Current Concepts on Immunity and Mastitis

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Take Home Messages

- The mammary gland is protected against mastitis-causing pathogens by several different immunological defence mechanisms
- The immune system of dairy cows has a diminished ability to fight disease during the transition period
- Oxidative stress is a major contributor to diminished immune capabilities and increased disease susceptibility in transition cows
- Micronutrients that enhance antioxidant defenses of mammary tissues can greatly impact the incidence and severity of mastitis

Mastitis

Mastitis is a significant disease of adult dairy cattle affecting up to 40 percent of cows within a herd at any given time. Recent surveys show that udder health problems are consistently the most frequent cause of morbidity with the US dairy cattle population (Table 1). The U.S. dairy industry loses an estimated $2 billion every year due to mastitis, with reduced milk production accounting for the majority of the total economic loss.

The incidence of mastitis is directly related to changes in the composition, magnitude, and efficiency of the mammary gland defense system. However, many different aspects of bovine mammary gland defenses are suboptimal during distinct periods of the lactation cycle, particularly around the transition period (Sordillo and Streicher, 2002). Most notably, the two weeks prior to calving through the first three weeks of lactation have long been recognized as a period when key host defense mechanisms alter dramatically. As a consequence, dairy cattle are more susceptible to mastitis during the periparturient period and through peak milk production. New intramammary infections occurring during the perinatal period are especially problematic as they may greatly impact the productive efficiency of dairy cattle in the ensuing lactation. Therefore, it is not surprising that considerable research efforts have

been focused on defining how mammary gland defenses change as a consequence of lactation cycle and understanding those factors that may contribute to immune-dysfunction during this critical period.

Table 1: Health Problems of US Dairy Cattle (*NAHMS Surveys)*

	1996	2002	2007
Mastitis/Udder Problems	13.4 ± 0.3	14.7 ± 0.3	16.5 ± 0.5
Lameness	10.5 ± 0.3	11.6 ± 0.3	14.0 ± 0.4
Infertility	11.9 ± 0.3	11.9 ± 0.3	12.9 ± 0.3
Retained fetal membranes	7.8 ± 0.2	7.8 ± 0.2	7.8 ± 0.2

Morbidity expressed as percentage of all cows in the US ± standard deviation of the mean. *http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/index.htm*

■ Mammary Gland Defense Mechanisms

The mammary gland is protected by a variety of defense mechanisms that can be separated into two distinct categories: innate immunity and specific immunity. Innate immunity, also known as nonspecific responsiveness, is the predominant defense during the early stages of infection (Rainard and Riollet, 2006). Nonspecific responses are present or are activated quickly at the site of infection by numerous stimuli; however, they are not augmented by repeated exposure to the same insult. Nonspecific or innate responses of the mammary gland are mediated by the physical barrier of the teat end, macrophages, neutrophils, natural killer (NK) cells, and by certain soluble factors (Table 2).

The specific or acquired mammary immune system recognizes specific determinants of a pathogen. Activation of specific mammary immune defenses results in the selective elimination of mastitis-causing pathogens (Sordillo and Streicher, 2002). Recognition of pathogenic factors is mediated by several lymphoid populations, macrophages, and antibody molecules (Tables 3 and 4). Because of the “memory” of certain lymphocytes, specific immune responses can be augmented by repeated exposure to a pathogen. Vaccination of dairy cattle against certain pathogens can occur if specific mammary immune mechanisms are effectively activated.

Table 2: Summary of Mammary Non-specific Defenses

Factor	Biological Function
Neutrophil	phagocytosis and intracellular killing of bacteria; secretion of antibacterial factors
Macrophage	phagocytosis and intracellular killing of bacteria
Natural Killer Cells	non-immune lymphocytes that secrete antibacterial proteins upon activation
Cytokines	pro-inflammatory and immunoregulatory factors
Complement	bacteriolytic and/or facilitates phagocytosis
Lysozyme	cleaves carbon bonds and disrupts bacterial cell walls
Lactoferrin	sequesters iron to prevent bacterial uptake; disrupts bacterial cell wall; regulates mammary leukocyte activity

