100.5	5.37	0.84
Day 53	95.8	113.5	84.6	13.21	0.06
Urea nitrogen, mg/dL					
Day 48	8.9	8.8	6.5	1.01	0.08
Day 53	10.2	10.7	10.3	1.99	0.92

Superscript letters (a, b, ab) indicate differences $P < 0.05$.
[1]0 = no abscesses; A = one or two small abscesses or abscess scars; A+ = one or more large, active abscesses.
[2]Highest treatment SEM reported.

numerical increase in AUC on day 42 for the CON steers, whereas otherwise CON and Zn-AA remained relatively consistent, with Zn-AA numerically greater than CON (P = 0.12) on average. There was an effect of treatment by day (P = 0.03) on maximum pH during the pre-challenge period which is likely driven by NON-steers having a lower maximum rumen pH early in the pre-challenge period. No treatment effects on AUC, minimum or maximum pH were noted on day 46 (day of feed restriction); however, on day 47 (grain challenge day) NON had lower maximum ruminal pH vs. CON, while no other comparisons were significant on this day. Although, maximum ruminal pH was lower in NON vs. CON steers on the grain feeding day the values are more typical for NON (6.31) vs. CON (6.70), likely reflecting the more normal feed intake pattern of NON for this 24-h period, while CON was still feed restricted for part of this period, resulting in abnormally high ruminal pH. This is similar to the day 47 maximum ruminal pH observed for Zn-AA steers (6.61).

Day (P = 0.03) did impact AUC during this post-challenge period, where day 48 had the greatest AUC, and AUC tended to decrease over the next 5 d, suggesting that the mild-acidosis challenge disrupted normal function of the rumen as intended. However, during the week following the challenge (days 48 to 53), no effects of treatment by day ($P \geq 0.22$) were observed, and treatment comparisons revealed no effects ($P \geq 0.26$) on AUC, minimum pH, or maximum pH. The lack of treatment differences suggests that feed restriction and grain challenge were not great enough to disrupt ruminal acid load. The difference between the success of the current approach and the challenge by Dohme et al., 2008, could be due to the differences in pre-challenge diet (5% forage vs. 45% to 60% forage, respectively), challenge substrate and allotment (10% of previous DMI from cracked corn vs. approximately 19% of previous DMI from ground barley and wheat, respectively), animal type (beef steers vs. lactating Holstein cows, respectively) or other factors that may have made the challenge completed in this study less severe. Interestingly, minimum pH pre-challenge was lower for cows receiving a diet with 45% forage (5.21; Dohme et al., 2009), and minimum pre-challenge pH was similar to the cows receiving 60% forage total mixed ration (TMR) (5.60) when compared to the than

the minimum pre-challenge pH of the steers in this study (5.56), although AUC < 5.8 was greater in this study. This reduced variability may also have influenced the response. Fecal pH was not different across treatments on day 48 (6.67 ± 0.177) or day 58 (6.70 ± 0.097; $P \geq 0.15$).

Steers in this study grew very well throughout the trial, gaining over 2.27 kg/d across the period. Average daily gain for the 109 d study was not different across treatments (Table 4; $P \geq 0.88$); however, CON tended to have lesser ADG ($P = 0.10$) during the RAC period (last 28 d on feed) compared to NON. Because of RAC period ADG, feed efficiency during the RAC period was lesser ($P = 0.03$) in CON-fed steers compared to the NON steers. Feed efficiency was not different ($P \geq 0.24$) between CON and Zn-AA steers at any point throughout the study. Final BW were not affected by treatment ($P \geq 0.74$).

