Effects of prepartum zinc-methionine supplementation on feed digestibility, rumen fermentation patterns, immunity status, and passive transfer of immunity in dairy cows

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ABSTRACT

The aim of this study was to determine the effects of prepartum supplementation of zinc-methionine (Zn-Met) on feed digestibility, rumen fermentation patterns, and immunity status in dams and passive immunity transfer in their calves. A randomized complete design was used in this study. Forty multiparous Holstein dairy cows in late pregnancy (60 d before the expected calving date) were blocked by parity (2.1 ± 0.3), body weight (651 ± 52 kg), and expected calving date, and randomly assigned to 1 of 4 treatments. Cows were supplemented with Zn as Zn-Met at 0, 20, 40, or 60 mg/kg of dry matter (DM) from 60 d before expected calving date to the calving day. Though the nutrient digestibility was not affected by Zn supplementation, DM intake, Zn digestibility, and Zn deposition increased linearly with increasing Zn-Met supplementation. Ruminal pH and molar proportion of individual volatile fatty acids were similar, whereas a linear decrease and increase were observed in ruminal ammonia and microbial crude protein concentration, respectively, with increasing Zn-Met supplementation. Maternal serum concentration of alkaline phosphatase, carboxypeptidase, Cu and Zn superoxide dismutase, and total antioxidant capacity were greater in cows supplemented with >40 mg of Zn/kg of DM compared with the control group. With increasing Zn-Met supplementation, maternal blood concentration of IL-1 decreased linearly, whereas IL-2 and IL-6 increased linearly, and no differences

were observed in IL-4. Concentration of nonesterified fatty acids and β -hydroxybutyric acids in maternal blood was similar between treatments. No difference was observed in colostrum composition with increasing Zn-Met supplementation. Concentration of Zn and immunoglobulins (including IgA, IgG, and IgM) in maternal blood did not differ among treatments. However, Zn concentration in colostrum and blood of calves increased linearly. The concentration of IgA and IgM in colostrum increased linearly with increasing Zn-Met supplementation, whereas no differences in immunoglobulins were observed in calf blood. In conclusion, Zn supplementation as Zn-Met at 40 mg/kg of DM may improve antioxidant activity of dam and potentially increase passive immunity transfer in calves.

Key words: zinc metabolism, zinc-dependent enzyme, antioxidant capacity, colostrum composition, immunoglobulin

INTRODUCTION

Zinc is an essential micronutrient in animals, as it is a cofactor of numerous enzymes. Zinc further plays an important role in protein synthesis, gene regulation, and immune function (Weiss, 2017). In dairy cows, Zn supplementation may enhance milk production and blood concentration of cytokines and immunoglobulins and decrease the SCC in lactating cows (Pfeiffer et al., 1977; Stefanidou et al., 2006; Cope et al., 2009). The Zn can also improve the health status of dairy calves (Wright and Spears, 2004). However, research on the effects of Zn supplementation in prepartum cows and subsequent postpartum performance is scarce. Because Zn is beneficial to the immune response (Stefanidou et al., 2006), we hypothesized that Zn supplementation may have potential benefits for passive immunity transfer through colostrum.

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Dietary Zn can be fed in inorganic or organic form. Usually, organic Zn has greater utilization efficiency (Wedekind et al., 1992; Garg et al., 2008). One plausible reason for this observation might be that more of the inorganic Zn than organic Zn readily reacts with other chemicals in the digestive tract, creating indigestible complexes (Uchida et al., 2001). Moreover, Zn metabolism may be different in organic form compared with inorganic form as reported by Wedekind et al. (1992). Zinc-methionine (Zn-Met) is an organic Zn source that is widely used in animals. Wedekind et al. (1992) found that both DMI and weight gain were greater in chicks supplemented with Zn-Met than ZnSO₄ or ZnO. Garg et al. (2008) reported greater fiber digestibility, Zn retention, DMI, ADG, and feed efficiency in lambs supplemented with Zn-Met compared with ZnSO₄. Moreover, cows supplemented with Zn-Met might experience reduced SCC (Kellogg et al., 2004; Sobhanirad et al., 2010). All of the previous studies demonstrated that Zn-Met should be a bioavailable Zn source in dairy cows.

However, the optimum dose of Zn-Met has not been well defined. Cope et al. (2009) reported an increase in milk yield and decrease in SCC in cows supplemented with organic Zn at 65 versus 40 mg/kg of DM. Kellogg et al. (2003) reported improved production and reproduction performance in cows supplemented at 360 mg of Zn/d, which was approximately equal to 20 mg of Zn/kg of DM. Hence, we set up an experiment to evaluate the effect of organic Zn dosage in the prepartum period on (1) DMI, nutrient digestibility in the total digestive tract, rumen fermentation characteristics, and Zn metabolism and (2) passive immunity transfer from dam to calves.

