Effects of hydroxy trace minerals on oxidative metabolism, cytological endometritis, and performance of transition dairy cows

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ABSTRACT

Multiparous Holstein cows ($n = 60$) were used to determine effects of supplementing hydroxy forms of Zn, Cu, and Mn compared with 2 other common supplementation strategies on oxidative metabolism, cytological endometritis, and performance of transition cows. After a 1-wk pretreatment period, cows were assigned randomly to 1 of 3 dietary treatments from 21 d before expected calving through 84 d postcalving. Dietary treatments administered by daily top-dressing included (1) inorganic sulfate forms of Zn, Cu, and Mn (ITM); (2) a blend (75:25) of sulfates and organic complexes of Zn, Cu, and Mn (ITM/OTM); and (3) hydroxy trace minerals (HTM) of Zn, Cu, and Mn. The resulting dietary concentrations of supplemental Zn, Cu, and Mn were similar among treatments and averaged 40, 10, and 27 mg/kg, respectively, before calving and 59, 15, and 40 mg/kg, respectively, after calving. Total concentrations of Zn, Cu, and Mn averaged 80, 16, and 62 mg/kg during the prepartum period and 102, 23, and 75 mg/kg, respectively, during the postpartum period. Overall, effects of treatment on milk yield and milk composition were not significant. Cows fed HTM during the prepartum period had higher body weight (BW) than those fed ITM during the prepartum period and had higher BW during the postpartum period than those fed the other treatments; however, BW change, body condition score, and body condition score change were not affected by treatment. Plasma total antioxidant capacity was lower in cows fed HTM than ITM but was not different from cows fed ITM/OTM. Cows fed HTM tended to have lower concentrations of plasma thiobarbituric acid reactive substances than those fed ITM during the whole study period, but plasma thiobarbituric acid reactive substances were not different between HTM and ITM/OTM. Plasma haptoglobin was lower in cows fed HTM than ITM/OTM at 1 wk postpartum. Endometrial cytology 7 d postcalving

and cytological endometritis as assessed on 1 d between 40 and 60 d postcalving was not affected by treatment. In conclusion, supplementation with HTM sources of Zn, Cu, and Mn modulated plasma variables related to oxidative metabolism compared with supplementation with ITM; however, HTM and ITM/OTM resulted in similar responses. Furthermore, the source of trace minerals did not affect performance or uterine health in this experiment.

Key words: trace minerals, oxidative stress, cytological endometritis

INTRODUCTION

Trace minerals are important for many critical biological functions including immunity, oxidative metabolism, nutrient and energy metabolism, and reproductive function in dairy cows (NRC, 2001; Spears and Weiss, 2008). The specific roles of Zn, Cu, and Mn have received substantial attention in dairy cows, and studies have been conducted to evaluate effects of Zn, Cu, and Mn on productive and reproductive performance (Siciliano-Jones et al., 2008; Formigoni et al., 2011; Nemec et al., 2012). Specifically, decreased Zn status was demonstrated to be associated with higher rates of metritis, mastitis, and retained placenta (Enjalbert et al., 2006). Copper supplementation during the prepartum period and early lactation resulted in less severe clinical responses to intramammary *Escherichia coli* challenge during early lactation (Scaletti et al., 2003). Nemec et al. (2012) reported that supplementation of organic chelates of Zn, Cu, and Mn enhanced both innate and humoral immune responses in lactating cows.

Oxidative stress is increased during the transition period due to high demands of metabolism (Sordillo and Aitken, 2009) and likely contributes to periparturient disorders, including metabolic diseases (Sharma et al., 2011). Bernabucci et al. (2005) reported that cows with higher plasma BHBA and NEFA concentrations had higher concentrations of reactive oxygen metabolites and thiobarbituric acid-reactive substances (TBARS) and lower levels of antioxidants in plasma during the transition period. Sordillo and Aitken (2009) suggested

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that oxidative stress during the transition period can be a major underlying cause of inflammatory and immune dysfunction in dairy cattle and that a significant relationship exists between antioxidant supplementation and decreased incidence of diseases around parturition. Because of their antioxidant function, trace minerals can contribute to counterbalancing oxidative stress, which could reduce metabolic and immune problems during the transition period.

Cytological endometritis (**CE**) is characterized by inflammation of the endometrium, which results in a significant reduction in reproductive performance in the absence of signs of clinical endometritis (Sheldon et al., 2009). Given the role of trace minerals in immune function, it is possible that their interaction with immunity and, hence, CE may underpin some of the responses of reproduction to trace mineral supplementation outlined above.

