

NUTRITIONAL INFLUENCES ON THE PREVALENCE AND SEVERITY OF MASTITIS IN DAIRY COWS

W.P. Weiss

Ohio Agricultural Research and Development Center, The Ohio State University
Wooster, Ohio, USA

The risk that a cow will develop mastitis is largely 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 for the energy and amino acids needed for basic 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 (5 point scale, 1= emaciated, 5 = obese) in early

lactation and reach their BCS nadir by 4 to 7 wk of lactation. Some cows start losing body condition several days 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 [(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 with 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 dry matter intake (DMI) during the prepartum period. DMI can decrease by more than 20% during the last 1-2 weeks of gestation. This large drop in intake causes cows to mobilize fat, which can infiltrate the liver and 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, which 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 (Knight, 2000) and higher concentrations of specific PUFA are related to improved neutrophil function (Kew et al., 2003). 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., 2004; Lessard et al., 2003) 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-3PUFA) 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 increases 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 impaired 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 show 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

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 lifespan 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 Figure 1.

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 do 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).

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 infections (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 providing 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 suggest 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.

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).

The current NRC recommendations for vitamin E appear adequate for most situations. Accumulating data suggest that higher intakes (>1000 IU/d) of vitamin E during the periparturient period may be beneficial (Baldi et al., 2000; Politis et al., 2004; Weiss et al., 1997). In those studies, prefresh cows fed 2000 to 4000 IU of vitamin E/day had improved mammary gland health compared with cows fed 1000 IU of vitamin E/day. Conversely, a study conducted on commercial farms in Sweden found no reduction in clinical mastitis or SCC in early lactation when cows were supplemented with approximately 2200 IU of vitamin E/day (Waller et al., 2007). Control cows in that study received between 150 and 2800 IU/day of supplemental vitamin E and the supplemental vitamin E was RRR-alpha-tocopheryl. The form of vitamin E used in the studies that showed a link between vitamin E supplementation and mastitis was all-rac-alpha-tocopheryl acetate. This form of vitamin E consists of 8 different stereoisomers whereas the tocopherol synthesized by living plants consists of only one isomer (RRR-alpha-tocopherol). The acetate form of this isomer is available commercially and can be used to provide supplemental vitamin E. Direct comparisons between vitamin E sources on their effects on mammary gland health are lacking. Cows that consume the same number of IU of vitamin E from RRR usually have higher concentrations of tocopherol in plasma than those fed all-rac vitamin E but that did not translate into improved neutrophil function (Weiss et al., 2009).

Under normal conditions, inorganic Se and Se-yeast appear similar with respect to neutrophil function. 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.

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 ascorbic acid concentrations in cows (Santos et al., 2001). Injecting ascorbic acid (IV) following intramammary challenge with endotoxin had only very limited effects on inflammation and other clinical signs in cows (Chaiyotwittayakun et al., 2002). We conducted an experiment to examine changes in ascorbic acid status following an intramammary challenge with *E. coli* and found significant correlations between vitamin C concentrations in milk and plasma and clinical signs of mastitis (Figure 2) (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; however, 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.

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.

References

- Baldi, A., G. Savoini, L. Pinotti, E. Monfardini, F. Cheli, and V. DellOrto. 2000. Effects of vitamin E and different energy sources on vitamin E status, milk quality and reproduction in transition cows. *J. Vet. Med. (Ser. A)*. 47:599-608.
- Berry, D.P., J.M. Lee, K.A. Macdonald, K. Stafford, L. Matthews, and J.R. Roche. 2007. Associations among body condition score, body weight, somatic cell count, and clinical mastitis in seasonally calving dairy cattle. *J. Dairy Sci.* 90:637-648.
- Compton, C.W.R., S. McDougall, K. Parker, and C. Heuer. 2007. Risk factors for peripartum mastitis in pasture-grazed dairy heifers. *J. Dairy Sci.* 90:4171-4180.
- Curtis, C.R., H.N. Erb, C.J. Sniffen, R.D. Smith, and D.S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. *J. Dairy Sci.* 68:2347-2360.
- Doepel, L., M. Lessard, N. Gagnon, G.E. Lobley, J.F. Bernier, P. Dubreuil, and H. Lapierre. 2006. Effect of postprandial glutamine supplementation on immune response and milk production in dairy cows. *J. Dairy Sci.* 89:3107-3121.
- Douglas, G.N., T.R. Overton, H.G. Bateman, H.M. Dann, and J.K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. *J. Dairy Sci.* 89:2141-2157.
- Erskine, R.J., R.J. Eberhart, P.J. Grasso, and R.W. Scholz. 1989. Induction of *Escherichia coli* mastitis in cows fed selenium-deficient or selenium-supplemented diets. *Amer J Vet Res.* 50:2093-2100.