MATERIALS AND METHODS

This study was conducted between October 2017 and January 2018 at Jiulonghe dairy farm in Baoding, P. R. China. The experimental protocol (YXG 1711) was approved by the Institutional Animal Care and Use Committee of Hebei Agricultural University.

Animals, Diets, and Experimental Design

Forty multiparous Holstein dairy cows in late pregnancy (60 d before expected calving date) were blocked by parity (2.1 ± 0.3 ; range from 2–3), BW (651 ± 52 kg), previous 305-d milk yield ($8,129 \pm 367$ kg), DMI, and expected calving date and randomly assigned to 4 treatments. The Zn-Met supplemental period lasted from 60 d before expected calving date to the day of calving. The actual supplemental period was $57.3 \pm$

Table 1. Ingredients and chemical composition of the experimental diets

Item	Diet	
	Dry period	Prepartum
Ingredient, % of DM		
Oat hay	24.8	25.8
Corn silage	15.6	15.6
Chinese wildrye hay	28.8	28.8
Corn DDGS ¹	3.06	4.06
Wheat bran	3.56	2.56
Cracked corn	5.00	3.50
Beet pellet	6.38	6.58
Soybean meal	4.48	5.78
Steam-flaked corn	3.30	2.30
Cottonseed meal	3.52	3.52
Premix ²	1.57	1.57
Chemical composition, DM basis		
NE _L , ³ Mcal/kg	1.34	1.34
CP, %	12.1	13.4
Ether extract, %	3.16	3.92
NDF, %	46.0	46.4
ADF, %	25.9	26.3
Ca, %	0.45	0.55
P, %	0.22	0.27
Zn, mg/kg	21.5	21.3

¹DDGS = dried distillers grains with solubles.

²Premix contained (per kg of DM): 800,000 IU of vitamin A, 180,000 IU of vitamin D₃, 7,000 mg of vitamin E, 45 mg of biotin, 300 mg of β -carotene, 600 mg of Cu, 1,000 mg of Fe, 1,800 mg of Mn, 20 mg of Co, 30 mg of Se, and 39 mg of I.

³Data were calculated according to NRC models (NRC, 2001).

4.55 d (range from 53–68 d) according to the actual calving date. Dairy cows were fed a TMR thrice daily at 0600, 1300, and 1900 h. The diet was formulated according to NRC (2001), and the ingredients and nutritional composition are shown in Table 1. Cows were changed to the prepartum diet from the dry period diet at 21 d before expected calving date. Cows were housed individually in tiestalls with ad libitum access to TMR (ensuring at least 5% refusals) and fresh water. The cows had outdoor exercise from 2200 to 0600 h daily prepartum. Only fresh water was accessible during the outdoor exercise periods. Feed refusals were cleaned up 3 times per day before each feeding.

Four treatments ($n = 10$ /treatment) were as follows: control (no Zn-Met supplemented in diet) and the control diet supplemented with Zn at 20, 40, or 60 mg/kg of DM as Zinpro Zn-Met complex (Zinpro Corp., Eden Prairie, MN). The Zn-Met was mixed with 72 g of ground corn and 18 g of molasses, split into 3 portions, and top dressed thrice daily immediately after feed offered. Cows from the control group received 72 g of ground corn and 18 g of molasses only by top dressing. The top dress for every cow was prepared weekly according to the DMI. Briefly, Zn-Met was weighed into a plastic container by cow, ground corn and molasses

for 40 cows for 7 d were mixed using a Hobart mixer (model H662, Hobart Corp., Troy, OH), and 90 g of mixture was added to each container.

Sampling and Data Collection

Feed offered and refusals of each cow were recorded daily during the experimental period (from 60 d before expected calving date to calving). Feed ingredients and TMR were collected weekly; feed refusals were sampled every last 3 d of the week, pooled by treatments, and oven-dried at 55°C for 48 h for DM content determination, and then ground to pass through a 1-mm screen (standard model 4 Wiley mill; Arthur H. Thomas, Philadelphia, PA) for chemical analyses. The DMI was calculated weekly as the difference between DM offered and DM refusals.

Apparent total-tract digestibility of nutrients was determined by total collection of the feces and urine from 31 to 33 d (29 to 27 d before the expected calving date) of the experiment (no outdoor exercise during total collection period). Feces and urine were collected separately by fitting cows with indwelling bladder catheters (75-mL capacity balloon, Bardex Foley Catheter, C. R. Bard Inc., Covington, GA) as described by Walter et al. (2012). Briefly, indwelling bladder catheters were inserted into bladder to separate urine and feces; urine was collected into a 20-L container via catheter, and feces were collected from the floor. Both feces and urine were collected and weighed daily on the sampling days. Thoroughly mixed urine samples were subsampled daily and mixed by cow after total collection. Approximately 40 mL of the subsampled urine was collected into a 50-mL centrifuge tube with screw cap and stored at –20°C until further analyses. About 2% of the feces were collected daily into an aluminum pan, mixed thoroughly by cow after total collection, oven-dried at 55°C, and then ground through a 1-mm screen (standard model 4 Wiley mill; Arthur H. Thomas) for chemical analyses.

