Title: Dietary zinc concentration and lipopolysaccharide injection affect circulating trace minerals, acute phase protein response, and behavior as evaluated by an ear-tag based accelerometer in beef steers.

Katherine R. VanValin*, Remy N. Carmichael-Wyatt*, Erin L. Deters*, Elizabeth M. Messersmith*, Katie J. Heiderscheit*, Katherine G. Hochmuth*, Trey D. Jackson*, Joshua M. Peschel†, Anna K. Johnson* and Stephanie L. Hansen*.

*Department of Animal Science, Iowa State University, Ames, IA 50011, USA

†Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA 50011, USA

1This work was funded in part by the Iowa Beef Industry Council

2Current address: Department of Animal and Food Sciences, University of Kentucky, Princeton, KY, 42445, USA

3Corresponding author: slhansen@iastate.edu
Abstract

To assess plasma trace mineral (TM) concentrations, the acute phase protein response, and behavior in response to a lipopolysaccharide (LPS) challenge, 96 Angus cross steers [average initial body weight (BW): 285 ± 14.4 kg] were sorted into two groups by BW (heavy and light; n = 48/group), fitted with an ear-tag based accelerometer (CowManager SensOor; Agis, Harmelen, Netherlands), and stagger started 14 d apart. Consecutive day BW were recorded to start the 24-d trial (d -1, 0). Dietary treatments began on d 0: common diet with either 30 (Zn30) or 100 (Zn100) mg supplemental Zn/kg DM (ZnSO4). On day 17 steers received one of the following injection treatments intravenously to complete the 2 × 3 factorial: 1) SALINE (~2-3 mL of physiological saline), 2) LOWLPS: 0.25 µg LPS/kg BW or 3) HIGHLPS: 0.375 µg LPS/kg BW. Blood, rectal temperature (RT), and BW were recorded on d 16 (-24 h relative to injection), and BW was used to assign injection treatment. Approximately 6, 24 (d 18), and 48 (d 19) h after treatment BW, RT, and blood were collected, and final BW recorded on d 24. Data were analyzed in Proc Mixed of SAS with fixed effects of diet, injection, diet × injection; for BW, RT, dry matter intake (DMI), plasma TM, and haptoglobin repeated measures analysis was used to evaluate effects over time. Area under the curve analysis determined by GraphPad Prism was used for analysis of accelerometer data. Body weight was unaffected by diet or injection (P ≥ 0.16), but there was an injection × time effect for DMI and RT (P < 0.05), where DMI decreased in both LPS treatments on d 16, but recovered by d 17, and RT was increased in LPS treatments 6 h post-injection. Steers receiving LPS spent less time highly active and eating than SALINE (P < 0.01). Steers in HIGHLPS spent lesser time ruminating, followed by LOWLPS and then SALINE (P < 0.001). An injection × time effect (P < 0.001) for plasma Zn showed decreased concentrations within 6 h of injection and remained decreased through 24 h before recovering by 48 h. A tendency for a diet × time effect (P = 0.06) on plasma Zn suggests plasma Zn repletion occurred at a greater rate in Zn100 compared to Zn30. These results suggest increased supplemental Zn may
alter rate of recovery of Zn status from an acute inflammatory event. Additionally, ear-tag-based accelerometers used in this study were effective at detecting sickness behavior in feedlot steers, and rumination may be more sensitive than other variables.

Key words: beef cattle, lipopolysaccharide, sickness behavior, stress, zinc

List of abbreviations

| Abbreviation | Description |
|--------------|-------------|
| LPS          | Lipopolysaccharide |
| TM           | Trace mineral |
| DM           | Dry matter |
| TMR          | Total mixed ration |
| BW           | Body weight |
| Zn30         | Steers receiving a common total mixed ration with 30 mg supplemental Zn/kg DM |
| Zn100        | Steers receiving a common total mixed ration with 100 mg supplemental Zn/kg DM |
| IV           | Intravenously |
| SALINE       | Steers receiving intravenous injection of 0.9% physiological saline |
| LOWLPS       | Steers receiving intravenous injection of 0.25 µg lipopolysaccharide/kg body weight. |
| HIGHLPS      | Steers receiving intravenous injection of 0.375 µg lipopolysaccharide/kg body weight |
| Abbreviation | Description                  |
|--------------|------------------------------|
| RT           | Rectal temperature           |
| RR           | Respiration rate             |
| CBC          | Complete blood count         |
| DMI          | Dry matter intake            |
| AUC          | Area under the curve         |
| NLR          | Neutrophil to lymphocyte ratio |
Introduction

The feedlot receiving period involves many stressors leading to increased disease incidence (Duff and Galyean, 2007). The National Animal Health Monitoring System estimates that bovine respiratory disease affects 21.2% of all beef cattle placed in feedlots (USDA, 2013). Bovine respiratory disease commonly affects cattle during the receiving period (Johnson and Pendell, 2017), and identifying morbid cattle early may lead to improved animal welfare through decreased morbidity and mortality (Janzen et al., 1984) and increased treatment efficacy (Ferran et al., 2011). Use of ear-tag-based accelerometers have been validated to assess time spent ruminating, eating, and activity in healthy dairy (Pereira et al., 2018) and beef cattle (Wolfger et al., 2015). However, less is known about the use of these technologies for detecting behavior alterations in sick feedlot cattle.

Symptoms of bovine respiratory disease can be mimicked by injection of lipopolysaccharide (LPS; Carroll et al., 2009b). Lipopolysaccharide, a component of the cell wall of most gram-negative bacteria (Zähringer et al., 1999), binds to the myeloid differentiation-2 and toll-like receptor-4 complex on the surface of mononuclear cells (Alexander and Rietcschel, 2001). Upon LPS binding, a series of Zn-dependent post-translational modifications are required for production of pro-inflammatory cytokines (Wan et al., 2014). Pro-inflammatory cytokines are a critical component of the immune response necessary for infection resolution (Mogensen, 2009). Hepatic Zn and Fe concentrations are increased in response to LPS in other species (Aydemir et al., 2012; Liuzzi et al., 2005), resulting in lesser circulating Zn and Fe. Additionally, urinary excretion of Cu and Zn increase in response to infectious bovine rhinotracheitis virus in cattle (Orr et al., 1990). Thus, as Zn appears to be utilized during the innate immune response, and trace mineral (TM) homeostasis is disrupted, there may be a greater need for dietary Zn during this time. Consulting feedlot nutritionists have
reported feeding 100 mg Zn/kg dry matter (DM; Samuelson et al., 2016), which is in excess of the NASEM (2016) recommended 30 mg Zn/kg DM, possibly due to the positive role of Zn in immune function. Thus, the objective of this study was to assess plasma TM concentrations, the acute phase protein response, and cattle behavior when given various doses of injected LPS and supplemented with either 30 or 100 mg Zn/kg DM. It was hypothesized that regardless of LPS dose, plasma TM concentrations and blood cell populations related to the acute phase response would decrease, but that increased supplemental Zn would lessen the severity of these changes. A secondary hypothesis was that ear-tag-based accelerometers would detect illness behaviors such as less time spent eating or ruminating in cattle treated with LPS.

**Materials and Methods**

All experimental procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC-18-226).

