Introduction
Oxidative stress occurs when the production of reactive oxygen metabolites (ROM) exceeds the capacity of the antioxidant system of the cell, tissue, or body. Certain nutrients act as antioxidants or are components of antioxidant enzymes and have a direct effect on oxidative stress. The prevalence and severity of several important health disorders in dairy cows including retained fetal membranes, udder edema, and mastitis appear to be related to oxidative stress. In addition, antioxidant nutrients can affect milk quality. Milk quality is usually defined in terms of mastitis. Milk with a low somatic cell count (SCC) and visibly normal appearance (no clots) is considered high quality. Although these measures are clearly important, the definition of high quality milk must be expanded. To ensure a continued growing demand by consumers for dairy products, milk and dairy products must also taste good. A consumer that has a bad tasting glass of milk may be hesitant to purchase milk again.

Antioxidant Systems
Normal cell metabolism, environmental insults, and inflammatory responses produce compounds called ROM or free radicals. The major ROM found in biological systems are superoxide, hydrogen peroxide, hydroxyl radical, and fatty acid radicals. These compounds can react with enzymes, cell membranes, and DNA causing cell damage or cell death. Because ROM are toxic to cells, the body has developed a sophisticated antioxidant system that relies on antioxidant nutrients (Table 1). Several trace minerals (as part of enzymes) and some vitamins are integral components of the antioxidant system. The system includes water soluble antioxidants and fat soluble antioxidants. Both water and fat soluble antioxidants are needed because free radicals are found in both areas of cells. A free radical located in a cell membrane cannot be neutralized by an antioxidant located in the cytosol. Known antioxidant pathways suggest that the requirements of antioxidant nutrients are interrelated. A deficiency of one antioxidant may increase the requirement of another nutrient. However, a deficiency of a particular antioxidant nutrient cannot be alleviated fully by another nutrient.

Simply because a nutrient is directly involved with the antioxidant system does not mean that supplementing diets with that nutrient will improve cow health. Cows need to consume a certain quantity of biologically available minerals and vitamins to maintain optimal status. A cow is in optimal status when she has adequate amounts of trace minerals and vitamins for maximal production and to maintain good health. When cows are below optimal status, supplementation of a biologically available form of the nutrient should elicit a positive response but once optimal status is obtained, no additional positive responses would be expected when the nutrient is supplemented. Indeed, excessive supplementation of some antioxidant nutrients may increase oxidative stress, decrease immune function, and increase health problems.

Mastitis and Antioxidants
Mastitis is still an extremely prevalent and expensive problem for dairy farmers. On well-managed farms, approximately 50 cases of clinical mastitis can be expected per 100 cow-years (assuming 305 d lactation). The total costs associated with clinical mastitis range from about $100 to $140 per case. An inflammatory response occurs after a pathogen invades the mammary gland. Substantial amounts of ROM are produced by certain types of immune cells during an inflammatory response to assist those cells in killing the pathogen. When those cells contain adequate amounts of antioxidants, the concentrations of ROM are kept in check, which allows those cells to kill additional bacteria before the immune cell is killed. When antioxidant capacity is limited, the lifespan of those immune cells is reduced and the infection can become established or severity of the infection can increase.
Studies examining the effects of vitamin A and B-carotene on mastitis measures have been inconsistent. Two (Dahlquist and Chew, 1985; Chew and Johnston, 1985) reported positive effects when cows were diets that approximately met NRC (2001) requirements for vitamin A and supplemented with 300 mg/d of B-carotene, and one study reported no effect (Oldham et al., 1991). Supplementation occurred during the dry period and early lactation. A likely reason for the difference between studies was differences in vitamin A and B-carotene status of the control cows. Control cows in the study that found no effect had high concentrations of plasma B-carotene (10 mg/L) whereas in one of the studies that reported an effect, plasma B-carotene was 2.5 mg/L. Jukola et al. (1996) suggested that plasma concentrations of B-carotene in dairy cows should be >3 mg/L to optimize udder health. LeBlanc et al. (2004), however, reported no statistical association between serum B-carotene concentrations and clinical mastitis during the first 30 DIM even though average B-carotene concentrations were below 3 mg/L. They did find that elevated serum retinol during the prepartum period was associated with a decreased risk of clinical mastitis during the first 30 DIM. A 100 ng/ml increase in serum retinol was associated with a 60% decrease in the risk for clinical mastitis. Information on vitamin A supplementation was not given by LeBlanc et al. Based on current data, feeding vitamin A in excess of the current NRC requirement (approximately 70,000 IU/d) has not been shown to reduce mastitis.

