SHORT COMMUNICATION

Response of lactating dairy cows fed different supplemental zinc sources with and without evaporative cooling to intramammary lipopolysaccharide infusion: metabolite and mineral profiles in blood and milk

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Abstract

The objective of this study was to determine the effect of evaporative cooling and dietary supplemental Zn source on blood metabolites, insulin and mineral concentrations, and milk mineral concentrations following intramammary lipopolysaccharide (LPS) infusion. Seventy-two multiparous Holstein cows were assigned to one of four treatments with a 2 × 2 factorial arrangement. Treatments included two environments: with or without evaporative cooling using fans and misters over the freestall and feedbunk, and two dietary sources of supplemental Zn: 75 mg/kg of dry matter (DM) supplied by Zn hydroxychloride (inorganic Zn; IOZ) or Zn hydroxychloride (35 mg of Zn/kg of DM) + Zn–Met complex (ZMC; 40 mg of Zn/kg of DM). A subset of cows ($n = 16$; 263 ± 63 d in milk) was infused with 10 µg of LPS or a saline control in the left or right rear quarters on day 34 of the environmental treatment. Individual milk samples collected from LPS-infused quarters at –4, 0, 6, 12, 24, 48, 72, 96, and 144 h relative to infusion were analyzed for minerals. Blood samples were collected at the same time with an additional sample collected at 3 h post-infusion to analyze glucose, nonesterified fatty acids (NEFA), insulin, and minerals. Cooling by time interactions ($P \leq 0.07$) were observed for plasma glucose, NEFA, and serum insulin. Compared with cooled cows, non-cooled cows had lower concentrations of plasma glucose except at 3 h following intramammary LPS infusion, greater serum insulin at 3 and 12 h, and lower plasma NEFA at 24 and 48 h after infusion. Relative to cooled cows, non-cooled cows tended ($P = 0.07$) to have lower serum K concentration and had lower ($P < 0.01$) serum Zn 6 h following infusion (cooling by time interaction: $P < 0.01$). Relative to ZMC cows, IOZ cows had greater ($P \leq 0.09$) concentrations of plasma Se, skim milk Na and Se, and skim milk Na to K ratio. Regardless of treatment, intramammary LPS infusion reduced ($P < 0.01$) serum or plasma concentrations of Ca, Mg, Zn, Fe, and Se, but increased ($P < 0.01$) their concentration in skim milk. In conclusion, deprivation of cooling resulted in more rapid and prolonged insulin release and influenced the systemic and mammary mineral metabolism during mammary inflammation induced by LPS of lactating dairy cows. Dietary supplementation of Zn–Met complex reduced blood and milk Se concentrations compared with cows fed Zn from an inorganic source.

Key words: cooling, dairy cow, intramammary lipopolysaccharide infusion, zinc

Abbreviations

DMI	dry matter intake
LPS	lipopolysaccharide
NEFA	nonesterified fatty acids
SCC	somatic cell count

Introduction

Heat stress not only negatively affects animals' production and reproduction but also compromises their health (West, 2003). During heat stress, the incidences of disease, especially mastitis, increase presumably because of the favorable environment for survival and growth of pathogens coupled with the impaired immune function of the cow (Kadzere et al., 2002). *Escherichia coli* is an environmental pathogen that causes mastitis in dairy herds (Wenz et al., 2001; Bradley, 2002). Lipopolysaccharide (LPS), a component of the structural membrane of Gram-negative bacteria, is an endotoxin responsible for disease pathogenesis caused by *E. coli*. Its presence within the mammary gland induces a series of immune cascade from local inflammation to systemic immune responses (Burvenich et al., 2003). These LPS-induced responses subsequently alter the energy and mineral metabolism of a dairy cow. Immunoactivation caused by intravenous or intramammary LPS infusion is related to increased insulin secretion and significant glucose consumption by the immune system (Waldron et al., 2006; Kvidera et al., 2017). Further, mammary inflammation induced by LPS is associated with reduced blood mineral concentrations, including Zn and Fe, in dairy cows, suggesting altered mineral metabolism (Lohuis et al., 1988). However, the impact of heat stress on energy and mineral metabolism during mastitis or LPS-induced mammary inflammation is still unclear.

