

# INFLUENCE OF VITAMIN E AND SELENIUM ON MASTITIS AND MILK QUALITY IN DAIRY COWS

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## INTRODUCTION

The primary function of vitamin E is as an antioxidant. Indeed, vitamin E is the most important antioxidant found in cellular membranes. Selenium is an integral component of the enzyme, glutathione peroxidase (GSHpx). That enzyme also is an important part of the cellular antioxidant system, but GSHpx is water soluble and is found in the cytosol of cells, not in cellular membranes. Although both nutrients are involved in the cellular antioxidant system, the nutrients are found in different cellular components. Because they have similar functions, their requirements are not independent. High dietary vitamin E reduces the requirement for selenium and vice versa. However, because of the differences in solubility and subsequent location within cells, the two nutrients are not completely interchangeable; excessive intake of one will not eliminate the need for the other.

During the last 10 years, our understanding of the importance of selenium and especially vitamin E for dairy cattle has increased tremendously. Scientific experiments have established that vitamin E and selenium can influence the function of certain immune cells, reduce calf mortality and morbidity, and improve reproductive and mammary gland health in adult dairy cows. This paper will review newer data regarding vitamin E, selenium, and dairy cows with the major emphasis on mastitis and immune function. Supplementation strategies based on economics, regulations, and animal response also will be discussed.

## ANTIOXIDANTS AND OXIDATIVE DAMAGE

Normal cell processes, environmental insults, and inflammatory responses produce compounds called reactive oxygen species or free

radicals. Environmental insults include solar radiation, tobacco smoke (for humans not cows), certain myco toxins, nitrates, and a host of other toxic compounds. The major free radicals found in biological systems are superoxide, hydrogen peroxide, hydroxyl radical, and fatty acid radicals. Hydrogen peroxide is found primarily in the cytosol of cells and fatty acid radicals are found primarily in cell membranes. Superoxide and hydroxyl radicals can be found in both cell components. Because free radicals are extremely toxic to cells, the body has developed a sophisticated antioxidant system (Table 1). Superoxide dismutase (an enzyme that contains copper and zinc) converts superoxide to hydrogen peroxide. Hydrogen peroxide is converted to water by the enzyme GSHpx. Those two enzymes effectively control most free radicals within the cytosol. Superoxide and the hydroxyl radical can migrate into cell membranes where they attack fatty acids (especially unsaturated fatty acids) and produce fatty acid radicals (a process called initiation). Fatty acid radicals then react with other fatty acids producing a chain reaction. Vitamin E, and to a lesser extent  $\beta$ -carotene, reacts with fatty acid radicals and stops the chain reaction.

Free radicals are highly reactive compounds because they are missing an electron. Free radicals can react with nucleic acids causing mutations, with enzymes and render them inactive, and with fatty acids in membranes causing membrane instability. Free radicals can eventually kill cells and damage tissues.

## IMMUNITY

The immune system can be partitioned into two broad categories: specific and nonspecific. Specific or acquired immunity is the basis of vaccination programs. Specific immunity occurs when animals develop or acquire immunity to a specific pathogen once it is exposed to the pathogen.

**Table 1: Antioxidant systems of mammalian cells.**

Component (location in cell)	Nutrients Involved	Function
Superoxide dismutase (cytosol)	Copper, zinc, and manganese	An enzyme that converts superoxide to hydrogen peroxide
Glutathione peroxidase (cytosol)	Selenium	An enzyme that converts hydrogen peroxide to water
Catalase (cytosol)	Iron	An enzyme (primarily found in the liver) that converts hydrogen peroxide to water
$\alpha$ -tocopherol (membranes)	Vitamin E	Breaks fatty acid peroxidation chain reactions
$\beta$ -carotene (membranes)	$\beta$ -carotene	Prevents initiation of fatty acid peroxidation chain reactions

Antibodies specific to that pathogen are produced and the immune system *memorizes* the antigenic properties of the pathogen so that an immune response can be initiated quickly when the host is exposed to the pathogen again. Lymphocytes and macrophages are the cells primarily involved with specific immunity. The nonspecific immune system is designed to protect the body from all antigens. Vaccination will not influence nonspecific immunity and the nonspecific immune system does not have a *memory*. Neutrophils are the cells most involved with nonspecific immunity.