**Experimental design and sample collection**

Ninety-six Angus crossbred steers, with an average initial body weight (BW) of 285 kg ± 14.4 S.D. were purchased from a single ranch and utilized in the present trial. Three days after arrival all steers were weighed, vaccinated with Vision 7 and Vista Once SQ (Merck Animal Health, Summit, NJ, USA), treated against parasites with Dectomax injectable (Zoetis Inc., Kalamazoo, MI, USA), and received a unique visual and electronic identification tag. The current study was part of an additional behavioral observation study, which required two unique observational groups. Additionally, two groups were needed to accommodate the schedule during the Lipopolysaccharide challenge. To establish the two unique groups of animals, steers were sorted by weight (n = 48
steers per group), and the two groups were stagger started 14 d apart on the 24-d experimental protocol (Table 1). The heavy group had an average initial BW of 295 kg ± 11.1 kg, and the light group had an average initial BW of 275 ± 9.6 kg. Upon arrival and until the start of the experimental period for each group, steers received a common total mixed ration (TMR; Table 2) supplemented with 30 mg Zn/kg DM from ZnSO₄.

Within group, consecutive day BW (d -1, d 0), were recorded to start the trial and steers were blocked by BW to pens (6 steers per pen). The average of the consecutive day BW for each individual animal was calculated, analyzed, and reported as d 0 BW. Pens were randomly assigned to one of two dietary treatments (4 pens/treatment) consisting of the common TMR supplemented with either 30 mg Zn/kg DM (Zn30) or 100 mg Zn/kg DM (Zn100) from ZnSO₄. Each group began receiving dietary treatments on d 0. Each pen was equipped with one GrowSafe bunk (GrowSafe systems Ltd., Airdrie, Alberta, CA), and an automatic waterer that was shared between two adjacent pens.

Lipopolysaccharide challenge

On d 16 for each group, BW were recorded and utilized to assign 2 steers per pen to one of three LPS challenge treatments to be given on the morning of d 17: 1) SALINE: 2.5-3 mL of 0.9% physiological saline (VetOne; MWI Veterinary Supply, Meridian, ID, USA) injected intravenously (IV) at the same volume that would have been injected in LPS treatments based on steer BW; 2) low dose LPS (E.coli O55:B5, Sigma-Aldrich, St. Louis, MO, USA; LOWLPS): 0.25 µg LPS/kg BW injected IV; 3) high dose LPS (HIGHLPS): 0.375 µg LPS/kg BW injected IV. Injection treatments were administered via jugular venipuncture using a winged infusion set that was pre-flushed with 2 mL of physiological saline. Upon administration of injection treatment, 6 mL of physiological saline was flushed through the infusion set. The LPS solution was made by dissolving LPS in sterile saline at a concentration of
50 µg/mL and passing through a 0.2 µm sterile non-charged syringe filter (Thermo Scientific, Waltham, MA, USA).

Rectal temperature (RT) and respiration rate (RR) were recorded on d 16. Respiration rates were determined by two individuals recording the visual respirations of each steer for 15 s while the steer stood in a chute, and the average of the two observed results were calculated and multiplied by 4 to determine the RR in breaths per minute. Baseline blood samples were also collected on d 16 via jugular venipuncture into either sodium heparin tubes, K2 EDTA tubes, or TM grade EDTA tubes for plasma, tubes with no additive for serum, or K2 EDTA tubes for whole blood (Becton Dickson and Company, Franklin Lakes, NJ, USA). Samples for serum were allowed to clot at room temperature for at least 2 h while samples for plasma were immediately placed on ice, until centrifugation at 1200 × g for 10 min.

On the morning of d 17 steers were weighed again immediately prior to treatment injections being given, to ensure consistency with d 16 BW that was used for LPS dose determination. Upon administration of the injection treatment steers were allowed to return to their home pens. Approximately 6 h following injection treatment administration on d 17, steers were briefly brought to the working facility for determination of RR, RT, and for blood collection as previously described. On the morning of d 18 and 19 (approximately 24 and 48 h post injection treatment, respectively) steers were weighed, RT recorded, and blood again collected as previously described. Steers were weighed again on d 24 to end the trial.

Steers were fitted with ear-tag based accelerometers (CowManager SensOor; Agis, Harmelen, Netherlands) that recorded activity, and determined time cattle spent being non-active, active, highly active, as well as time spent eating, and ruminating, and ear surface temperature. Data
from the 24 h challenge period (d 17) was used for assessing the effects of dietary and injectable treatment on behavior as assessed by the ear-tag accelerometers.

**Blood sample analysis**

Plasma TM concentrations (Cu, Fe, and Zn) were determined from samples collected into TM grade EDTA tubes on d 16, 17, 18, and 19. A subset of steers was used for the analysis of plasma TM, which included one randomly selected steer/injection treatment within a pen (n = 3 steers/pen; n = 8 steers/treatment combination in total). Sample preparation and TM analysis of plasma was conducted as described by Pogge et al. (2014). Whole blood samples collected on d 17 were refrigerated at 4°C overnight and transported on ice to the Iowa State University Veterinary Diagnostic Laboratory for complete blood count (CBC) analysis with automated differential, for determination of blood cell populations. Serum haptoglobin concentrations were determined from serum collected at approximately -24, 24, and 48 h relative to injection treatment administration using a commercially available kit (Life Diagnostics, West Chester, PA, USA).

**Feed analysis and determination of dry matter intake**

Weekly TMR samples were collected throughout the trial for each dietary treatment. The TMR samples were dried in a forced-air oven for 48 h for DM determination. Daily DMI was determined by applying a DM correction to daily individual animal as-fed feed disappearance values collected via GrowSafe bunks. Individual daily dry matter intake (DMI) are reported for d 15, 16, 17, 18, and 19, to determine pre-challenge DMI (d 15,16) and assess the effects of the LPS challenge (d 17-19) on DMI. Dried TMR samples were ground to fit through a 2-mm screen in a Retsch ZM 100 grinding mill (Retsch GmbH, Haan, Germany). A composite sample was made for each dietary treatment within each group from the weekly dried and ground TMR samples. Composite samples
were sent to Dairyland Laboratories (Arcadia, WI, USA) for wet chemistry analysis to determine organic matter (method 942.05; AOAC, 1996), crude protein (method 990.03; AOAC, 1996), ether extract (method 920.39; AOAC, 1996), and neutral detergent fiber (method 2002.04; AOAC, 2005). For TM analysis a 1.0 g sample of the dried and ground TMR composites were microwave digested (CEMS MARSXpress, Matthews, NC, USA) with 5 mL of TM grade nitric acid, following digestion sampled were diluted to 10% nitric acid with deionized water and analyzed via inductively coupled plasma atomic emission spectrometry (Optima 7000 DV, PerkinElmer, Waltham, MA, USA)

**Statistical analysis**

Body weight, DMI, RT, plasma TM, and serum haptoglobin were analyzed as repeated measures using the Mixed procedure of SAS (SAS version 9.4, SAS Inst. Inc., Cary, NC). Steer was the experimental unit and the model included the fixed effects of group, diet, injection, and time and the interactions among diet, injection and time. Day of trial (time) was the repeated effect. Day 0 BW were used as a covariate in the analysis of BW and DMI data. Except for serum haptoglobin, diet× injection × time was not significant for any variable (P ≥ 0.23) and was removed from those models. Covariance structures were selected based on the lowest corrected Akaike Information Criterion and were heterogeneous Toeplitz for RT and DMI, unstructured for BW data and serum haptoglobin, and autoregressive (1) for plasma TM concentrations. Rectal temperature and serum haptoglobin data were log transformed to meet normality assumptions, and back transformed means and SEM are presented. Day 17 RR were covariate adjusted using d 16 RR. The CBC data were analyzed using the using the Mixed procedure of SAS, with steer as the experimental unit and the fixed effects of diet, injection treatment and group, and the interaction of diet and injection treatment. Behavioral data recorded from ear-tag-based accelerometers were recorded as
minutes/hour. Area under the curve (AUC) analysis (GraphPad Prism 8, Graph Pad Software, San Diego, CA, USA) was performed on hourly behavior data from the 24 h challenge day (d 17). Area under the curve values for each behavior was analyzed using the Mixed procedure of SAS. All data were examined for outliers using Cook’s D. Significance was declared at $P \leq 0.05$ and tendencies were declared as $P \geq 0.06$, but $\leq 0.10$.

