Role of Vitamin E and Selenium in Host Defense Against Mastitis

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ABSTRACT

Vitamin E and Se are essential nutrients that share common biological activities. Deficiencies in either of these micronutrients have been related in increased incidence and severity of mastitis. A known physiological consequence of α-tocopherol or Se deficiency is reduced neutrophil activity. Vitamin E and the Se-containing enzyme, glutathione peroxidase, are antioxidants that protect neutrophils from the destructive action of toxic oxygen molecules necessary for intracellular kill of ingested pathogens. Dietary supplementation of cattle with Se results in a more rapid neutrophil influx into milk following intramammary bacterial challenge and increased intracellular kill of ingested bacteria by neutrophils. Dietary supplementation of early lactation cows with vitamin E results in increased bactericidal activity by bovine blood neutrophils. Recently completed trials have shown that subcutaneous injections of vitamin E approximately 10 and 5 d prior to calving successfully elevated neutrophil α-tocopherol concentrations during the periparturient period and negated the suppressed intracellular kill of bacteria by neutrophils that is commonly observed at calving. 

(Key words: vitamin E, selenium, mastitis)

Abbreviation key: FIA = Freund's incomplete adjuvant, GSH-Px = glutathione peroxidase.

INTRODUCTION

Vitamin E and Se are essential micronutrients that share common biological activities. Vitamin E and the Se-containing enzyme, glutathione peroxidase (GSH-Px; EC 1.11.1.9), are integral parts of the antioxidant system present in most mammalian cells (21). Tissue concentrations of Se are correlated with GSH-Px activity and are directly related to dietary intake (34). Plants grown on Se-deficient soils generally do not provide adequate dietary Se to meet nutritional requirements of dairy cows. Selenium-deficient soils are geographically widespread, and approximately two-thirds of dairy cows in the United States are in areas with soils known to be deficient in Se (38). A major source of vitamin E for dairy cows is forages (26, 33), but the concentration of α-tocopherol in forages decreases as plants mature. Substantial loss of vitamin E also occurs when feedstuffs are processed and stored (28). Total confinement of dairy herds (zero grazing) results in increased feeding of locally grown and stored forages that may be low in both vitamin E and Se. Therefore, the possibility is great that dietary vitamin E and Se are inadequate in many dairy herds.

The first direct evidence that deficiencies in vitamin E and Se are related to mammary health was reported nearly a decade ago (37). Since that initial report, the role of vitamin E and Se in host defenses against mastitis has been researched using a variety of experimental models and designs. Earliest trials concentrated on dietary vitamin E and Se deficiencies that resulted in increased incidence of naturally occurring clinical mastitis (36, 37). Subsequently, field surveys throughout the world confirmed the practical importance of dietary vitamin E and Se supplementation of dairy cows in maintaining udder health (2, 3, 15, 32, 45). Experimental challenge and in vitro experiments have recently revealed probable
mechanisms of action for which vitamin E and Se are involved in nonspecific host defenses (14, 17, 19, 23, 24). The purpose of this paper is to review recent studies on the relationships among vitamin E, Se, and mammary health parameters.

**Dietary Vitamin E and Se**

**Incidence of IMI and Clinical Mastitis**

Research during the last decade has shown that the incidence and severity of mastitis are related to the vitamin E and Se status of a dairy herd. Dietary vitamin E and Se supplementation of dairy cows reduced the rates and duration of clinical mastitis. The first controlled study on the effects of vitamin E and Se supplementation on clinical mastitis was reported by Smith et al. (37). Cows receiving diets supplemented with 740 IU/d of vitamin E throughout the dry period had a 37% lower incidence of clinical mastitis at calving than did cows fed dry period diets without vitamin E supplementation. Injection of .1 mg of Se/kg of BW at 21 d prior to calving had no effect on incidence of clinical mastitis. Neither dietary supplementation of vitamin E nor injection of Se affected the percentage of quarters infected at calving. A synergistic effect of vitamin E and Se on duration of clinical signs was measured. Cows supplemented with both vitamin E and Se had shorter duration of clinical signs than cows supplemented with either micronutrient alone.

Dietary vitamin E and Se also affected prevalence of IMI at calving in first lactation cows (36). A prepartum diet (60 d) was supplemented to provide 2 IU/d of vitamin E/kg of BW and 2 μg/d of Se/kg of BW. Supplemented primiparous cows also were injected subcutaneously at 21 d prepartum with .1 mg of Se/kg of BW. During lactation, concentrate for the experimental group was supplemented with 88 IU of vitamin E/kg and .3 mg of Se/kg. Prepartum and lactation diets for control heifers were not supplemented with either vitamin E or Se. Prevalence of IMI in supplemented heifers at calving was reduced by 42% compared with that of controls. Incidence of clinical mastitis during the first 4 d of lactation was reduced by 57% in supplemented heifers compared with that of controls. Although rates of IMI during lactation did not differ between groups, milk SCC in supplemented cows were lower than that in controls (Figure 1).