Optimal protection of the mammary gland from new intramammary infections requires that both innate and acquired protective factors interact in a highly coordinated fashion. However, there exist numerous genetic, physiological, and environmental factors that can compromise host defense mechanisms during the functional transitions of the mammary gland. For example, emphasis on genetic selection to maximize milk production has increased metabolic stresses associated with milk synthesis and secretion and a negative correlation exists between milk production capacity and resistance to mastitis. Total confinement housing, increased cow densities per unit area, and use of bedding materials that support bacterial growth also can have a marked impact on the susceptibility of dairy cattle to new intramammary infections by overwhelming important local defense mechanisms. Whereas the concept that immunocompromised animals are more susceptible to disease is well established, it is unlikely that disease susceptibility caused by increased production demands on food-producing animals will wane as animal agriculture strives to compete within a global economy. Intensity of dairy cattle management and genetic selection to increase milk production will continue and most likely result in additional immunological stresses being placed on dairy cows. Such stresses will undoubtedly lead to increased problems associated with mastitis. Completely eliminating any form of stress is impractical, and an alternative approach to reduce the influence of stress

on disease susceptibility is to modify the host response to the stressor. If stress-induced changes in host immunity predispose dairy cattle to disease, then methods of up-regulating the immune response during distinct periods of stress should increase disease resistance. The challenge that confronts researchers now is to gain a better understanding of the complex interactions between the pathogenesis of bacteria, host responses needed to eliminate the pathogens from the mammary gland, and ways to enhance the immune potential of these factors before disease is established.

Table 3: Summary of Mammary Cellular Specific Defenses

Factor	Biological Function
T Lymphocytes	
CD4+ (helper)	Cytokine production; memory cells following antigen recognition
CD8+ (cytotoxic)	lysis of damaged host cells; production of cytokines that down-regulate certain leukocyte functions
γδ T cells	biological role in the mammary gland is speculative
B Lymphocytes	
mature B cells	displays membrane-bound antibody molecules to facilitate antigen presentation; memory cells following antigen interactions
plasma cell	terminally differentiated B lymphocytes that synthesize and secrete antibody against a specific antigen
Macrophage	antigen presentation in conjunction with MHC

Table 4: Summary of Mammary Soluble Specific Defenses

Factor	Biological Function
Antibodies	
IgG1	selectively transported into mammary secretions; opsonizes bacteria to enhance phagocytosis
IgG2	transported into secretions during neutrophil diapedesis; opsonizes bacteria to enhance phagocytosis
IgA	associated with the fat portion of milk; does not bind complement or opsonize particles; can cause agglutination, prevent bacterial colonization, and neutralize toxin
IgM	efficient at complement fixation, opsonization, agglutination and toxin neutralization; only opsonic for neutrophils in the presence of complement

■ Oxidative Stress in Transition Cows

Dairy cows undergo substantial metabolic and physiological adaptations during the transition from pregnancy to lactation that are thought to contribute dysfunctional host defense mechanisms (Sordillo, 2005). Physiological stresses associated with rapid differentiation of secretory parenchyma, intense mammary gland growth, and the onset of copious milk synthesis and secretion are accompanied by a high energy demand and an increased oxygen requirement. This increased oxygen demand augments the production of oxygen-derived reactants, collectively termed reactive oxygen species (ROS). Although molecular oxygen is required for normal cellular functions in mammals, excess accumulation of ROS can cause cell and tissue injury and can lead to a condition referred to as oxidative stress (Sordillo and Aitken, 2008). Host tissues do have several enzymes and small molecules that can reduce ROS to less reactive metabolites and it is this antioxidant capability that will help to protect cells from the damaging effects of oxidative stress. Therefore, the imbalance between increased production of ROS and reduced availability of antioxidant defenses around the time of parturition results in increased oxidative stress during this transitional period. Oxidative stress is thought to be a significant underlying factor to dysfunctional host immune and inflammatory responses that can increase the susceptibility of dairy cattle to a variety of health disorders, particularly during the transition period (Sordillo and Aitken, 2008).