Mineral nutrition affects the incidence, duration, and severity of diseases. Zinc is an essential micromineral for dairy cattle and adequate Zn supplementation is positively related to disease resistance (Weiss, 2002; Spears and Weiss, 2008). The dietary source of supplemental Zn also influences mammary health, but inconsistent results have been reported. Kellogg et al. (2004) reported a positive impact of supplementation of Zn-Met complex compared with ZnSO₄ or Zn oxide for reducing milk somatic cell count (SCC). In contrast, Whitaker et al. (1997) reported no effect of dietary Zn source on udder health. Although most of those studies focus on milk SCC and disease incidence, there is still a lack of understanding of how different sources of supplemental Zn affect metabolic and mineral responses during mammary inflammation or mastitis in lactating dairy cows. Therefore, we hypothesized that heat stress and dietary source of supplemental Zn (Zn-Met complex vs. Zn hydroxychloride) alter metabolic responses and mineral metabolism after LPS infusion into lactating quarters of dairy cows. The objective of the current study was to evaluate the effects of deprivation of evaporative cooling and replacing a portion of the dietary inorganic source of supplemental Zn with Zn-Met complex on blood metabolites, insulin and mineral concentrations, and milk mineral concentrations of lactating dairy cows to an intramammary LPS infusion during summer.

Materials and Methods

This study was conducted during the summer at the Dairy Research Center, the University of Georgia—Tifton campus. Prior to beginning the trial, experimental protocols were approved by

the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia.

The cows used for the intramammary LPS infusion were a subset of cows from an experiment previously reported by Weng et al. (2018). Briefly, 72 multiparous Holstein cows were randomly assigned to four treatments with a 2 × 2 factorial arrangement. Treatments included two environmental conditions: evaporatively cooled or not cooled, and two dietary supplemental Zn sources: 75 mg of supplemental Zn/kg of DM supplied by Zn hydroxychloride (IOZ [inorganic Zn], Micronutrients, Indianapolis, IN) or 75 mg of supplemental Zn/kg of DM supplied by Zn hydroxychloride (35 mg/kg of DM) + Zn-Met complex (40 mg/kg of DM, ZMC, Zinpro Corporation, Eden Prairie, MN). Before the initiation of the environmental treatments, all cows were pre-fed their respective dietary treatments for 12 wk. Cows were fed the same total mixed ration once daily at 1300 hours, and each Zn supplement was mixed with the total mixed ration diet before feeding. Daily intake was measured using a Calan Broadbent feeding system (American Calan Inc., Northwood, NH). The ingredient composition and chemical composition of the diets were reported by Weng et al. (2018). After the initiation of the environmental treatment, non-cooled cows were deprived of evaporative cooling for 12 wk, and cooled cows remained under evaporative cooling. The evaporative cooling system consisted of misters attached to the front face of fans over feedbunks and freestalls. Cows were housed in adjacent pens according to their environmental treatments. The average temperature humidity index was 77.6 ± 3.8 and 77.8 ± 3.8 (mean ± SD) in cooled and non-cooled pens, respectively. Cows were milked three times each day at 0800, 1600, and 2400 hours. The milk yield of individual cows was recorded at each milking (Alpro, DeLaval, Kansas City, MO).

The procedures of intramammary LPS infusion and milk yield, dry matter intake (DMI), and hematological profiles during intramammary LPS infusion were described in a companion paper (Marins et al., 2019). Briefly, the intramammary infusion of LPS or saline was performed on day 34 of the environmental treatment. Twenty cows (*n* = 5 per treatment) with negative milk sample cultures and SCC below 200,000 cells/mL from both rear quarters collected 3 d before infusion were selected for the infusion study. The infusion procedure was started 2 h after milking at 0800 hours. The left rear quarter was infused with 10 µg of *E. coli* O111:B4 LPS (Sigma-Aldrich, St. Louis, MO; Greco et al., 2015) dissolved in 5 mL pyrogen-free saline solution, and the right rear quarter was infused with 5 mL pyrogen-free saline solution. Individual quarter milk samples (50 mL) were collected at -12, -4, 0, 6, 12, 24, 48, 72, 96, and 144 h relative to infusion for milk composition analysis or stored at -20 °C for mineral analysis. Blood samples were collected to obtain serum and plasma at the same time points with an additional sample collected at 3 h post-infusion. There were four cows with SCC above 200,000 cells/mL in at least one of the rear quarters at -12, -4, or 0 h relative to infusion of LPS or saline. Thus, the data and sample collected from these cows were eliminated from final analyses, resulting in *n* = 4, 4, 5, and 3 cows for cooled cows fed IOZ, non-cooled cows fed IOZ, cooled cows supplemented with ZMC, and non-cooled cows supplemented with ZMC, respectively. The remaining 16 cows averaged 263 ± 63 d in milk, 3.2 ± 1.4 parity, and 66.7 ± 55.5 × 10³ cells/mL for SCC of milk collected at -12, -4, and 0 h before infusion (± SD).