Results of field surveys on relationships among mammary health, vitamin E, and Se also have reflected benefits of vitamin E and Se supplementation. Erskine et al. (15) reported a negative correlation between the percentage of quarters infected with major pathogens and mean herd GSH-Px activity in whole blood. Serum α-tocopherol did not differ between high and low SCC herds (15). Atroshi et al. (2, 3) reported α-tocopherol and Se concentrations in blood and milk of Finnish cows; cows with clinical mastitis had lower erythrocyte GSH-Px activity and lower milk and plasma α-tocopherol concentrations than uninfected herdmates. In contrast, Ropstad et al. (32) reported that Norwegian herds with high Se status had higher frequencies of treatments for clinical mastitis and higher milk SCC than herds with lower Se status.

In a survey of Ohio herds, vitamin E and Se were related to rate of clinical mastitis and bulk tank milk SCC (45). High serum Se concentrations were associated with reduced rates of clinical mastitis and low bulk tank milk SCC (Figure 2). Concentration of Se in serum was correlated positively to concentration of Se in the diet until cows consumed more than 5 mg/d of Se. Above this value, serum Se was
Neutrophil Function

Dietary supplementation of mammals with vitamin E and Se is important to maintain host defense mechanisms, including antibody production, cell proliferation, cytokine production, prostaglandin metabolism, and neutrophil function [reviewed by Smith (35)]. Most information concerning the bovine host defense deals with neutrophil function. Neutrophils are considered to be a primary defense mechanism to bacterial infections in mammals. The importance of neutrophils in host defense against bovine IMI is well documented (13). Incidence and severity of clinical signs associated with IMI depend on responsiveness of neutrophils (13). Therefore, herd management practices that result in optimal vitamin E and Se status of dairy cows also optimize neutrophil responses and increase resistance to IMI.

The earliest trials to determine effects of vitamin E and Se on neutrophil function were performed on species other than the cow. Neutrophils that were from either vitamin E-deficient or Se-deficient mice (20) or humans (5, 8, 9) had impaired bactericidal activities. The respiratory burst by neutrophils is characterized by marked changes in oxygen metabolism that result in increased production of superoxide and hydrogen peroxide (4). Although neutrophil-generated oxygen metabolites are necessary in antimicrobial defense mechanisms, these free radicals also can damage the neutrophil and surrounding tissues (43). Vitamin E and GSH-Px both are cellular antioxidants that protect against the cytotoxic capabilities of oxygen metabolites. Vitamin E protects at the membrane, whereas GSH-Px activity is in the cytosol. Glutathione peroxidase converts hydrogen peroxide to water and lipid hydroperoxides to the corresponding alcohol (6). Vitamin E inhibits autoxidation of polyunsaturated fatty acids in neutrophil membranes (5, 6). Vitamin E is localized in cellular membranes in close proximity to the mixed function oxidase enzymes that initiate the production of free radicals.

Benefits of Se supplementation on speed of neutrophil response to mammary irritation was tested by experimental challenge of quarters with mastitis pathogens. Erskine et al. (14) infused Escherichia coli into mammary quarters of cows fed diets supplemented with Se (.14 ppm of Se) or unsupplemented (.04 ppm of Se). The supplemented cows had more rapid SCC response following challenge, maintained lower bacterial colony-forming units per milliliter of milk, eliminated IMI more rapidly, and had less severe clinical signs than did unsupplemented cows. Selenium status of cows also had an effect on the ability of milk neutrophils to kill mastitis pathogens in vitro (17). Neutrophils collected from cows fed Se-supplemented diets had increased intracellular kill of bacteria, enhanced viability, and reduced extracellular hydrogen peroxide concentration compared with neutrophils harvested from milk of cows fed Se-deficient diets. Intracellular kill of bacteria also was greater in neutrophils isolated from blood from cows supplemented with parenteral Se than in neutrophils from cows without supplemental Se (19). Ability of neutrophils to phagocytize bacteria was independent of Se (17, 19, 23).

