NUTRITIONAL EFFECTS ON IMMUNE FUNCTION AND MASTITIS IN DAIRY COWS?

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The risk that a cow will develop mastitis is a function of pathogen load at the teat end and the cow's ability to prevent a bacterial infection from becoming established in the mammary gland. Nutrition indirectly affects teat end exposure via changes in the amount of manure produced and by altering characteristics of manure (e.g., moisture concentration, pH), but effects on mastitis would probably be small. Conversely, nutrition can have significant effects on the immune system thereby affecting infection rate and severity of mastitis. The highest rates of mastitis generally occur at or shortly after parturition (Smith et al., 1985). Early lactation is also the time when most cows experience short-term malnutrition, i.e., intake of nutrients does not meet nutrient requirements. The immune system, as any physiological system, does not function optimally during periods of malnutrition. In addition, the immune system has high requirements for specific nutrients and when these nutrients are not provided in adequate amounts, immune function may suffer. This review will concentrate on nutritional influences on immune function and mastitis during the periparturient period.

Energy and Protein

During late gestation and early lactation, dry matter intake (DMI) by dairy cows is quite low whereas nutrient demand, especially post-partum is extremely high. This leads to cows being in negative protein and energy balance. Body fat and protein are mobilized by the cow to provide the energy and amino acids needed for maintenance functions and to produce milk. The protein deficient is short-lived because: 1) protein intake by cows can be increased easily by increasing the concentration of protein in the diet and 2) labile body protein reserves are depleted quickly and once they are exhausted, milk production will decrease to match protein supply. An immune response can include antibody production and cellular proliferation both of which require amino acids. However, compared to the kilogram quantity of milk protein produced daily by early lactation cows, the amino acid needs of the immune system are small. No direct data are available showing that mitigating the moderate protein deficiency that occurs in early lactation improves immune function and increases resistance to mastitis. However, one study reported very modest beneficial effects on immune function when peripartum cows were infused with 300 g of glutamine per day (Doepel et al., 2006) but this likely has little practical significance. If protein nutrition is adequate for milk production in early lactation, it likely is adequate for proper immune function.

The energy deficient experienced by most cows lasts much longer than the protein deficient and usually starts a few days before calving and continues for several weeks after parturition. Body energy reserves in a cow are usually much greater than body protein reserves, and it is very difficult to increase energy intake in early lactation via diet changes. Normal, healthy cows lose 0.25 to 0.5 body condition score (BCS) units in early lactation and reach their BCS nadir by 4 to 7 wk of lactation. Some cows start losing body condition several day or even a few weeks before calving, continue losing condition after calving and lose more than 1 BCS

unit in early lactation. This severe negative energy balance is either a consequent of health disorders (e.g., milk fever, retained fetal membranes, or metritis) or will lead to health problems (e.g., ketosis and displaced abomasum). Negative energy balance has also been identified as a risk factor for mastitis.

The degree of negative energy balance experienced by cows is correlated with immune function. Various measures of energy balance such as calculated energy balance, plasma concentrations of non-esterified fatty acids (NEFA) and B-hydroxy-butyrate (BHBA) were negatively correlated with concentrations of antibodies in plasma and milk SCC in early lactation cows (van Knegsel et al., 2007). In that study, all treatment average energy balances were reasonable and based on BHBA and NEFA cows were not suffering from clinical ketosis. Experimentally-induced negative energy balance in steers (DMI was severely restricted) did not negatively affect neutrophil function (Perkins et al., 2001) but neutrophils from cows naturally afflicted with subclinical or clinical ketosis had reduced functionality (Zerbe et al., 2000). An epidemiological study found that high concentrations of plasma ketones or a loss of more than 0.5 BCS units were significant risk factors for the development of udder edema which then was a risk factor for the development of clinical mastitis (Compton et al., 2007); however, they also found that low concentrations of NEFA was associated with **increased** risk of mastitis. In support of that finding, (Berry et al., 2007) reported that increased BCS loss was associated with lower SCC. During the peripartum period, negative energy balance and elevated concentrations of NEFA and BHBA coincides with numerous other events including hormonal changes, hypocalcemia, and changes in vitamin status, therefore it is not possible to determine unequivocally that energy balance direct affect on immune function. However, enough data are available to strongly suggest that excessive mobilization of body fat and the associated increase in NEFA and BHBA during the peripartum period contributes to immunosuppression. Management and dietary practices that should help reduce excessive body condition loss include:

1. Prevent cows from becoming too fat in late lactation and the dry period. This may require a pen dedicated to fat lactating cows so that they can be fed a low energy diet. Excess energy consumption is a common problem during the dry period because dry cows only require about 14 Mcal of NEL/day. To meet, but not exceed, the energy requirement a diet based on less digestible feeds is needed so that the rumen gets full before overconsumption of NEL occurs.