Substantial interest has focused on the potential to enhance the function of dairy cows by feeding trace mineral sources with greater bioavailability (NRC, 2001; Andrieu, 2008) or to maintain high function by feeding lower overall levels of trace minerals using these more bioavailable sources (Nocek et al., 2006). Indeed, recent research conducted by our research group suggested that trace mineral amount and source can modulate aspects of oxidative metabolism (Yasui et al., 2009). Hydroxy trace mineral sources (Micronutrients Inc., Indianapolis, IN) have shown higher bioavailability than sulfates in steers (Spears et al., 2004) and weanling pigs (Fry et al., 2012), but have not been evaluated in dairy cattle. In addition, the role of these and other trace mineral sources in the etiology of CE has not been elucidated. Therefore, the objectives of this experiment were to evaluate a new source of trace minerals with potentially higher bioavailability and determine whether trace mineral source affects aspects of oxidative metabolism, CE, and performance of cows during the transition period and early lactation.

MATERIALS AND METHODS

Experimental Animals, Treatments, and Procedures

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee (Ithaca, NY) before the onset of the experiment. Sixty Holstein cows entering their second lactation or greater from the Cornell University Teaching and Research Center Dairy were moved to individual tie-stalls and enrolled in this experiment 28 d before expected calving and fed the same prepartum basal diet (Table 1). Cows were assigned to 1 of 3 treatments, with randomization restricted by previous lactation 305-d

mature-equivalent milk production. Treatments were initiated 21 d before expected calving and continued through 84 d postcalving and consisted of the following: (1) inorganic sources based on sulfates of Zn, Cu, and Mn (**ITM**); (2) a blend (75:25) of sulfates and organic AA complexes of Zn, Cu, and Mn (**ITM/OTM**); and (3) hydroxy trace minerals (**HTM**) of Zn, Cu, and Mn (IntelliBond; Micronutrients Inc.). The blend ratio for the ITM/OTM treatment was determined based upon our observations of common strategies for trace mineral supplementation in the dairy industry. The target concentrations of supplemental Zn, Cu, and Mn across treatments were the same for both prepartum and postpartum periods: 60, 15, and 40 mg/kg, respectively. Final total concentrations of Zn, Cu, and Mn based upon actual DMI during the prepartum and postpartum periods are provided in Table 2. Final total concentrations were similar across treatments and averaged 80, 16, and 62 mg/kg during the prepartum period and 102, 23, and 75 mg/kg during the postpartum period for Zn, Cu, and Mn, respectively. The ration fed to cows during the early dry period before enrollment in this experiment was formulated to contain 75 mg of Zn/kg and 20 mg of Cu/kg (Mn information not available); all supplemental Zn, Cu, and Mn was from sulfate-based sources. Treatments were administered as premixes with trace mineral sources blended with calcium carbonate, rice hulls, and mineral oil (premix amount was 28 g/d for all treatments during the prepartum and period and 70 g/d for all treatments during the postpartum period) once daily by top-dressing and mixed well by hand into the top of each cow's daily allotment of TMR to ensure consumption.

Ingredient and chemical compositions of the diets fed during the experiment are described in Table 1. Formulated dietary ingredients and composition were typical of diets fed in the Northeastern United States, except that the mineral supplements fed omitted Zn, Cu, Mn, Se, Co, and I. All 3 experimental top-dress premixes also contained Se, Co, and I at targeted concentrations in the final TMR of 0.3 mg/kg (added), 0.4 mg/kg (added), and 0.8 mg/kg (added), respectively. All nonforage ingredients were blended by a commercial feed mill into separate concentrate mixtures and diet mixing at the farm consisted of mixing the component forages with the appropriate concentrate mixture. Fresh feed was provided each morning at 0800 h,orts were weighed and recorded daily, and water was available at all times. Samples of forages and concentrate mixtures were obtained weekly throughout the experiment, and DM content determined by drying at 55°C until constant weight. Amounts of individual feed components in the TMR were adjusted weekly based on changes in the DM content of these feed components.

Table 1. Ingredient and chemical composition (DM basis, % of DM unless otherwise noted) of experimental diets