Maternal blood samples were collected once daily from a jugular vein before morning feeding at d 53 (7 d before the expected calving date; the actual calving date was 2.68 ± 0.52 d before the expected calving date) of the experimental period. Calves were weighed immediately after birth, fed 2 L of colostrum within 2 h, and bled at 24 h after birth. Vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) with Na heparin or without any additive (cat. no. 367614) were used to collect plasma and serum, respectively. Both plasma and serum were stored at –20°C for further analyses. Within 2 h after birth, approximately 120 mL of colostrum was divided into 3 portions, placed in 50-mL

screw-cap centrifuge tubes, and then stored at –20°C for further analyses.

Ruminal fluid was collected before morning feeding at d 30 before the expected calving date by a trained person, using an oral stomach tube as described by Shen et al. (2012). Ruminal pH was measured immediately after collection, squeezed through 4 layers of cheesecloth, divided into 4 portions, and stored at –20°C for further analyses. Two subsamples of 5 mL of ruminal fluid were mixed with 1 mL of 25% (wt/vol) HPO_3 and 1 mL of 1% (wt/vol) H_2SO_4 for VFA and $\text{NH}_3\text{-N}$ determination, respectively (Shen et al., 2018). One subsample was stored at –20°C directly for the analysis of ruminal Zn. The remaining subsamples of 40 mL of ruminal fluid were placed into a 50-mL screw-cap centrifuge tube for the analysis of microbial CP (MCP).

Sample Analyses

The feed, refusals, and feces were analyzed for DM, ash, ether extract, and CP (methods 930.15, 942.05, 920.39, and 996.11, respectively) according to AOAC International (2005). The OM content was calculated as $\text{OM} = 100\% - \text{ash} \%$. The content of NDF was measured as described by Van Soest et al. (1991) using heat-stable α -amylase and sodium sulfite, and ADF of feeds and feces was determined according to AOAC International (2005; method 973.18). Both NDF and ADF were expressed inclusive of residual ash (Mertens, 2002). Concentration of ruminal VFA was determined by GC (GC-14B, Shimadzu, Kyoto, Japan; 30 m $\times$ 0.32 mm $\times$ 0.25 mm; column temperature, 110°C; injector temperature, 180°C; and detector temperature, 180°C) as described by Shen et al. (2019). Concentration of ruminal $\text{NH}_3\text{-N}$ was determined using the method of Rhine et al. (1998), and ruminal MCP content was determined as described by Cotta and Russell (1982), with differential centrifugation used to isolate MCP. Flame atomic absorption spectroscopy was used to determine the Zn of feed offered, refusals, feces, urine, colostrum, ruminal fluid, and the maternal and calf serum as described by Osorio et al. (2016).

Concentration of serum alkaline phosphatase (ALP), carboxypeptidase, Cu and Zn superoxide dismutase (Cu-Zn SOD), total antioxidant capacity (T-AOC), and IL-1, 2, 4, and 6 were measured using commercial ELISA kits (Biosino Biotechnology Co. Ltd., Beijing, China). Concentrations of plasma nonesterified fatty acid (NEFA) and BHB were determined using commercial colorimetry kits (Nanjing Jiancheng Bioengineering Inc., Nanjing, China). Colostrum composition, including lactose, fat, protein, total solids, and nonfat

Table 2. Effects of Zn supplementation on prepartum DMI and feed digestibility in dairy cows

Item ¹	Zn, mg/kg of DM				SEM	P-value ²	
	0	20	40	60		L	Q
DMI, kg/d	10.1 ^b	10.5 ^b	10.7 ^b	12.2 ^a	0.35	0.01	0.14
Apparent total-tract digestibility, %							
OM	78.4	77.1	79.2	74.3	1.49	0.20	0.28
NDF	54.3	58.1	60.8	59.6	2.43	0.16	0.37
ADF	47.0	52.1	53.2	52.9	2.17	0.12	0.28
CP	71.9	74.9	76.8	75.2	2.25	0.30	0.36
EE	86.8	87.8	89.5	88.1	0.93	0.24	0.27
Ca	35.5	37.1	38.5	37.5	2.83	0.59	0.68
P	35.8	36.6	39.0	39.0	2.24	0.29	0.88
Zn metabolism							
Zn intake, mg/d	207 ^d	455 ^c	700 ^b	970 ^a	7.2	0.01	0.15
Fecal Zn, mg/d	109 ^d	143 ^c	184 ^b	223 ^a	3.4	0.01	0.47
Urinary Zn, mg/d	3.97	3.99	4.05	4.06	0.075	0.38	0.98
Zn retention, ³ mg/d	94.9 ^d	308 ^c	512 ^b	743 ^a	8.1	0.01	0.30
Zn digestibility, %	47.6 ^d	68.6 ^c	73.8 ^b	77.0 ^a	0.73	0.01	0.01
Zn retention, %	45.7 ^d	67.7 ^c	73.2 ^b	76.6 ^a	0.73	0.01	0.01