Results

There were no diet × injection × time, diet × time, injection × time, or diet × injection effects for BW ($P > 0.10$; Figure 1A). There was a group effect for BW, where group one (337 ± 1.6 kg) was heavier than group two (329 ± 1.7 kg; $P = 0.001$). There were no diet × injection × time, diet × time, or diet × injection effects ($P \geq 0.10$) for DMI. There was an injection × time effect for challenge period DMI ($P < 0.01$; Figure 1B), where DMI was similar across treatments for d 15, 16, 18 and 19 ($P \geq 0.10$); while on challenge day (d 17) HIGHLPS and LOWLPS were similar and lesser than SALINE ($P < 0.001$). There was a group effect for DMI ($P = 0.02$), where group one (7.46 ± 0.19 kg/d) had lesser DMI during the challenge period than group two (8.12 ± 0.20 kg/d).

There were no diet × injection × time, diet × time, diet × injection, or group effects for RT ($P \geq 0.10$). There was an injection × time effect ($P = 0.04$; Figure 1C) where RT were similar ($P \geq 0.10$) 24 h prior to injection, while at 6, 24, and 48 h post injection RT in HIGHLPS and LOWLPS were similar ($P \geq 0.10$). At 6 and 24 h post injection RT were greater ($P < 0.05$), and at 48 h post injected, tended to be greater ($P = 0.10$) in HIGHLPS compared to SALINE. However, LOWLPS tended to be greater ($P < 0.10$) than SALINE at 6 h post injection but was similar to SALINE at 24 and 48 h post injection ($P > 0.10$). There was a tendency ($P = 0.10$; Figure 1D) for a diet × injection effect for 6 h post injection RR where within Zn100, HIGH and LOWLPS treatments were greater than SALINE ($P < 0.05$), while no
injection effects within Zn30 were noted ($P > 0.10$). There was also a group effect for RR ($P = 0.006$), where group one ($46 \pm 1.9$ breathes/min) was lesser than group two ($54 \pm 2.0$ breathes/min).

There were no diet × injection × time effects ($P > 0.10$) for plasma TM concentrations. There were no diet × time, injection × time, diet × injection, diet or injection effects for plasma Cu ($P > 0.10$; data not shown). Plasma Cu concentrations were affected by time ($P < 0.001$) relative to injection treatment administration, where plasma Cu concentrations were lesser 6 h post injection compared to all other timepoints and averaged 0.98, 0.93, 0.98, and 1.01 $\pm$ 0.024 mg/L for -24, 6, 24, and 48 h relative to injection, respectively. Plasma Cu concentrations were also lesser in group two ($0.88 \pm 0.034$ mg/L) compared to group one ($1.11 \pm 0.034$; $P < 0.01$). There was no diet × time or diet × injection effect for plasma Fe ($P > 0.10$). There was an injection × time effect ($P = 0.001$; Figure 2A) for plasma Fe where 24 h prior to injection, concentrations were lesser in LOWLPS compared to SALINE ($P = 0.05$), but were similar across other treatments ($P > 0.10$), while 6 h after injection, Fe concentrations were similar between treatments ($P > 0.10$). At 24 and 48 h after injection, plasma Fe concentrations were decreased in both LPS treatments compared to SALINE ($P < 0.05$).

There was no diet × injection effect for plasma Zn concentrations ($P = 0.16$). There was an injection × time effect ($P < 0.01$; Figure 2B) for plasma Zn where 24 h prior to injection, concentrations were lesser in LOWLPS compared to SALINE ($P = 0.004$), but were similar in all other treatments ($P > 0.10$). Regardless of Zn treatment, LOW and HIGHLPS plasma Zn concentrations were decreased 6 and 24 h post injection compared to SALINE ($P < 0.001$), and tended to be lesser ($P = 0.07$) in HIGHLPS compared to SALINE at 48 h post injection, while LOWLPS was similar ($P = 0.11$) to SALINE at 48 h post injection. There was a diet × time effect ($P = 0.03$; Figure 2C) for plasma Zn where concentrations were decreased ($P < 0.001$) at 6 h post injection but by 48 h post injection concentrations were greater ($P = 0.01$) in Zn100 compared to Zn30.
There was a diet × injection × time effect for serum haptoglobin concentrations ($P = 0.03$; Figure 2D). Serum haptoglobin concentrations were similar across all treatments at -24 h relative to injection ($P > 0.10$). At 24 and 48 h post injection haptoglobin concentrations were increased in Zn100-SALINE compared to Zn30-SALINE ($P < 0.01$). However, at 24 h post injection SALINE treatments had lesser haptoglobin concentrations than LPS-treated steers ($P < 0.01$). At 48h post injection Zn30-HIGHLPS had increased haptoglobin concentrations compared to Zn100-LOWLPS ($P = 0.04$), whereas Zn100-HIGHLPS and Zn30-LOWLPS were intermediate ($P = 0.97$). Serum haptoglobin concentrations were lesser in Zn30-SALINE than all LPS-treated steers ($P < 0.001$), and Zn100-SALINE had lesser haptoglobin concentrations than Zn30-HIGHLPS, Zn100-HIGHLPS, and Zn30-LOWLPS ($P < 0.01$), and tended to be lesser than Zn100-LOWLPS ($P = 0.07$) at 48 h post injection administration.

Table 3 reports CBC data measured at 6 h post injection. There was a diet × injection effect ($P = 0.01$) for hemoglobin concentrations in which Zn100-HIGHLPS was lesser than Zn30-HIGHLPS and Zn100-SALINE ($P < 0.05$). However, Zn30-HIGHLPS tended to be greater than Zn30-LOWLPS ($P = 0.06$). There was a diet × injection effect ($P = 0.007$) for hematocrit, where within Zn100, HIGHLPS tended to be lesser ($P = 0.07$) than LOWLPS and was lesser than SALINE ($P = 0.02$), and within Zn30, HIGHLPS tended to be greater than LOWLPS and SALINE ($P < 0.10$). Furthermore, Zn100-HIGHLPS was lesser than Zn30-HIGHLPS ($P = 0.006$) while all other treatment combinations were similar ($P \geq 0.15$).

