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The effect of injectable trace minerals (selenium, copper, zinc, and manganese) on peripheral blood leukocyte activity and serum superoxide dismutase activity of lactating Holstein cows

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ABSTRACT

The objective of this study was to evaluate the effect of subcutaneous supplementation of 300 mg of zinc, 50 mg of manganese, 25 mg of selenium, and 75 mg of copper on peripheral blood leukocyte activity and serum β-hydroxybutyrate (BHBA) concentrations at 10 ± 2 days in milk (DIM), and on serum superoxide dismutase (SOD) activity during the transition period and subsequent lactation of multiparous Holstein cows. A total of 250 multiparous cows were randomly allocated into one of two treatments groups, namely, trace mineral supplemented (TMS) or control. Cows in the TMS group were injected at 230 and 260 days of gestation, and 35 days postpartum. Serum SOD activity was measured at enrollment, and 10, 60 and 100 DIM. Serum BHBA concentration and leukocyte function were assessed at 10 DIM. Overall serum SOD activity for TMS and control was 16.01 and 12.71 U/mL, respectively. The interaction between treatment and time of serum collection was significant. Additionally, overall serum SOD activity was 12.85 and 14.78 U/mL for cows diagnosed with mastitis and unaffected cows, respectively. Treatment did not affect leukocyte function. For parity >2, TMS cows had lower serum BHBA concentrations than control cows; BHBA concentrations were 0.41 and 0.27 mmol/L for control and TMS cows, respectively. In conclusion, cows diagnosed with mastitis had decreased serum SOD activity, and trace mineral supplementation increased serum SOD activity although leukocyte function was not affected by supplementation.

Introduction

The transition period (defined as the period from 3 weeks before to 3 weeks after calving) is extremely challenging for the dairy cow (Drackley, 1999). It has been reported that the concentration of some trace minerals are affected during the transition period, especially around the time of parturition (Goff and Stabel, 1990; Xin et al., 1993; Meglia et al., 2001).Trace minerals play an important role in dairy cow immune function (Shankar and Prasad, 1998), fertility (Rabiee et al., 2010), and growth (Enjalbert et al., 2006). Polymorphonuclear leukocyte function and bactericidal efficiency is compromised during the transition period (Burvenich et al., 2003). Trace mineral status, especially selenium (Se) and zinc (Zn), affects neutrophil function in postpartum cows and affects neutrophil adhesion and superoxide production (Meglia et al., 2001; Cebra et al., 2003).

Recently, we conducted a large field trial using 1400 dairy cows from three farms located in upstate New York to evaluate the effect of a trace mineral product containing Zn, Se, manganese (Mn) and copper (Cu) at 230 and 260 days of gestation, and 35 days postpartum on production, fertility and health traits. Supplementation did not affect milk production or fertility; however, we observed a significant positive effect on udder health for multiparous cows, decreasing somatic cell count (SCC) and the incidence of clinical mastitis (Machado et al., 2013). Nevertheless, the effect of injectable trace mineral on the transition cow's immune and antioxidant systems remains unknown.

Metabolic demands associated with the transition period increase the production of reactive oxygen species (Sordillo et al., 2009) and may lead to oxidative stress (Miller et al., 1993). Superoxide dismutases (SOD) are enzymes that are involved in the antioxidant system and are Mn, Cu and Zn dependent (Andrieu, 2008). The enzymes are considered to be the first line of antioxidant defense, converting superoxide anion (O_2^-) to hydrogen peroxidase (H_2O_2), which is not a free radical (Michiels et al., 1994). Although there is evidence for a role of other antioxidants in udder health,

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Table 1
Chemical composition (mineral and vitamins) of pre-fresh and fresh cow diets.

