**Nutrition, Immunity and Mastitis**

*In financial terms, mastitis is the most costly disease in the dairy industry, totaling approximately $1.8 billion dollars lost annually. Cost of a single case of mastitis ranges from $104 to $200.*

**Take Home Messages**

- Mastitis is the result of a bacterial invasion of the mammary gland where the immune system is either weakened or overwhelmed.
- The immune system has many different components, both pathogen specific and nonspecific, which can reduce or eliminate bacterial invasion of the mammary gland; all of which are sensitive to nutritional status of animal.
- Of all the possible nutrients that could affect immunity and mastitis incidence, vitamin E and selenium, zinc and vitamin A have been well documented.
- Physiologic changes associated with the dry period and initiation of lactation predispose the cow to increased mastitis susceptibility.
- Proper nutritional management of the transition cow in conjunction with pathogen reduction in the cow’s environment are critical mastitis preventive measures.

**Introduction**

In financial terms, mastitis is the most costly disease in the dairy industry, totaling approximately $1.8 billion dollars lost annually. Cost of a single case of mastitis ranges from $104 (Hoblet et al. 1991) to $200 (NMC, 1996). These losses are a result of reduced milk production, discarded milk, replacement costs, extra labor, treatment and veterinary service costs. Increased prevalence of mastitis also results in greater risk of antibiotic residues in human food as well as milk quality issues.

Mastitis is an inflammation of the mammary gland. The term, inflammation describes the response of a tissue or organ to injury. The purpose of inflammation is to destroy or neutralize infectious agents and associated toxins, thus allowing the gland to return to normal function. Bacterial invasion of the mammary gland occurs by bacteria entering the teat sphincter and moving into the teat cistern and beyond. Bacterial presence within the udder results in the movement of white blood cells into the gland to help fight the disease. An uninfected mammary gland will maintain a low total cell count (< 25,000 to 200,000 cells/ml), with most cells being macrophages. Macrophages can be viewed as special surveillance cells, constantly monitoring for the presence of foreign particles or microorganisms. Once gland tissue becomes infected, numerous neutrophils will be drawn to the mammary gland, resulting in increased somatic cell counts.

Outcome from bacterial invasion of the udder depends upon pathogenicity of the bacterial species involved and competency of the cow’s immune system. In the best case scenario, the bacteria are cleared without subsequent colonization of mammary tissues. Successful bacterial colonization of mammary tissue can result in a wide spectrum of disease outcomes, ranging from subclinical (e.g., no obvious change to udder or milk) to peracute clinical (e.g., severe systemic disease symptoms with dramatic changes to udder and milk secretion) mastitis (NMC, 1996). Contagious mastitis pathogens such as *Streptococcus agalactia*, *Mycoplasma bovis* and *Staphylococcus aureus* are most often associated with subclinical mastitis infections, recognized as elevated somatic cell counts (>200,000 cells/ml). Coliform bacteria such as *E. coli* and *Klebsiella sp.* are most often associated with acute clinical mastitis cases. The interaction between bacterial pathogenicity and immune response dictates the ultimate disease severity and duration. For example, differing strains of *Staphylococcus aureus*, based on virulence factors associated with capsular polysaccharides, can result in subclinical as well as acute clinical mastitis and everything in between.

The objective of this presentation is to provide an overview of how nutrition during the dry period and early lactation can influence a cow’s susceptibility to mastitis. Emphasis will be on how diet influences the immune system.
Immune Defenses Against Mastitis

The immune system is a highly specialized, coordinated set of cells and tissues that have a primary role of body surveillance for foreign antigens. Foreign antigens are any macromolecule (protein, lipid, polysaccharide) or microorganism (bacteria, virus, mold, protozoan) that does not contain a special host-specific identification code recognized as “self”. Many pathogenic organisms have evolved exquisite mechanisms to evade the host immune system and facilitate disease propagation. *Staphylococcus aureus* can survive within phagocytic cells or become walled off within mammary tissue, thus evading immune detection and preventing its elimination.

The immune system is often viewed solely as specialized white blood cells (leukocytes) that either engulf and destroy (e.g., phagocytosis) invading microorganisms (cell-mediated immunity) or respond to vaccines to produce antibody (humoral immunity). Over looked components of the immune system are physical barriers and non-specific immunity. The immune system can be viewed as a three-tiered defense starting with physical barriers and non- specific and specific immune responses. Physical barriers and non-specific immune responses comprise the innate or natural immunity. These immune responses are not antigen specific, nor do they have any memory response. Cell mediated and humoral immune responses comprise active immunity and are antigen specific and have memory.