There was a diet × injection effect for lymphocytes ($P = 0.04$), where Zn100-SALINE was greater than Zn30-SALINE ($P = 0.02$), but lymphocytes were lesser in LPS treatments regardless of dietary treatment ($P < 0.001$). Furthermore, the HIGHLPS treatments tended to be lesser than Zn30-LOWLPS ($P < 0.10$), and all other treatments were similar ($P > 0.10$). There was a diet × injection effect ($P = 0.03$) for monocytes, where monocytes were greatest in SALINE regardless of dietary
treatment ($P < 0.001$), Zn30-LOWLPS and Zn100 LPS treatments were similar ($P > 0.10$) while monocytes were lesser in Zn30-HIGHLPS ($P < 0.01$). There was a tendency ($P = 0.08$) for a diet × injection effect for eosinophils, where eosinophils tended to be lesser in Zn100-HIGHLPS compared to Zn30-HIGHLPS ($P = 0.07$) and within Zn30, HIGHLPS tended ($P = 0.07$) to be greater than LOWLPS and was greater than SALINE ($P = 0.01$). All other diet and treatment combinations were similar ($P > 0.10$). There was a diet × injection effect for the neutrophil to lymphocyte ratio (NLR; $P = 0.04$) where within Zn100 NLR was greater in LPS treatments vs. SALINE ($P < 0.05$), while within Zn30, NLR was similar across injection treatment ($P > 0.10$). There was an injection effect for white blood cells, platelets, neutrophils, and basophils where all decreased due to LPS treatments ($P < 0.001$) but were not affected by dietary Zn treatment ($P > 0.10$).

Ear tag based accelerometer data are reported in Table 4. There was a tendency for a diet × injection effect ($P = 0.08$) for AUC for the time spent classified as non-active in which within Zn30 HIGHLPS and LOWLPS exhibited greater time being non-active compared to SALINE ($P < 0.001$), but were similar to each other ($P = 0.14$). There were no diet × injection effects for any other accelerometer data ($P > 0.10$). Within Zn100 HIGHLPS spent more time non-active compared to SALINE ($P < 0.001$), but was similar to LOWLPS ($P = 0.19$), but LOWLPS tended to be greater than SALINE ($P = 0.08$). Time spent active AUC was not affected by diet, injection, or the interaction ($P > 0.10$). There was an injection effect for time spent highly active AUC in which HIGHLPS and LOWLPS were lesser than SALINE ($P < 0.001$). Steers within HIGHLPS and LOWLPS had lesser AUC values for time spent eating compared to SALINE (injection $P = 0.004$), and Zn30 steers tended to have a greater AUC for time spent eating compared to Zn100 (diet $P = 0.06$). For time spent ruminating HIGHLPS had the lowest AUC, LOWLPS were intermediate, and SALINE had the greatest AUC (injection $P < 0.001$). Ear surface temperature AUC was unaffected by injection ($P = 0.25$) but was greater in Zn100 vs. Zn30 (diet $P = 0.004$).
Discussion

Bovine respiratory disease is estimated to cost the US feedlot industry 908 million USD due to death loss (Peel, 2020). Injection of LPS can be utilized as a model to induce an inflammatory response similar to that caused by bovine respiratory disease (Carroll et al., 2009b). Zinc is critical in the immune system, and providing 100 mg of supplemental Zn/kg DM tended to decrease morbidity due to respiratory disease in newly weaned steers (Galyean et al., 1995). Current requirements to prevent Zn deficiency in beef cattle were established at 30 mg Zn/kg DM (NASEM, 2016), but a survey of consulting feedlot nutritionists has suggested Zn is often supplemented at 100 mg Zn/kg DM (Samuelson et al., 2016). This research was conducted to evaluate the response to LPS in steers receiving diets supplemented with Zn based on either the NASEM (2016; 30 mg Zn/kg DM) or industry recommendations (100 mg Zn/kg DM).

Cattle in the present study exhibited hallmark responses to LPS, including decreased DMI (McMahon et al., 1998), increased RR (Carroll et al., 2009b; Plessers et al., 2015), and increased RT (Carroll et al., 2009a). That DMI was not impacted by dietary Zn treatment is not surprising, as steers in both dietary treatments had similar and adequate Zn status based on plasma Zn concentrations (Kincaid, 2000). Due to the transient effects of LPS, no differences were observed in BW in the present trial. Roberts et al. (2002) also noted no effect of supplemental Zn concentration on growth performance in response to an LPS challenge in pigs. However, marginally Zn deficient rats exhibited lesser BW gain in response to an LPS challenge (Shea-Budgell et al., 2006). Thus, it would be interesting to evaluate the effect of LPS administration on growth performance in animals with marginal Zn status.
In the present study RR was increased in LPS treated animals receiving the Zn100 diet, while RR was unaffected by injection treatment within the Zn30 treatment. Respiratory rates have increased in cattle 1 h post administration of LPS (Carroll et al., 2009b; Plessers et al., 2015); whereas in the present study RR was evaluated at 6 h post injection. The increase in RT 6 h post injection administration observed in LPS treated steers is in agreement with others (Carroll et al., 2009b; Carroll et al., 2015).

Decreased concentrations of blood leukocytes, neutrophils, lymphocytes, and monocytes are a signature response to LPS in beef cattle (Carroll et al., 2015), likely due to these cell types migrating to peripheral tissues (Cybulsky et al., 1988). However, an increased NLR has been associated with poorer outcomes in critically ill humans (De Jager et al., 2010). The NLR of healthy adult cattle should be 1:2 (Jones and Allison, 2007). In the present study, all means for NLR were ≥ 0.50 (1:2); however, within steers receiving LPS, the Zn100 treatment had an increased NLR compared to Zn30 animals. Due to the acute nature of the LPS challenge, it is not possible to assess the effects of the increased NLR in Zn100 LPS treated animals on growth performance and health.

Decreased monocyte concentrations in response to an endotoxin challenge in humans (Krabbe et al., 2001), can lead to decreased production of pro-inflammatory cytokines (Van der Poll et al., 1996). However, monocytes can also be stimulated by Zn to release pro-inflammatory cytokines (Haase and Rink, 2007). Steers in the Zn100-HIGHLPS group had greater concentrations of monocytes than steers in Zn30-HIGHLPS, which may suggest increased supplemental Zn is supporting the inflammatory response to LPS. However, it is unclear if this improvement in monocyte concentrations in the Zn100-HIGHLPS steers would result in a more rapid recovery to an inflammatory challenge.
The acute phase protein haptoglobin has been studied as a marker of acute and chronic inflammation or disease in cattle (Carroll et al., 2009b). Plasma haptoglobin concentrations were increased in beef steers receiving LPS relative to saline, 24 h relative to treatment administration and remained elevated through 72 h post treatment administration (Lippolis et al., 2017). Serum haptoglobin was increased by 24 h post injection in the present study. Work in dairy cows suggests timing of peak haptoglobin concentrations relative to LPS administration and concentration of haptoglobin is LPS dose dependent (Jacobsen et al., 2004). However, in the present study haptoglobin concentrations were increased in LPS steers 24 h post injection and were similar 48 h post injection, except for Zn30-HIGHLPS steers which were further increased at 48 h post injection. Interestingly, some monocytes are able to take up haptoglobin and store it within the cytoplasm (Wagner et al., 1996). In the present study Zn30-HIGHLPS steers had less than half the concentration of monocytes compared to other LPS treated animals. This may suggest monocyte uptake of haptoglobin is responsible for the decreased haptoglobin concentrations observed by other treatments at 48 h post injection. Steers in the Zn100-HIGHLPS exhibited a response similar to LOWLPS steers regardless of supplemental Zn concentration. This suggests increased supplemental Zn may help to attenuate the cellular immune response in cattle experiencing more severe illness.

