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Effect of Selenium Source on Selenium Status, Neutrophil Function, and Response to Intramammary Endotoxin Challenge of Dairy Cows*

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ABSTRACT

The effects of feeding dry and early lactation dairy cows diets with selenate or selenized yeast (Se-yeast) on concentrations of Se in serum, milk, and newborn calves, neutrophil function, and inflammatory response were determined. At 60 d before anticipated calving until approximately 30 d in milk (DIM), cows were fed diets that contained 0.3 mg of supplemental Se/kg of DM from sodium selenate or Se-yeast. Diets also contained 0.2% supplemental S (as sulfate) because it has been shown to reduce absorption of Se by dairy cows. The concentration of Se in serum at calving and 28 DIM was about 1.4 times greater for cows fed Se-yeast than for those fed selenate. Serum concentrations decreased 45 and 23% from dry-off to calving for cows fed selenate or Se-yeast, respectively. Selenium concentrations in serum from newborn calves were also about 1.4 times greater when the dams were fed Se-yeast. Concentrations of Se in colostrum and milk were about 1.8 times greater when cows were fed Se-yeast. Blood neutrophils were isolated from cows at 28 DIM and were used in an in vitro kill assay. Selenium treatment did not affect bacterial kill or the percentage of neutrophils that phagocytized bacteria. At approximately 28 DIM, one quarter from each cow was infused with a solution containing endotoxin. Peak body temperature (40.7°C) occurred 6 h postinfusion, and peak somatic cell count (6.5 $\log_{10}$/mL) occurred at 12 h postinfusion. Neither measure was influenced by Se treatment.
(**Key words:** selenium, neutrophils, mastitis, dairy cow)

Abbreviation key: GSH-px = glutathionine peroxidase; **Se-Met** = seleno-methionine.

INTRODUCTION

Selenium can be supplemented to cattle diets in inorganic (usually sodium salts of selenite or selenate) or organic forms (Se-yeast). Selenium-yeast is produced by growing specific strains of yeast in a Se-enriched media. Although the distribution of Se compounds in yeast varies among sources, Seleno-methionine (**Se-Met**) is usually the predominant form of Se, but selenocysteine and numerous other seleno-compounds are also found (Rayman, 2004).

Concentrations of Se in serum and whole blood have been used as an index of Se status because, in general, increased concentrations of Se in serum or whole blood have been related to reduced SCC, reduced mastitis, and improved neutrophil function (Smith et al., 1984; Erskine et al., 1987; Weiss et al., 1990a; Cebra et al., 2003). Cattle fed Se-yeast usually have higher concentrations of Se in whole blood, serum (or plasma), and milk than do those fed inorganic Se (Nicholson et al., 1991b; Knowles et al., 1999; Ortman and Pehrson, 1999; Gunter et al., 2003). Studies that found relationships between immunological or clinical measures and blood concentrations of Se used inorganic Se. Whether the typically higher concentrations of Se in blood when cows are fed Se-yeast reflect improved Se status (i.e., improved disease resistance) compared with cows fed inorganic Se is unknown.

Factors other than Se intake and Se source can influence Se concentrations in tissues and clinical and immunological responses. Increased intake of sulfate by cows reduces apparent absorption of Se (from selenate), resulting in reduced plasma concentrations of Se (Ivancic and Weiss, 2001). Different systems are used for intestinal absorption of Se-Met (same mechanism as used from methionine) and inorganic Se (Vendeland et al., 1992). Sulfate is less likely to interfere with absorption of Se from Se-yeast than with absorption of inorganic Se. Vitamin E and Se are involved with cellular antioxidant status and increased intake of vitamin E can reduce responses to Se supplementation (Hogan et al., 1990).

We hypothesized that cows fed Se-yeast during the dry period and early lactation would have higher con-

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Table 1. Ingredient composition of diets fed during the experiment (% of DM).

Ingredient	Dry period	Prefresh period	Lactation period
Grass silage	41.5	—	—
Grass hay, long	15.0	10.0	—
Corn silage	26.0	30.0	35.0
Alfalfa silage	—	25.0	20.0
Corn grain, ground	5.70	22.30	22.20
Soybean hulls	6.90	7.55	—
Soybean meal, 44% CP	3.20	3.00	7.00
Soybean meal, treated[1]	—	—	5.10
Animal-vegetable fat	0.17	0.35	0.33
Soybeans, whole roasted	—	—	8.00
Magnesium sulfate	0.65	0.84	0.81
Calcium sulfate	0.36	0.42	0.45
Dicalcium phosphate	0.04	0.03	0.20
Trace mineral salt	0.17	0.20	0.40
Sodium bicarbonate	—	—	0.24
Selenium premix[2]	0.14	0.14	0.14
Mineral-vitamin premix[3]	0.17	0.17	0.13

[1]Surepro, Land O'Lakes, Inc. (St. Paul, MN).