Physical barriers of the udder are anatomic features of the teat and associated structures that pose a physical blockade to invading bacteria at the teat sphincter, the point of entry. These anatomic features include the teat skin, teat sphincter muscle and keratin plug. Teat skin that has abrasions, cracks or is chapped increases contagious bacteria colonization of the skin greatly increasing bacterial numbers around the teat sphincter and thusly increasing risk of bacterial penetration through the teat duct. Following milking the teat duct is dilated, greatly increasing the risk of bacterial penetration. Contraction of the teat sphincter takes time, which is why providing cows fresh feed following milking is promoted. This practice allows time for the teat sphincter to constrict, closing off the teat opening, before cows return to their stalls and have direct contact with the environment. The keratin plug is produced by skin lining the teat duct. Keratin is gummy, has bacteriostatic activity and completely occludes the teat canal.

Other non-specific immune responses include phagocytic cells (i.e., somatic cells), inflammatory response, complement cascade and lactoferrin. Phagocytic cells of various types are by in far the most important mediator of mastitis infections. All though there are a number of cell types, neutrophils and macrophages account for the majority of phagocytic cells in mastitis infections. Macrophages play multiple roles in coordinating activation of the specific immune response. After engulfing a foreign antigen, macrophages will present these on their cellular surface to stimulate lymphocytes to respond.

The inflammatory response produces much of the signs associated with clinical mastitis, heat, redness and swelling of the udder. Inflammation is a response to activated macrophages resulting in increased permeability of blood vessels allowing fluids, minerals, proteins (albumin and immunoglobulins) to move into the infection site. Neutrophils are then attracted to the site and move from surrounding blood vessels. Complement proteins also move into the inflamed area and promote phagocytosis and killing of bacteria by neutrophils and macrophages. Lactoferrin is a specialize protein synthesized in the udder that binds iron making it unavailable for bacterial growth, especially coliform bacteria.

Lymphocytes are specialized leukocytes that are involved in the active immune response, which includes cell mediated and humoral immunity. T-lymphocytes coordinate and stimulate the immune response as well provide cytotoxic cells. B-lymphocytes are responsible for the production of antibody (i.e., immunoglobulin). Both T and B lymphocytes respond only to a very specific antigen, thus the term specific immunity. When T and B lymphocytes respond, in addition to generating clones of their effector (active) cells, they produce memory cells. These memory cells are retained for periods of time allowing the animal to respond more immediately if the same antigen is encountered. This is the function premise behind vaccination protocols, generation of memory cells to a specific pathogenic agent.

### Nutritional Factors Impacting Mastitis

All of the essential nutrients, e.g., energy, protein, macrominerals, microminerals and vitamins, can influence some aspect of immune function (Table 1). Nutritional status can have direct and indirect effects on immune function.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-specific (Innate)</th>
<th>Specific (Active)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components</td>
<td>Epithelium, Keratin plug, Teat sphincter, Phagocytic cells, Inflammatory response, Lactoferrin Complement</td>
<td>Lymphocytes, T &amp; B Antibodies Ag Presenting Cells</td>
</tr>
<tr>
<td>Ag Specific</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Memory Response</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Nutrition Sensitive?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
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Table 1. Components of the immune system and their characteristics and responsiveness to nutrition.
Direct Nutritional Effects

Specific nutrients can influence one or more of the immune defenses previously described. Based on known effects on epithelial differentiation and growth, protein, vitamin A and zinc all influence epithelial health and can impact physical barriers of the udder. Protein status will also influence the integrity of the smooth muscle teat sphincter. Quality and quantity of the keratin plug may be influenced by protein, zinc and vitamin A.