When a pathogen invades the mammary gland of a cow, a cascade of events occurs. First, neutrophils from the blood are drawn to the infection site. Neutrophils are the first line of defense after a pathogen invades the body. The function of a neutrophil is to engulf (phagocytize) and then kill bacteria. After a neutrophil engulfs a bacterium, a chemical reaction called a respiratory burst occurs. This produces a high concentration of free radicals. These free radicals help kill the bacteria, but if not controlled, they can damage and kill the neutrophil also. The life span of neutrophils is short; each neutrophil can engulf 5 to 20 bacteria before the cell is killed. As part of the inflammatory response, macrophages also are drawn into the infection site. These cells can kill bacteria directly but more importantly they initiate the acquired immune response. Antibodies are produced against the bacteria and lymphocytes are drawn to the infection site. The immunological response to some mastitis pathogens can be increased by vaccination (e.g., J-5

vaccine). The combined efforts of neutrophils, macrophages, lymphocytes, and antibodies help to eliminate the invading pathogen.

#### **Effects of Vitamin E and Selenium on Immunity in Cows**

The research on vitamin E and immunity in dairy cows has concentrated on neutrophil function. Vitamin E supplementation has consistently improved neutrophil function in dairy cows (Table 2). The results from the two experiments that used fresh cows are noteworthy. The nonspecific immune system is depressed during the peripartum period and cows are extremely susceptible to intramammary gland infections at this time. Both studies found that vitamin E supplementation eliminated the depression in neutrophil function associated with parturition. The clinical and practical significance of this is discussed later.

Selenium supplementation of cows that were deficient in selenium has also consistently improved neutrophil function. Neutrophils from cows fed 0.1 ppm of supplemental selenium killed mastitis pathogens more effectively than did neutrophils from cows fed no supplemental selenium (Grasso et al., 1990). When cows were challenged experimentally with *E. coli*, the influx of neutrophils into the infection site was more rapid in cows fed supplemental selenium than for those fed no selenium (Erskine et al., 1989; 1990). When cows were challenged with *S. aureus* (this produced a less virulent response than *E. coli*) selenium

**Table 2: Research results on the effect of vitamin E supplementation on neutrophil function in dairy cows.**

Type of cow	Supplementation	Response	Ref.
Lactating cow, 30 days in milk	1000 IU/day of dietary vitamin E during the dry period and 500 IU/day during the first 30 days of lactation	<ul style="list-style-type: none"> <li>• Phagocytosis not affected</li> <li>• Ability to kill <i>S. aureus</i> and <i>E. coli</i> was improved</li> </ul>	Hogan et al., 1990
Fresh cow (<3 days in milk)	3000 IU of vitamin E injected at 10 and 5 days before anticipated calving.	<ul style="list-style-type: none"> <li>• Phagocytosis not affected</li> <li>• Ability to kill <i>E. coli</i> was improved</li> </ul>	Hogan et al., 1992
Dairy cows from 4 wk pre to 5 wk postpartum	3000 IU/d of dietary vitamin E from 4 wk pre to 8 wk postpartum + 3000 IU of vitamin E injected 1 wk prepartum	<ul style="list-style-type: none"> <li>• Neutrophil chemotaxis (movement into infection site) was improved</li> </ul>	Politis et al., 1996
Fresh cows (<7 days in milk)	3000 IU/d of dietary vitamin E from 4 wk pre to 8 wk postpartum + 3000 IU of vitamin E injected 1 wk prepartum	<ul style="list-style-type: none"> <li>• Overall neutrophil function was improved</li> </ul>	Politis et al., 1995