^{a-d}Different superscripts within a row represent significant differences ($P < 0.05$).

¹Apparent total-tract digestibility was measured during 27 to 29 d before the expected calving date; EE = ether extract.

²L and Q = linear and quadratic effects of Zn supplementation dose (0, 20, 40, 60 mg/kg of DM).

³Zn retention = Zn intake – fecal Zn – urinary Zn.

solids, was determined using a MilkoScan FT 120 (Foss Electric, Hillerød, Denmark). Commercial ELISA kits [MBS702497 (detection range: 1.56–100 µg/mL), MBS282896 (detection range: 0.16–10.00 µg/mL), and MBS285353 (detection range: 31.25–2,000 µg/mL), respectively; MyBioSource Inc., San Diego, CA] were used to determine IgA, IgM, and IgG concentrations of colostrum and calf serum IgA, IgM, and IgG, respectively, as described by Ding et al. (2019). The interassay coefficients of variation were lower than 10%, and the intraassay coefficients of variation were lower than 12%.

Calculations and Statistical Analyses

Apparent digestibility of nutrients was calculated as described by Beckman and Weiss (2005) using the following equation: nutrient digestibility = $100\% - [(feces\ weight, kg) \times (fecal\ nutrient\ content, \%)/(DMI, kg) \times (feed\ nutrient\ content, \%)]\%$. Data were analyzed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) for a randomized complete design. Treatment was the fixed effect, and the cow was the random effect in the MIXED model. The PDIFF option adjusted by the Tukey method was included in the LSMEANS statement to account for multiple comparisons among treatments when the F test was significant. Specific preplanned contrasts were used to determine the linear and quadratic responses to increasing Zn-Met dose. Treatment effects were declared significant at $P \leq 0.05$,

and trends were discussed at $0.05 < P < 0.10$ unless otherwise stated.

RESULTS

Apparent total-tract digestibility of OM, CP, NDF, ADF, ether extract, Ca, and P were not affected, whereas DMI increased linearly ($P < 0.05$) with increasing amounts of Zn-Met (Table 2). The magnitude of DMI increase was more pronounced with 60 mg/kg of DM (+21%) than with 20 (+4%) and 40 mg/kg of DM (+5.9%) compared with the control. The Zn intake and fecal Zn linearly ($P < 0.01$) increased with increasing Zn-Met supplementation dosage. Fecal Zn discharge increased linearly ($P < 0.01$) as well. However, urinary Zn excretion was not affected by Zn-Met supplementation. Total-tract digestibility of Zn linearly increased ($P < 0.01$) with increasing amounts of Zn-Met. Likewise, the amount of Zn deposited in dairy cows increased linearly by 48.1, 60.2, and 67.6% with 20, 40, and 60 mg of Zn/kg of DM, respectively.

No treatment effect was observed in the concentration of ruminal Zn (Table 3). With increasing Zn-Met supplementation, ruminal concentration of ammonia decreased ($P < 0.01$), whereas the concentration of MCP increased ($P < 0.01$) linearly. Ruminal pH did not differ among treatments, whereas the concentration of total VFA increased linearly ($P < 0.01$) with increasing Zn-Met supplementation. No treatment effect was observed on molar proportion of ruminal acetate

Table 3. Effects of Zn supplementation on rumen fermentation in prepartum dairy cows

Item ¹	Zn, mg/kg of DM				SEM	P-value ²	
	0	20	40	60		L	Q
Rumen Zn, mg/L	0.53	0.55	0.53	0.57	0.016	0.20	0.53
NH ₃ -N, mg of N/mL	10.6 ^a	9.85 ^{ab}	8.84 ^{bc}	7.98 ^c	0.380	0.01	0.95
MCP, mg/mL	9.75 ^c	10.2 ^c	11.6 ^b	12.8 ^a	0.35	0.01	0.35
pH	6.94	6.90	6.87	6.93	0.079	0.87	0.49
Total VFA, mM	96.6 ^c	101.6 ^{bc}	102.4 ^b	109.0 ^a	4.27	0.01	0.67
Acetate, %	65.2	66.1	66.2	66.9	0.86	0.22	0.93
Propionate, %	18.4	18.3	18.3	18.5	0.61	0.92	0.91
Butyrate, %	9.4	8.5	8.2	7.6	0.43	0.06	1.00
Acetate:propionate ratio	3.55	3.61	3.63	3.63	0.162	0.75	0.87

^{a-c}Different superscripts within a row represent significant differences ($P < 0.05$).