Phagocytic cells are influenced by a number of nutrients, including copper, zinc, selenium and vitamins A and E. These nutrients may influence cell phagocytic capacity, bactericidal activity or both. A number of in vitro studies have documented effects of these nutrients on phagocyte function. A landmark clinical study at Ohio State University showed additional supplementation of vitamin E decreased clinical signs and duration of mastitis (Smith et al., 1984). Subsequent studies by these researchers and others, with selenium and vitamin E supplementation, showed decreased incidence and severity of mastitis in supplemented cows (Erskine et al., 1987, Hogan et al., 1990, Weiss et al., 1997). Improvements with vitamin E and selenium supplementation were attributed to improved phagocytic cell activity and function. Cows that were not able to maintain a serum vitamin E concentration of 3.0 μg/ml were 9.4 times more likely to have mastitis. Based on these positive results, the NRC (2001) increased the vitamin E requirement for dry and lactating dairy cows from 15 to 80 IU/kg DM and 15 to 20 IU/kg DM, respectively. A general recommendation is to feed 1000 IU vitamin E per day to dry cows. Some consultants have increased this level to 2,000 or even 4,000 IU/day for herds experiencing mastitis, metritis or retained placenta problems. Selenium supplementation is defined by FDA regulations and set at 0.3 mg/kg diet or 3 mg/d for dry cows. This is generally adequate without excessive amounts of interfering substances (e.g., iron, sulfur) and higher concentrations of vitamin E. With the yeast-based organic selenium supplement now approved, one may want to incorporate a portion of their selenium supplement with selenomethionine, especially if interfering substances are present.

Copper has also been shown to affect phagocytic function, but its impact on cell mediated and humoral immunity has been variable in cattle. Current recommendations are to maintain copper between 10 and 15 ppm of the diet. If excessive interfering minerals such as molybdenum, sulfur, iron or zinc are present, dietary levels may be increased slight. It would be best to perform some testing to determine actual status in the animals prior to increasing dietary copper as it potentially can be detrimental.

Lymphocytes activity can be influenced by energy, protein, zinc and vitamin A. Antibody production is influenced by energy, protein, copper, zinc, selenium and vitamins A, D and E. Supplementation of vitamin A and beta-carotene to attain a higher blood concentration of vitamin A has been shown to reduce mastitis incidence (Chew et al., 1982). Vitamin A requirements for dry cows have been increased from 76 IU/kg BW to 110 IU/kg BW to account for the improved animal health and mastitis reduction effects. Supplementation of vitamin D was shown to improve titers to J-5 vaccination by improving T-helper cell stimulation of immune response. Currently the NRC (2001) recommends vitamin D at 30 IU/kg BW for dry cows. This has not changed from the previous version, but is higher than what would be considered sufficient levels to maintain adequate blood concentrations (17 IU/kg BW).

One of the most studied nutrients relative to mastitis and immune function is zinc. Zinc is an active component of more than 90 metalloenzymes and has significant effects on gene expression and cellular growth. A summary of 12 lactation trials addressing zinc supplementation showed a 33% reduction in somatic cell count (Kellogg, 1990, Socha and Tomlinson, 2002). Many of these studies were comparing a chelated form of zinc to inorganic zinc supplementation. However, not all organic forms of zinc showed positive effects on mastitis. No differences were observed when cows were fed 260 mg inorganic zinc compared to 200 mg zinc proteinate and 60 mg inorganic zinc (Whitaker et al., 1997). There are differences in bioavailability among even the organic forms of minerals. For pregnant cows the NRC recommends 22.8 ppm zinc in the diet or approximately 300 mg/day. This is a marked decline in dietary zinc concentration as a result of documented differences in bioavailability of the mineral from feed ingredients.

Indirect Effects - Metabolic Disease

Not only can dietary nutrients have a direct impact on immune function and susceptibility to mastitis, but they can indirectly increase cow susceptibility to mastitis through their impact on periparturient metabolic diseases. All essential nutrients can induce one or more metabolic diseases when either deficient or in excess in the transition diet (Van Saun, 1991). Hypocalcemia (milk fever) has been shown to slow the closure of the teat sphincter. Cows with milk fever are 8.1 times more likely to have mastitis and 9 times more likely to have a coliform mastitis event as a result (Curtis et al., 1983). Mastitis was also associated with ketosis and retained placenta (Oltenacu and Ekesbo, 1994, Emanuelson et al., 1993). Cows with fatty infiltration of the liver have been shown to be slower in clearing E. coli from their mammary gland (Hill et al., 1985).