Vitamin E supplementation of diets increased intracellular kill of Staphylococcus aureus and E. coli by bovine blood neutrophils but had no effect on phagocytic index (23).
The recommended dietary and blood concentrations of vitamin E and Se as discussed relate to maintenance of host defenses to protect against infections. Optimal blood concentrations of antioxidants may be greater during status of a herd. The recommended and legal upper limit for Se concentration in dairy cow rations is .3 ppm (29), which corresponds to an approximate intake of 3 mg/d for dry and 6 mg/d for lactating Holsteins. Little data exist to suggest that dietary Se greater than .3 ppm additionally enhances host defenses against mastitis. However, factors exist that interfere with Se absorption in the intestinal tract, such as presence of sulfates, nitrates, and high concentrations of dietary Ca. To ensure that herd Se status is adequate, blood samples from a representative group of cows in the herd should be analyzed. We recommend that whole blood concentrations of Se should be at least .2 μg/ml but not exceed 1 μg/ml (equivalent lower and upper plasma concentrations are .07 and .5 μg/ml, respectively).

The NRC (29) established requirement for dietary vitamin E is 15 IU/kg of DMI for both dry and lactating cows, which is equivalent to consumption of 150 and 300 IU/d for dry and lactating cows, respectively. This dietary concentration of vitamin E should prevent overt signs of vitamin E deficiencies, such as muscular dystrophy, but the beneficial effects of vitamin E on mammary health imply that greater intake is warranted. We recommend that both dry and lactating cows consume 1000 IU/d of vitamin E. Our recommendations are based on the significant reductions in IMI, clinical mastitis, and milk SCC that were observed when cows were supplemented with enough additional vitamin E to achieve this consumption level (23, 24, 36, 37, 44, 45, 46, 47). For cows fed stored forages, vitamin E may need to be supplemented at 1000 IU/d for dry cows and at 500 IU/d for lactating cows, dependent on forage quality and DMI. Plasma concentrations of greater than 3.5 to 4 μg/ml of α-tocopherol are considered to be adequate, as evidenced by the relationship between intracellular kill of bacteria by neutrophils and plasma vitamin E concentrations ([44]; Figure 4).

**PARENTERAL VITAMIN E**

**Periparturient Period**

The recommended dietary and blood concentrations of vitamin E and Se as discussed relate to maintenance of host defenses to protect against infections. Optimal blood concentrations of antioxidants may be greater during...
periods of stress (7), such as parturition. Plasma vitamin E concentrations in dairy cows are normally lowest when rates of IMI are highest and when neutrophil functions are depressed during the periparturient period (22, 25, 44, 45). The decrease in plasma α-tocopherol during the periparturient period is related to changes in consumption of vitamin E and to decreased transport capacity for the vitamin in plasma (45, 46, 47). Concentration of plasma α-tocopherol typically decreases 7 to 10 d prior to calving and remains low during the first 2 to 3 wk of lactation, even when the dietary vitamin E offered to cows is constant throughout this period (Figure 5). Administration of vitamin E to late gestation cows other than in feed was tested as a means of preventing a drop in plasma concentrations of vitamin E (44, 46). Parenteral administration of vitamin E successfully elevated α-tocopherol concentrations in plasma and neutrophils during late gestation and early lactation periods. Cows receiving parenteral vitamin E were injected subcutaneously with 3000 IU of vitamin E (all-rac-α-tocopherol) at 10 and 5 d prior to anticipated calving. Cows injected with vitamin E had greater plasma α-tocopherol concentrations 5 d after the first injection, at calving, and 1 wk after calving than did cows injected with placebo (44, 46). Neutrophils from cows injected with vitamin E had greater intracellular kill of bacteria at calving than did neutrophils from cows injected with a placebo (Figure 6). Neither phagocytic index nor percentage of neutrophils phagocytizing differed between cows injected with vitamin E or with a placebo. Dietary vitamin E during the dry period had less effect on neutrophil function at calving than did parenteral vitamin E.

Impaired neutrophil function in cows during the periparturient period has been thoroughly documented (18, 25). Impairment of neutrophil microbicidal mechanisms and membrane-associated activities of chemokinesis and ingestion were reported in cows during wk 1 after calving (25). Specifically, neutrophil functions associated with the oxidative burst of metabolism were altered. These neutrophil impairments were similar to depressed neutrophil activity in vitamin E-deficient animals (9, 23). Cows that received vitamin E injections maintained intracellular kill by blood neutrophils and neutrophil concentrations of vitamin E (24). Subcutaneous injections of vitamin E approximately 10 and 5 d prior to calving negated the suppression of in vitro intracellular kill of E. coli by neutrophils at calving. Intracellular kill by blood neutrophils from treated cows was constant from calving to wk 4 of lactation. Neutrophils from cows injected with the placebo had depressed intracellular kill at calving compared with their values at 2 and 4 wk postpartum.