Item	Prepartum diet	Postpartum diet
Ingredient		
Corn silage, processed	42.40	39.16
Wheat straw	25.12	2.04
Legume silage	—	16.31
Ground shelled corn	—	7.51
Soybean meal	—	5.10
Wheat middlings	—	4.52
Corn germ meal	8.18	3.60
Distillers grains (with solubles)	6.99	5.12
Citrus pulp	—	2.99
Cereal fines	—	2.94
Soy Chlor ¹	3.26	—
Amino Plus ²	3.22	—
Soybean hulls	3.15	1.79
Canola meal	3.21	2.59
Blood meal	—	1.31
Molasses	1.04	1.19
Bypass fat ³	—	1.10
Alimet ⁴	—	0.10
Urea	—	0.33
Calcium carbonate	2.18	0.84
Calcium sulfate	0.56	—
Sodium bicarbonate	—	0.82
Potassium-magnesium-sulfate ⁵	0.23	—
Potassium chloride	—	0.08
Monocalcium phosphate	0.20	—
Salt	0.11	0.40
Magnesium oxide	0.09	0.11
Vitamin A, D, E premix ⁶	0.05	0.03
Vitamin E premix ⁷	0.02	—
Rumensin ⁸	0.01	0.01
Biotin 1%	—	0.06
Chemical composition ($\pm$ SD ⁹)		
CP	12.4 (1.02)	16.8 (0.52)
Soluble protein (% of CP)	32.7 (5.34)	43.3 (3.84)
ADF	29.1 (1.96)	21.4 (0.71)
NDF	47.6 (3.9)	33.9 (1.1)
Starch	18.1 (1.9)	24.7 (1.3)
Sugar	4.38 (0.89)	5.70 (0.80)
Ether extract	2.95 (0.33)	3.80 (0.16)
Ash	7.30 (0.44)	6.50 (0.47)
Ca	1.25 (0.10)	0.84 (0.06)
P	0.39 (0.03)	0.42 (0.01)
K	1.12 (0.05)	1.47 (0.06)
Mg	0.35 (0.01)	0.32 (0.02)
Na	0.14 (0.03)	0.45 (0.04)
Cl	0.52 (0.02)	0.48 (0.07)
S	0.29 (0.03)	0.24 (0.01)
Fe, mg/kg	232.2 (21.8)	205.1 (26.0)
Zn, mg/kg	39.4 (3.7)	43.1 (6.7)
Cu, mg/kg	6.0 (1.4)	7.7 (1.3)
Mn, mg/kg	35.0 (4.3)	35.1 (5.2)
DCAD, mEq/100 g of DM ¹⁰	2.0 (2.2)	29.0 (1.6)

¹Anionic feed supplement (West Central Cooperative, Ralston, IA).²Rumen-undegradable protein supplement (AGP Inc., Omaha, NE).³Prilled SFA (Cargill, Minnetonka, MN).⁴D,L-2-Hydroxy-4-methylthiobutanoic acid (Novus International Inc., St. Louis, MO).⁵Contained 18.8% K, 11.5% Mg, and 22.9% sulfate.⁶Contained 30,464 IU of vitamin A/kg, 5,862 KIU of vitamin D/kg, and 93,784 IU of vitamin E/kg.⁷Contained 499,400 IU of vitamin E/kg.⁸Contained 200 g of monensin sodium/kg (Elanco Animal Health, Greenfield, IN).⁹Standard deviation calculated from 5 and 7 samples for prepartum and postpartum diet, respectively.¹⁰Calculated as mEq of [(Na + K) - (Cl + S)]/100 g of DM (NRC, 2001).

Table 2. Basal diet concentrations, supplementation rates, and final total calculated diet concentrations of Zn, Cu, and Mn for cows fed varying sources during the periparturient period and early lactation

Item	Diet ¹		
	ITM	ITM/OTM	HTM
Prepartum period			
Actual DMI, kg/d	15.0	14.8	14.8
Zn			
Basal diet, mg/kg	39.4	39.4	39.4
Supplemented, mg/d	600	600	600
Supplemented, mg/kg (calculated)	40.0	40.5	40.5
Final total concentration, mg/kg (calculated)	79.4	79.9	79.9
Cu			
Basal diet, mg/kg	6.0	6.0	6.0
Supplemented, mg/d	150	150	150
Supplemented, mg/kg (calculated)	10.0	10.1	10.1
Final total concentration, mg/kg (calculated)	16.0	16.1	16.1
Mn			
Basal diet, mg/kg	35.0	35.0	35.0
Supplemented, mg/d	400	400	400
Supplemented, mg/kg (calculated)	26.7	27.0	27.0
Final total concentration, mg/kg (calculated)	61.7	62.0	62.0
Postpartum period			
Actual DMI, kg/d	25.4	24.5	25.8
Zn			
Basal diet, mg/kg	43.1	43.1	43.1
Supplemented, mg/d	1,500	1,500	1,500
Supplemented, mg/kg (calculated)	59.0	61.2	58.1
Final total concentration, mg/kg (calculated)	102.1	104.3	101.2
Cu			
Basal diet, mg/kg	7.7	7.7	7.7
Supplemented, mg/d	375	375	375
Supplemented, mg/kg (calculated)	14.8	15.3	14.5
Final total concentration, mg/kg (calculated)	22.5	23.0	22.2
Mn			
Basal diet, mg/kg	35.1	35.1	35.1
Supplemented, mg/d	1,000	1,000	1,000
Supplemented, mg/kg (calculated)	39.4	40.8	38.7
Final total concentration, mg/kg (calculated)	74.5	75.9	73.8

¹ITM = inorganic sources based upon sulfate forms of Zn, Cu, and Mn; ITM/OTM = a blend (75:25) of trace minerals from sulfates and organic complexes of Zn, Cu, and Mn; HTM = hydroxy forms of Zn, Cu, and Mn.