2. Avoid a large decrease in DMI during the prepartum period. DMI can decrease by more than 20% during the last 1 – 2 wk of gestation. This large drop in intake causes cows to mobilize fat which can infiltrate the liver cause fatty liver and ketosis. The drop in intake can be mitigated by feeding a less digestible diet to far-off dry cows so that average DMI for a Holstein cow during the dry period is around 25-26 lbs/day (~12 kg). Cows with high DMI during the early dry period tend to have a greater decrease in DMI during late gestation than do cows that have more moderate DMI during the early dry period (Douglas et al., 2006). The peripartum decrease in DMI can also be moderated by feeding a well-balanced prefresh diet (e.g., 30 to 35% NDF, 30 to 40% concentrate with good forage). Intake by specific animals can be reduced when pens are overcrowded. Make sure pens containing prefresh animals have adequate bunk space and stalls.

3. Promote a rapid increase in energy intake post calving which usually requires a rapid increase in DMI. Feeding excessive grain (i.e., starch) or fat to increase the energy density of diets (i.e., Mcal/kg) usually is counterproductive because it often reduces DMI. Feeding a well-balanced diet based on high quality forage, that contains moderate concentrations of fiber (approximately

30% NDF) and starch (22 to 25%) and <5% total fat improves DMI. Overcrowding fresh cows also restricts their intake.

Energy Source (Specific Fatty Acids)

Neutrophils and other types of immune cell have high concentrations of polyunsaturated fatty acids (PUFA) in their membranes and higher concentrations of specific PUFA are related to improved neutrophil function. In nonruminants, fatty acid profiles of cells reflect the diet composition but in ruminants, dietary unsaturated fatty acids are often biohydrogenated to saturated fatty acids making it difficult to substantially change fatty acid profiles of cells. In two separate studies with transition cows from the same group (Lessard et al., 2003; Lessard et al., 2004) the exact opposite response to fat supplements was observed. In one study lymphocyte proliferation was enhanced when flax seed was fed (a source of n-3 PUFA) compared with cows fed soybeans (a source of n-6 PUFA) but in the other study, cows fed soybeans had enhanced lymphocyte proliferation.   At this time, no compelling data are available to support feeding specific types of fat to improve mammary gland health and reduce mastitis.

Calcium and Other Minerals Related to Hypocalcemia

Cows with milk fever are much more likely to get clinical mastitis than cows without milk fever (Curtis et al., 1985) because:

1. Calcium is required for muscle contractions and the teat sphincter of cows with hypocalcemia may not contract as quickly or as completely as for cows with normal blood Ca increasing the risk of bacterial invasion.
2. Cows with hypocalcemia spend more time lying down which increase teat end exposure
3. Cows with milk fever have higher concentrations of plasma cortisol than normal cows (Horst and Jorgensen, 1982) and cortisol suppresses immune function.
4. Ca status of monocytes is impared in cows with milk fever (Kimura et al., 2006). When monocytes are activated intracellular Ca is released but the amount of Ca released is less in cows with milk fever. This reduces the ability of the monocyte to function properly.

Available data clearly shows that preventing subclinical and clinical milk will reduce the prevalence of mastitis in early lactation. Dietary concentrations of Ca, phosphorus, magnesium, potassium, chloride, sulfur, and vitamin D are related to milk fever. One approach is to feed slightly less Ca to dry cow than their requirement. The marginal Ca deficiency increases mobilization of Ca from bone. Another approach is to feed an anionic diet (elevated concentrations of chloride and sulfur without elevated concentrations of sodium and potassium. This induces metabolic acidosis which is then compensated by mobilizing phosphate from the bone bringing Ca with it. If possible, avoid feeding diets with excessive concentrations of K and make sure dietary Mg is adequate (>0.25% of diet DM).

