Mastitis, Mammary Gland Immunity, and Nutrition

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INTRODUCTION

Although intensive research and prevention measures have been carried out for decades to control bovine mastitis, it continues to cause the biggest economic impact to the dairy industry worldwide. Severe economic losses, which are estimated to be approximately $200/cow/yr in the United States, are due to reduced milk production, discarded milk, replacement costs, extra labor, treatment, and veterinary service costs (Smith and Hogan, 2001). In addition, mastitis increases the risk of antibiotic residues in milk due to treatments and decreases milk quality due to an increase in somatic cell count, as well as proteolytic and lipolytic enzymes, which increase the enzymatic breakdown of milk protein and fat (National Mastitis Council, 1991).

Intramammary infections (**IMI**) result in a typical inflammatory response characterized by an influx of somatic cells composed primarily of neutrophils accompanied by variable numbers of macrophages and lymphocytes (Rainard and Riollet, 2003). The response is driven by the action of a variety of inflammatory mediators including cytokines, chemokines, prostaglandins, and leukotrienes; all of which play pivotal roles in mammary gland defenses by mediating and regulating inflammation and immunity. The ensuing inflammatory response induced by entry of bacteria into the mammary gland is variable; the intensity typically dictating an outcome ranging from successful elimination of the pathogen to establishment of chronic infection.

There are 2 groups of bacterial pathogens that can cause mastitis in dairy cows: contagious pathogens such as *Streptococcus agalactiae*, *Mycoplasma bovis,* and *Staphylococcus aureus*, and environmental pathogens such as *Escherichia coli* and *Klebsiella* sp. Contagious microorganisms reside on the skin of the teat and interior of the udder, and are transmitted from cow-to-cow during the milking procedure through milking equipment and milker's hands. Environmental pathogens are found in the environment, such as in the cattle shed and milking parlor; and, in an opportunistic way, enter the teat canal when the cow comes in contact with a contaminated environment (Table 1). The severity of the mastitis depends on the animal response to the insult, for example the entrance and installation of the pathogen inside the gland, and on virulence factors present on the bacteria.

There are various genetic, physiological, and environmental factors that can compromise host defense mechanisms during the functional transitions of the mammary gland (Sordillo, 2005). Nowadays, the lactating cow has been genetically selected to produce more milk, which is the basis of the dairy industry. However this increase in milk volume metabolically stresses dairy cows and affects mammary gland immunity by impairing defense mechanisms decreasing the resistance to mastitis (Heringstad et al., 2003). In addition, the milking procedure can cause trauma to teat and tissues, making it easier for the invasion and colonization of mastitis causing pathogens in the mammary gland.

Housing is also a factor that aggravates the incidence of mastitis, first due to excess numbers of animals in a limited space; and also because of the use of bedding material that easily allows for bacterial survival and growth, which over exposes animals and challenges their immune defense mechanisms (Hogan and Smith, 2003). Prevention of intramammary infections leads to better milk quality, which in turn is beneficial for the dairy industry because high quality milk means extended shelf life, increased cheese yield, and increased consumption of dairy products.

There are several factors both infectious and non-infectious, that can cause bovine mastitis; and there is an increase in the evidence that nutritional factors are associated with mastitis in cows and heifers (Heinrichs et al., 2009). Despite all the measures taken by dairy producers to prevent intramammary infections in their herds, it is not always clearly understood that there is a strong relationship between nutrition and susceptibility to mastitis. When the consumption of minerals and vitamins by dairy cows is not optimal, it can have negative effects on immunity. Animals become more susceptible to diseases such as mastitis, because a depressed immune system is not able to fight off bacteria that invade the udder.