Dry matter contents of TMR were used in calculation of DMI for the corresponding week. Weekly samples of forages, concentrate mixtures, and TMR were composited into 4-wk composite samples and submitted to a commercial laboratory for Cornell Net Carbohydrate and Protein System/Cornell-Penn-Miner profiles (Cumberland Valley Analytical Services, Hagerstown, MD). Samples were analyzed for DM (method 930.15; AOAC International, 2000), CP (method 990.03; AOAC International, 2000), soluble protein (Krishnamoorthy et al., 1982), ADF (method 973.18; AOAC International, 2000), NDF (Van Soest et al., 1991), starch (Hall, 2009), sugar (DuBois et al., 1956), ether extract (method 2003.05; AOAC International, 2006), ash (method 942.05; AOAC International, 2000), and minerals (method 985.01; AOAC International, 2000).

Cows were milked twice daily (0900 and 2100 h) and milk yields were recorded at all milkings for the 84-d postpartum treatment period. Milk samples were collected on the same day each week from both milkings.

Samples were composited and stored at 4°C with a preservative (bronopol tablet; D & F Control Systems, San Ramon, CA) until analyzed (Dairy One Cooperative Inc., Ithaca, NY) within 24 h for fat, true protein, lactose, TS, and MUN using infrared analysis (method 972.160; AOAC International, 2000), and SCC by an optical fluorescent method (method 978.26; AOAC International, 2000). Body condition scores (1 to 5 scale; Wildman et al., 1982) and BW were measured weekly from the preliminary period through the whole treatment period. Gait scores were measured once during the week before assignment to treatment and at 4, 8, and 12 wk postpartum using a 5-point scale (1 = normal to 5 = severely lame; Sprecher et al., 1997).

Plasma Sampling and Analyses

Samples of blood were obtained from each cow via coccygeal blood vessel puncture on 1 d before assignment to treatment and once weekly from 21 d prepartum.

tum through 84 d postpartum. Plasma was harvested following centrifugation ($2,800 \times g$ for 15 min at 4°C), snap-frozen in liquid N₂, and stored at -20°C until analysis. Plasma samples collected from -3 wk prepartum through 12 wk postpartum were analyzed for total antioxidant capacity (TAC) and TBARS. Plasma samples collected from 1 wk postpartum through 8 wk postpartum were analyzed for concentrations of haptoglobin (Hp). Plasma TAC was measured colorimetrically using a commercial kit (Antioxidant Assay Kit; Cayman Chemical Co., Ann Arbor, MI). The assay relied on the ability of antioxidants present in the samples to inhibit the oxidation of 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS). The capacity to prevent ABTS oxidation was compared with a water-soluble tocopherol analog and was expressed as millimolar concentrations. Plasma TBARS in samples was measured fluorometrically using a commercial kit (TBARS Assay Kit; Cayman Chemical Co.). In the assay, an adduct formed by the reaction of malondialdehyde in the samples and thiobarbituric acid under high temperature and acidic conditions was measured and expressed as micromolar concentrations. Plasma concentrations of Hp in samples were measured by enzymatic analysis (Phase Range Haptoglobin Assay; Tridelta Diagnostics Ltd., Morris Plains, NJ). In the assay, hemoglobin peroxidase activity is preserved by the binding of Hp in the samples to hemoglobin; the amount of activity was directly proportional to the amount of Hp in the sample. Spectrophotometric and fluorometric measurements were conducted using a VersaMax tunable microplate reader (Molecular Devices LLC, Sunnyvale, CA) and a Synergy HT Multi-Mode microplate reader (BioTek Instruments Inc., Winooski, VT), respectively.

Endometrial Cytology

Evaluation of endometrial cytology by low-volume lavage (Gilbert et al., 2005) was determined on all cows at 7 d postcalving (first lavage) and on 1 d between 40 and 60 d (second lavage) postcalving as previously described (Cheong et al., 2011). The perineum of the cow was cleansed and a 64-cm Flex Tip sterile plastic infusion pipette (Exodus Breeders Corp., York, PA) was manipulated through the cervix into the uterus. Sterile saline solution (20 mL) was injected into the uterus and agitated gently via the rectum; then, a sample of the fluid was aspirated. The recovered fluid was centrifuged ($58 \times g$; 3 min; room temperature) using a cytocentrifuge directly onto a glass slide. After drying, the slides were fixed and stained using a rapid Romanowsky-type staining procedure and examined at $400\times$ magnification. Two hundred cells were counted from each slide,

and results were expressed as the percentage of PMNL in total cells (excluding erythrocytes). All the slides were read, masked to treatment by the same investigator. The percentage of PMNL was compared among treatments as a continuous variable for both first and second lavage. Incidence of CE diagnosed with a cut-off point of 10% PMNL (Cheong et al., 2011) was dichotomously analyzed in second lavage.

Statistical Analyses

Data from several cows were removed before data analysis because of health reasons. One cow assigned to ITM/OTM was removed from the entire study because she stepped on 2 teats during the prepartum period; therefore, the number of cows available for the prepartum analysis was 20, 19, and 20 for ITM, ITM/OTM, and HTM treatments, respectively. In addition, 5 cows were removed from the postpartum data set due to severe health problems following parturition: 1 cow from the ITM treatment was removed because of the development of retained placenta, ketosis, and displaced abomasum; 3 cows were removed from the ITM/OTM treatment (1 cow because of ketosis and hypocalcaemia; 1 cow due to ketosis, mastitis, and displaced abomasum; and 1 cow because of retained placenta and displaced abomasum); and 1 cow from the HTM treatment because of dystocia, retained placenta, and fever.