¹MCP = microbial crude protein.

²L and Q = linear and quadratic effects of Zn supplementation dose (0, 20, 40, 60 mg/kg of DM).

or propionate or acetate:propionate ratio. The molar proportion of butyrate tended ($P < 0.06$) to decrease linearly with increasing Zn-Met supplementation.

With increasing supplementation of Zn-Met, the concentrations of ALP, carboxypeptidase, Cu-Zn SOD, and T-AOC increased linearly ($P < 0.01$) in maternal blood (Table 4). Maternal blood concentration of IL-1 decreased ($P < 0.05$), whereas IL-2 increased ($P < 0.05$) and IL-6 tended ($P = 0.06$) to increase linearly with increasing Zn-Met supplementation. However, adding Zn-Met in the diet of dry cows had no effect on maternal blood concentration of IL-4, NEFA, or BHB.

Colostrum composition is shown in Table 5. No treatment effect was observed in colostrum composition except a trend ($P = 0.10$) toward increased lactose concentration. With increasing Zn supplementation, colostrum lactose percentage increased by 11.6, 6.8, and 44.2%, respectively.

Blood concentrations of Zn and immunoglobulins (including IgA, IgG, and IgM) of the dam were not

affected by Zn-Met supplementation (Table 6). Colostrum concentration of Zn, IgA, and IgM increased linearly ($P < 0.05$). However, though the calf serum concentration of Zn linearly ($P < 0.05$) increased with increasing amount of Zn-Met, the immunoglobulin concentration did not differ among treatments. The percentage increase in colostrum Zn (17.6, 29.7, and 40.7% for the supplementation of Zn at 20, 40, and 60 mg/kg of DM, respectively) and calf blood (0.5, 22.7, and 40.1% for the supplementation of Zn at 20, 40, and 60 mg/kg of DM, respectively) was similar except for the dose of 20 mg/kg of DM.

DISCUSSION

In the present study, Zn was added into the diet of dry cows in the form of Zn-Met at the doses of 0, 20, 40, and 60 mg of Zn/kg of DM. As an organic Zn source, feeding Zn-Met to dairy cows showed improvement of productive performance and health status in lactat-

Table 4. Effects of Zn supplementation on blood indicators in prepartum dairy cows

Item ¹	Zn, mg/kg of DM				SEM	P-value ²	
	0	20	40	60		L	Q
ALP, U/L	83.6 ^b	87.1 ^b	92.8 ^a	91.9 ^a	1.25	0.01	0.11
Carboxypeptidase, U/L	52.1 ^b	57.2 ^{ab}	65.8 ^a	66.5 ^a	2.62	0.01	0.40
Cu-Zn SOD, ng/mL	107 ^b	121 ^a	133 ^a	130 ^a	4.5	0.01	0.09
T-AOC, U/mL	8.8 ^b	9.7 ^{ab}	11.4 ^a	10.8 ^a	0.59	0.01	0.22
IL-1, ng/L	140	133	117	108	9.6	0.02	0.92
IL-2, pg/mL	604	654	699	692	29.8	0.04	0.36
IL-4, ng/L	11.1	12.8	12.7	13.3	0.93	0.15	0.56
IL-6, ng/L	51.2	52.5	60.7	71.5	7.48	0.06	0.54
NEFA, mmol/L	323	310	303	301	9.6	0.12	0.55
BHB, μ mol/L	7.01	6.61	6.80	7.08	0.46	0.86	0.48

^{a,b}Different superscripts within a row represent significant differences ($P < 0.05$).

¹ALP = alkaline phosphatase; Cu-Zn SOD = Cu and Zn superoxide dismutase; T-AOC = total antioxidant capacity; NEFA = nonesterified fatty acid.

²L and Q = linear and quadratic effects of Zn supplementation dose (0, 20, 40, 60 mg/kg of DM).

Table 5. Effects of Zn supplementation on the composition of colostrum in dairy cows

Item	Zn, mg/kg of DM				SEM	P-value ¹	
	0	20	40	60		L	Q
Fat, %	5.64	6.75	6.78	5.80	0.599	0.86	0.12
Protein, %	18.1	17.4	19.4	13.6	1.95	0.21	0.22
Lactose, %	1.90	2.12	2.03	2.74	0.294	0.10	0.42
SNF, %	20.3	21.0	19.9	18.0	1.68	0.32	0.46
TS, %	25.9	28.7	28.3	23.0	2.74	0.47	0.17

¹L and Q = linear and quadratic effects of Zn supplementation dose (0, 20, 40, 60 mg/kg of DM).

ing cows by increasing milk yield and decreasing SCC (Kellogg et al., 2004). However, the effect of Zn-Met in periparturient cows has been scarcely evaluated.