Erskine, R.J., R.J. Eberhart, and R.W. Scholz. 1990. Experimentally induced *Staphylococcus aureus* mastitis in selenium-deficient and selenium-supplemented dairy cows. *J Amer Vet Med Assoc.* 51:1107-1111.

Harmon, R.J., and P.M. Torre. 1994. Copper and zinc: Do they influence mastitis? Pages 54-65 in *Proc. Natl. Mast. Council.*

Horst, R.L., and N.A. Jorgensen. 1982. Elevated plasma cortisol during induced and spontaneous hypocalcemia in ruminants. *J. Dairy Sci.* 65:2332-2337.

Jukola, E., J. Hakkarainen, H. Saloniemi, and S. Sankari. 1996. Blood selenium, vitamin E, vitamin A, and B-carotene concentrations and udder health, fertility treatments and fertility. *J. Dairy Sci.* 79:838-845.

Kew, S., T. Banerjee, A.M. Minihane, Y.E. Finnegan, C.M. Williams, and P.C. Calder. 2003. Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects. *Am J Clin Nutr.* 77:1278-1286.

Kimura, K., T.A. Reinhardt, and J.P. Goff. 2006. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. *J. Dairy Sci.* 89:2588-2595.

Knight, J.A. 2000. Review: Free radicals, antioxidants, and the immune system. *Annals Clin Lab Sci.* 30:145-158.

Lessard, M., N. Gagnon, D.L. Godson, and H.V. Petit. 2004. Influence of parturition and diets enriched in n-3 or n-6 polyunsaturated fatty acids on immune response of dairy cows during the transition period. *J. Dairy Sci.* 87:2197-2210.

Lessard, M., N. Gagnon, and H.V. Petit. 2003. Immune response of postpartum dairy cows fed flaxseed. *J. Dairy Sci.* 86:2647-2657.

Michal, J.J., L.R. Heirman, T.S. Wong, B.P. Chew, M. Frigg, and L. Volker. 1994. Modulatory effects of dietary B-carotene on blood and mammary leukocyte function in periparturient dairy cows. *J. Dairy Sci.* 77:1408-1421.

Oldham, E.R., R.J. Eberhart, and L.D. Muller. 1991. Effects of supplemental vitamin A and B-carotene during the dry period and early lactation on udder health. *J. Dairy Sci.* 74:3775-3781.

Perkins, K.H., M.J. Vandehaar, R.J. Tempelman, and J.L. Burton. 2001. Negative energy balance does not decrease expression of leukocyte adhesion or antigen-presenting molecules in cattle. *J. Dairy Sci.* 84:421-428.

Politis, I., I. Bizelis, A. Tsiaras, and A. Baldi. 2004. Effect of vitamin E supplementation on neutrophil function, milk composition and plasmin activity in dairy cows in a commercial herd. *J Dairy Res.* 71:273-278.

Santos, M.V., F.R. Lima, P.H.M. Rodrigues, S.B.M. Barros, and L.F.L. daFonseca. 2001. Plasma ascorbate concentrations are not correlated with milk somatic cell count and metabolic profile in lactating and dry cows. *J. Dairy Sci.* 84:134-139.

Scaletti, R.W., D.S. Trammell, B.A. Smith, and R.J. Harmon. 2003. Role of dietary copper in enhancing resistance to *Escherichia coli* mastitis. *J. Dairy Sci.* 86:1240-1249.

Smith, K.L., J.S. Hogan, and W.P. Weiss. 1997. Dietary vitamin E and selenium affect mastitis and milk quality. *J. Anim. Sci.* 75:1659-1665.