Several recent studies have documented important changes in the antioxidant potential and pro-oxidant status in the transition dairy cattle (Bernabucci et al., 2005; Castillo et al., 2005; Sordillo et al., 2007). Antioxidants can be found as water-soluble or lipid-soluble molecules that are localized transiently throughout tissues and various cell types. Given the multiplicity of antioxidant pathways, their centrality in the prevention of oxidative stress, and the influences of diet on overall antioxidant capacity, it is important to be able to quantitatively measure the total antioxidant capacity or antioxidant power within biological specimens. Impairment of blood and milk leukocyte function has long been linked with increased susceptibility to mastitis around the time of calving when oxidative stress is increased. However, remarkably few studies have examined in any detail the redox status of important immune cell populations during this time. Results from our laboratory indicate that the antioxidant potential of isolated peripheral blood mononuclear cells (PBMC) remained relatively constant from 3 weeks prior to calving and through calving, but dropped significantly ($P < 0.05$) by 21 days in milk (Figure 1) (Sordillo et al., 2007). These findings are consistent with reports in both humans and dairy cows that showed a relationship between the physiological changes during the periparturient period with a loss in overall antioxidant potential in several different tissue compartments (Bernabucci et al., 2002; Gitto et al., 2002).

Lower antioxidant potential as a consequence of lactation stage can result from an excess accumulation of ROS, a depletion of antioxidant defenses, or a combination of both. One way to determine if ROS-mediated damage is occurring within host tissues is to measure end products of free radical oxidative processes. For example, when ROS react with polyunsaturated fatty acids, lipid peroxidation occurs. Peroxidation of lipids within cellular membranes can lead to changes in fluidity and cause damage to intracellular organelles. The determination of lipid hydroperoxide levels in plasma would be an indication of early stages of this lipid peroxidation damage. We showed that measurement of lipid hydroperoxides increased significantly ($P < 0.05$) from calving through the first 3 weeks of lactation when compared to the pre-partum measurements (Figure 2) (Sordillo et al., 2007). These findings are consistent with other reports in periparturient animals where lipid hydroperoxides and biomarkers of lipid peroxidation, such as thiobarbituric acid-reactive substances (TBARS), were found to increase from calving and through 25 DIM (Bernabucci et al., 2005; Castillo et al., 2005).

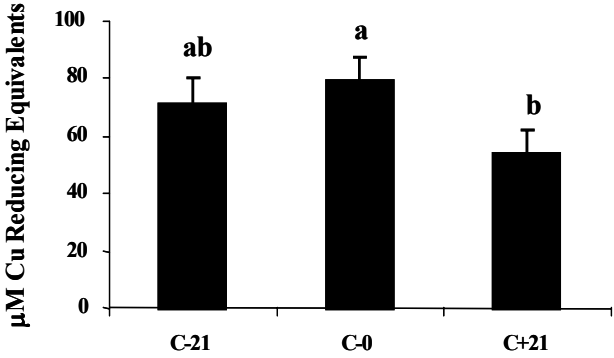

Figure 1. An assay was used to determine total antioxidant potential of PBMC obtained from transition cows and was based upon the reduction of Cu^{++} to Cu^{+} by the combined action of all antioxidants present in the sample. Changes in total antioxidant potential of white blood cells obtained approximately 21 d prior to calving (C-21), at calving (C-0), and 21 d after calving (C+21). Data are expressed as least square means ± SE. [a,b]Bars with different superscripts differ (P< 0.05).

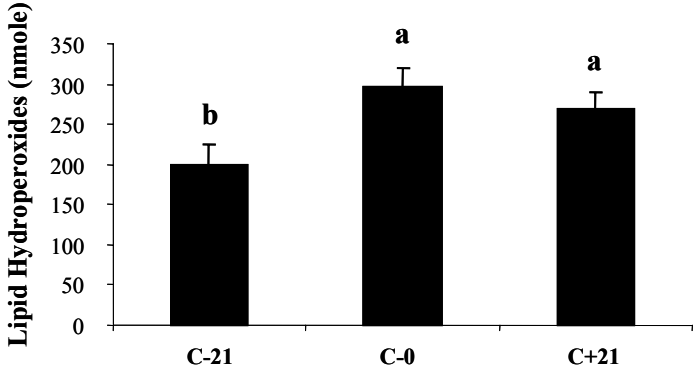

Figure 2. Changes in lipid hydroperoxide levels in plasma samples obtained approximately 21 d prior to calving (C-21), at calving (C-0), and 21 d after calving (C+21). Data are expressed as least square means ± SE. [a,b]Bars with different superscripts differ (P< 0.05).