Cows supplemented with Zn at 60 mg/kg of DM increased DMI in the present study. Previous studies in either heifers or lactating cows showed that Zn supplementation from 5 to 65 mg/kg of DM had no effect on DMI (Arelovich et al., 2000; Cope et al., 2009). In contrast, a reduction in DMI with Zn supplementation was reported in steers (Malcolm-Callis et al., 2000) and in dairy goats (Salama et al., 2003). A study using a sheep model found that by increasing the Zn-Met in diet from 0 to 0.4, 0.8, and 1.2 g/d (equal to 0, 48, 98, and 136 mg of Zn/kg of DM), DMI linearly increased from 770 to 826, 816, and 881 g/d (Jafarpour et al., 2015). Nayeri et al. (2014) suggested that the observed differences in DMI among studies may be due to the breed, production stage, level and source of Zn, and treatment duration. Chen et al. (2000) and Mantzoros et al. (1998) found that Zn supplementation can enhance the leptin level in humans by increasing the IL-2 level. Leptin is associated with appetite and plays an

important role in regulating DMI in dairy cows (Liefers et al., 2003). In the present study, though the concentration of leptin was not measured, the increased DMI may be the result of Zn increasing IL-2, which in turn increases leptin.

Zinc is an essential component in digestive enzymes; hence, its dietary supplementation might improve activities of digestive enzymes in the gut. In pigs, Hu et al. (2012) found that Zn supplementation at a dose of 62.5 mg/kg of DM improved the activities of protease, amylase, lipase, trypsin, and chymotrypsin. Using an in vitro study, Martinez and Church (1970) found that at low doses (5 mg/L), Zn increased cellulose digestibility, whereas at high doses, it decreased cellulose digestibility (20 mg/L). Moreover, Zn-Met was also reported to have a negative effect on nutrient digestibility compared with inorganic Zn (Caldera et al., 2019). Thus, it was expected that Zn-Met supplementation would have a negative effect on nutrient digestibility. However, in the present study, supplementing 0 to 60 mg of Zn/kg of DM had no effect on ruminal Zn concentration, which may explain the lack of an effect of Zn supplementation

Table 6. Effects of Zn supplementation on the concentration of Zn and immunoglobulins in maternal blood, colostrum, and calf blood

Item	Zn, mg/kg of DM				SEM	P-value ¹	
	0	20	40	60		L	Q
Maternal blood							
Zn, $\mu\text{mol/L}$	10.3	11.9	12.1	12.0	0.72	0.14	0.30
IgA, $\mu\text{g/mL}$	2.54	2.94	2.95	2.98	0.261	0.27	0.49
IgG, $\mu\text{g/mL}$	21.8	23.3	21.5	24.9	2.30	0.47	0.68
IgM, $\mu\text{g/mL}$	4.77	5.50	5.48	5.43	0.550	0.45	0.49
Colostrum							
Zn, mg/L	6.04 ^b	6.07 ^b	7.41 ^a	8.46 ^a	0.404	0.01	0.23
IgA, mg/mL	12.0	13.8	17.3	17.3	2.03	0.06	0.67
IgG, mg/mL	76.7 ^b	79.7 ^b	90.5 ^a	89.8 ^a	3.09	0.01	0.57
IgM, mg/mL	21.4	23.0	28.0	27.9	4.50	0.26	0.86
Calf blood							
Zn, $\mu\text{mol/L}$	9.1 ^d	10.7 ^c	11.8 ^b	12.8 ^a	0.32	0.01	0.39
IgA, mg/mL	2.24	2.27	2.36	2.36	0.176	0.57	0.94
IgG, mg/mL	28.8	28.8	29.4	29.1	1.42	0.80	0.92
IgM, mg/mL	4.17	4.13	4.19	4.15	0.226	0.98	1.00

^{a-d}Different superscripts within a row represent significant differences ($P < 0.05$).

¹L and Q = linear and quadratic effects of Zn supplementation dose (0, 20, 40, 60 mg/kg of DM).

on nutrient digestibility. Similarly, a lack of effect on DM digestibility has been reported in heifers by Arelovich et al. (2000), and Salama et al. (2003) reported greater digestibility of DM, OM, and CP in dairy goats supplemented with Zn.