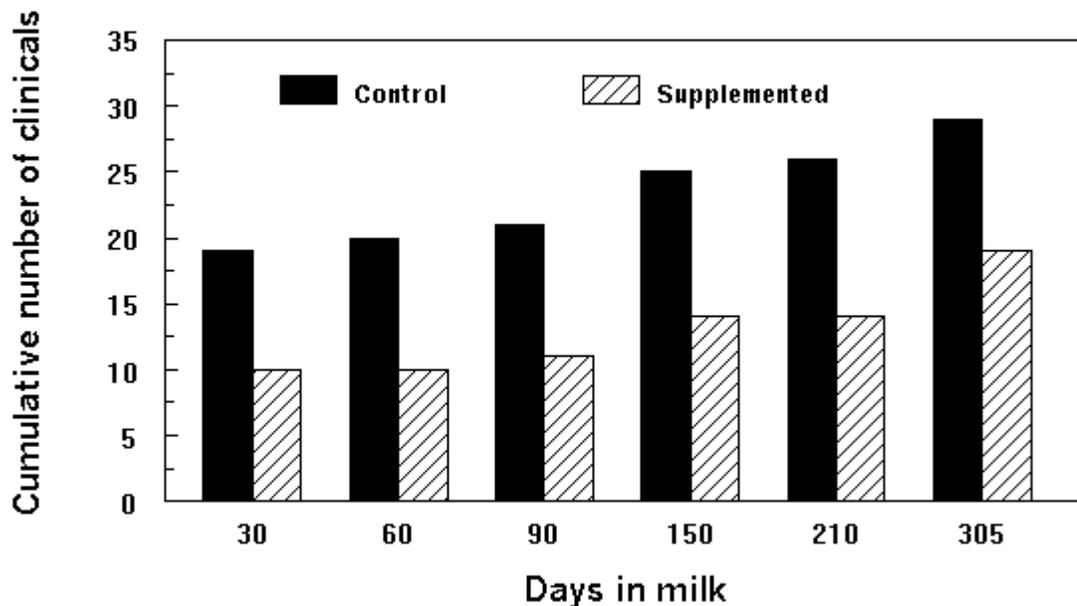
supplementation did not affect influx of neutrophils. Neutrophils from cows supplemented with 0.3 ppm of selenium during the dry period and first 30 days of lactation had a greater killing ability against *E. coli* and *S. aureus* (Hogan et al., 1990).

The effects of vitamin E and selenium on acquired immunity in dairy cattle have not been studied extensively. In the few studies conducted, inconsistent responses have been reported. In some studies immunoglobulin titers in blood and/or milk were increased with vitamin E or selenium supplementation but in other studies no response was found.

## VITAMIN E, SELENIUM, AND MAMMARY GLAND HEALTH

Mastitis is an extremely prevalent and costly disease. Surveys have found that in well-managed dairy herds, approximately 50 cases of clinical mastitis can be expected per 100 cows annually. Each case of clinical mastitis costs between \$100 and \$140 (Hoblet et al., 1991). Those costs include veterinary and drug costs, lost production, and dumped milk. For a well-managed herd of 100 cows, clinical mastitis would cost about \$6,000 per year. The cost of subclinical mastitis is more

difficult to quantify but most experts agree that subclinical mastitis costs the average dairy farmer more than does clinical mastitis. The total cost of mastitis (lost production and treatment costs) is estimated to be \$150 to \$200/cow per year or about \$17,500 annually for an average 100 cow herd. Smith et al. (1984) were the first to report that supplemental vitamin E and selenium reduced clinical mastitis. That study was conducted in Ohio where the soil concentration of selenium is very low, and used dry cows fed hay-based diets (should be very low in vitamin E). Cows were either injected with a placebo or 50 mg of selenium at 21 days before calving and were fed either 0 or 1000 IU/day of supplemental vitamin E (dl- $\alpha$ -tocopheryl acetate). Selenium without supplemental vitamin E reduced the incidence and duration of clinical mastitis, but the largest response was caused by vitamin E with or without selenium. A subsequent study compared mammary gland health of cows fed no supplemental selenium or vitamin E with that of cows fed diets with 0.1 ppm of supplemental selenium and 1000 IU/day of supplemental vitamin E during the dry period and 0.3 ppm of supplemental selenium and approximately 700 IU of supplemental vitamin E during lactation (Smith, 1986). Selenium and vitamin E supplementation reduced mammary gland infections by 42% and reduced clinical mastitis by 32%. The largest response was found during the first