Supplemental vitamin E and/or Se has been shown to reduce prevalence and severity of mastitis and reduce SCC (Malbe et al., 1995; Smith et al., 1984; Weiss et al., 1997; Wichtel et al., 1994). In general, supplementation rates were 0.3 ppm Se and 500 IU/d of supplemental vitamin E during lactation and 1000 IU/d during the dry period. Erskine et al. (1989) reported that feeding cows 2 mg of supplemental Se/day starting 3 months before calving and throughout lactation reduced the severity and duration of mastitis caused by experimentally challenging cows with Escherichia coli. Control cows in that study were fed a diet with 0.04 ppm Se. In a similar study, Erskine et al. (1990) found no effects of supplemental Se on mastitis when cows were experimentally challenged with Staphylococcus aureus. Weiss et al. (1997) reported that feeding 4000 IU of supplemental vitamin E/day during the last 14 days of the dry period reduced clinical mastitis and new infections at calving compared with cows fed 1000 IU/d. An important feature of that study was that cows were fed a low Se diet (0.1 ppm) and had low concentrations of plasma Se (0.048 mg/L). The current NRC recommendation for vitamin E and Se appear adequate for most situations. Accumulating data suggests that higher intakes of vitamin E during the periparturient period (>1000 IU/d) may be beneficial.

Diets with 20 ppm supplemental copper have been shown to reduce the severity of mastitis following an E. coli challenge compared to diets with 7 ppm (Scaletti et al., 2000). Heifers that received no supplemental copper after weaning and then were fed a diet with no supplemental copper from 84 d prepartum to 108 d postpartum had more infected quarters during lactation than did animals fed 20 ppm supplemental copper from 84 d pre to 107 d post partum (Harmon and Torre, 1994). Tomlinson et al. (2002) summarized results of 12 experiments and reported an overall significant reduction (196,000 vs. 294,000) in SCC when Zn-Met was supplemented (about 200 mg of Zn/d in 5 experiments and about 380 mg of Zn/d in 7 studies). In that summary, 4 of the experiments used a control diet that did not meet NRC (2001) requirements for Zn. Whitaker et al. (1997) compared the effects of providing supplemental Zn from a mixture of Zn proteinate (250 mg of Zn/day) and inorganic Zn (140 mg/day) or from all inorganic sources (390 mg of Zn/day). Diets contained approximately 50 ppm total Zn (about 25 ppm supplemental and 25 ppm from basal diet). Source of Zn had no effect on infection rate, new infections, clinical mastitis and SCC. More experiments similar to Whitaker et al. are needed to determine whether increasing Zn intake of cows is directly related to mammary gland health. Currently available data suggests that diets should contain about 20 ppm of copper (assuming no antagonists) and 50 to 60 ppm of Zn. Obtaining at least a portion of the supplemental zinc from zinc methionine may be beneficial.