Chemical composition (dry matter basis)	Pre-fresh	Lactation
Crude protein (%)	14.00	15.70
Soluble protein (% CP)	30.00	29.00
Acid detergent fiber (%)	26.40	20.50
Neutral detergent fiber (%)	43.00	33.00
Calcium (%)	1.35	0.86
Phosphorus (%)	0.40	0.43
Magnesium (%)	0.35	0.31
Potassium (%)	1.19	1.35
Sodium (%)	0.18	0.49
Sulfur (%)	0.43	0.27
Copper (ppm)	15.00	20.00
Iron (ppm)	292.00	188.00
Manganese (ppm)	85.00	63.00
Selenium (ppm)	0.66	0.80
Zinc (ppm)	74.00	103.00
Molybdenum (ppm)	0.90	1.20

Pre-fresh diets were fed from 3 week prepartum through parturition, and lactation diets were fed from parturition through week 35 postpartum.

the effect of SOD status on mammary gland health deserves further research (Sordillo and Aitken, 2009).

A high serum concentration of postpartum β-hydroxybutyrate (BHBA) is an indicator of negative energy balance in the transition period, and it is associated with increased fat mobilization in early lactation (Ingvartsen and Andersen, 2000). It has been previously reported that Cu supplementation increased metabolism of adipose tissue in steers, due to an increased response to hormones responsible for lipolysis (Engle et al., 2000). To the best of our knowledge, there is no information regarding what effect Mn, Zn, Se, and Cu supplementation during the transition period has on postpartum BHBA concentrations.

The objective of this study was to evaluate the effect of subcutaneous supplementation of 300 mg of Zn, 50 mg of Mn, 25 mg of Se, and 75 mg of Cu (Multimin 90, Multimin North America) at 230 and 260 days of gestation, and 35 days postpartum on peripheral blood leukocyte activity and serum BHBA concentration at 10 ± 2 days in milk (DIM), and serum SOD activity during the transition period, and subsequent lactation of multiparous Holstein cows.

Material and methods

Farms and management

This study was conducted at a dairy farm located near Ithaca, New York. Cows were enrolled from 24 August until 29 September 2011; the follow-up period continued until 4 July 2012. The farm milked 3300 Holstein cows three times daily in a double 52-stall parallel milking parlor. The cows were housed in freestall barns, with concrete stalls covered with mattresses and bedded with manure solids. All cows were offered a total mixed ration (TMR) consisting of approximately 55% forage (corn silage, haylage and wheat straw) and 45% concentrate (corn meal, soybean meal, canola, cottonseed and citrus pulp) on a dry matter basis. The diet was formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% fat corrected milk. Nutrient contents of the diets are described in Table 1.

Samples of TMR from pre-fresh and lactation diets were obtained on two different days of a given week during the study period. The samples were combined into 2-day composite samples and submitted (for both pre-fresh and lactation diets) to a commercial laboratory (Dairy One Cooperative) for wet chemistry analysis. Samples were analyzed for dry matter, crude protein, acid detergent fiber, neutral detergent fiber, and macro and micro minerals.

Study design and treatments

A randomized field trial study design was used; 250 cows were randomly allocated by study entry date into one of two treatments groups: trace mineral supplemented (TMS) or control. Randomization was completed in Excel (Microsoft) and imported into the farm's Dairy Comp 305 program (Valley Agricultural Software). Cows that were randomly assigned to the treatment group received three injections of 5 mL of Multimin 90 at 230 and 260 days of gestation, and 35 days postpartum; each injection contained 300 mg Zn, 50 mg Mn, 25 mg Se, and 75 mg Cu.

Body condition scores (BCS) were determined for all study cows at 230 days of gestation and at 35 ± 3 DIM by a single investigator masked to the treatment group using a five-point scale with a quarter-point system (Edmonson et al., 1989). Serum samples were collected at 230 ± 3 and 260 ± 3 days of gestation, 10 ± 2, 60 ± 3, and 100 ± 3 DIM. For evaluation of peripheral blood neutrophil function, blood was collected at 10 ± 2 DIM and processed for leukocyte activity within 15 h.

The proposal was approved by the Cornell University Animal Care and Use Committee (2009-0001) and owner consent was obtained before the study was started.