In the present study, the linear increase in Zn digestibility with increasing Zn supplementation was expected because the organic form is more stable in the digestive tract and readily absorbable (Uchida et al., 2001). The digestibility of Zn ranged from 47.6 to 77% in this study, which agreed with a previous study (range from 45.4 to 76.1%; Balabánová et al., 2014). Ziegler et al. (1989) reported that urinary Zn concentration was not affected by Zn supplementation, and Spears and Kegley (2002) found no significant difference in urinary Zn excretion when supplemented with either inorganic (ZnO) or organic Zn (Zn-Met). Therefore, lack of differences in urinary Zn concentration among treatments was consistent with other studies.

An interesting finding in this study was that, though the Zn supplementation increased significantly, the ruminal soluble Zn did not differ among treatments. One possible explanation could be that most of the supplemented Zn was used by the ruminal bacteria. Eryavuz and Dehority (2009) reported greater increase in Zn concentration in both cellulolytic and total bacteria after 48 h of *in vitro* incubation in the supplemented group compared with the control (4.8 vs. 129.5×10^7 /mL for cellulolytic bacteria and 17.6 vs. 224.0×10^7 /mL for total bacteria). In the present study, though the amount of ruminal bacteria was not detected, the ruminal ammonia and MCP concentration decreased and increased linearly, respectively. Hence, lack of differences in ruminal Zn concentration among treatments may be explained by greater ruminal bacteria in cows supplemented with more Zn. Less ammonia and greater MCP concentration in sheep supplemented with Zn-Met has also been reported by Ashry et al. (2012). Another possible explanation for the lack of difference in ruminal soluble Zn concentration among treatments could be the solubility of Zn-Met. The Zn-Met used in the present study was water soluble, and thus may have flowed with the fluid fraction of the rumen contents, resulting in a short residence time in the rumen. The rumen samples were collected before the morning feeding, which in theory should have been the longest period of time between meals and allowed the most time for nutrients associated with the fluid fraction to leave the rumen.

Ruminal pH and total VFA concentration are important indicators to evaluate rumen fermentation. Usually, the ruminal pH is negatively associated with DMI and total VFA concentration (Shen et al., 2018). In the present study, DMI and total VFA concentration were

greater in cows supplemented with 60 mg of Zn/kg of DM compared with the other treatments, whereas ruminal pH did not differ among treatments. Both saliva contamination and sampling locations in the rumen could affect ruminal pH. However, in this study, the rumen sampling was taken by the same person and followed the same procedure, thus minimizing the effect of nontreatment factors on results. The reason for similar ruminal pH was not clear. Ruminal pH is associated with total VFA concentration and saliva excretion; hence, adding Zn-Met to dairy cows may have some potential effect on saliva excretion. Ishii et al. (1999) suggested that Zn, together with some Zn-dependent enzymes, plays an important role in saliva excretion. Even though our data cannot sufficiently support this hypothesis, we speculate that the lack of differences in ruminal pH together with greater total VFA concentration may be explained by greater saliva excretion in cows supplemented with Zn at 60 mg/kg of DM. Greater VFA concentration with no differences in ruminal pH in sheep supplemented with Zn-Met compared with ZnSO₄ was also reported by Ashry et al. (2012).

Alkaline phosphatase and carboxypeptidase are Zn-dependent enzymes that can be used to evaluate Zn status in dairy cows (Arelovich et al., 2000; Yan et al., 2002). In the present study, activity of ALP and carboxypeptidase was greater in cows supplemented with 40 and 60 mg of Zn/kg of DM, suggesting that 40 mg or more of Zn/kg of DM as Zn-Met may be beneficial in improving the activity of these Zn-dependent enzymes.

The concentration of Cu-Zn SOD and T-AOC activity are commonly used to evaluate the antioxidant status in animals (Zhang et al., 2013; Xu et al., 2014). The linear increase of Cu-Zn SOD and T-AOC concentration with an increase in the amount of Zn-Met in the present study suggested a greater antioxidant capacity in cows receiving greater amounts of Zn-Met. Previously, researchers reviewing the role of Zn in mice and monkeys reported its importance in improving the antioxidant capacity (Stefanidou et al., 2006). In the present study, the levels of Cu-Zn SOD and T-AOC were similar and significantly greater in cows supplemented with Zn (as Zn-Met) at ≥ 40 mg/kg of DM than the control, suggesting that 40 mg/kg of DM should be high enough to provide greater antioxidant capacity.