■ Mastitis and Oxidative Stress

The ability to control the degree of oxidative stress can be effective in ameliorating the severity of several pro-inflammatory-based diseases, such as mastitis. For example, it is well established that certain antioxidant micronutrients, such as selenium (Se), can dramatically impact the progression of acute coliform mastitis (Sordillo and Aitken, 2008). This was best illustrated by the earlier studies by Smith et al., (1984) who showed that dairy cattle with existing deficiencies in Se had more severe clinical symptoms of coliform mastitis when compared to cows supplemented with adequate levels of this micronutrient. While supplementing dairy cattle with antioxidants is now a widely accepted management practice to avoid deficiencies, it is important to note that the underlying mechanisms for the benefits of Se are not completely known. Better understandings of how Se exerts its beneficial effects are needed for several reasons. Negative energy balance and increased production demands during the transition period results in an accumulation of ROS that far exceeds the cow's current antioxidant capabilities when supplemented with the maximum allowable (non-toxic) levels of Se. This trend of increased oxidative stress will likely continue as cows continue to be selected for increased milk production and therefore, the ways in which animals receive safe levels of antioxidant supplementation will need to change accordingly. Unfortunately, there is no information to suggest how Se plasma levels may translate to increased antioxidant potential within targeted cell populations of the mammary gland. The lack of information concerning which seleno-metabolites are critical for optimal health benefits has hampered the design of nutritional regimes that would maximize the effectiveness of either organic or inorganic sources of Se.

■ Conclusions

Oxidation and the production of free radicals are an integral part of aerobic metabolism. Considerable evidence supports the contention, however, that oxidative stress during the periparturient and early lactation period may contribute to a number of health disorders in dairy cattle. Dairy cattle management practices and emphasis on genetic selection to maximize milk production has increased the metabolic stresses associated with parturition and the onset of copious milk synthesis and secretion. The antioxidant requirements of cows will likely increase as production demands continue to escalate within the dairy industry. The performance of high producing dairy cows can be optimized to a certain extent by supplementing diets with optimal levels of micronutrients with antioxidant capabilities. However, oxidative stress continues to be a problem in transitions cows. Innovative approaches are needed to enhancing the antioxidant defense mechanisms of dairy cows during times of increased metabolic demands. A better understanding of how

antioxidants may preventative immune dysfunction and prevent oxidative damage to host tissues may lead to more effective strategies to control health disorders in the transition dairy cow.

References

Bernabucci, U., B. Ronchi, N. Lacetera and A. Nardone, 2002. Markers of Oxidative Status in Plasma and Erythrocytes of Transition Dairy Cows During Hot Season. J. Dairy Sci. 85: 2173-2179.

Bernabucci, U., B. Ronchi, N. Lacetera and A. Nardone, 2005. Influence of Body Condition Score on Relationships Between Metabolic Status and Oxidative Stress in Periparturient Dairy Cows. J. Dairy Sci. 88: 2017-2026.

Castillo, C., J. Hernandez, A. Bravo, M. Lopez-Alonso, V. Pereira and J.L. Benedito, 2005. Oxidative status during late pregnancy and early lactation in dairy cows. The Veterinary Journal 169: 286-292.

Gitto, E., R.J. Reiter, M. Karbownik, D.X. Tan, P. Gitto, S. Barberi and I. Barberi, 2002. Causes of oxidative stress in the pre- and perinatal period. Biol Neonate 81: 146-157.

Rainard, P. andC. Riollet, 2006. Innate immunity of the bovine mammary gland. Vet Res 37: 369-400.

Smith, K.L., J.H. Harrison, D.D. Hancock, D.A. Todhunter and H.R. Conrad, 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. J Dairy Sci 67: 1293-1300.

Sordillo, L.M., 2005. Factors affecting mammary gland immunity and mastitis susceptibility. Livestock Production Sciences 98: 89-99.

Sordillo, L.M. andS.L. Aitken, 2008. Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunopathol. in press.

Sordillo, L.M., N. O'Boyle, J.C. Gandy, C.M. Corl and E. Hamilton, 2007. Shifts in thioredoxin reductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. J Dairy Sci 90: 1186-1192.

Sordillo, L.M. andK.L. Streicher, 2002. Mammary gland immunity and mastitis susceptibility. J Mammary Gland Biol Neoplasia 7: 135-146.