Table 1: Differences between contagious and environmental mastitis

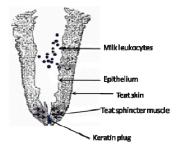|	Contagious	Environmental
Microorganisms	*Staphylococcus aureus* *Streptococcus agalactiae* *Mycoplasma bovis*	*Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Streptococcus uberis* *Streptococcus disgalactiae* *Enterococcus faecalis* *Enterococcus faecium* *Candida, yeast* *Corynebacterium pyogenes*
Primary source	Udders of infected cows	The environment of the cow
Indicators of problem	Bulk tank SCC > 300,000 cells/ml; Frequent cases of clinical mastitis, often in the same cows; and Bacterial culturing results shows *S. agalactiae* and/or *S. aureus* infections.	High rate of clinical mastitis, usually in early lactation or during hot weather; Large numbers of dry cows that have mastitis during the early dry period; and Increase in the herd's somatic cell.
Control	Develop program to prevent the spread of bacteria at milking time. Eliminate existing infections by treating all cows at dry-off and culling chronic cows.	Reduce the number of bacteria to which the teat end is exposed. Improve cleanliness of cow surroundings, especially in late dry period and at calving. Improve pre-milking procedures to ensure clean, dry teats are being milked.

Researchers have studied the relationship between the supplementation of trace minerals and vitamins and immune system function and mammary gland health. Several studies have shown that susceptibility to intramammary infections can be influenced by the levels of vitamins A and E and minerals such as Se, Cu, and Zn in the diet. The results obtained from those experiments were used to establish the daily intake requirements of minerals and vitamins for dairy cows, and are published in the most recent dairy cattle version of the National Research Council (NRC, 2001).

IMMUNITY OF THE MAMMARY GLAND

Defense mechanisms can be divided into innate immunity and acquired immunity. The innate immunity, also known as non-specific immunity, is the predominant defense during early stages of infection. This first defense is activated quickly at the site of infection by numerous stimuli; however, it does not have *memory* and is not increased by repeated exposure to the same insult. This non-specific defense is initiated at the teat end, which is a physical barrier, and continues with immune cells such as macrophages, neutrophils, natural killer (**NK**) cells, and by other soluble factors, such as cytokines.

On the other hand, the acquired immunity also known as specific immunity, is the basis of vaccination. It recognizes specific determinants of a pathogen that activate a selective response leading to its elimination. Antibodies, macrophages, and lymphocytes recognize pathogenic factors and incite a response. This type of immunity has memory, and is increased by repeated exposure to the same pathogen.

As part of the innate immune system, physical barriers of the udder, including teat skin, teat sphincter muscle, and keratin plug, work together to prevent bacterial entrance (Figure 1). However, failure in their normal function results in intramammary infection. Abrasion and cracks on teat skin favor bacterial colonization, increasing the risk of bacterial entrance, mainly between milking procedures when the duct is dilated. Another protective factor on the teat is the keratin plug, which is secreted by cells that line the teat. This substance has bactericidal properties, and also entraps and prevents the upward movement of bacteria into the mammary gland decreasing the chance of intramammary infections, consequently decreasing somatic cell count (**SCC**) (Kellogg et al., 2004).

Figure 1: Physical barriers of the mammary gland

For the best protection of the mammary gland against intramammary infections, innate and acquired immune systems must interact in a synchronized fashion. Some pathogens have developed mechanisms to avoid the host immune system and survive causing disease. *Staphylococcus aureus,* for example, survives inside phagocytic cells or become walled off within mammary tissue; therefore escaping immune detection and preventing its elimination (Hensen et al., 2000). In addition some animal factors, such as stage of lactation, may interfere with the quality of the immune response. The transition period is a good example, where hormonal changes and stresses lead to depression of the non-specific immune system (Sordillo and Streicher, 2002).

Dairy cows are more susceptible to mastitis at the time around parturition, when the keratin plug in the teat breaks down and the teat canal is open for the entrance of mastitis pathogens (Figure 2). In addition to impaired neutrophil function, decreased lymphocyte responsiveness to mitogen stimulation contributes to this increased susceptibility to new intramammary infections (Mallard et al., 1998). Increased production of reactive oxygen species (**ROS**), the so called free radicals, is expected in late pregnancy, parturition, and initiation of lactation due to metabolic demands on these stages of lactation (Sordillo and Aitken, 2009). New intramammary infections during the periparturient period have a significant impact on milk production efficiency due to the damage on milk producing cells (Oliver and Sordillo, 1988).