Plasma concentrations of glucose (Autokit Glucose, Wako Chemicals USA, Inc., Richmond, VA), nonesterified fatty acids (NEFA; HR Series NEFA-HR(2), Wako Chemicals USA, Inc.), and insulin (Mercodia Bovine Insulin ELISA, Mercodia AB, Uppsala, Sweden) from samples collected at -4, 0, 3, 6, 12, 24, 48, 96, and

144 h relative to intramammary LPS infusion were determined by colorimetric or enzyme-linked immunosorbent assay according to the manufacturers' procedures. The intraassay and interassay coefficient of variation (CV) were 3.5% and 4.9% for glucose, 1.3% and 3.6% for NEFA, and 3.4% and 4.1% for insulin. Concentrations of Zn, Fe, and Cu of serum samples collected at -4, 0, 3, 6, 12, 24, 48, and 72 h relative to intramammary LPS infusion were measured by atomic absorption (AAnalyst 100, Perkin Elmer, Waltham, MA), and the intraassay CV was 1.3%, 2.0%, and 4.5%, respectively. Concentrations of Na, K, Ca, and Mg of serum samples collected at -4, 0, 3, 6, 12, 24, 48, and 72 h relative to intramammary LPS infusion were determined at the Veterinary Diagnostic Laboratories of the University of Georgia (Tifton, GA) using an Advia 1800 Chemistry System (Siemens Medical Solutions USA, Inc., Malvern, PA).

Milk samples collected from LPS-infused quarters were thawed at room temperature and then centrifuged at $3,000 \times g$ for 15 min at 4 °C to obtain skim milk. Analyses to measure skim milk mineral concentrations (Na, K, Ca, Mg, Fe, Cu, Zn, and Se) and plasma Se concentration of samples collected between -4 and 72 h relative to intramammary LPS infusion were performed at Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI) using an Agilent inductively coupled plasma mass spectrometry according to the method described by Wahlen et al. (2005). The Cu concentration of skim milk in most of the samples was below the detection limit (0.02 µg/mL), thus not included in the statistical analysis.

Plasma or serum concentrations of metabolites, insulin and minerals, and skim milk concentrations of minerals were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). The SAS models included cooling, supplemental Zn source, time relative to infusion, and their respective interactions. Cow nested within treatment was included as a random variable, and time was included as a repeated measure. Least squares means $\pm$ SEM are reported, and significance and tendency were declared when $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

Results

Weng et al. (2018) reported the performance data of cows fed different dietary treatments under different environments in detail. Relative to cooled cows, non-cooled cows carried more heat load, as suggested by greater ($P < 0.01$) respiration rates (64.8 vs. 73.9 breath/min) and vaginal temperature (39.03 vs. 39.94 °C). As a result, non-cooled cows had lower ($P < 0.01$) milk yield (25.9 vs. 35.4 kg/d) and DMI (21.6 vs. 26.7 kg/d) compared with cooled cows. Supplemental Zn source did not affect these measures ($P > 0.25$). Milk yield and composition and hematological profiles during intramammary LPS infusion were reported previously (Marins et al., 2019). Cooled cows produced more ($P \leq 0.05$) milk from -7 to -1 d and at 2, 3, 4, 5, and 7 d relative to intramammary LPS infusion, but tended ($P \leq 0.08$) to have greater milk yield at 1 and 6 d after intramammary LPS infusion compared with non-cooled cows. Treatments had no apparent impact on milk SCC. Relative to cooled cows, non-cooled cows had lower ($P < 0.03$) blood concentrations of neutrophils and lymphocytes at 3 h after LPS infusion. Supplemental Zn source had no ($P \geq 0.13$) impact on these measurements (Marins et al., 2019).