Antioxidant Nutrients

Reviews are available discussing relationships between immune function and minerals and vitamin of ruminants in greater detail than is presented here (Sordillo, 2005; Weiss and Spears, 2005)   Substantial amounts of free radical are produced during an inflammatory response such as that which occurs when the mammary gland becomes infected. When adequate

antioxidants are present, free radicals are kept in check which increases the life span of certain immune cells.   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. Cells and animals have developed sophisticated systems to control oxidative stress. Components of the antioxidant system include enzymes (many of which contain metal cofactors), vitamins, and numerous other compounds. A simplified version of the antioxidant system is shown in Table 1 and Figure 1.

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

Component (location in cell)	Nutrients Involved	Function
Superoxide dismutase (cytosol)	Copper and zinc	An enzyme that converts superoxide to hydrogen peroxide
Superoxide dismutase (mitochondria)	Manganese and zinc	An enzyme that converts superoxide to hydrogen peroxide
Ceruloplasmin (water phase)	Copper	An antioxidant protein, may prevent copper and iron from participating in oxidation reactions
Glutathione peroxidase (cytosol)	Selenium	An enzyme that converts hydrogen peroxide to water
Catalase (cytosol)	Iron	An enzyme (primarily in liver) that converts hydrogen peroxide to water
Ascorbic acid (cytosol)	Vitamin C	Reacts with several types of ROM
α-tocopherol (membranes)	Vitamin E	Breaks fatty acid peroxidation chain reactions
β-carotene (membranes)	β-carotene	Prevents initiation of fatty acid peroxidation chain reactions

Vitamin A and B-Carotene

The effects of vitamin A and B-carotene on mastitis measures have been inconsistent. Some studies have found positive effects on neutrophil and lymphocyte function when cows are supplemented with approximately 70,000 IU/d of vitamin A or 300 to 600 mg of B-carotene (Michal et al., 1994) but in a clinical study similar treatments had no effect on mammary gland health (Oldham et al., 1991). A likely reason for different responses among studies is differences in vitamin A and B-carotene status of the control cows. (Jukola et al., 1996) suggested that plasma concentrations of B-carotene in dairy cows should be >3 mg/L to optimize udder health. Currently available data does not support feeding vitamin A in excess of the current NRC requirement (approximately 70,000 IU/d) to improve mammary gland health.   Supplemental B-carotene may have some benefit if cows are in low B-carotene status (i.e., fed a diet based largely on weathered, low quality hay).

Cytosol

Catalase (Fe)

$$O_2^{\bullet} \xrightarrow{\text{SOD}} H_2O_2 \xrightarrow{\text{GSH-Px}} H_2O$$

(Cu, Zn)   (Se)