The immune defense of the mammary gland against intramammary infections is directly related to the action of somatic cells, mainly neutrophils. The entrance of microorganisms into the mammary gland initiates a cascade of events. Initially neutrophils, the first line of defense, migrate from the blood stream to the infected site, where they phagocytize and kill intruding organisms by a chemical process called respiratory burst (Figure 3). This event releases a high concentration of free radicals, which assist in killing bacteria, but can also lead to tissue damage and the cell's death (Figure 4).
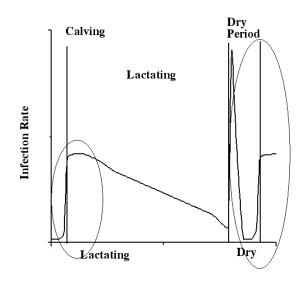
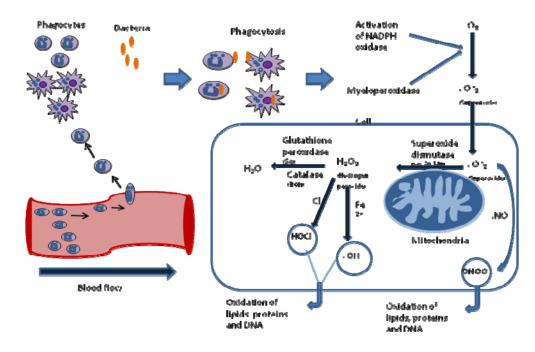**Figure 2:** Mastitis susceptibility by stage of lactation

Figure 3: Schematic representation of respiratory burst

In addition to neutrophils, macrophages are also directed to the site of infection where they help kill the bacteria and initiate the acquired immune response. At this stage of response, lymphocytes are stimulated and initiate production of antibodies against the bacteria, and also migrate to the site of infection. The cell's interaction helps eliminate the invading pathogen and resolve the infection. This immune response, with an influx of cells into the gland and release of immune mediators such as cytokines and chemokines, for example Tumor Necrosis Factor (**TNF**)-α and Interleukin (**IL**) 8, increases blood vessel permeability allowing fluids to move into the infected site and producing an inflammatory response with the clinical signs of mastitis: heat, redness, pain, and swelling of the udder.

Important free radicals present in biological systems are superoxide, hydrogen peroxide, hydroxyl radical, and fatty acid radicals (Smith et al., 1984). Because they are very toxic to cells, the body has an antioxidant system where superoxide is converted to hydrogen peroxide by the enzyme superoxide dismutase; which contains Cu, Zn, and Mn. Hydrogen peroxide is then converted to water by the enzyme glutathione peroxidase, which depends on Se to work. By these 2 processes, these enzymes control most free radicals in the cytosol of cells. Superoxide and hydroxyl radicals can migrate into cell membranes and attack fatty acids, producing fatty acid radicals. These fatty acid radicals can react with other fatty acids and produce a chain reaction.

IMPACT OF NUTRITION ON MASTITIS

Antioxidants and trace minerals play important roles in immune function, which in turn can influence some aspects of health in transition dairy cows. Vitamin A and Zn influence epithelial health, can impact physical defense barriers of the udder, and also alter the quality and quantity of the keratin plug. Phagocytic cells are influenced by a number of nutrients, including Cu, Zn, Se, and vitamins A and E. Copper can affect phagocytic function, with variable impacts on cell mediated and humoral immunity in cattle. Lymphocyte activity can be influenced by energy, protein, Zn, and vitamin A. Antibody production is influenced by energy, protein, Cu, Zn, Se, and vitamins A, D, and E. The killing ability of immune cells is shown to be increased by nutritional supplementation with Vitamin E, which has consistently been shown to improve neutrophil function in dairy cows (Politis et al., 2004). Deficiency or excess of essential nutrients in the transition cow diet can induce one or more metabolic diseases (Van Saun, 1991). In addition, dietary nutrients can increase susceptibility to mastitis through their impact on periparturient metabolic diseases.
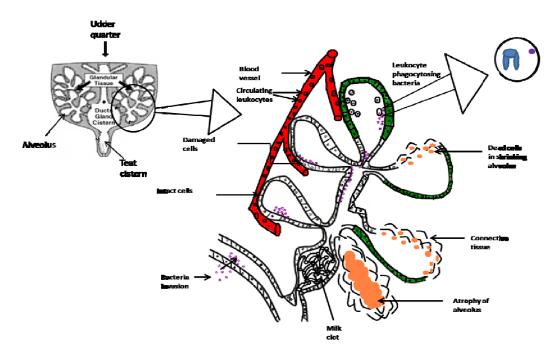
Figure 4: Schematic representation of mastitis development in an infected udder