In the current study, supplemental Zn source and its interactions with cooling and/or time had no impact on plasma concentrations of glucose, NEFA, and insulin ($P > 0.15$,

Supplementary Table S1). Compared with cooled cows, non-cooled cows had lower ($P < 0.01$) plasma glucose concentrations except at 3 h following intramammary LPS infusion (cooling $\times$ time interaction: $P = 0.07$, Figure 1). There was also a cooling by time interaction ($P < 0.01$) for serum insulin concentration. Non-cooled cows tended ($P = 0.09$) to have a greater serum insulin concentration immediately before infusion; circulating insulin increased in both groups of cows following intramammary LPS infusion with greater ($P < 0.05$) serum insulin concentration at 3 and 12 h after infusion for non-cooled compared with cooled cows (Figure 1). Relative to cooled cows, non-cooled cows had lower ($P < 0.05$) plasma NEFA concentrations at 24 and 48 h after intramammary LPS infusion (cooling $\times$ time interaction: $P = 0.02$, Figure 1).

Regardless of treatment, intramammary LPS infusion reduced ($P < 0.01$) serum or plasma concentrations of K, Ca, Mg, Zn, Fe, and Se, and their concentrations reached nadirs around 3 to 12 h following infusion (Supplementary Table S1 and Supplementary Figure S1). In contrast, LPS infusion had no ($P > 0.10$) impact on serum concentrations of Na and Cu (Supplementary Table S1 and Supplementary Figure S1). Supplemental Zn source and its interaction with cooling or time had no impacts on blood mineral concentrations, except that cows supplemented with IOZ tended ($P = 0.09$) to have a greater plasma concentration of Se relative to those supplemented with ZMC (Supplementary Table S1). Regardless of the time, cooled cows tended to have greater serum K concentrations ($P = 0.07$) compared with non-cooled cows (Supplementary Table S1). There was also a cooling by time interaction ($P < 0.01$) for serum Zn concentration because of the higher ($P < 0.01$) serum Zn concentrations for cooled cows compared with non-cooled cows at 6 h following intramammary LPS infusion (Figure 2). Although there was a cooling by time interaction ($P = 0.02$) for serum Fe concentrations, no treatment effect was identified at any time points by PDIF or SLICE function of SAS.

Intramammary LPS infusion dramatically affected skim milk concentrations of minerals. Following infusion, concentrations of Na, Ca, Mg, Zn, Fe, and Se increased (time effect: $P < 0.01$) and peaked approximately 6 to 24 h after infusion (Supplementary Table S2 and Supplementary Figure S2). In contrast, the skim milk K concentration decreased (time effect: $P < 0.01$) and reached the nadir at 12 h after intramammary LPS infusion, which resulted in an increased (time effect: $P < 0.01$) ratio of Na to K (Supplementary Table S2 and Supplementary Figure S2). No interactions between supplemental Zn source and time were observed, but cows fed IOZ tended ($P \leq 0.07$) to have greater skim milk concentrations of Na and Se and greater ($P = 0.04$) skim milk Na to K ratio compared with cows supplemented with ZMC (Supplementary Table S2). Regardless of the supplemental Zn source, non-cooled cows tended ($P = 0.07$) to have greater skim milk K concentration compared with cooled cows (Supplementary Table S2). Further, there was cooling by time interactions ($P = 0.05$) for skim milk Fe concentration, because Fe concentration in skim milk of cooled cows tended ($P = 0.08$) to be greater at 6 h but lower at 12 ($P = 0.09$) and 24 ($P = 0.05$) h following intramammary LPS infusion compared with non-cooled cows (Figure 2).

Discussion

In the current study, no cooling by supplemental Zn source interactions were observed for any measurements. Due to the occurrence of subclinical mastitis (SCC > 200,000 cells/mL) prior to the LPS infusion, several cows were eliminated from the final

Figure 1. The plasma concentrations of glucose and NEFA and serum concentration of insulin of cows exposed to evaporative cooling (solid diamonds [♦]) or not (open diamonds [○]) after intramammary LPS infusion. ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$.

analysis which reduced the number of animals per treatment. Therefore, it is important to recognize this limitation that may impede our ability to detect any potential environment by diet interactions. Despite significant glucose consumption by mammary inflammation, plasma glucose concentration is transiently increased following intramammary LPS challenge (Waldron et al., 2006; Vernay et al., 2012; Gross et al., 2018). A similar increase in blood glucose concentration was observed at 12 h following intramammary LPS infusion in the current study. This is likely due to the enhanced hepatic glycogenolysis and gluconeogenesis (reviewed by Moyes, 2015). In the present study, the plasma glucose concentration of non-cooled cows remained lower before and after intramammary LPS infusion except at 3 h after infusion compared with cooled cows, which may reflect the lower DMI resulting from heat stress.