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A known physiological parameter that influences neutrophil function in vivo is plasma cortisol concentrations. Changes in neutrophil activity at calving are preceded by increased corticosteroid in blood (18). These responses can be induced experimentally by injections of exogenous ACTH that increase blood cortisol and decrease antibacterial activity of blood-derived neutrophils (31). Nockels (30) recently reported that injection of ACTH also reduced vitamin E content in neutrophils. Chan et al. (12) reported that, during infection and other periods of inflammation, an increased destruction of tocopherol in phagocytes can be expected, and treatment of the animal with high doses of vitamin E will have a beneficial effect. These data imply that parenteral vitamin E may temper the adverse effects of increased cortisol on neutrophil vitamin E content. Concentration of vitamin E in neutrophils was related linearly to killing ability of neutrophils at parturition (44). In summary, parenteral vitamin E prior to calving successfully elevated plasma and neutrophil α-tocopherol concentrations and maintained intracellular kill of bacteria by neutrophils when dietary supplementation did not maintain these parameters.

**Vitamin E as Adjuvant in an *E. coli* J5 Vaccine**

Immunological responses to specific mastitis pathogens can be heightened by vaccination. Vitamin E has immunoenhancing properties when incorporated into vaccines. The addition of all-rac-α-tocopherol acetate into vaccine adjuvants enhanced immune responses in poultry, sheep, and mice compared with activity following use of conventional adjuvants (1, 16, 27, 39, 40, 41). Positive attributes ascribed to vitamin E in adjuvant systems include detoxification of reactive oxygen radicals generated at the sites of injection during antigen processing and presentation by immune cells (39).

Vitamin E recently was evaluated as an adjuvant in an *E. coli* (O111:B4) J5 mastitis vaccine. Twenty cows were assigned to five groups of 4 cows balanced by breed, parity, and milk production. Cows in four groups were vaccinated with an *E. coli* J5 bacterin. Vaccinations were at drying off, 30 d after drying off, and within 48 h after calving. The *E. coli* J5 bacterin contained 5 ml of $10^9$ boiled cells/ml of *E. coli* J5. Vaccine adjuvants differed among groups. The four treatment adjuvants were 5 ml of Freund's incomplete adjuvant (FIA; Difco Laboratories, Detroit, MI), 5 ml of vitamin E (250 IU/ml of α-tocopherol), 2.5 ml of FIA plus 2.5 ml of vitamin E, and 5 ml of PBS. All immunizations were subcutaneous on the upper part of the rib cage just posterior to the scapula. Cows in one group were unimmunized controls.

The use of FIA can result in lumps of abscesses at the site of injection (42). Adjuvants containing a mixture of FIA and vitamin E successfully vaccinated sheep without creating lumps or abscesses at the injection site (42). Replacement of one-half of the FIA with vitamin E reduced the swelling at *E. coli* J5 injection sites but did not eliminate the occurrence of lumps detected by palpation. The percentage of injections (n = 12) that resulted in swelling at the injection site by treatment group were FIA, 58%; FIA plus vitamin E, 33%; vitamin E, 0%; and PBS, 0%.

Vitamin E alone was not an effective adjuvant. Serum and milk IgG titers in cows vaccinated with the vitamin E adjuvant vaccine did not differ from those in cows vaccinated with the placebo adjuvant (Figures 7 and 8). Vitamin E alone also had no immunoenhancing effects when tested in other species, but an equal mixture of FIA and vitamin E resulted in a greater humoral response than did FIA alone. Adjuvants containing a mixture of FIA plus vitamin E resulted in higher peak titers and greater persistence of protection than did FIA.
Mammary secretion IgG titers to *Escherichia coli* J5 lipopolysaccharide in cows either not vaccinated (control; •) or vaccinated with whole cell *E. coli* J5 plus Freund's incomplete (FIA; ◦), vitamin E (E; ▽), FIA and E (◇), or PBS (□) adjuvant. Samples were taken at drying off (D − 0), at calving (C + 0), and 21 d into lactation (C + 21). Values are covariant-adjusted least squares means of four cows.

Vitamin E and FIA is promising, but the immunoenhancing properties were considerably less than in other species (39, 40, 41).

**CONCLUSIONS**

Vitamin E and GSH-Px are both cellular antioxidants that protect against the cytotoxic capabilities of oxygen metabolites produced by neutrophils in responses to bacterial IMI. Deficiencies in either of these micronutrients results in impaired bactericidal activity of neutrophils and increased mastitis. Dietary levels should maintain basal tissue concentrations adequate to defend against oxidation by free radicals produced during normal physiological events. Parenteral vitamin E can augment dietary supplementation 1) when feed intake or nutrient absorption is reduced, 2) when management practices restrict dietary supplementation, and 3) when events occur that result in heightened free radical production.

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