Vitamin E and Selenium

Vitamin E is a lipid soluble antioxidant that protects against lipid peroxidation initiated by free radicals and has been shown to play an important role in immune response and health of dairy cows (Spears and Weiss, 2008). Blood levels of vitamin E decrease as parturition approaches and remain low for several days postpartum (Weiss et al., 1994). The effect of supplementation of vitamin E on health and reproduction of dairy cows has been reviewed by Allison and Laven (2000). In this review they conclude that there is enough evidence to surmise that supplementation with Vitamin E decreases incidence and/or duration of clinical mastitis in dairy cows. Studies have shown that Vitamin E is directly associated with neutrophil function in dairy cows by enhancing neutrophil function, improving the killing ability of blood neutrophils during the periparturient period (Hogan et al., 1992). In addition, vitamin E supplementation during the transition period prevented a decline in neutrophil superoxide anion production, interleukin-1 (**IL-1**) production, and MHC-II expression by blood monocytes after parturition; as well as prevented a decrease in chemotactic responsiveness of neutrophils beginning 2 wk prior to and continuing for 4 wk after parturition (Politis et al., 1995, 1996).

Selenium is an essential component of the enzymes glutathione peroxidase and thioredoxin reductase located in the cytosol of the cells; which function in preventing oxidative stress (Mustacich and Powis, 2000). In addition, Se is also considered to have a protective effect on phagocytic cells from autoxidative damage during the respiratory burst. Leakage of free radicals from the phagolysosomes, or failure to detoxify these products, could affect the microbicidal and metabolic functions of phagocytic cells (Larsen, 1993). It has been stated that Se concentration in colostrum is 4 times higher than in milk (Underwood and Suttle, 1999). Grasso et al. (1990) have demonstrated that neutrophils, from cows supplemented with Se, killed mastitis pathogens more efficiently compared to cows not fed with supplemental Se. Erskine et al. (1989), in a similar study, showed that cows supplemented with Se and challenged with *Escherichia coli* had a faster influx of neutrophils to the site of the infection compared with non-supplemented cows.

Studies have shown that supplementation of Se and vitamin E to dry cows reduced the duration of mastitis and incidence of clinical mastitis, with vitamin E alone being a good supplementation in preventing mastitis in early

lactation (Weiss et al., 1997; Smith et al., 1984). In addition to the benefit of supplementing dairy cows with Vitamin E and Se in reducing clinical mastitis, it improves milk quality by decreasing somatic cell counts and reduces oxidative flavor problems in milk by increasing vitamin E content in milk (Weiss et al., 1994).

The best way to assess vitamin E and Se status in dairy cows is by collecting blood samples. In normal cows, one third of Se is found in plasma and two-thirds in the red blood cells. The serum or plasma concentration mainly represents current Se status and is more sensitive to short-term changes in the diet (Ullrey, 1987). In contrast, whole blood Se mainly represents the supplementation 1 to 2 mo earlier, as Se is incorporated into the glutathione peroxidase in the red blood cells during erythropoiesis (Underwood and Suttle, 1999). Plasma Se increases shortly after intramuscular administration of Se, but the content of the red blood cells will not change for several weeks. Therefore, whole blood Se is the best method to assess Se status, but plasma is also adequate (Smith et al., 2000).

In conclusion, supplementation with vitamin E and Se improves the immune function of dairy cows, decreasing the incidence of mastitis.

β -Carotene and Vitamin A

The role of vitamin A and β-carotene in prevention of animal diseases is well documented. Vitamin A is necessary for all cellular division and differentiation (Herdt and Stowe, 1991), and plays a key role in inhibition of keratinization. Deficiency of vitamin A culminates with hyperkeratinization of the secretory epithelium, increasing the susceptibility to diseases (Reddy and Frey, 1990).