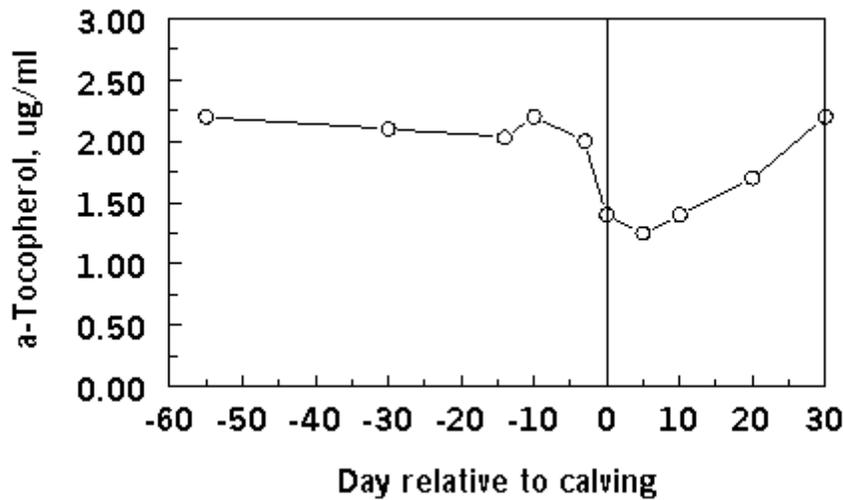
**Figure 1: The cumulative number of cases of clinical mastitis at various stages of lactation for cows fed no supplemental vitamin E and selenium or fed supplemental vitamin E and selenium (Smith, 1986).**

3 months of lactation (Figure 1). A clinical trial conducted in Canada with similar vitamin E supplementation found no effect of vitamin E on mammary gland health (Batra et al., 1992). Cows in that study had extremely low concentrations of selenium in the blood suggesting that selenium was probably deficient.

#### **Vitamin E During the Periparturient Period**

Many experiments have shown that plasma concentrations of  $\alpha$ -tocopherol in dairy cows are low at parturition (Figure 2). As discussed earlier, cows are immunosuppressed during the time when plasma concentrations of vitamin E are low. Because of the beneficial effects of vitamin E on neutrophil function, we postulated that providing extra vitamin E during the periparturient period may reduce the incidence of mastitis. We conducted an experiment in which all cows were fed diets that contained 0.1 ppm of supplemental selenium. One group was fed a diet that provided 150 IU/day of supplemental vitamin E during the dry period, another group was fed a diet that provided 1000 IU/day of supplemental vitamin E during the dry period, and a third group was fed a diet that provided 1000 IU/day of supplemental vitamin E from dry-off until 2 weeks before anticipated calving. During the last 2 weeks prepartum those cows were fed a diet that provided 4000 IU/day of supplemental

vitamin E (Weiss et al., 1997). The plasma concentrations of  $\alpha$ -tocopherol followed expected trends for cows fed diets that provided 150 or 1000 IU/day of supplemental vitamin E. Cows fed the high amount of vitamin E during the prefresh period did not show the expected decrease in plasma  $\alpha$ -tocopherol. The prevalence of clinical mastitis during the first week of lactation was 37, 14, and 0% of quarters for first lactation cows fed the low, intermediate, and high concentrations of vitamin E. For multiparous cows, the prevalence of clinical mastitis was 18, 18, and 4% for the three treatments, respectively. Compared with the low vitamin E treatment, the 1000 IU/day treatment reduced clinical mastitis by 30% and the 4000 IU/day treatment reduced clinical mastitis by 80%. A recently completed field study (W. P. Weiss, unpublished data) found that the rate of intramammary gland infections in cows fed 1000 IU/day or 5000 IU/day of vitamin E was not different. The percent infected quarters was 40% for both treatments. Cows in the field study were fed diets that contained at least 0.3 ppm of selenium and, based on plasma concentrations of selenium, the cows were in adequate selenium status.