To assess the effects of LPS on behavior, cattle were equipped with ear-tag-based accelerometers, which assign each minute to a behavior, based on proprietary algorithms (CowManager SensOor; Wolfger et al., 2015). These tags have been validated for eating and rumination behavior in healthy dairy cows but were unable to detect differences in activity level (Pereira et al., 2018). In the present study the ear-tag system was able to detect a difference in time spent eating in LPS treated animals relative to SALINE but not between the two LPS treatments, which was expected given the similar decrease in DMI of LPS treated animals. Technology capable of detecting decreased feed intake of cattle could be a useful tool for the cattle industry, as decreased
feed intake is commonly associated with many cattle disorders. Technology able to detect subtle changes in animal behavior and or physiology due to illness could help improve morbidity and mortality in beef production.

Although very few differences due to LPS were noted in blood parameters or DMI. The ear-tags were able to detect a difference between LOWLPS and HIGHLPS for time spent ruminating. This may suggest time spent ruminating is a more robust measure for detecting sickness in cattle. In dairy calves administered extremely low doses of LPS (0.025 vs 0.05 µg/kg BW), time spent ruminating was similar between LPS doses when determined by a human observer (Borderas et al., 2008). Decreased time spent ruminating is LPS dose dependent; thus, the magnitude of this decrease may affect a specific technologies ability to detect changes in time spent ruminating. This highlights the need for a greater understanding of the sensitivity of algorithms used to determine behavior in ear-tag based accelerometers. Early detection of illness is feedlot cattle is critical to limiting the economic impact of diseases such as bovine respiratory disease.

Cattle receiving LPS spent increased time classified as non-active, and less time being highly active than those receiving saline. Interestingly, there was no difference for time spent active between steers receiving LPS or saline. This may be due to the algorithms utilized by the ear-tags to determine behaviors not being sensitive enough to detect subtle differences in activity as compared to the extremes of non-active and highly active. Pereira et al. (2018) determined that time spent active as determined by CowManager SensOors was lowly correlated (r = 0.20) with visual observations. Thus, ear-tag based accelerometer technology may be more sensitive at detecting changes in certain behaviors such as time spent ruminating compared to other behaviors such as time spent active.
In response to the LPS challenge plasma TM data followed a classic immune response known as nutritional immunity. Nutritional immunity is described as decreased circulating concentrations of Fe and Zn and increased circulating concentrations of Cu in response to inflammation or a pathogen, resulting in the sequestration of TM that are critical to pathogen survival (Hood and Skaar, 2012). In the present study plasma Zn concentrations were markedly decreased in steers receiving LPS within 6 h of injection administration and remained depressed through 24 h after injection administration. It is interesting that plasma Zn concentrations exhibited a similar decrease regardless of dietary Zn or LPS concentrations. It is likely concentrations of interleukin-6 were also increased, as interleukin-6 induced up-regulation of the Zn importer ZIP-14 is responsible for the decrease in plasma Zn during nutritional immunity (Liuzzi et al., 2005). In response to the influx of Zn, the intracellular Zn binding protein, metallothionein (MT) is also upregulated (Liu et al., 1991).

In the present study, plasma Zn depletion was not affected by LPS injection concentration, which suggests this response was not further exacerbated by the more potent LPS concentration used in this study, and that the lesser dose of LPS used in this study was sufficient to induce nutritional immunity. In ZIP-14 knockout mouse models, LPS induced hypozincemia is not observed (Aydemir et al., 2012), highlighting the importance of ZIP-14 in LPS induced hypozincemia. However, future work is needed to understand the Zn transport mechanisms that are involved in the movement of extracellular Zn in response to immune activation in ruminants, and how this may be affected by supplemental Zn concentration.

Although plasma Zn concentrations were similar and adequate in both dietary treatments, based on the reference ranges developed by Kincaid (2000; adequate 0.8-1.4 mg/L), plasma Zn concentrations are not generally a sensitive indicator of TM status. Plasma Zn concentrations can be increased or decreased when the homeostatic mechanisms controlling plasma Zn are overwhelmed,
but subtle differences in Zn status may not be detected in plasma (Hambidge, 2003). The tendency for dietary Zn concentration to affect plasma Zn recovery following LPS challenge differentially suggests Zn100 steers may have had circulating Zn available for metabolic processes supporting growth more quickly than Zn30 steers. However, the biological relevance of this small change is unclear. Thus, future work should be conducted to 1) better understand the timing of fluctuations in plasma Zn in response to an LPS challenge, and 2) understand the implications of greater plasma Zn concentrations following immune activation, as it relates to animal growth and health.

Iron concentrations also decrease in circulation following immune challenge, resulting in increased concentrations of intracellular Fe and decreased plasma Fe. In rats treated with LPS, this decrease occurred at 8 h post LPS administration (Duvigneau et al., 2008). In the present study plasma Fe concentrations were unaffected by LPS at 6 h post injection administration, but were markedly decreased by 24 h. While ZIP-14 can also facilitate cellular uptake of non-transferrin bound Fe, increased transcript abundance of several proteins involved in Fe homeostasis, including hepcidin, divalent metal transporter-1, ferritin, and transferrin receptor-1 (Aydemir et al., 2012) may be responsible for LPS induced hypoferremia. Thus, it is likely the expression of the major proteins involved in maintaining Fe homeostasis were altered to sequester Fe in response to LPS injection in the present trial.

Administrating LPS to steers at two concentrations (0.25 or 0.375 µg LPS/kg BW) had similar effects on circulating TM concentrations, DMI, and BW. Thus, a single, lesser concentration of LPS can be utilized to induce symptoms of illness, and influence circulating TM concentrations. However, time spent ruminating was impacted by LPS concentration; suggesting concentration of LPS may impact some parameters of animal behavior. Feeding dietary Zn at industry recommendations had minimal impact on markers of illness or performance assessed in this study, possibly due to the
short duration of the LPS challenge. However, feeding increased concentrations of Zn to cattle prior to and during an LPS challenge may allow for a faster realignment of Zn homeostasis following an LPS challenge, based on rate of plasma Zn increases post challenge. Future work should evaluate proteins involved in transport and storage of TM would allow for a greater understanding of the mechanisms behind the LPS disruption of TM homeostasis, potentially allowing for more strategic TM supplementation strategies for sick cattle.

Disclosures

The authors have no conflicts of interest to disclose.

Literature Cited

Alexander, C., and E. T. Rietschel. 2001. Invited review: bacterial lipopolysaccharides and innate immunity. J. endotoxin res 7:167-202. doi: 10.1177/09680519010070030101.

Association of Official Analytical Chemists (AOAC). 1996. Official methods of analysis, 16th ed. AOAC Int., Rockville, MD, USA.

Association of Official Analytical Chemists (AOAC). 2005. Official methods of analysis, 18th ed. AOAC Int., Rockville, MD, USA.

Aydemir, T. B., S. M. Chang, G. J. Guthrie, A. B. Maki, A. B. Ryu, M. S. Karabiyik, A. B. Maki, M. S. Ryu, A. F. Karabiyik, and R. J. Cousins. 2012. Zinc transporter ZIP14 functions in hepatic zinc, iron and glucose homeostasis during the innate immune response (endotoxemia). PloS one. 7: e48679. doi: 10.1371/journal.pone.0048679.