Vitamin C (ascorbic acid) is probably the most important water soluble antioxidant in mammals. Most forms of vitamin C are extensively degraded in the rumen, therefore the cow must rely on tissue synthesis of vitamin C. Cows can synthesize vitamin C and it is not considered an essential nutrient for cattle. The concentration of ascorbic acid is high in neutrophils (important immune cells with respect to
mastitis) and increases as much as 30-fold when the neutrophil is stimulated. Santos et al. (2001) reported that plasma ascorbic acid concentrations in dairy cows were not correlated with somatic cell count (SCC). However, the range in SCC was limited (67,000 to 158,000/ml) and cows were only sampled once. Correlation analysis is a very weak statistical test for this type of data. Another experiment evaluated the therapeutic use of ascorbic acid following intramammary challenge with endotoxin (Chaiyotwittayakun et al., 2002). One quarter from each cow was infused with endotoxin and the ascorbic acid was injected IV at 3 and 5 hours post challenge (25 g/dose). Vitamin C therapy had only limited effects on clinical signs. The protocol used in this experiment (i.e., endotoxin challenge) was not ideal for evaluating ascorbic acid therapy. Ascorbic acid concentrations are very high in neutrophils—probably to protect those cells from ROM produced when the cell tries to kill pathogens. An experiment that used live bacterial challenge would provide more definitive data on the value of ascorbic acid as a therapy for mastitis. We recently conducted an experiment to examine changes in ascorbic acid status following an intramammary challenge with E. coli (Weiss et al., 2004). We observed significant correlations between vitamin C status and clinical signs. Large decreases in vitamin C status were related to longer duration of clinical mastitis and larger decreases in milk production following challenge were associated with larger decreases in vitamin C status (Figure 1). Data from this experiment does not mean that increasing vitamin C status of cows will reduce the prevalence or severity of mastitis. We do not know whether lower vitamin C status allowed severity of mastitis to increase or whether increased severity depleted body vitamin C. Current data do not support the use of vitamin C to prevent or help cure mastitis.

Antioxidants and Retained Fetal Membranes
Various surveys report that about 9% of all calvings in the U.S. resulted in retained fetal membranes (RFM). The estimated total cost associated with RFM range from about $100 to $280/case. Accumulating evidence strongly suggests that in many cases, RFM is an oxidative stress disease. The vitamin C concentration in maternal and fetal placental tissue is about 50% lower when cows have RFM than when they do not (Kankofer, 2001). Kimura et al. (2002) reported that neutrophils from cows with RFM had significantly less killing ability than neutrophils from cows without RFM. Neutrophils from Se-deficient cows have lower killing ability than neutrophils from Se-adequate cows (Hogan et al., 1990). Supplementation of pro-oxidant nutrients (i.e., available iron) tends to increase the prevalence of RFM (Miller et al., 1997). Supplementation of certain antioxidant nutrients can reduce the prevalence of RFM. The majority of studies in which the control diet contained less than 0.1 ppm total Se have found that the incidence of RFM is reduced when Se is supplemented via the diet (0.1 to 0.3 ppm) or via injections (about 50 mg of Se given 21 d prepartum), but Se supplementation had limited or no effect in studies in which the control diet contained more than 0.1 ppm Se. The effect of Se supplementation on RFM is influenced by the vitamin E status of cows. The incidence of RFM for cows that have low vitamin E status often is not influenced by Se supplementation (Harrison et al., 1984). In a more recent study, a statistical association was found between the concentration of tocopherol in serum of periparturient cows and risk of RFM (LeBlanc et al., 2004). They found that for every 1 ug/ml unit increase in serum tocopherol concentration (samples taken during the 1 wk before calving), the risk for RFM decreased by 21%. Cows that had a serum tocopherol:cholesterol ratio (ug tocopherol/ml divided by mg of cholesterol/ml) of <2 were three times as likely to have RFM than cows with a ratio >2 and cows with a ratio of <2.5 were twice as likely to have RFM as cows with a ratio >2.5. In that study (LeBlanc et al., 2004) no associations were found between serum retinol and RFM or serum B-carotene and RFM.