Data for DMI and milk yield were averaged to weekly means before analysis. Baseline values collected during the pretreatment week before assignment to treatment were used as covariates for DMI, BW, BCS, gait score, plasma TAC, and plasma TBARS concentration. Previous 305-d mature equivalent milk yields were used as covariates for milk yield and milk composition. Weekly variables were analyzed using PROC MIXED of SAS (version 9.1; SAS Institute Inc., Cary, NC) for a completely randomized design with repeated measures. Model terms were the fixed effects of covariate, treatment, week, and the interaction of treatment and week. The random effect was cow nested within treatment. For each weekly variable, cow was subjected to 4 covariance structures (first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, and heterogeneous compound symmetry) and the structure yielding the smallest Akaike information criterion was selected. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom. Covariates were retained in the model if $P < 0.10$. Consistent with our primary objective of comparing supplementation of HTM with 2 other common approaches for trace mineral supplementation to dairy cattle, single degree of freedom contrasts were used to test HTM versus ITM and HTM versus ITM/OTM.

The percentage of PMNL in the total cells in low-volume lavage was analyzed as a continuous variable for data collected during the first and second lavage. Incidence of CE was analyzed as a dichotomous variable using the cut point described above for second lavage. Statistical significance was declared at $P < 0.05$ and trends were discussed at $0.05 < P < 0.10$. Least squares means and standard error of the mean are reported throughout.

RESULTS

Production results are presented in Table 3. Overall effects of HTM supplementation compared with either ITM or ITM/OTM on DMI, milk yield, and milk components (fat, true protein, and lactose) were not significant; however, cows fed HTM tended to have greater postpartum DMI than cows fed ITM/OTM (25.8 vs. 24.5 kg/d; $P = 0.07$). An interaction of treatment and week existed ($P = 0.02$; Figure 1) for milk yield; however, differences of individual treatments were apparent only at wk-5 postpartum for the comparison of HTM versus ITM. This interaction was also present and similar for yields of 3.5% FCM ($P = 0.03$) and lactose ($P = 0.05$; data not shown). Neither SCC nor concentrations of MUN were affected by treatment (Table 3).

The effects of trace mineral source on BW, BW change, BCS, and BCS change along with gait score are presented in Table 4. Cows fed HTM had higher BW

Figure 1. Milk yield for cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation. Values are LSM; $n = 19$ for inorganic sources based upon sulfates (ITM), $n = 16$ for 75:25 blend of sulfates and organic complexed sources (ITM/OTM), and $n = 19$ for hydroxy trace minerals (HTM); SEM averaged 1.7 kg/d; the P -value for the interaction of treatment $\times$ week was 0.02.

than those fed ITM during the prepartum period (765 vs. 719 kg; $P = 0.02$). During the postpartum period, cows fed HTM had higher BW than cows fed either ITM ($P = 0.02$) or ITM/OTM ($P = 0.05$). Despite these differences in BW, BW change was not affected by treatment. Neither BCS nor BCS change during either the prepartum or postpartum periods was affected by treatment. Gait scores were not affected by treatment.

Responses of plasma variables to supplementation with various sources of trace minerals are detailed in

Table 3. Least squares means for DMI, milk yield, and milk composition for cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation

Item					P-value		
	Treatment ¹				Treatment contrast		
	ITM	ITM/OTM	HTM	SEM	HTM vs. ITM	HTM vs. ITM/OTM	Trt $\times$ Wk ²
DMI, kg/d							
Prepartum	15.0	14.8	14.8	0.3	0.68	0.93	0.68
Postpartum	25.4	24.5	25.8	0.5	0.59	0.07	0.93
Milk, kg/d	45.3	45.7	46.6	1.1	0.37	0.54	0.02
Fat, %	3.35	3.29	3.29	0.06	0.44	0.98	0.92
Fat, kg/d	1.48	1.45	1.49	0.05	0.88	0.53	0.11
Protein, %	3.01	2.96	3.01	0.05	0.97	0.49	0.71
Protein, kg/d	1.34	1.33	1.37	0.04	0.45	0.42	0.19
Lactose, %	4.71	4.70	4.66	0.04	0.29	0.39	0.94
Lactose, kg/d	2.14	2.16	2.18	0.05	0.54	0.76	0.05
SCC ($\times 1,000$)	347	285	382	67	0.68	0.28	0.74
Urea N, mg/dL	11.0	11.8	11.2	0.3	0.64	0.14	0.84
TS, %	12.0	11.9	11.9	0.1	0.43	0.96	0.67
TS, kg/d	5.38	5.35	5.46	0.11	0.57	0.47	0.16
3.5% FCM, ³ kg/d	43.6	43.3	44.3	1.2	0.64	0.52	0.03
ECM, ⁴ kg/d	43.2	42.9	44.0	0.9	0.47	0.36	0.18

¹ITM = inorganic sources based upon sulfates; ITM/OTM = a blend (75:25) of sulfates and organic sources; HTM = hydroxy trace minerals.