Borderas, T. F., A. M. De Passillé, and J. Rushen. 2008. Behavior of dairy calves after a low dose of bacterial endotoxin. J. Dairy. Sci 86: 2920-2927. doi: 10.2527/jas.2008-0926.
Carroll, J. A., J. D. Arthington, and C. C. Chase Jr. 2009a. Early weaning alters the acute-phase reaction to an endotoxin challenge in beef calves. J. Anim. Sci. 87: 4167-4172. doi: 10.2527/jas.2009-2016.

Carroll, J. A., R. R. Reuter, C. C. Chase Jr., S. W. Coleman, D. G. Riley, D. E. Spiers, J. D. Arthington, and M. L. Galyean. 2009b. Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. Innate Immun. 15: 81-89. doi: 10.1177/1753425908099170.

Carroll, J. A., N. C. Burdick, R. R. Reuter, C. C. Chase Jr., D. E. Spiers, J. D. Arthington, and S. W. Coleman. 2011. Differential acute phase immune responses by Angus and Romosinuano steers following an endotoxin challenge. Domest. Anim. Endocrin. 41: 163-173. doi: 10.1016/j.domaniend.2011.06.002.

Carroll, J. A., N. C. Burdick Sanchez, L. E. Hulbert, M. A. Ballou, J. W. Dailey, L. C. Caldwell, R. C. Vann, T. H. Welsh Jr., and R. D. Randel. 2015. Sexually dimorphic innate immunological responses of pre-pubertal Brahman cattle following an intravenous lipopolysaccharide challenge. Vet. Immunol. Immunop.166: 108-115. doi: 10.1016/j.vetimm.2015.06.009.

Cybulsky, M. I., D. J. McComb, and H. Z. Movat, 1988. Neutrophil leukocyte emigration induced by endotoxin. Mediator roles of interleukin 1 and tumor necrosis factor alpha 1. J. Immunol. 140: 3144-3149.

De Jager, C. P., P. T. Van Wijk, R. B. Mathoera, J. De Jongh-Leuvenink, T. Van Der Poll, and P. C. Wever. 2010. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. Crit. Care. 14: R192. doi: 10.1186/cc9309.

Duff, G. C., and M. L.Galyean. 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85: 823-840. doi: 10.2527/jas.2006-501.

Duvigneau, J. C., C. Piskernik, S. Haindl, B. Kloesch, R. T. Hartl, M. Hüttemann, I. Lee, T. Ebel, R. Moldzio, M. Gemeiner. 2008. A novel endotoxin-induced pathway: upregulation of heme oxygenase 1, accumulation of free iron, and free iron-mediated mitochondrial dysfunction. Lab. Invest. 88: 70-77. doi: 10.1038/labinvest.3700691.
Evans, S. S., E. A. Repasky, and D. T. Fisher, D. T. 2015. Fever and the thermal regulation of immunity: the immune system feels the heat. Nature Rev. Immunol. 15: 335. doi: 10.1038/nri3843.

Fernandez, M. S., S. C. Pearce, N. K. Gabler, J. F. Patience, M. E. Wilson, M. T. Socha, J. L. Torrison, R. P. Rhoads, and L. H. Baumgard. 2014. Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. Animal 8, 43-50. doi: 10.1017/S1751731113001961.

Ferran, A. A., P. L. Toutain, and A. Bousquet-Melou. 2011. Impact of early versus later fluoroquinolone treatment on the clinical, microbiological and resistance outcomes in a mouse-lung model of Pasteurella multocida infection. Vet. Microbiol. 148: 292-297. doi: 10.1016/j.vetmic.2010.09.005.

Galyean, M. L., S. A. Gunter, R. A. Berrie, K. J. Malcolm-Callis, F. K. Brazle, and H. W. Essig. 1995. Effects of zinc source and level and added copper lysine in the receiving diet on performance by growing and finishing steers. Prof. Anim. Sci. 11: 139-148. doi: 10.15232/S10807446(15)32578-X.

Haase, H., and L. Rink. 2007. Signal transduction in monocytes: the role of zinc ions. Biometals 20: 579-585. doi: 10.1007/s10534-006-9029-8.

Hambidge, M. 2003. Biomarkers of trace mineral intake and status. J. Nutr. 133:48S–955S. doi: 10.1093/jn/133.3.948S.

Hiss, S., M. Mielzen, R. M. Bruckmaier, and H. Sauerwein. 2004. Haptoglobin concentrations in blood and milk after endotoxin challenge and quantification of mammary Hp mRNA expression. J. Dairy. Sci. 87: 3778-3784. doi: 10.3168/jds.S0022-0302(04)73516-X.

Hood, M. I., and E. P. Skaar. 2012. Nutritional immunity: transition metals at the pathogen–host interface. Nat. Rev. Microbiol. 10: 525. doi: 10.1038/nrmicro2836.

Jacobsen, S., P. Andersen, T. Toelboell, and P. M. Heegaard. 2004. Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. J. Dairy. Sci. 87: 3330-3339. doi: 10.3168/jds.S0022-0302(04)73469-4.
Janzen, E. D., P. H. G. Stockdale, S. D. Acres, and L. A. Babiuk. 1984. Therapeutic and prophylactic effects of some antibiotics on experimental pneumonic pasteurellosis. Can. Vet. J. 25: 78.

Johnson, K. K., and D. L. Pendell. 2017. Market impacts of reducing the prevalence of bovine respiratory disease in United States beef cattle feedlots. Frontiers. in. Vet. Sci. 4: 189. doi: 10.3389/fvets.2017.00189.

Jones, M. L., and R. W. Allison. 2007. Evaluation of the ruminant complete blood cell count. Vet. Clin. N Am-Food A. 23: 377-402. doi: 10.1016/j.cvfa.2007.07.002.

Kaplan, J. H., and S. Lutsenko. 2009. Copper transport in mammalian cells: special care for a metal with special needs. J. Biol. Chem. 284: 25461-25465. doi: 10.1074/jbc.R109.031286.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. J. Anim. Sci. 77: 1-10. doi: 10.2527/jas2000.77E-Suppl1x.

Krabbe, K. S., H. Bruunsgaard, C. M. Hansen, K. Møller, L. Fonsmark, J. Qvist, P. L. Madsen, G. Kronborg, H. O. Andersen, and P. Skinhøj, P. 2001. Ageing is associated with a prolonged fever response in human endotoxemia. Clin. Diagn. Lab. Immunol. 8: 333-338. doi: 10.1128/CDLI.8.2.333-338.2001.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2016.. A procedure to estimate glucose requirements of an activated immune system in steers. J. Anim. Sci., 94: 4591-4599. doi: 10.2527/jas.2016-0765.

Lippolis, K. D., R. F. Cooke, K. M. Schubach, R. S. Marques, and D. W. Bohnert. 2017. Effects of intravenous lipopolysaccharide administration on feed intake, ruminal forage degradability, and liquid parameters and physiological responses in beef cattle. J. Anim. Sci., 95: 2859-2870. doi: 10.2527/jas.2017.1502.

