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Short communication: Effects of supplementing diets of Holsteins with copper, zinc, and manganese on blood neutrophil function

A. M. Dietz, W. P. Weiss, M. J. Faulkner, and J. S. Hogan[1]
Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

ABSTRACT

The effects of supplementing diets with sulfate or glycinate Cu, Zn, and Mn on blood neutrophil function were examined in 27 late-lactation Holstein cows having a mean (± standard deviation) days in milk at time of neutrophil assays of 216 ± 31 d. Cows were assigned to 9 blocks of 3 and were grouped by parity, milk production, and days in milk. Cows within each block were randomly assigned to 1 of 3 treatments: (1) control diet devoid of supplemental Cu, Zn, and Mn; (2) diet supplemented with Cu, Zn, and Mn via sulfates; and (3) diet supplemented with Cu, Zn, and Mn via glycinate form. All cows were initially fed a control total mixed ration with basal mineral concentrations of 8 mg/kg of Cu, 35 mg/kg of Zn, and 35 mg/kg of Mn for 30 d. During the treatment period, cows fed diets with mineral supplementation via sulfates or glycinate forms had target total dry matter dietary concentrations of 18 mg/kg of Cu, 60 mg/kg of Zn, and 60 mg/kg of Mn for 30 d. Control cows were fed the control diet devoid of supplemental minerals for an additional 30 d. In vitro neutrophil functions were measured after 30 d on experimental or control diets. Percentage of neutrophils phagocytizing, intracellular kill, and phagocytic index did not differ among treatments. Serum concentrations of Cu, Zn, and Mn were also not affected by dietary treatment after 30 d. Results from this study demonstrated that dietary Cu, Zn, and Mn supplemented either as sulfates or glycinate form for 30 d had no effect on either in vitro blood neutrophil function or serum concentrations of Cu, Zn, and Mn in late-lactation Holstein cows.
Key words: copper, zinc, manganese, neutrophils

Short Communication

Many factors contribute to the functional status of bovine neutrophils that ultimately affect mammary gland host defenses and prevention of mastitis. Nutritional status of the cow influences host defenses and effects the duration and severity of disease (Scaletti et al., 2003; Goff, 2006; Heinrichs et al., 2009). Microminerals, such as Cu, Zn, and Mn, have been shown to be beneficial in improving udder health by reducing SCC and decreasing the incidence of mastitis in the periparturient and early lactation periods (Scaletti et al., 2003; Siciliano-Jones et al., 2008). Copper, Zn, and Mn are essential for the functionality of many enzymes and structural and cellular proteins (Nocek et al., 2006). The essential nature of these minerals in the antioxidant role of enzymes mediating phagocytosis and killing of bacteria by bovine neutrophils was recently reviewed by Overton and Yasui (2014). Traditionally, microminerals have been supplemented via sulfates; however, organic supplementation has been reported to increase bioavailability and absorption of microminerals in the gut (Andrieu, 2008). Enhancing mineral absorption may have a positive effect on neutrophil function due to increased accessibility to minerals in blood. Studies have shown that organic supplementation of microminerals has been associated with improvements in milk yield, clinical udder score, and *Escherichia coli* counts in milk compared with inorganic and unsupplemented groups following an *E. coli* challenge in early-lactation animals (Scaletti and Harmon, 2012). Although the influence of dietary supplementation of various essential minerals on host defenses and mammary health in early-lactation cows have been reported, little information is available on the benefits of supplementing dietary inorganic and organic sources of minerals on health parameters, including neutrophil function, in late-lactation cows. Therefore, the objective of the current study was to examine the effects of sulfate or glycinate dietary supplementation of Cu, Zn, and Mn in late-lactation cows on in vitro bovine neutrophil phagocytosis and intracellular kill.

Twenty-seven late-lactation Holstein cows in the Ohio Agriculture Research and Development Center Krauss Dairy Herd were used to measure the effects of Cu, Zn, and Mn supplementation on bovine neutrophil function. All procedures using animals were approved through The Ohio State Institutional Animal

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[1]Corresponding author: hogan.4@osu.edu