Serum insulin concentration also increased following intramammary LPS infusion, consistent with previous studies (Waldron et al., 2006; Vernay et al., 2012; Gross et al., 2018).

Figure 2. The serum concentration of Zn and skim milk concentration of Fe of cows exposed to evaporative cooling (solid diamonds [♦]) or not (open diamonds [○]) after intramammary LPS infusion. ** $P \leq 0.01$, * $P \leq 0.05$, † $P \leq 0.10$.

The mechanism of the increased insulin secretion following mammary inflammation by LPS is unknown, but may be in response to increased glucose and enhanced pancreatic sensitivity to glucose stimulation. Compared with cooled cows, non-cooled cows had a more rapid and prolonged increase in serum insulin concentration, suggesting a more sensitive pancreatic insulin release during inflammation. During lactation, heat-stressed cows have reduced glucose concentration but have constant or increased plasma insulin concentration (Wheelock et al., 2010; Koch et al., 2016), indicating that factors rather than glucose concentration influence insulin secretion under heat stress conditions. The data from the current study suggest that the elevated insulin secretion of heat-stressed lactating dairy cows is related to inflammatory responses and immune activation. Regardless of treatments, intramammary LPS infusion resulted in reduced plasma NEFA concentration at 12 h post-infusion, which may be a result of the increased circulating insulin. The increased NEFA concentration at 24 h following LPS infusion of cooled cows suggests adipose tissue mobilization (Moyes, 2015), which is likely a secondary response because of the reduced DMI following infusion. In contrast, a similar response was not observed in non-cooled cows potentially because of the inhibitory effects of heat stress on body fat mobilization. In lactating dairy cows, heat stress is reported to inhibit adipose tissue mobilization despite the substantially decreased DMI (Wheelock et al., 2010; Lamp et al., 2015).

Interestingly, deprivation of evaporative cooling tended to decrease K concentration in serum but tended to increase skim milk K concentration. In cattle, the sweating rate increases as ambient temperature increases, and there is a greater loss of

K than of Na during sweating (Jenkinson and Mabon, 1973). This may be one mechanism that explains the lower plasma K concentration of non-cooled cows than cooled cows observed in the current study. The mammary gland is believed to have evolved from the apocrine-like skin gland associated with hair follicles (Capuco and Akers, 2009), similar to the apocrine sweat gland of cattle. Thus, the increased milk K concentration of non-cooled cows than cooled cows may resemble the greater K loss during sweating of heat-stressed cattle. Because mammary epithelial integrity is not significantly altered by deprivation of evaporative cooling (Weng et al., 2018), this phenomenon may also indicate greater Na⁺-K⁺-ATPase activity of the basolateral membrane of mammary epithelial cells (Shennan and Peaker, 2000).

The Na to K ratio in milk is a reliable marker for mammary epithelial integrity (Stelwagen et al., 1999). In the current study, supplementation of ZMC lowers the Na to K ratio in skim milk compared with IOZ, suggesting lower mammary epithelial permeability. This is consistent with previous studies that reported replacing a portion of inorganic sources of Zn with amino acids-chelated Zn improved epithelial integrity of the mammary gland in lactating dairy cows (Weng et al., 2018) and the ileum of growing pigs (Pearce et al., 2015). The advantage of amino acids-chelated Zn on epithelial integrity may be due to the greater bioavailability over inorganic source of Zn (Spears, 2003). It is also reported that microminerals influence immunity partly by their involvement in the antioxidant system (Andrieu, 2008; Spears and Weiss, 2008). For example, both Zn and Se act as cofactors in anti-oxidative enzymes to neutralize free radicals. Zinc serves as a structural cofactor of Cu/Zn superoxide dismutase and promotes the synthesis of metallothionein (Spears and Weiss, 2008); Se is an integral part of Se containing glutathione peroxidases (Brigelius-Flohé and Maiorino, 2013). In the present study, feeding ZMC resulted in lower Se concentrations in both plasma and skim milk relative to IOZ, potentially suggesting that supplementation of amino acids-chelated Zn reduces Se requirement because of the greater bioavailability compared with inorganic source of Zn. If this phenomenon will alter the activities of anti-oxidative enzymes is unknown but deserves further investigation.