²Treatment $\times$ week interaction.

³FCM = $(0.4324 \times \text{kg of milk}) + (16.216 \times \text{kg of milk fat})$.

⁴ECM = $(0.323 \times \text{kg of milk}) + (12.82 \times \text{kg of fat}) + (7.13 \times \text{kg of protein})$.

Table 4. Least squares means for BW, BW change, BCS, and BCS change and gait score for cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation

Item	Treatment ¹			SEM	P-value		
					Treatment contrast		Trt × Wk ²
	ITM	ITM/OTM	HTM		HTM vs. ITM	HTM vs. ITM/OTM	
BW, kg							
Prepartum	719	740	765	14	0.02	0.20	0.48
Postpartum	638	644	682	14	0.02	0.05	0.19
BW change, kg							
Prepartum	8.6	10.6	4.4	11.0	0.77	0.68	
Postpartum	−21.6	−42.5	−33.9	12.6	0.47	0.62	
BCS							
Prepartum	3.34	3.44	3.37	0.07	0.87	0.73	0.99
Postpartum	2.86	2.79	2.86	0.05	0.82	0.24	0.84
BCS change							
Prepartum	−0.08	−0.06	−0.09	0.07	0.87	0.73	
Postpartum	−0.25	−0.35	−0.22	0.07	0.82	0.24	
Gait score	1.76	1.79	1.57	0.11	0.22	0.17	0.93

¹ITM = inorganic sources based upon sulfates; ITM/OTM = a blend (75:25) of sulfates and organic sources; HTM = hydroxy trace minerals.

²Treatment × week interaction.

Table 5 and Figure 2. Cows fed HTM had lower plasma TAC than cows fed ITM during the overall study period (1.93 vs. 2.14 mM; $P = 0.03$), the prepartum period (1.84 vs. 2.09 mM; $P = 0.03$), and the postpartum period (1.95 vs. 2.16 mM; $P = 0.04$). Plasma TAC was not different for cows fed HTM versus ITM/OTM. Cows fed HTM tended to have lower plasma TBARS concentration during the entire study period compared with cows fed ITM (1.95 vs. 2.11 μ M; $P = 0.07$), but differences were not significant when analyzed separately for the prepartum and postpartum periods. Plasma TBARS concentration was not different in cows fed HTM compared with ITM/OTM. Plasma Hp concentration was not affected by treatment when assessed during the entire postpartum period; however, cows fed HTM tended to have lower Hp concentration than cows fed ITM/OTM during wk-1 postpartum (0.90 vs. 1.26 mg/mL; $P = 0.10$).

The percentages of PMNL in both first and second lavage were not affected by treatments and the incidence of CE as assessed at second lavage was not affected by treatment (Table 6). One second lavage from 1 cow fed the ITM/OTM treatment was not obtained because of cervix deformity for unknown reasons, which resulted in 15 cows total for the ITM/OTM treatment.

DISCUSSION

Target and final concentrations of supplemental Zn, Cu, and Mn were virtually identical across the 3 treatments during the postpartum period (Table 2). During the prepartum period, the final concentrations of supplemental Zn, Cu, and Mn were lower than targeted

because cows consumed more of the prepartum diet than anticipated. However, prepartum DMI was virtually identical across treatments; therefore, final dietary concentrations of trace minerals of interest were lower than expected but similar among treatments (Table 2).

Effects of feeding HTM on milk yield and milk composition were not significant, with the exception of a treatment by time interaction for milk yield (Figure 1) and FCM yield (data not shown); this interaction was driven primarily by differences for cows fed HTM versus ITM at wk-5 postcalving. This is the first experiment to assess the effects of HTM supplementation on the performance of dairy cows during the periparturient period and early lactation; however, a meta-analysis of research conducted using organic forms of trace minerals has shown increased milk yield (Rabiee et al., 2010) when these forms are supplemented, although responses are heterogeneous. The organic forms are considered to have greater bioavailability than inorganic (sulfate or oxide) forms (Ammerman et al., 1995); therefore, we anticipated that feeding HTM sources that have been shown in steers (Spears et al., 2004) to have higher bioavailability than sulfates might also increase milk yield.