$^1O_2$ Vit C   Vit C ✗   PH-GSHpx

$PL^{\bullet} \longrightarrow PL$

$^1O_2$ BC ✗ H_2O_2 Vit E
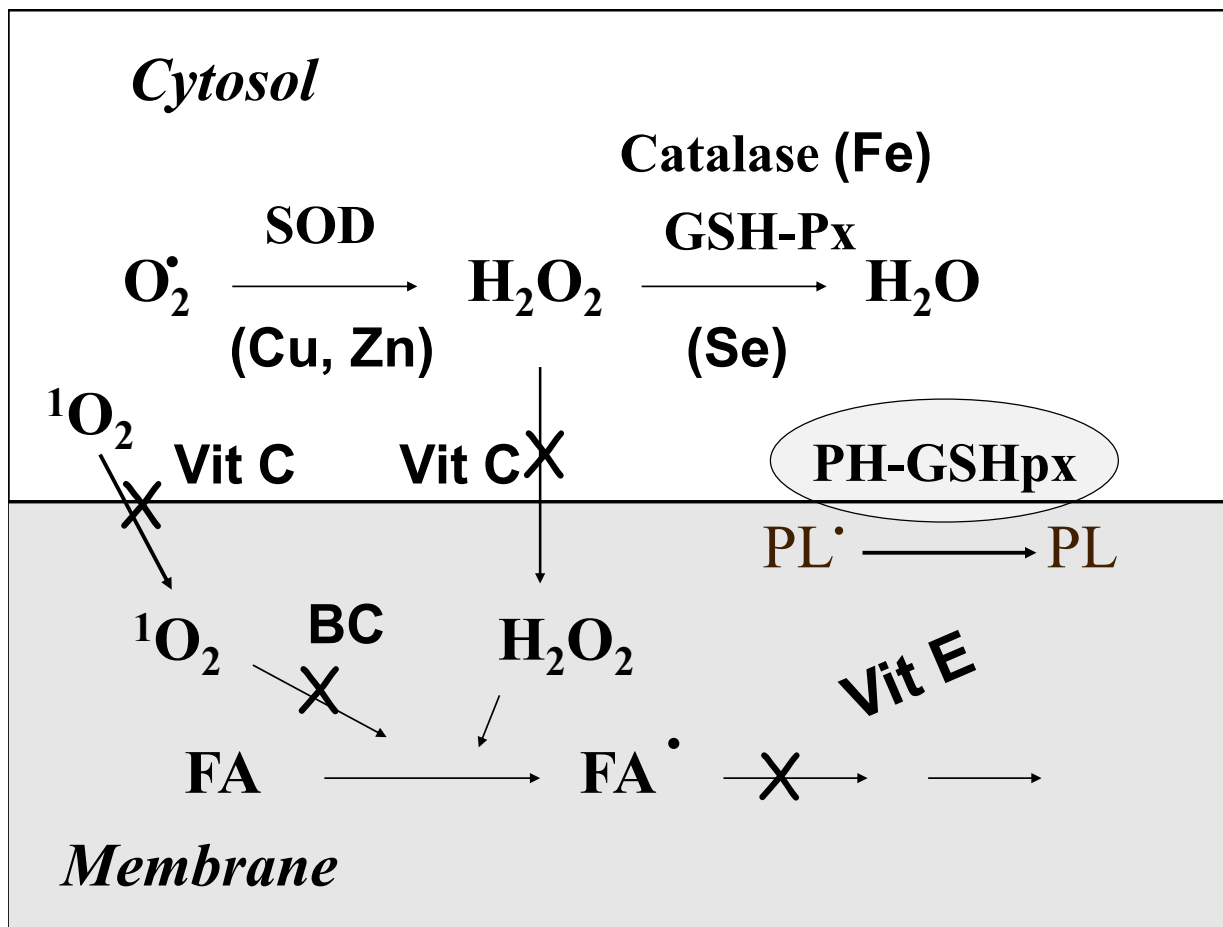
$FA \longrightarrow FA^{\bullet}$ ✗

Membrane

Figure 1. Simplified depiction of the cellular antioxidant system showing relationships with antioxidant nutrients. Enzymes: SOD = superoxide dismutase, GSH-px = glutathione peroxidase, PH-GSHpx= phospholipid hydroperoxide glutathione peroxidase. Nutrients: BC = B-carotene, Virt C = vitamin C (ascorbic acid), Vit E = vitamin E (tocopherol). Other: FA = fatty acid. $FA^{\bullet}$ = FA radical, $O_2^{\bullet}$ = superoxide, $^1O_2$ = singlet oxygen; PL = phospholipid; $PL^{\bullet}$ = PL radical.

Copper and Zinc

Cows and heifers fed diets with 20 ppm supplemental copper had less severe mastitis following a mammary gland challenge (*E. coli*) and fewer natural infection than animals fed diets with about 8 ppm (Harmon and Torre, 1994; Scaletti et al., 2003). 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 (between 200 and 380 mg of Zn/d). 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 supplemental Zn from a mixture of Zn proteinate and inorganic Zn or from all inorganic sources. Source of Zn had no effect on infection rate, new infections, clinical mastitis and SCC. Currently available data suggests that diets should contain about 15 to 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.