Smith, K.L., D.A. Todhunter, and P.S. Schoenberger. 1985. Environmental mastitis: Cause, prevalence, prevention. *J. Dairy Sci.* 68:1531-1553.

van Knegsel, A. T.M., G. de Vries Reilingh, S. Meulenberg, H. van den Brand, J. Dijkstra, B. Kemp, and H. K. Parmentier. 2007. Natural antibodies related to energy balance in early lactation dairy cows. *J. Dairy Sci.* 90:5490-5498.

Waller, K.P., C.H. Sandgren, U. Emanuelson, and S.K. Jensen. 2007. Supplementation of RRR- α -tocopheryl acetate to periparturient dairy cows in commercial herds with high mastitis incidence. *J. Dairy Sci.* 90:3640-3646.

Weiss, W.P., and J.S. Hogan. 2007. Effects of vitamin C on neutrophil function and responses to intramammary infusion of lipopolysaccharide in periparturient dairy cows. *J. Dairy Sci.* 90:731-739.

Weiss, W.P., J.S. Hogan, and K.L. Smith. 2004. Changes in vitamin C concentrations in plasma and milk from dairy cows after an intramammary infusion of *Escherichia coli*. *J. Dairy Sci.* 87:32-37.

Weiss, W.P., J.S. Hogan, D.A. Todhunter, and K.L. Smith. 1997. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. *J. Dairy Sci.* 80:1728-1737.

Weiss, W.P., J.S. Hogan, and D.J. Wyatt. 2009. Relative bioavailability of all-rac and RRR vitamin E based on neutrophil function and total α -tocopherol and isomer concentrations in periparturient dairy cows and their calves. *J. Dairy Sci.* 92:720-731.

Zerbe, H., N.R. Schneider, W. Leibold, T. Wensing, T.A.M. Kruip, and H.J. Schuberth. 2000. Altered functional and immunophenotypical properties of neutrophilic granulocytes in postpartum cows associated with fatty liver. *Theriogenology.* 54:771-786.