Regardless of the dietary or environmental treatments, intramammary LPS infusion reduced the serum concentrations of K without changing the Na concentration. Similarly, Ohtsuka et al. (1997) reported that cows with severe coliform mastitis had lower serum K concentrations compared with healthy animals. Intramammary LPS infusion also reduced concentrations of macrominerals (Ca and Mg) and microminerals (Zn, Fe, and Se) in the blood but simultaneously increased their concentrations in skim milk. Mammary inflammation induced by LPS is associated with disrupted mammary epithelial junction, as indicated by the increased skim milk Na to K ratio following LPS infusion. The impaired mammary epithelial integrity enables the paracellular transport between blood and milk based on the concentration gradient, which may partially explain the altered milk and blood mineral concentrations (Oliver and Calvinho, 1995). However, since the skim milk concentrations of Ca and Mg were 2- to 3-fold greater than serum before infusion, macromineral transport from blood to milk through the paracellular route is unlikely. Because intravenous LPS infusion also decreases serum macromineral (Waldron et al., 2003) and micromineral concentrations (Lohuis et al., 1988; Erskine and Bartlett, 1993; Hennigar and McClung, 2016), reduced serum or plasma mineral concentrations observed in the current study may result from the systemic inflammation induced by intramammary LPS infusion. LPS-induced

inflammation is related to decreased gut motility; therefore, the reduced intestinal absorption of mineral may be a potential mechanism for the lower blood macromineral and micromineral concentrations following intramammary LPS infusion (Lohuis et al., 1988; Waldron et al., 2003). Additionally, an endotoxin shock is associated with hepatic Ca sequestration and increased intracellular Ca concentration in liver cells, which is not only suggested to be associated with various endotoxin-induced metabolic disorders but also partly explains the decrease in blood Ca concentrations (Sakaguchi and Furusawa, 2006).

In the current study, because the blood micromineral concentrations were greater than skim milk prior to infusion, the paracellular transport from blood to milk may contribute to the decreased blood concentrations and increased skim milk concentrations of microminerals. Relative to cooled cows, non-cooled cows had a greater reduction in serum Zn concentrations following intramammary LPS infusion, potentially suggesting greater tissue sequestration of blood Zn to cope with inflammation-related oxidative stress under heat stress. However, this hypothesis has never been tested and deserves further investigation. Intramammary LPS infusion significantly reduced the milk yield of dairy cattle (Marins et al., 2019), which may be the primary mechanism of the increased concentrations of macromineral and micromineral in skim milk collected from the LPS-infused quarter due to a concentrating effect. After LPS infusion, the increase in skim milk Fe is delayed in non-cooled cows compared with cooled cows. The mechanism of this delay is not clear but may be associated with altered lactoferrin production. Future study needs to focus on the impact of heat stress on mammary gland immune responses to mammary infection or inflammation.

Conclusions

Following intramammary LPS infusion, non-cooled cows maintained lower plasma glucose concentrations but had a more rapid and prolonged increase in serum insulin concentration compared with cooled cows. These data may indicate that heat-stressed lactating dairy cows have more sensitive pancreatic insulin release induced by inflammation. Compared with cooled cows, non-cooled cows had a greater reduction in serum Zn concentration and a delayed increase in skim milk Fe concentration after intramammary LPS infusion. Because both Zn and Fe are related to immune responses, these data potentially suggest that heat stress affects the systemic and mammary immune responses during mammary inflammation. Future studies in this area are required. The results of our current trial suggest that dietary sources of supplemental Zn have no apparent impact on the metabolic and mineral responses of cows to intramammary LPS infusion. However, the reduced plasma and milk concentrations of Se in cows supplemented with Zn-Met complex compared with IOZ suggest a potential interaction between the requirements of Zn and Se in lactating dairy cows.

Supplementary Data

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

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Conflict of interest statement

D.J.T. and J.M.D. are employed by Zinpro Corporation. The remaining authors are associated with The University of Georgia and have no actual or potential conflicts of interest.

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