Selenium and Vitamin E

Supplemental vitamin E and/or Se has been shown to reduced prevalence and severity of mastitis (Smith et al., 1997). Based on mammary challenge experiments, the positive effects of Se were greater when clinical responses are more severe (i.e., E. coli vs. S. aureus challenge) (Erskine et al., 1989; Erskine et al., 1990). The positive effects of supplemental Se on mammary gland health are well-established; a more recent question concerns source of supplemental Se. In the U.S. supplemental Se can be provided by sodium selenate or selenite (inorganic) or by Se-yeast (organic). Cows fed Se-yeast usually have higher concentrations of Se in plasma, whole blood, and milk compared with cows fed an equal amount of inorganic Se but neutrophil function has not been affected by Se source (Weiss and Hogan, 2005). When Se antagonists are present (e.g., sulfate) obtaining a portion of Se from Se-yeast, especially during the dry period and early lactation should be beneficial. The exact quantity of vitamin E needed by peripartum cows is not known; however feeding more than 1000 IU/d during this period probably is beneficial.

The NRC requirement for vitamin E is about 500 IU/day for lactating cows and 1000 IU/day for dry cows. Essentially no new research has been conducted evaluating vitamin E requirements for lactating cows but several experiments have been conducted with dry cows. Increasing vitamin E supplementation during the transition period (2 or 3 wk prepartum until 1 or 2 wk post partum) has improved measures of immune function or improved mammary gland health in several, but not all, studies (Weiss et al., 1997; Baldi et al., 2000; Politis et al., 2004; Persson Waller et al., 2007) (Figure 2). Supplementation rates during the transition period ranged from 2000 to 4000 IU/day. No study has shown any negative effects of high supplementation rates in the prefresh period. Therefore the only known cost is the cost of the additional vitamin E but because the supplementation period is short (a few weeks) and the potential payoff is high (reduced mastitis and reduced retained placenta) increasing vitamin E supplementation during the prefresh period is justified. Increasing vitamin E supplementation during the entire dry period, however is not justified. A recent study (Bouwstra et al., 2010) evaluated the effects of feeding 3000 IU of vitamin E/day (controls were fed approximately 130 IU/day) during the dry period. On 3 of 5 farms, more cases of mastitis occurred when cows were fed **high** vitamin E and on 2 farms little difference in mastitis was observed between treatments (Figure 3). Overall, they reported that feeding high vitamin E increased the risk of mastitis by 1.7X compared with the control. Although I have some technical concerns regarding the paper (e.g., methods used to diagnose mastitis), the paper clearly shows no benefit of increasing amounts of vitamin E and potentially it might have negative effects. Vitamin E supplementation during the dry period should be limited to 1000 IU/day.
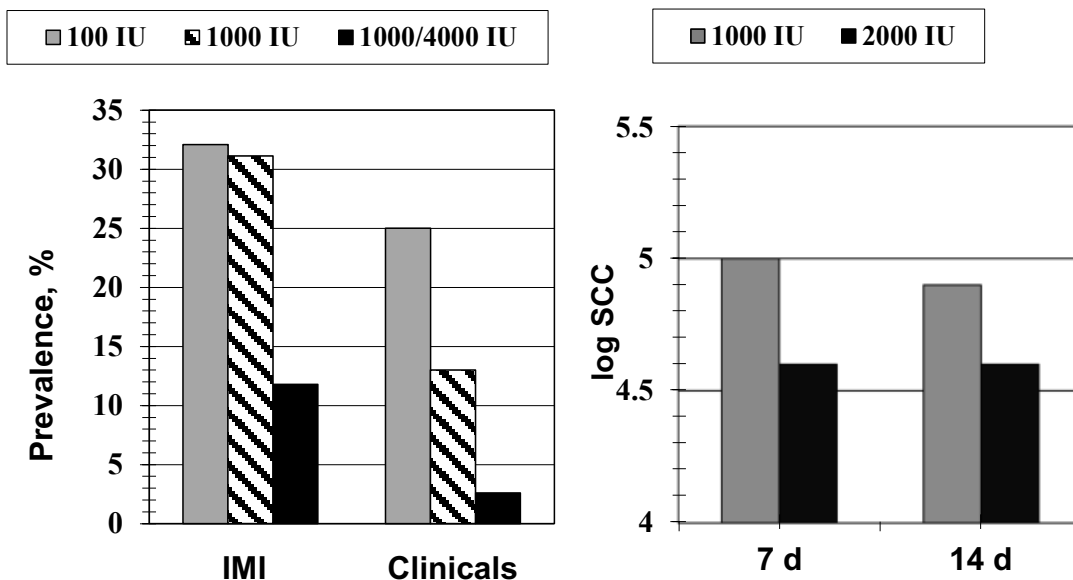
Figure 2. Effects of extra vitamin E during the prefresh (approximately last 2 wk of gestation) on prevalence of intramamary gland infection (IMI), clinical mastitis, and somatic cell count (SCC). The panel on the left (Weiss et a., 1976) has data from an experiment where cows were fed either 100 IU/d or 1000 IU of supplemental vitamin E during the entire dry period or 1000 IU/d until 14 d prepartum and then fed 4000 IU/d. The extra vitamin E greatly reduced IMI and clinical mastitis in early lactation (first 21 days). The panel on the right (Baldi et al., 2000) is from an experiment in which cows were fed either 1000 or 2000 IU of supplemental vitamin E/day during the last 14 d of gestation. The extra vitamin E reduced SCC during the first 14 d of the following lactation.