Liu, J., Y. P. Liu, L. Sendelbach, and C. Klaassen. 1991. Endotoxin induction of hepatic metallothionein is mediated through cytokines. Toxicol. Appl. Pharm. 109: 235-240. doi: 10.1016/0041-008X(91)90171-A.
Liuzzi, J. P., L. A. Lichten, S. Rivera, R. K. Blanchard, T. B. Aydemir, M. D. Knutson, T. Ganz, and R. J. Cousins. 2005. Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. P. Natl. Acad. Sci. 102: 6843-6848. doi: 10.1073/pnas.0502257102.

McMahon, C. D, T. H. Elsasser, D. R. Gunter, L. G. Sanders, B. P. Steele, and J. L. Sartin. 1998. Estradiol/progesterone implants increase food intake, reduce hyperglycemia and increase insulin resistance in endotoxic steers. J. Endocrinol. 159: 469-478. doi: 10.1677/joe.0.1590469.

Mogensen, T. H. 2009. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin. Microbiol. Rev., 22: 240-273. doi: 10.1128/CMR.00046-08.

National Academies of Sciences, Engineering, and Medicine. 2016. Nutrient requirements of beef cattle. National Academies Press, Washington, DC.

Netea, M. G., B. J. Kullberg, and J. W. Van der Meer. 2000. Circulating cytokines as mediators of fever. Clin. Infect. Dis. 31: S178-S184. doi: 10.1086/317513.

Niedermayer, E. K., O. N. Genther-Schroeder, D. D. Loy, and S. L. Hansen. 2018. Effect of varying trace mineral supplementation of steers with or without hormone implants on growth and carcass characteristics. J. Anim. Sci. 96: 1159-1170. doi: 10.1093/jas/skx063.

Orr, C. L., D. P. Hutcheson, R. B. Grainger, J. M. Cummins, and R. E. Mock. 1990. Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. J. Anim. Sci., 68: 2893-2900. doi: 10.2527/1990.6892893x.

Peel, D. S. 2020. The effect of market forces on bovine respiratory disease. Vet. Clin. Food. Anim. 36: 497-508. doi: 10.1016/j.cvfa.2020.03.008.

Pereira, G. M., B. J. Heins, and M. I. Endres. 2018. Validation of an ear-tag accelerometer sensor to determine rumination, eating, and activity behaviors of grazing dairy cattle. J. Dairy. Sci., 101: 2492-2495. doi: 10.3168/jds.2016-12534.

Plessers, E., H. Wyns, A. Watteyn, B. Pardon, P. De Backer, and S. Croubels. 2015. Characterization of an intravenous lipopolysaccharide inflammation model in calves with respect to the acute-phase response. Vet. Immunol. Immunop. 163: 46-56. doi: 10.1016/j.vetimm.2014.11.005.
Pogge, D. J., M. E. Drewnoski, and S. L. Hansen. 2014. High dietary sulfur decreases the retention of copper, manganese, and zinc in steers. J. Anim. Sci. 92: 2182-2191. doi: 10.2527/jas.2013-7481.

Roberts, E. S., E. V. Heugten, K. Lloyd, G. W. Almond, and J. W. Spears. 2002. Dietary zinc effects on growth performance and immune response of endotoxemic growing pigs. Asian-australasian journal of animal sciences 15: 1496-1501. doi: 10.5713/ajas.2002.1496.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: the 2015 New Mexico State and Texas Tech University survey. J. Anim. Sci. 94: 2648-2663. doi: 10.2527/jas.2016-0282.

Shea-Budgell, M., M. Dojka, M. Nimmo, D. Lee, and Z. Xu. 2006. Marginal zinc deficiency increased the susceptibility to acute lipopolysaccharide-induced liver injury in rats. Exp. Biol. Med. 231: 553-558. doi: 10.1177/153537020623100509.

Suttle, N. 2010. Mineral Nutrition of Livestock, 4th ed. CABI. Wallingford, UK.

USDA. 2013. Feedlot 2011 Part IV: Management Practices on U.S. Feedlots with a Capacity of 1,000 or More Head. USDA-APHIS-VS-CEAH-NAHMS, Fort Collins, CO.

Van Der Poll, T., S. M. Coyle, L. L. Moldawer, and S. F. Lowry. 1996. Changes in endotoxin-induced cytokine production by whole blood after in vivo exposure of normal humans to endotoxin. J. Infect. 174: 1356-1359. doi: 10.1093/infdis/174.6.1356.

Wan, Y., M. J. Petris, and S. C. Peck. 2014. Separation of zinc-dependent and zinc-independent events during early LPS-stimulated TLR4 signaling in macrophage cells. FEBS letters, 588: 2928-2935. doi: 10.1016/j.febslet.2014.05.043.

Wagner, L., A. Gessl, S. B. Parzer, W. Base, W. Waldhäusl, and M. S. Pasternack. 1996. Haptoglobin phenotyping by newly developed monoclonal antibodies. Demonstration of haptoglobin uptake into peripheral blood neutrophils and monocytes. J. Immunol. 156: 1989-1996.

Wolfger, B., E. Timsit, E. A. Pajor, N. Cook, H. W. Barkema, and K. Orsel. 2015. Accuracy of an ear tag-attached accelerometer to monitor rumination and feeding behavior in feedlot cattle. J. Anim. Sci. 93: 3164-3168. doi: 10.2527/jas.2014-8802.
Zähringer, U., B. Lindner, and E. T. Rietschel. 1999. Chemical structure of lipid A: recent advances in structural analysis of biologically active molecules. Endotoxin in health and disease 93.
Table 1. Experimental timeline

| Day\(^1\) | Activity\(^2\) |
|-----------|----------------|
| -1        | BW             |
| 0         | BW, assign to and start dietary treatments |
| 16        | BW, blood, RT, RR, assign to injection treatments |
| 17 (challenge) | BW, injection treatment administration; 6 h post injection administration: blood, RT, RR |
| 18 (1 d)  | BW, blood, RT |
| 19 (2 d)  | BW, blood, RT |
| 24        | BW             |

\(^1\) Day is relative to the start of each group, and the two groups were started on the experimental timeline 14 d apart, values in parenthesis are d relative to challenge.

\(^2\) BW: body weight, RT: rectal temperature, RR: respiration rate.
Table 2. Common total mixed ration (TMR)

| Ingredient                                           | DM, % |
|------------------------------------------------------|-------|
| Corn silage                                          | 40    |
| Sweet Bran¹                                          | 40    |
| Dried distillers grains with solubles                | 10    |
| Vitamin and mineral pre-mix²                         | 5     |
| Supplemental Zn pre-mix³                             | 5     |
| Analyzed composition⁴                                |       |
| DM                                                   | 54.0  |
| OM                                                   | 93.3  |
| NDF                                                  | 32.6  |
| CP                                                   | 17.7  |
| EE                                                   | 3.87  |
| Zn, mg/kg diet DM⁵                                    | 81    |

¹Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE).

²Vitamin and mineral pre-mix provided per kilogram of diet DM: 0.15 mg Co (cobalt carbonate), 20 mg Mn (manganese sulfate), 0.1 mg Se (sodium selenite), 30 mg Zn (zinc sulfate), 0.5 mg I (calcium iodate). Remaining contributed (as % of total diet DM): dried distillers grains 3.04%, limestone 1.5%, vitamin A and E premix 0.11% (2.200 IU vitamin A and 25 IU vitamin E), salt 0.31%, urea 0.3%, Rumensin 90 0.015%

³Supplemental Zn pre-mix provided 5% diet DM as dried distillers grains with solubles and contributed 70 mg supplemental Zn/kg DM from ZnSO₄ to the Zn100 dietary treatment. The Zn30 dietary treatment received 5% diet DM as dried distillers grains in place of the supplemental Zn pre-mix.