Use and Care Committee (Columbus, OH), Animal Use Protocol: 2014A00000059. Cows were assigned to 9 blocks of 3 based on parity, milk production, and DIM. Four blocks were composed of primiparous cows, and 5 blocks were composed of multiparous cows. Average (±SD) milk production was 31.4 ± 6.3 kg during the pretreatment period and DIM was 216 ± 31 d at time of neutrophil assays. Blocks of cows were moved to the pretreatment pen on alternating days over an 18-d period so that each block was sampled on a separate day for neutrophil assays corresponding to d 31 on dietary treatments. Cows were fed a TMR once daily through the pretreatment and treatment periods with a target of 3 to 5% refusal. During the pretreatment period, all cows were fed a diet without supplementation with a commercial source of Cu, Zn, and Mn. Dietary concentrations of Cu, Zn, and Mn were 8.0, 35.0, and 35.0 mg/kg of dietary DM, respectively, for 30 d and cows were housed in freestalls. After this period, cows were moved into tiestalls and placed on 1 of the 3 treatment diets for an additional 30 d (Table 1). Cows within blocks were randomly assigned to 1 of 3 treatments by pulling slips of paper out of a container: (1) devoid of supplemental Cu, Zn, and Mn; (2) supplemented with Cu, Zn, and Mn via sulfates; and (3) supplemented with Cu, Zn, and Mn via glycinate form (B-Traxim, Pancosma, Geneva, Switzerland). The unsupplemented treatment cows, which served as the control, remained on basal control Cu, Zn, and Mn dietary concentrations. The sulfate and glycinate treatments were supplemented with target total dietary concentrations of 18.0, 60.0, and 60.0 mg/kg of DM of CU, Zn, and Mn, respectively. Details of ingredient composition of diets and nutrient composition of diets and forages supplied by diets are in Table 2. Feed component samples were collected weekly and DM (100°C oven for 24 h) was calculated and recorded. Feed samples were kept frozen at −20°C until the trial was complete. Once the trial was completed, composite samples were prepared for each feed component: corn silage, alfalfa silage, dry hay, and 3 treatment grain mixes. Composite samples were composed of 2 to 4 consecutive sample dates and sent to a commercial analytic laboratory (Cumberland Valley Analytical Services; Hagerstown, MD) for analyses.

Blood (50 mL) was collected from each animal in a block on the same day via jugular vein with a 16-gauge, 38.1-mm needle. Neutrophils were isolated according to Carlson and Kaneko (1973). Blood was collected in sterile 60-mL syringes (BD Luer-Lok, Becton Dickinson Microbiology Systems, Cockeysville, MD) containing 5 mL of EDTA anticoagulant (0.7% NaCl, 1.5% EDTA disodium salt: dehydrate, 0.0132 *M* KH_2PO_4 and 0.0132 *M* Na_2HPO_4; adjusted to pH 6.6). Forty milliliters of blood was dispensed into 50-mL conical bottom centrifuge tubes (Corning Inc., Corning, NY) and centrifuged (Sorvall RT7, RTH-750 rotor; Newtown, CT) for 45 min at 4°C and 1,294 × *g*. The plasma, buffy coat, and upper 2 mL of red blood cells were discarded, leaving approximately 8 mL of packed cells remaining. Remaining red blood cells were lysed by adding 30 mL of 0.03 *M* NaCl to the tube and mixing for 90 s by hand. Eight milliliters of 0.63 *M* NaCl was added and mixed to bring saline solution back to equilibrium. After lysis, cells were centrifuged for 15 min at 4°C and 748 × *g*. The supernatant was removed, neutrophil pellet was washed with 5 mL of Hanks' Balanced Salt Solution (**HBSS**; modified, without phenol red and sodium bicarbonate; Sigma-Aldrich, St. Louis, MO) and then centrifuged again for 15 min at 4°C and 748 × *g*. Again, the supernatant was removed and 1 mL of HBSS was added to resuspend the pellet. Viability of isolated cells was measured using a trypan blue exclusion procedure. Briefly, 0.05% trypan blue (Sigma-Aldrich) solution was added to 10 μL of neutrophil suspension, and 100 cells mounted on a hemocytometer (Bright-line hemocytometer; Reichert, Buffalo, NY) were counted at

Table 1. Ingredient composition of the diet[1] (% of DM) for cows fed unsupplemented control diets or sulfate or glycinate Cu-, Zn-, and Mn-supplemented diets for 30 d

Ingredient	Control	Sulfate	Glycinate
Corn silage	29.0	29.0	29.0
Alfalfa silage	29.0	29.0	29.0
Alfalfa hay	5.0	5.0	5.0
Corn dried distillers grains	9.2	9.2	9.2
Ground corn	19.2	19.2	19.1
Soybean meal, 48% CP	6.5	6.5	6.5
Animal/vegetable fat	0.36	0.36	0.36
Limestone	0.65	0.65	0.65
Magnesium oxide	0.05	0.05	0.05
Iodized salt	0.42	0.42	0.42
Selenium premix[2]	0.14	0.14	0.14
Vitamin mix[3]	0.46	0.18	0.18
Cu sulfate	—	0.003	—
Zn sulfate	—	0.006	—
Mn sulfate	—	0.006	—
Glycinate Cu[4]	—	—	0.003
Glycinate Zn[4]	—	—	0.009
Glycinate Mn[4]	—	—	0.010

[1]The control diet was fed to all cows for 30 d before the experiment and to the control group during the 30-d experiment. The sulfate and organic treatments provided all supplemental Cu, Zn, and Mn as either sulfate or as glycinates and were fed only during the experimental phase.

[2]Sodium selenate, 200 mg/kg.

[3]Contained 735 kIU of vitamin A/kg, 270 kIU of vitamin D/kg, 4,400 IU of vitamin E, and 135 mg of biotin (Rovimix Biotin, DSM Nutritional Products, Heerlen, the Netherlands) per kilogram.

[4]B-TRAXIM 2C (Pancosma S. A., Geneva, Switzerland). The products contained 270,000, 260,000, and 220,000 mg of Cu, Zn, and Mn/kg, respectively.