Beta-carotene, a precursor of vitamin A, functions as an antioxidant reducing superoxide formation within the phagocyte (Sordillo et al., 1997) and can directly enhance immunity with reproductive and mammary benefits (Chew, 1993).Vitamin A is also related to immunity and mastitis. It plays an important role in maintaining epithelial tissue health and preserving the integrity of the mucosal surface (Sordillo et al., 1997); which may contribute in preventing the entrance of mastitis causing pathogens into the mammary gland.

Studies have shown that blood concentrations of vitamin A and β-carotene in dairy cows start to decline around 15 d before calving, reaching its lowest level at parturition (Oldham et al., 1991), due mainly to reduced dry matter intake (**DMI**) and transfer to colostrum (Weiss, 1998).

Zinc and Copper

Zinc plays an important role in maintaining health and integrity of skin due to its role in cellular repair and replacement, and by increasing the speed of wound healing (Sordillo et al., 1997). In addition to this healing effect, it has been suggested that Zn reduces somatic cell count due to its role in keratin formation. Zinc plays a critical role in function and effectiveness of some immune components. It is an essential component of several enzymes involved in the synthesis of DNA and RNA, and has an antioxidant role by being part of a group of elements that induces the synthesis of metallothionein, which binds to free radicals (Prasad et al., 2004). As a component of the enzyme superoxide dismutase, it can stabilize cell membrane structures (Reddy and Frey, 1990). According to Goff and Stable (1990), Zn levels in dairy cows decrease at parturition due to a decrease in DMI, transfer of Zn to colostrum, increased stress at this time, and return to baseline levels within 3-5 d postpartum. In addition, during *Escherichia coli* induced mastitis, the blood concentration of Zn declines, suggesting an antibacterial mechanism by which Zn is made less available for bacterial growth (Erskine and Bartlett, 1993). However, there are very few studies on Zn supplementation with very limited clinical data on the effect of dietary Zn on mammary gland health.

Copper has also been associated with immune function. It is a component of the enzyme ceruloplasmin, which is synthesized in the liver, that assists in iron absorption and transport. Furthermore, Cu is an important part of superoxide dismutase, an enzyme that protects cells from the toxic effects of oxygen metabolites released during phagocytosis. Both functions may be important in reducing the incidence of mastitis during the periparturient period. Copper supplemented to heifers starting 60 d pre-calving and continuing to 30 d postpartum decreases the severity of *Escherichia coli* induced mastitis cases (Scalleti et al., 2003). In addition cows supplemented with Cu had fewer infected

quarters and fewer intramammary infections caused by major mastitis pathogens and no difference in prevalence of coagulase-negative *Staphylococcus* mastitis at calving (Scalleti et al., 2003).

Zinc and Cu play important roles in removing superoxide radicals (free radicals) from the body. These radicals can disrupt cellular membranes and cause cellular damage leaving the mammary gland more susceptible to infection, scarring, and lost milk production.

CONCLUSIONS

Mastitis continues to be one of the most common diseases of dairy cattle that result in a negative economic impact for the dairy industry. Currently dairy cow management focuses on maximizing milk production, which has increased the incidence of health disorders due to failure to control the production and elimination of free radicals; that metabolically activate tissues resulting in oxidative stress and subsequently leads to health issues in dairy cattle. The periparturient period is a period of immune suppression where oxidative stress has a significant impact on the health of dairy cows. One of the main factors in controlling mastitis is maintaining proper nutritional status of the animals to prevent health disorders.

Results from several studies have shown that nutrition and management can affect mammary gland immunity, and consequently mastitis. These studies show the importance of vitamins and minerals, such as vitamin E, Se, Cu, and Zn for proper maintenance of immunity and health of the mammary gland. However, supplementation of diets should be preceded by testing feed to determine the natural levels of vitamins and minerals. In this case, when supplementation is necessary, it becomes important to allow for optimal immunity, mainly in the periparturient period. Mastitis incidence can be reduced by controlling nutritional and management aspects. Improving the animal's immune system to increase its effectiveness against invading mastitis pathogens is an important point in controlling mastitis.

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