There were no differences between treatments ($P \geq 0.12$) for white blood cell, fibrinogen, lymphocytes, neutrophils, monocytes, eosinophils or basophils on day 48 or 53 (Table 6). There were no differences in neutrophil-to-lymphocyte ratio on day 48, but tended to be lesser ($P = 0.07$) in CON compared to Zn-AA on day 53, while CON and NON were not different ($P = 0.21$). The combination of neutrophilia and lymphopenia is a marker of inflammation and physiological stressors (Roland et al., 2014), although the total neutrophil and lymphocyte counts were within normal ranges (Roland et al., 2014). A typical neutrophil:lymphocyte in adult cattle is approximately 0.5 (Roland et al., 2014) which is similar to the range found in this study (0.41 to 0.57). There were no treatment differences in plasma concentrations of Zn and Fe ($P \geq 0.23$; Table 6) on day 48 or 53, or differences in plasma Cu on day 48 (1 d post-challenge; $P \geq 0.66$). But consistent with neutrophil:lymphocyte, but CON had lesser plasma Cu than Zn-AA on day 53 (6 d post-challenge; $P = 0.02$), but was not different from NON ($P = 0.98$). Increased plasma Cu could be an indicator of increased ceruloplasmin (a Cu-containing acute phase protein), as 90% of circulating Cu can be found in ceruloplasmin, but was not measured in this study. There were no additional indicators of inflammation in these steers, and no differences in performance, suggesting that if additional inflammation was occurring post-challenge, it was mild. A liver sample was collected from each carcass at the packing plant for trace mineral analysis (Table 7). Liver copper and Zn concentrations were not different ($P \geq 0.20$) between treatments. While liver Fe concentration was lesser ($P = 0.04$) in CON compared to NON, liver Fe concentrations in CON and Zn-AA-fed steers were not different ($P = 0.15$). Despite being fed additional dietary Zn, an increase in liver Zn concentration was not expected, as liver Zn is not reflective of Zn status (Kincaid, 2000).

There were no differences between NON and CON or CON and Zn-AA in serum chemistry ($P \geq 0.12$) post-challenge, except for serum alkaline phosphatase, which was lesser in CON than Zn-AA steers ($P = 0.05$) on day 48 (1 d post-acidosis challenge), but not day 53 ($P = 0.39$). As alkaline phosphatase is a Zn-metalloprotein, this difference may be a response to increased dietary Zn in these steers as observed by others (Spears, 1989), especially given that no differences in alkaline phosphatase were noted due to liver abscess severity.

A post hoc analysis of blood data was conducted by separating steers into those with 0, A, or A+ abscess scores (Table 8). This analysis revealed a trend for gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) to increase in A+ steers across the trial, beginning to separate most around day 53 of study. This further supports the potential value of these two markers of hepatic damage as potential tools to identify cattle experiencing liver abscesses. In a smaller clinical case, Abdelaal et al. (2014) evaluated serum chemistry in feedlot cattle and buffalo with liver abscesses and found that some animals exhibited elevated concentrations of AST and GGT and also reported lesser albumin concentrations, similar to the current study, but found that all animals had increased white blood cell (**WBC**), which was not found in the current study. Similarly, Lechtenberg and Nagaraja (1991) found increased WBC, fibrinogen, bilirubin, and GGT in steers with experimentally induced liver abscesses. Macdonald et al. (2018) similarly report lesser serum albumin over time in bulls with liver abscesses, while also reporting increased AST along with several other parameter changes such as testosterone, glucose, and cortisol that collectively may indicate liver damage. It is unclear why cattle in this experiment only demonstrated some of the more common changes in serum metabolites, but again, small numbers of cattle, liver abscess severity, and sampling time points may have led to some differences.

Overall, liver abscess formation tended to be decreased in Zn-AA vs. CON, though no differences in live animal performance were noted due to Zn treatment. However, a few blood parameters, including aspartate aminotransferase, gamma-glutamyl transferase, and basophils, appear to be indicators of liver abscess development, likely reflecting progressive liver damage as abscesses develop. To further discern differences, additional research with larger experimental units is necessary to better determine the effect of Zn on liver abscess formation.

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Conflict of interest statement

Lundy-Woolfolk, Genther-Schroeder, and Hansen declare no conflicts of interest. Branine is an employee of Zinpro Corp. and reviewed the manuscript but offered only grammatical suggestions for improvement. The content was at the discretion of the Iowa State University researchers.

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