Few studies have reported effects of supplemental trace minerals on milk composition. The responses to feeding more bioavailable trace minerals vary across studies: no response for percentages and yield of milk components (Uchida et al., 2001), increased percentage of protein (Kincaid and Socha, 2004), increased yield of protein (Siciliano-Jones et al., 2008), and increased yield of fat and protein (Ballantine et al., 2002; Kellogg et al., 2003; Griffiths et al., 2007). The reason for the discrepancy among studies is unknown, although the

Table 5. Plasma total antioxidant capacity (TAC), thiobarbituric acid reactive substances (TBARS), and haptoglobin (Hp) in cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation

Item					P-value		Trt × Wk ²
	Treatment ¹			Treatment contrast			
	ITM	ITM/OTM	HTM	SEM	HTM vs. ITM	HTM vs. ITM/OTM	
TAC, mM							
Whole study period ³	2.14	2.07	1.93	0.07	0.03	0.17	0.94
Prepartum period ⁴	2.09	1.93	1.84	0.08	0.03	0.45	0.85
Postpartum period ⁵	2.16	2.09	1.95	0.08	0.04	0.19	0.87
TBARS, μM							
Whole study period ³	2.11	1.98	1.95	0.07	0.07	0.80	0.40
Prepartum period ⁴	1.47	1.49	1.38	0.06	0.28	0.19	0.74
Postpartum period ⁵	2.26	2.11	2.10	0.09	0.15	0.91	0.23
Hp, mg/mL							
Postpartum period ⁶	0.83	0.91	0.78	0.09	0.65	0.28	0.66
1 wk postpartum ⁷	1.05	1.26	0.90	0.16	0.47	0.10	—

¹ITM = inorganic sources based upon sulfates; ITM/OTM = a blend (75:25) of sulfates and chelated sources; HTM = hydroxy trace minerals.

²Treatment × week interaction.

³Represents plasma samples collected weekly from 3 wk prepartum through 12 wk postpartum.

⁴Represents plasma samples collected weekly from 3 wk prepartum through 1 wk postpartum.

⁵Represents plasma samples collected weekly from 1 wk postpartum through 12 wk postpartum.

⁶Represents plasma samples collected weekly from 1 wk postpartum through 8 wk postpartum.

⁷Represents plasma samples collected from 1 wk postpartum only.

responses in component yields appear to be more related to milk production responses rather than changes in milk composition. A meta-regression analysis indicated that milk fat and protein percentages were not influenced by supplementation with a mixture of organic complexes of trace minerals, whereas protein and fat yields in milk were increased (Rabiee et al., 2010).

Few studies have reported temporal patterns of plasma markers related to oxidative stress during the periparturient period and early lactation. Plasma TAC represents gross antioxidant status, including both water- and fat-soluble antioxidants (Rice-Evans and Miller, 1994). Temporal patterns depicted in Figure 2 for plasma TAC (panel A) suggest that TAC was slightly greater during the postpartum period than the prepartum period. These results differ somewhat from those reported by Castillo et al. (2006) who reported that TAC was slightly greater during wk-1 postcalving than wk-4 and -8 postpartum. Reasons for the difference in patterns between our results and those of Castillo et al. (2006) are not readily apparent, although performances of cows (both milk yield and DMI) were substantially (40 to 50%) greater in our study. Furthermore, supplementation levels and strategies for vitamin and trace mineral nutrition were not described in the other study.

Plasma TBARS is considered to represent a composite number of lipid oxidative end products, including malondialdehyde (Bernabucci et al., 2005). Plasma TBARS concentrations appeared to decrease slightly as parturition approached, but then increased steadily

during the postpartum period (Figure 2, panel B). This pattern is similar to those reported by Bernabucci et al. (2005) and Wullepit et al. (2012). In contrast, Castillo et al. (2006) reported that plasma malondialdehyde, which should largely reflect TBARS as suggested above, was elevated at wk-1 postpartum compared with subsequent weeks in lactation.

In the current experiment, plasma TAC was lower in cows fed HTM than cows fed ITM during the whole, prepartum, and postpartum study periods (Table 5). Plasma TBARS concentration tended to be lower in cows fed HTM than cows fed ITM during whole study period (Table 5). Although the treatment by time interaction was not significant, it appears that the overall trend was mostly driven by the patterns observed later in the postpartum period (Figure 2, panel B). This would support the concept that differences in response variables related to oxidative metabolism may require supplementation over a longer period of time. Our research group determined that feeding organic chelates of Zn, Cu, and Mn at NRC or commercial (2× of NRC) levels decreased plasma TBARS concentration right after intramammary LPS challenge in mid-lactation cows compared with sulfate forms (Yasui et al., 2009). Lower plasma TAC in cows fed HTM may be due to less demand for antioxidant activity as a result of low plasma TBARS concentration or utilization of antioxidants that in turn contributed to the decreased TBARS concentration, although the temporal patterns of response in Figure 2 suggest that responses of TAC to

Figure 2. Plasma total antioxidant capacity (TAC; panel A), thio-barbituric acid reactive substances (TBARS; panel B), and haptoglobin (Hp; panel C) for cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation. Values are LSM; $n = 19$ for inorganic sources based upon sulfates (ITM), $n = 16$ for 75:25 blend of sulfates and organic complexed sources (ITM/OTM), and $n = 19$ for hydroxy trace minerals (HTM); SEM averaged 0.10 mM, 0.12 μ M, and 0.14 mg/mL for TAC, TBARS, and Hp, respectively. The P -values for the effect of treatment were 0.09, 0.15, and 0.55 for TAC, TBARS, and Hp, respectively. The P -values for the effect of week were <0.01 for all variables and the P -values for the interaction of treatment and week were >0.40 for all variables.