Antioxidants and Milk Flavor
Most fluid milk is judged to have a good flavor up to 14 d of storage but off-flavor of milk is still an important problem. Oxidized flavor (OF) is described as cardboard-like, metallic, or tallowy, and can develop over time because of improper storage and handling of the milk. In certain situations, OF can be detected in milk almost immediately following milking. The fatty acid profile of milk fat is a major factor in the development of OF. Oxidized flavor is more likely in milk that has a high concentration of polyunsaturated fatty acids such as linoleic or linolenic acid. The concentrations of those two fatty acids in milk can be increased by feeding certain oilseeds or rumen protected oils. As the use of these types of products increase, OF may become a larger problem.
Some antioxidants (for example, Cu) can increase susceptibility to oxidized flavor development, others reduce susceptibility. Milk with high concentrations of Cu is extremely susceptible to the development of OF, especially if the milk also is high in polyunsaturated fatty acids (Timmons et al., 2001). Milk Cu is not highly correlated with Cu intake, but at very high dietary Cu concentrations (about 80 ppm) milk Cu is elevated (Dunkley et al., 1968). The vitamin E concentration of milk is correlated with vitamin E intake, but large changes in intake of vitamin E elicit only modest changes in milk vitamin E. Usually less than 2% of the vitamin E consumed by a cow is secreted in her milk (Figure 2). Because of the low transfer of dietary vitamin E to milk, substantial amounts of vitamin E must be consumed by cows to reduce OF. In one study cows fed micronized soybeans and 8000 IU of vitamin E/d still produced OF milk (Charmley and Nicholson, 1994). Available data are not conclusive regarding the amount of dietary vitamin E needed to prevent OF when oilseeds are fed, but generally at least 3000 IU of vitamin E/d is recommended when OF is a problem.

Conclusions
Appropriate supplementation of antioxidant nutrients (especially vitamin E, Se, Cu, and Zn) can help reduce the prevalence of some important health disorders in dairy cows. In general, current NRC recommendations (plus a small safety factor) appear adequate for these nutrients. Peripartum cows may benefit from higher intakes of vitamin E than currently recommended.

References


Table 1. Some of the antioxidant systems found in mammalian cells.

<table>
<thead>
<tr>
<th>Component (location in cell)</th>
<th>Nutrients Involved</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (cytosol)</td>
<td>Copper and zinc</td>
<td>An enzyme that converts superoxide to hydrogen peroxide</td>
</tr>
<tr>
<td>Superoxide dismutase (mitochondria)</td>
<td>Manganese and zinc</td>
<td>An enzyme that converts superoxide to hydrogen peroxide</td>
</tr>
<tr>
<td>Ceruloplasmin (water phase)</td>
<td>Copper</td>
<td>An antioxidant protein, may prevent copper and iron from participating in oxidation reactions</td>
</tr>
<tr>
<td>Glutathione peroxidase (cytosol)</td>
<td>Selenium</td>
<td>An enzyme that converts hydrogen peroxide to water</td>
</tr>
<tr>
<td>Catalase (cytosol)</td>
<td>Iron</td>
<td>An enzyme (primarily in liver) that converts hydrogen peroxide to water</td>
</tr>
<tr>
<td>Ascorbic acid (cytosol)</td>
<td>Vitamin C</td>
<td>Reacts with several types of ROM</td>
</tr>
<tr>
<td>α-tocopherol (membranes)</td>
<td>Vitamin E</td>
<td>Breaks fatty acid peroxidation chain reactions</td>
</tr>
<tr>
<td>β-carotene (membranes)</td>
<td>β-carotene</td>
<td>Prevents initiation of fatty acid peroxidation chain reactions</td>
</tr>
</tbody>
</table>
Figure 1. Relationship between the decrease in milk vitamin C (ascorbic acid (AA)) concentration (24 h post challenge compared with prechallenge) and decrease in milk production caused by an intramammary challenge with E. coli (Weiss et al., 2004).
Figure 2. Effect of vitamin E intake on concentrations of vitamin E in milk and efficiency of transfer of consumed vitamin E to milk vitamin E. Treatments are in IU of supplemental vitamin E (all-rac a-tocopheryl acetate) per kg of dry matter intake. Transfer efficiency calculated as milligrams of vitamin E consumed/d divided by milligrams of vitamin E secreted in milk/d times 100 (Weiss and Wyatt, 2003).