No new data are available refuting current NRC requirements for vitamin E except during the prefresh period. Increasing vitamin E intake to between 2000 and 4000 IU/day can help reduce mastitis, retained fetal membranes and perhaps metritis. New data suggest over supplementation for the entire dry period (3000 IU/day) may be a risk factor for mastitis and should be avoided.

Vitamin C

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, but cows can synthesize vitamin C and it is not considered an essential nutrient for cattle. The concentration of ascorbic acid is high in neutrophils and increases as much as 30-fold when the neutrophil is stimulated. Within a limited range (67,000 to 158,000 cells/ml), SCC was not correlated with plasma vitamin C concentrations in cows (Santos et al., 2001). Injecting ascorbic acid following

intramammary challenge with endotoxin had very limited effects on inflammation and other clinical signs in cows (Chaiyotwittayakun et al., 2002). We found significant correlations between measures of ascorbic acid status and clinical signs of mastitis caused by *E. coli* challenge (Figure 4) (Weiss et al., 2004). That does not mean that increasing vitamin C status of cows will reduce the prevalence or severity of mastitis. A follow up experiment was conducted to determine whether feeding supplemental vitamin C to periparturient cows would enhance neutrophil function and reduce the inflammatory response following an endotoxin challenge (Weiss and Hogan, 2007). We were successful in enhancing ascorbic acid status of cows, but supplemental vitamin C had no effect on neutrophil function or inflammation. Based on current data, vitamin C is not recommended for either prophylactic or therapeutic treatment of mastitis.
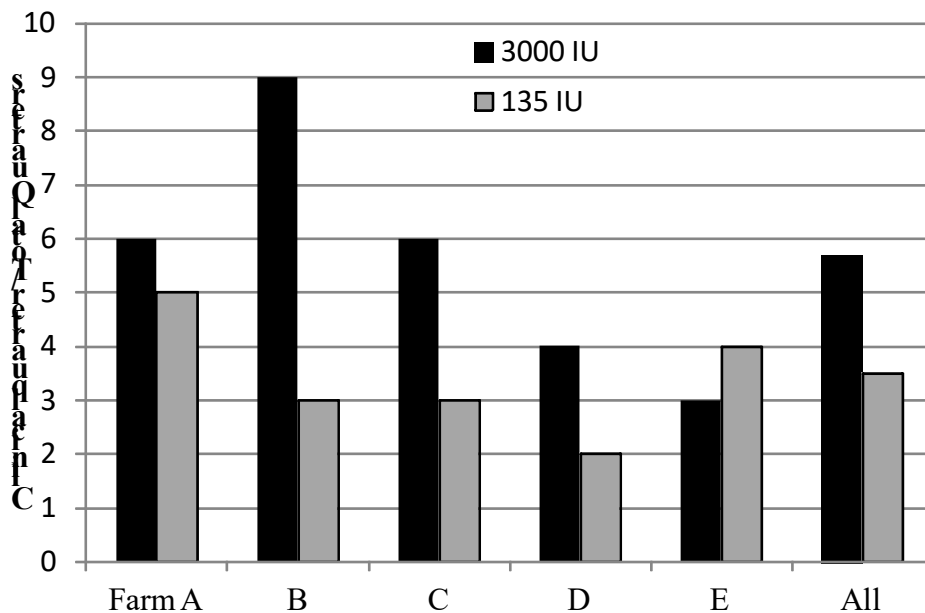
Figure 3. Rate of clinical mastitis in cows fed 135 or 3000 IU of supplemental vitamin E during the dry period on 5 different farms. Figure derived from Bouwstra et al.(2010).