**Figure 2: Plasma concentrations of  $\alpha$ -tocopherol in dairy cattle during the dry and early lactation periods [reprinted from Weiss et al.(1990) with permission].**

### Vitamin E, Selenium, and Milk Quality

Besides the economic benefit of reduced clinical mastitis, supplemental vitamin E and selenium also may increase the value of milk by improving milk quality. Weiss et al. (1990) reported that plasma concentrations of selenium had a high negative correlation with bulk tank somatic cell counts. A mean plasma concentration of selenium of 0.07  $\mu\text{g}/\text{ml}$  was associated with a SCC of 316,000 and a herd with a mean concentration of selenium of 0.09  $\mu\text{g}/\text{ml}$  had a SCC of 200,000. Vitamin E and selenium supplementation reduced the number of cows with SCC greater than 200,000 by almost 70% (Smith, 1986). Although not generally considered a measure of milk quality, supplementing dry cows with vitamin E substantially increases the vitamin E content of colostrum and that may improve calf health. Supplementing lactating cows with vitamin E increases the vitamin E content of milk slightly and may aid in reducing oxidative flavor problems.

### ASSESSING VITAMIN E AND SELENIUM STATUS

The concentration of selenium in whole blood or plasma is a reliable indicator of selenium status. The activity of GSHpx in whole blood also can be used to assess selenium status but interlaboratory variation is a problem, thus each laboratory must develop its own recommended ranges. For the normal cow, about one-third of the selenium in whole blood is in the plasma and two-thirds is in the red cells. Selenium is incorporated into red cells only when the cell is made; therefore, selenium content of the red cell reflects selenium intake 1 to 3 months previously. The selenium in plasma mainly represents a transport pool and reflects the current status. For example, plasma selenium will increase shortly after selenium is injected but the selenium content of red cells will not change for several weeks. Whole blood (red cells and plasma) reflects longer term status but is somewhat sensitive to recent changes in selenium nutrition. Because of

**Table 3: Recommended concentrations of selenium in plasma (or serum) and whole blood of dairy cows.**

Classification	Plasma or serum (µg/ml)	Whole blood (µg/ml)
Adequate	>0.075	>0.20
Marginal	0.05 to 0.075	0.14 to 0.20
Deficient	<0.05	<0.14

these reasons, whole blood selenium is the preferred method of assessing selenium status, but plasma (or serum) also is acceptable. The recommended values are in Table 3 and are based largely on reduction in the prevalence of retained fetal membranes and mammary gland health.

Much recent research has focused on using plasma concentrations of  $\alpha$ -tocopherol to assess the vitamin E status of cows. We have found that when plasma concentrations of  $\alpha$ -tocopherol in cows at parturition were less than 3 µg/ml, the probability of having clinical mastitis during the first week of lactation was 9 times greater than when plasma concentrations were greater than 3 µg/ml. In another experiment we found that neutrophil function was maximized when plasma concentration of  $\alpha$ -tocopherol was at least 3.5 µg/ml. Based on these data, we recommend that cows **at calving** have  $\alpha$ -tocopherol concentrations in their plasma of at least 3 to 3.5 µg/ml. The concentrations of  $\alpha$ -tocopherol and lipids in plasma are correlated (high plasma lipids = high  $\alpha$ -tocopherol). Because of the correlation, that recommendation is not reliable for cows at other stages of lactation.

## RECOMMENDATIONS

### Selenium

Essentially all dairy animals raised in the Midwestern US should be fed the maximum allowable amount of supplemental selenium (current FDA regulation is 0.3 ppm). The costs associated with that strategy is small. Potential benefits include reduced prevalence of retained fetal membranes (estimated cost of \$100 to \$120/case), reduced clinical mastitis (estimated cost \$120/case), and reduced SCC (potential quality bonuses for milk). Diets fed to animals at all stages of life (calves, heifers, and lactating and dry cows) should be supplemented with 0.3 ppm of Se. Often heifers are not supplemented