Interleukins are cytokines secreted by different cells, including monocytes, lymphocytes, and macrophages, and play an important role in immune regulation. Interleukin-1, IL-2, and IL-6 can promote the growth and differentiation of B cells and the secretion of antibodies, improving immune competence. However, some researchers believe that these cytokines also indicate body infection. In the present study, no infection was observed during the experiment. Although the mater-

nal blood concentration of immunoglobulins was not affected, the greater IgA and IgG concentrations in colostrum also illustrated an improved immune status in Zn-Met-supplemented cows. Thus, the linearly increased blood concentration of IL-2 and IL-6 with increasing Zn-Met supplementation reflected an improved immune status of dairy cows. This agreed with a previous review (Stefanidou et al., 2006). Both NEFA and BHB are important in evaluating the lipid metabolism in ruminants. In the present study, the lack of differences in maternal serum concentrations of NEFA and BHB among treatments illustrated that Zn supplementation may have no effect on lipid metabolism. Similarly, Zn supplementation did not affect NEFA and BHB in previous research (Nayeri et al., 2014).

Zinc concentration in colostrum did not vary between cows supplemented with Zn at different levels (Cope et al., 2009), which is inconsistent with our results. In the present study, though the Zn concentration in maternal blood did not differ, the concentration in colostrum increased linearly with increasing Zn-Met supplementation. Serum Zn represents only 0.1% of whole-body Zn; its concentration is regulated by homeostatic mechanisms and maintains balance even over a 10-fold Zn intake change (Roohani et al., 2013). Thus, serum Zn concentration could be used to reflect Zn deficiency but is not sensitive enough to reflect the increasing of Zn supplementation in healthy animals. In the present study, Zn concentration was not affected in maternal serum, even though we expected a linear increase in colostrum with increasing Zn-Met supplementation. The greater Zn concentration in colostrum of cows supplemented with Zn-Met was consistent with greater Zn transfer from blood to colostrum in our study. Wright and Spears (2004) found that blood Zn concentration increased in calves with diets supplemented with greater amounts of Zn. The linear increase in blood Zn concentrations in calves was consistent with previous studies, though Zn was supplemented via diet in the previous study (Wright and Spears, 2004) and via colostrum in the present study.

Colostrum is important in passive immunity transfer, and early feeding of colostrum provides immunoglobulins to calves, which improves their immunity (Quigley and Drewry, 1998). Interestingly, the concentration of immunoglobulins did not differ in maternal blood, although it linearly increased in colostrum (except for IgG) with increasing Zn-Met supplementation. Similar results were also observed in cows supplemented with mannan oligosaccharides. Greater immunoglobulin concentrations were detected in colostrum, but not in maternal or calf blood (Franklin et al., 2005). In a review, Stefanidou et al. (2006) reported that Zn can improve the immune response by stimulating activity of

immune cells. Thus, we expected the immunoglobulin concentration to be greater in both colostrum and maternal blood with Zn-Met supplementation. However, Blecha et al. (1981) showed that even under a CP restriction of more than 50%, maternal serum concentration of immunoglobulins did not differ, which suggested that there may be potential pathways that ensure the immunoglobulin concentration remains stable.

It is well established that passive transfer of immunity in calves is mainly through colostrum feeding instead of through placental transfer (Hough et al., 1990); hence, we were confounded by the inconsistent tendency of IgA, IgM, and IgG concentrations between colostrum and calf blood in the present study. In another review, Weaver et al. (2000) reported that the passive immunity transfer could be affected by many factors such as timing of colostrum ingestion, the method and volume of colostrum administration, the immunoglobulin concentration of colostrum ingested, and the age of the dam. In our study, we fed 2 L of colostrum within 2 h after birth, followed by blood sampling after 24 h, whereas previous studies that reported differences in immunoglobulins fed 1 L of colostrum every 8 or 12 h (Blecha et al., 1981; Hough et al., 1990). The lack of differences in calf immunoglobulin concentrations could be explained by either the lower volume of colostrum fed or the longer interval between feeding and blood sampling. Notwithstanding the aforementioned observations, the greater colostrum IgA and IgM concentrations of cows supplemented with Zn-Met suggested a potential benefit in passive immunity transfer. Because Zn supplemented as Zn-Met at 40 and 60 mg/kg of DM had no difference in colostrum IgA and IgM, but were both significantly greater than the control, supplementation of 40 mg of Zn/kg DM should be adequate for passive immunity transfer.

CONCLUSIONS

Though nutrient digestibility was not affected, the results of this study demonstrated improved DMI and antioxidant capacity with Zn-Met supplementation. With increasing Zn-Met supplementation, colostrum concentration of IgA and IgM increased linearly, which suggested potential benefits in passive immunity transfer. However, similar observations were not found for the concentration of IgA and IgM in calf blood, which may be due to the low volume of colostrum fed, as well as the long interval between colostrum feeding and blood sampling. Based on the present results, supplementing prepartum cows with 40 mg of Zn-Met/kg of DM will potentially improve the passive transfer of immunity. Further studies on the effect of Zn-Met supplementation on passive immunity transfer are desirable.

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