HTM supplementation existed across the entire treatment period, whereas responses of TBARS appeared to occur during the latter part of the treatment period. Further research is needed to elucidate the relationship between antioxidant status and oxidative stress response variables.

Concentrations of Hp in plasma were greatest during wk 1 and decreased steadily throughout the postpartum period (Figure 2, panel C). Effects of source of Zn, Cu, and Mn on plasma Hp concentration were not significant over the entire postpartum period; however, cows fed HTM tended to have lower plasma Hp concentration than those in the ITM/OTM treatment during the first week after parturition (Table 5). Acute-phase proteins such as Hp, serum amyloid A, and C-reactive protein are plasma proteins that increase in concentration following stimulus conditions such as infection, inflammation, or trauma (Eckersall, 2000). Haptoglobin is a notable acute-phase protein in ruminants, with a negligible circulating level in normal animals, but increases over 100-fold upon stimulation (Eckersall, 2000). Because cows fed HTM tended to have lower plasma Hp concentrations during the first week postcalving than cows fed ITM/OTM, it suggests that cows fed HTM had decreased systemic inflammation during the first week postcalving. Although it has been shown that serum Hp concentration within a week after parturition can be an indicator or risk factor to predict development uterine diseases such as metritis and CE in dairy cows (Huzzey et al., 2009; Chan et al., 2010; Dubuc et al., 2010), the lack of treatment differences in uterine cytology suggests that elevated Hp concentrations immediately postpartum may not be a major risk factor for subsequent development of CE.

Effects of sources of Zn, Cu, and Mn on the extent of influx of PMNL to the uterus at 7 d and 1 d between 40 and 60 d postpartum were not significant (Table 6). The reason for the lack of response of uterine cytology to various sources of trace minerals in the current study is unknown. Our results showed around 40% of PMNL influx at 7 d postpartum among all treatments. Gilbert et al. (2007) showed the median proportion of PMNL at 0, 7, and 21 d postpartum was 37, 20, and 41%, respectively. Therefore, all of the cows in the current study may have had somewhat higher percentages of PMNL compared with those in previous research. During the early postpartum period, increased infiltration of the uterus with PMNL may indicate that cows in this study had relatively robust immune function. This speculation is supported by an overall low ($\sim 35\%$) subsequent incidence of CE in cows in this experiment. Previous research (Gilbert et al., 2005) conducted on commercial dairy farms determined that CE rates ranges from 37 to 74% of cows.

Table 6. Percentages of neutrophils in first and second uterine lavage and incidence of cytological endometritis (CE) in second uterine lavage from cows fed varying sources of Zn, Cu, and Mn during the periparturient period and early lactation

% of PMNL ¹	Treatment ²				P-value		
					Treatment contrast		
	ITM	ITM/OTM	HTM	SEM	Trt ³	HTM vs. ITM	HTM vs. ITM/OTM HTM
First lavage ⁴	40.4	40.2	41.2	6.2	0.99	0.92	0.90
Second lavage ⁵	12.5	9.9	8.9	2.4	0.48	0.24	0.77
Cows with CE, no. ⁶	7	5	7	—	0.97		
Cows without CE, no.	12	10	12	—			

¹Percentage of PMNL in total 200 cells, excluding erythrocytes.

²ITM = inorganic sources based upon sulfates; ITM/OTM = a blend (75:25) of sulfates and chelated sources; HTM = hydroxy trace minerals.

³Treatment.

⁴Lavage at 7 d postpartum.

⁵Lavage at 1 d between 40 and 60 d postpartum.

⁶Number of cows that developed cytological endometritis (CE; diagnosed positive if more than 10% PMNL were counted in second lavage).

Consistent with the result of PMNL percentage, treatments effects on incidence of CE at 40 to 60 d postpartum were also not significant (Table 4) and the overall incidence was relatively low, as indicated above. These results suggest that trace mineral source at the levels of trace mineral supplementation studied does not affect CE. Other studies clearly have illustrated relationships of CE with Ca status (Martinez et al., 2012) and energy status (Hammon et al., 2006), suggesting that Zn, Cu, and Mn nutrition are less important nutritional factors in the etiology of CE.

CONCLUSIONS

Supplementation of hydroxy sources of Zn Cu, and Mn during the transition period and early lactation modulated plasma markers related to oxidative metabolism, resulting in trends for decreased TBARS concentration and decreased TAC compared with supplementation of these minerals as sulfates. Overall effects of treatment on milk yield and composition were not significant. Differences between hydroxy sources and a blend of sulfates and organic complexes of Zn, Cu, and Mn were not significant for virtually all outcome variables. Trace mineral source did not affect endometrial cytology and the incidence of CE in this experiment.

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