Case definition

Retained fetal membranes, metritis, ketosis, displaced abomasum and clinical mastitis were diagnosed and treated by trained farm personnel who followed a specific diagnostic protocol designed by veterinarians from the Ambulatory and Production Medicine Clinic, Cornell University. Farm personnel were masked to the treatments. The presence of retained fetal membranes was defined as failure to release fetal membranes within 24 h of calving. Metritis was defined as the presence of fetid, watery, red-brown uterine discharge and rectal temperature >39 °C. Ketosis was defined as high concentrations of ketone bodies (≥1470 μmol/L) in urine using Ketostix (Bayer). Displaced abomasum diagnosis made by the farm personnel was confirmed by veterinarians. Clinical mastitis was defined as abnormal changes in the udder and milk, such as watery appearance, flakes and clots. Data regarding health traits during the follow-up period were extracted from the farm's DairyComp 305 database, and all health events were considered as a single event variable.

Peripheral blood leukocyte function, serum SOD activity and serum concentration of BHBA

Leukocyte phagocytic activity was evaluated at 10 DIM using Phagotest Kit (Orpegen Pharma) containing fluorescein-labeled opsonized *Escherichia coli* (*E. coli* – FITC), following the manufacturer's instructions. Granulocyte oxidative burst was determined quantitatively with Bursttest Kit (Orpegen Pharma) following the manufacturer's instructions. Cells were analyzed with a FACSCalibur flow cytometer (Becton-Dickinson) using a 488 nm argon-ion laser. Ten thousand events were collected for each cell population (neutrophils or monocytes). Neutrophil activity assays were only performed on the first 200 cows that entered the study; one cow was excluded from these analyses due to a laboratory error.

Serum SOD activity was assessed using Superoxide Dismutase Assay Kit (Cayman Chemical Company), following the manufacturer's instructions. Serum SOD activity was measured at 230 ± 3 days of gestation, and 10 ± 2, 60 ± 3, and 100 ± 3 DIM.

Serum concentration of BHBA was measured using the Auto kit 3-HB (Wako Chemicals), following the manufacturer's instructions. Serum concentration of BHBA was measured at 10 ± 2 DIM. Serum concentrations of BHBA were only performed on the first 200 cows that entered the study.

Statistical analyses

To facilitate data analysis and interpretation, the variables BCS at enrollment (BCS1 = 1 if BCS was < 3; BCS1 = 2 if BCS = 3; BCS1 = 3 if BCS > 3), BCS at 10 ± 2 DIM (BCS2 = 1 if BCS at 10 ± 2 DIM was less than 3; BCS2 = 2 if BCS = 3 BCS2 = 3 if BCS > 3), and Disease (Disease = 0 if the cow did not have any health event during the follow up period; Disease = 1 if the cows had at least one health event) were created. Descriptive statistics analysis was undertaken in SAS using the FREQ procedure (SAS Institute). The statistician was not blinded to the treatments. The experimental unit was the cow.

Two mixed general linear models were fitted to the data using the MIXED procedure of SAS. The dependent variable evaluated in these analyses was serum SOD activity. The model assumption of normally distributed residuals was satisfied by visual evaluation of the distribution plot of the studentized residuals. The data were longitudinally collected and comprised a series of repeated measures of the dependent variable throughout the four time points of serum collection: at 230 days of pregnancy (enrollment), and 10 ± 2, 60 ± 3, and 100 ± 3 DIM. To account appropriately for repeated measures, the error term was modeled by imposing a first-order autoregressive covariance structure for all statistical models.

The independent variables offered to the first model were: treatment, BCS1, parity, and time of serum collection. The independent variables offered to the second model were: BCS1, BCS2, parity, ketosis, mastitis, metritis, retained placenta, displaced abomasum and time of serum collection. Two-way and three-way interactions between treatment and health parameters, parity and time of serum collection were offered to the models. Furthermore, variables and their respective interaction terms in all models were retained in the model only when they had a significant effect ($P < 0.05$).

To assess the effect of treatment, parity, disease, retained placenta, metritis, mastitis, displaced abomasum and ketosis on the proportion of neutrophils or