[2]The premix in diets with selenate contained 200 mg of Se from sodium selenate/kg. The premix in diets with Se-yeast contained 10% Alkosel (2000 mg of Se/kg; Lallemand, Inc., Milwaukee, WI) and 90% ground corn.

[3]For dry cow and prefresh diets, premix contained 3230 mg of Cu (copper sulfate), 4650 mg of Zn (zinc sulfate), 3400 kIU of vitamin A, 980 kIU of vitamin D, and 23 kIU of vitamin E/kg. For the lactation diets, the premix contained 6370 mg of Cu, 13,250 mg of Zn, 3740 kIU of vitamin A, 1140 kIU of vitamin D, and 19 kIU of vitamin E/kg.

centrations of Se in blood serum than cows fed selenate and that these higher concentrations would reflect improved Se status. The increased Se status would result in improved neutrophil function and an enhanced inflammatory response. Because of the relationships between Se and vitamin E and between Se and sulfate, we further hypothesized that these differences would be magnified when the basal diets provided less than recommended amounts of vitamin E and excessive amounts of sulfate.

MATERIALS AND METHODS

Holstein cows (n = 22) and heifers (n = 18) were grouped by parity and anticipated calving date into 20 blocks of 2 animals each. At 60 d before anticipated calving, cows were dried off, and cows and heifers moved to one of two identical group pens. The diets fed to both groups were identical except one contained Se-yeast (Alkosel, Lallemand Inc., Milwaukee, WI) and the other contained sodium selenate (Tables 1 and 2). Both diets were formulated to provide 0.3 mg of supplemental Se/kg. The diets were formulated to meet the requirements (NRC, 2001) of a dry cow except that they provided only about 500 IU of supplemental vitamin E/d [50% less than NRC (2001) recommendations] and contained 0.2% supplemental S (from a mixture of calcium and magnesium sulfate). Cows remained in their respective pens until approximately 1 wk before anticipated calving. They were then moved into individual box stalls and fed a prefresh period diet (Tables 1 and 2), but the source and concentration of supplemental Se remained the same as that fed during the dry period. Approximately 3 d after calving, cows were moved to individual tie stalls and fed a lactation diet (Tables 1 and 2). Cows received the same source and concentration of supplemental Se as they did during the dry period. When moved to the tie stalls, cows were fed once daily and milked twice daily. Milk samples (a.m. and p.m.) were taken once weekly during the first 21 DIM and analyzed for fat and true protein using infrared analysis (DHI Cooperative, Inc., Columbus, OH). Animals were weighed on –60 d, when moved to the maternity stalls (approximately 3 d before calving), 3 d postcalving, and on 7 d after they were challenged with LPS (approximately 35 DIM). All calves were weighed 3 d after birth, and female calves (n = 9 and 8, respectively, for selenate and Se-yeast groups) were weighed at weaning. During the first 3 d after birth, calves were fed only colostrum from their dam (4 L/d divided into 2 equal feedings). Female calves were fed a common diet (milk replacer and starter) from 3 d of age until weaning. Weaning occurred when calves consumed 0.7 kg of a DM from a starter grain mix.

At approximately 28 (SD = 7.1) DIM, either the right or left front mammary quarter from each cow was infused with LPS by intramammary infusion via the teat canal. Infusions were 3 h after morning milking. Only uninfected quarters were infused. Concentrated LPS was purchased (*Escherichia coli* O26:B6, Sigma Chemical Co., St. Louis, MO), diluted in PBS, and sterilized by passage through 0.2-μm pore filters. Challenge inoculum was 10 μg of LPS in 10 mL of PBS. Milk samples were collected from challenged and unchallenged quarters at 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h after challenge to determine speed and magnitude of intramammary neutrophil response. The SCC per milliliter of milk were determined with a Bentley Somatocount 150 milk somatic cell counter (Bentley Instruments, Inc., Chaska, MN). Samples from clinical quarters were diluted 1:10 and 1:50 (milk:PBS, vol/vol) for counting. Data were expressed as $\log_{10}$ SCC/mL of milk. Rectal temperatures were measured immediately before challenge and at each time that quarter foremilk samples were collected post-challenge.

Neutrophil phagocytosis and intracellular kill of *Escherichia coli* 487 were determined from blood neutrophils isolated immediately prior to intramammary LPS challenge. Phagocytosis and intracellular kill of bacteria by neutrophils were measured by modifica-