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

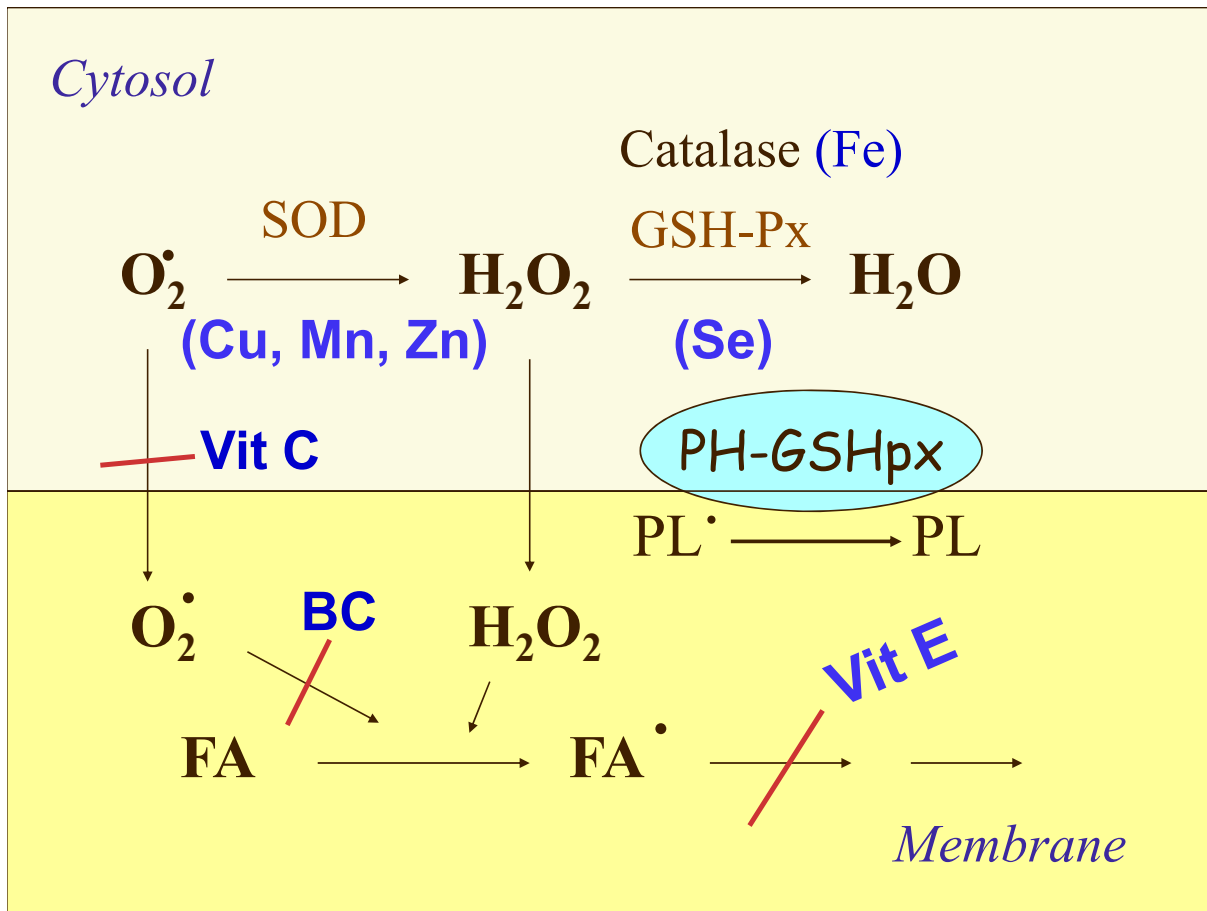


Figure 1. Simplified version of cellular antioxidant system showing relationships with antioxidant nutrients. Enzymes: SOD = superoxide dismutase, GSH-px = glutathione peroxidase, PH-GSHpx= phospholipid hydroperoxide glutathione peroxidase. BC = B-carotene, Vit C = vitamin C (ascorbic acid), Vit E = vitamin E (tocopherol). FA = fatty acid.

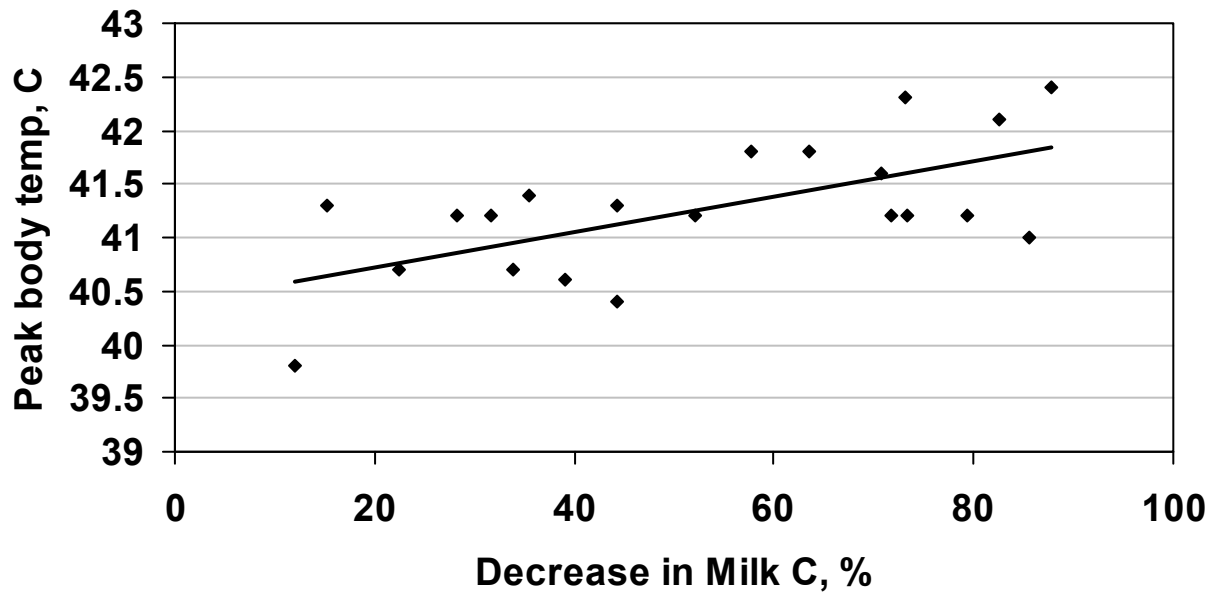


Figure 2. Relationship between concentration of ascorbic acid in milk and body temperature of cows following an infusion of *E. coli* into the mammary gland. As concentration of vitamin C in milk decreased more, febrile response was greater (Weiss et al., 2004).