⁴DM and Zn analysis was performed by the Hansen laboratory, and OM, NDF, CP, and EE were determined by Dairyland Laboratories (Arcadia, WI, USA).

⁵Analysis shown for Zn30, Zn100 analyzed at 139 mg Zn/kg DM.
Table 3. Effect of supplemental Zn concentration and lipopolysaccharide injection on complete blood counts (CBC) approximately 6 h after injection administration.

| Item                  | SALINE<sup>1</sup> | LOWLPS<sup>1</sup> | HIGHLPS<sup>1</sup> | SALINE<sup>1</sup> | LOWLPS<sup>1</sup> | HIGHLPS<sup>1</sup> | SEM   | Diet | Injection | D × I |
|-----------------------|---------------------|--------------------|----------------------|---------------------|--------------------|----------------------|-------|------|-----------|-------|
| Whole blood           |                     |                    |                      |                     |                    |                      |       |      |           |       |
| WBC, 10<sup>3</sup>/µl<sup>3,4</sup> | 11.32<sup>a</sup>   | 5.83<sup>b</sup>   | 4.32<sup>c</sup>     | 12.12<sup>a</sup>   | 4.65<sup>b</sup>   | 4.65<sup>c</sup>     | 0.542 | 0.84 | <0.001   | 0.66  |
| Neutrophils, 10<sup>3</sup>/µl<sup>3,4</sup> | 3.72<sup>a</sup>    | 2.05<sup>b</sup>   | 1.42<sup>c</sup>     | 3.42<sup>a</sup>    | 2.16<sup>b</sup>   | 1.82<sup>c</sup>     | 0.208 | 0.53 | <0.001   | 0.49  |
| Lymphocytes, 10<sup>3</sup>/µl | 5.98<sup>b</sup>    | 3.42<sup>c</sup>   | 2.44<sup>c</sup>     | 7.27<sup>a</sup>    | 2.68<sup>c</sup>   | 2.53<sup>c</sup>     | 0.422 | 0.50 | <0.001   | 0.03  |
| NLR<sup>5</sup>       | 0.65<sup>bc</sup>   | 0.78<sup>ab</sup>  | 0.63<sup>bc</sup>    | 0.50<sup>c</sup>    | 0.92<sup>a</sup>   | 0.88<sup>a</sup>     | 0.136 | 0.22 | 0.003    | 0.04  |
| Monocytes, 10<sup>3</sup>/µl<sup>4</sup> | 0.67<sup>a</sup>    | 0.17<sup>b</sup>   | 0.05<sup>c</sup>     | 0.82<sup>a</sup>    | 0.15<sup>b</sup>   | 0.13<sup>b</sup>     | 0.079 | 0.07 | <0.001   | 0.04  |
| Respiration rate, BPM<sup>6</sup> | 51.3<sup>xy</sup>   | 48.6<sup>xy</sup>  | 49.2<sup>xxy</sup>   | 43.7<sup>x</sup>    | 54.4<sup>x</sup>   | 53.8<sup>y</sup>     | 3.40  | 0.74 | 0.40      | 0.10  |

<sup>1</sup>Zn30: 30 mg supplemental Zn/kg DM; Zn100: 100 mg supplemental Zn/kg DM; SALINE: physiological saline I.V. on d 17; LOWLPS: 0.25 µg LPS/kg BW on d 17, HIGHLPS: 0.375 µg LPS/kg BW on d 17.

<sup>2</sup>D × I: diet × injection.

<sup>3</sup>Means within a row with differing superscripts were different (a,b,c; P ≤ 0.05), or tended to be different (x, y,z; P > 0.06, but < 0.10).

<sup>4</sup>Data have been log transformed, and back transformed means and SE are presented.

<sup>5</sup>Neutrophil to lymphocyte ratio, calculated as neutrophil concentration/lymphocyte concentration. Two steers were removed as outliers.

<sup>6</sup>BPM: Breaths per minute
Table 4. Effect of supplemental Zn concentration and lipopolysaccharide injection on behavior as determined by an ear-tag based accelerometer.

| Behavior, AUC | Diet 1 | Injection 2 | P-value 3 |
|--------------|--------|-------------|-----------|
|              | Zn30   | Zn100       | SEM       | SALINE   | LOWLPS   | HIGHLPS   | SEM       | Diet    | Inj. | D × I |
| Non-active   | 616    | 597         | 22.7      | 466      | 640      | 713       | 26.8      | 0.53    | <0.001 | 0.08  |
| Active       | 170    | 186         | 7.5       | 166      | 188      | 180       | 9.0       | 0.13    | 0.22   | 0.68  |
| Highly active| 160    | 169         | 8.5       | 208a     | 145b     | 140b      | 10.2      | 0.40    | <0.001 | 0.17  |
| Eating       | 38.9   | 24.1        | 5.62      | 49.8a    | 25.3b    | 19.5b     | 6.76      | 0.06    | 0.004  | 0.49  |
| Rumination   | 391    | 398         | 18.7      | 488a     | 381b     | 314c      | 22.5      | 0.79    | <0.001 | 0.54  |
| Surface ear temperature | 431    | 471         | 9.9       | 458      | 460      | 436       | 11.9      | 0.004   | 0.25   | 0.71  |

1Zn30: 30 mg supplemental Zn/kg DM; Zn100: 100 mg supplemental Zn/kg DM.

2SALINE: physiological saline I.V. on d 17; LOWLPS: 0.25 µg LPS/kg BW on d 17, HIGHLPS: 0.375 µg LPS/kg BW on d 17.
3 D × I: diet × injection.

4 AUC: Area under the curve.

5 D × I: Zn30 HIGHLPS and LOWLPS exhibited greater non-active AUC compared to SALINE ($P < 0.001$), but were similar to each other ($P = 0.14$).
Figure 1. Influence of injection (SALINE, LOWLPS: 0.25 μg LPS/kg BW or HIGHLPS: 0.375 μg LPS/kg BW), dietary treatment (30 or 100 mg supplemental Zn/kg DM) and day of study on A) body weight (P ≥ 0.10). Influence of injection treatment and time relative to injection treatment administration on B) DMI (P < 0.01), and C) rectal temperature (P = 0.04). Effect of injection treatment and dietary treatment (P = 0.10) on D) respiration rate determined 6 h after injection administration. Within a panel, data with differing superscripts are different (a, b, c; P ≤ 0.05) or tend to be different (x, y, z; 0.06 ≤ P ≤ 0.10).

Figure 2. Influence of injection treatment (SALINE, LOWLPS: 0.25 μg LPS/kg BW or HIGHLPS: 0.375 μg LPS/kg BW) and time relative to injection treatment administration on plasma trace mineral concentrations: A) plasma Zn (P < 0.001), B) plasma Fe (P < 0.001). Dietary treatment (30 or 100 mg supplemental Zn/kg DM) and time relative to injection treatment administration effect on: C) plasma Zn concentrations (P = 0.06). Influence of injection and dietary treatment combination and time relative to injection treatment administration on serum haptoglobin concentrations: D) diet × injection × time; P = 0.03.