Vitamin D

Because of the interest in vitamin D for human health, vitamin D for dairy cows is also being re-evaluated. Although adequate data are not available to quantitatively adjust the current NRC vitamin D requirement (approximately 20,000 IU/day), data are available suggesting potential benefits from increasing supplementation rates. Studies with humans and limited research with bovine cells have shown that vitamin D has important roles in immune function and that blood concentrations of 25-OH vitamin D (humans) required for maximal immune response was greater than concentrations required for optimal Ca metabolism (Lippolis, 2011). Whether this is true for dairy cows will require new studies. Dairy cows housed inside without exposure to sun and fed vitamin D at NRC recommendations had significantly lower plasma concentrations of 25-OH vitamin D than cows fed no supplemental vitamin D but housed outside in the summer with extensive sun exposure (Hymøller et al., 2009). We do not know the optimal

concentration of plasma 25-OH vitamin D but current supplementation rates may not provide for maximal concentrations (Nelson et al., 2016).
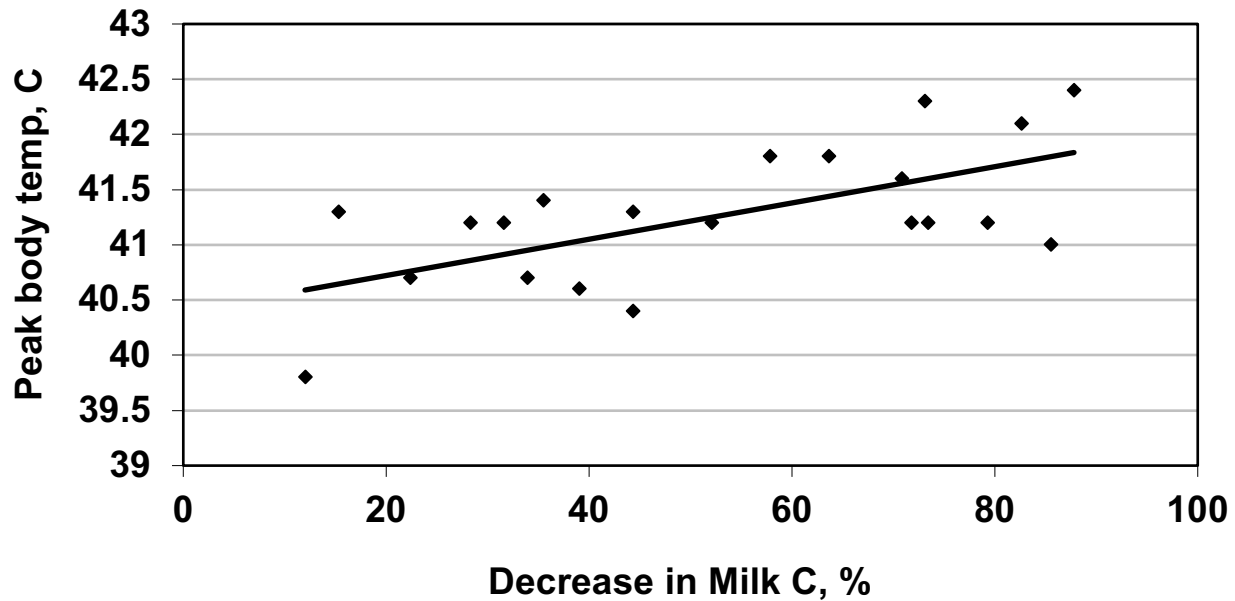

Figure 4. Relationship between concentration of ascorbic acid in milk and body temperature of cows following an infusion of E.coli into the mammary gland. As concentration of vitamin C in milk decreased more, febrile response was greater (Weiss et al., 2004).

Conclusions

To improve mammary gland health:

1. Feed and manage late lactation and dry cows to maintain proper body condition. Avoid a large decrease in feed intake around parturition and a large loss in BCS in early lactation.

2. Prevent hypocalcemia via proper mineral nutrition for dry cows.

3. Feed adequate, but not excessive amounts of trace minerals and vitamins. Selenium and vitamin E are especially critical. Consider increasing vitamin E supplementation during the prefresh period.

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