properly and are in marginal selenium status when they calve. Sodium selenite and sodium selenate are the two approved sources of supplemental selenium for animal diets. Limited data suggests that the selenate may have a higher bioavailability than selenite (FDA, 1987). In most situations, feeding 0.3 ppm provides adequate selenium, but occasionally that amount is not adequate. Certain conditions (high sulfate in the feed or water, excessive dietary copper, zinc, or iron, and diets with very high or very low concentrations of calcium) reduce the availability of selenium or increase its requirement. In these situations, collect some blood samples and assess the selenium status. If animals are deficient and the maximum legal amount of selenium is being fed, consider injections of selenium. Good results have been obtained when 50 mg of selenium was injected into Holstein cows approximately 3 weeks prepartum.

### Vitamin E

Most concentrate feeds contain very little vitamin E. Raw soybeans can be a good source of vitamin E, but roasting destroys most of the vitamin E. Fresh green forage is an excellent source of vitamin E and may contain more than 100 IU/lb. of dry matter. After forage is cut, the concentration of vitamin E in the plants decreases rapidly. Most wilted silages have less than 30% the vitamin E found in fresh plants. Hay that was cured quickly, may contain about 20% of the vitamin E found in fresh plants, but if the curing period was extended, hay may have very little or no vitamin E. A Midwestern type diet (corn silage, alfalfa silage, alfalfa hay, corn grain and soybean meal) for lactating and dry cows typically exceeds the current NRC recommendation for vitamin E (7 IU/lb. of DM). However, numerous research studies have shown that the NRC recommendation for vitamin E is inadequate to maintain good mammary gland health. Based on current data, we recommend that all dry cows not consuming fresh forage be fed 1000 IU/day of supplemental vitamin E. If the diet is at least 50% fresh forage (pasture), supplemental vitamin E is

probably not necessary. We think that lactating cows should be fed about 500 IU/day of supplemental vitamin E when fed stored forages. If cows are consuming at least 25% of their diet as pasture, supplemental vitamin E is probably not needed. When cows were in marginal selenium status, very positive results were obtained when dry cows within 2 weeks of calving were fed 4000 IU/day of supplemental vitamin E. No positive results were found when high levels of vitamin E were fed to peripartum cows that were adequate in selenium. If adequate blood concentrations of selenium cannot be obtained because of interfering compounds, extra vitamin E supplementation during the peripartum period may be beneficial.

Based on vitamin E prices in Ohio, feeding 1000 IU/day of vitamin E for 60 days and feeding 500 IU/day for 305 days will cost about \$7 to \$8 per cow per 365 days (about 2 cents per cow-day). The benefit associated with this program can be calculated based on the expected decrease in clinical mastitis (30%), a normal incidence rate of clinical mastitis in well-managed herds that have controlled contagious mastitis (50 cases/100 cows/365 days), and the average cost of clinical mastitis (\$120). For a 100 cow herd the potential benefit of this supplementation program is \$1800 while the cost is \$750. That benefit includes only clinical mastitis; the potential of reduced retained fetal membranes, reduced SCC, and potentially improved calf health are not considered. The practice of feeding supplemental vitamin E at the rates of 1000 IU/day to dry cows and 500 IU/day to lactating cows can be justified based on economics. The practice of feeding 4000 IU/day during the prefresh period (approximate cost of \$3/cow for 14 days) should be considered carefully. We recommend that practice only when selenium status is marginal and when clinical mastitis at calving is a problem.

## CONCLUSIONS

Supplemental vitamin E and selenium improve immune function of dairy cattle, especially during the peripartum period. An inadequate intake of selenium and vitamin E is related with an increased incidence of retained fetal membranes, mammary gland infections, and clinical mastitis. Feeding diets with 0.3 ppm of supplemental selenium to all classes of cattle and feeding 1000 IU/day of supplemental vitamin E to dry cows and springing heifers and 500 IU/day to lactating cows improves immunity, reduces

the incidence of clinical mastitis, and reduces SCC. Selenium status of cows can be evaluated based on blood concentrations. Evaluation of the vitamin E status of cows is more difficult, but plasma concentrations of  $\alpha$ -tocopherol in cows at parturition appear to have clinical value.

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