Role of Dry Period and Periparturient Nutrition

From a disease perspective, the cow is susceptible to infection when any factors exist that cause the teat sphincter to remain open. Physiological conditions at dry off and calving create this situation making the cow at highest risk for becoming infected at these times.

- At dry off – milk flow is acutely terminated resulting in building pressure within the gland which frequently results in milk leaking from the gland. Milk is a good culture media for bacteria that may enter the teat end and
move into the gland duct system.

- At calving – cows are initiating milk flow, and their immune system is frequently compromised. The teat end is often slow to close following milking due to Ca deficiency.

In both situations, pathogen load in environment is a key factor in causing disease. Dry cow therapy in all four quarters in all cows remains the most effective method of reducing mastitis.

The immune system of the dairy cow has been shown to decline in responsiveness during the transition period (Goff and Horst, 1997; Mallard et al., 1998). A compromised immune system may lead to increased incidence of metritis, mastitis or other infectious disease process. Although it is thought that hormonal and metabolic factors may play a primary role in this physiologic immune suppression, it can be further suppressed by nutritional insults. Mastectomy of cows prior to initiation of lactation did not totally eliminate the observed decline in immune function, suggesting other factors are involved (Kimura et al., 2002, Goff and Kirmura, 2002).

Nutritional management of the transition cow has undergone intensive research over the past decade, with many changes brought forth in the new NRC (2001) publication. Daily requirements for glucose, amino acids, fatty acids and calcium for an early lactation cow (4 days postpartum; 65 lbs milk, 4.7% fat and 4.2% protein) are 2.7, 2.0, 4.5 and 6.8 times greater, respectively, than those needed for pregnancy (Bell, 1995). These differences represent changes in nutrient requirements over a period of only 1-to-2 weeks and occur during a period of lowest dry matter intake, highlighting the tremendous metabolic alterations necessary to adequately support lactation. It must be remembered these metabolic changes associated with transition take place in all animals, including those that are well-fed. These metabolic changes, however, may become uncoordinated or exaggerated when maternal supplies of energy, protein or both are inadequate, thus leading to metabolic disease problems.

From the dam’s perspective, gestational micromineral and vitamin losses to fetus and colostrum may significantly affect her reserves and their metabolic function, especially when mineral and vitamin supplementation is reduced or interrupted during the dry period. The severity and duration of negative energy and protein balance the cow experiences postpartum may also have an adverse impact on immune function. Mastectomy reduced, but did not eliminate, declines in vitamin and energy metabolites associated with calving (Goff et al., 2002). However, periparturient hypocalemia was eliminated by mastectomy. These data would indicate that metabolic alternations are not entirely due to nutrient losses via the udder in early lactation. A critical control point in maintaining adequate nutrient intake through the tumultuous transition period is to maximize dry matter intake (Goff and Horst, 1997). If intake can not be maintained, then dietary nutrient levels must be adjusted to account for the lower intake.

### Beyond Nutritional Management

Even with the great strides forward with research on the nutritional management of the transition cow, we have not solved problems of periparturient disease or reduced reproductive performance. We still have much room for improvement in improving milk production efficiency, especially in states east of the Mississippi river. One consistent frustration with managing transition cows is the seemingly lack of consistency in response to a given program. To this end, Drackley (1999) stated: “Why do vastly different nutrition and management programs produce similarly good, or similarly poor, transition success?”

Drackley (2001) best answered his own question in suggesting it is beyond our singular approach focusing on nutrition and includes how a given nutrition program is delivered and the environment in which it is consumed. Overcrowding, exposure to pathogens, changing social organization, among other situations can induce stress-mediated physiologic and metabolic changes. Animals alter how they utilize and partition available nutrients in response to these stress situations, which may compromise availability of nutrients to support productive functions. Other metabolic responses to stress result in increased fat mobilization leading to greater potential for fatty liver disease, wasting of muscle tissue, and immune suppression. A number of physiologic and metabolic responses to stress result in a decline in dry matter intake further compromising nutrient availability to support production. It is believed the effects of stress are additive, thereby as stress situations accumulate, greater physiologic and metabolic changes occur ultimately resulting in abnormality seen as metabolic dysfunction or infectious disease. These stress responses will be more exaggerated in animals consuming an imbalanced diet, but may also overwhelm an animal consuming an adequate diet. The foundation for a successful transition period is your ability to provide a properly formulated close-up ration within a “stress-free” environment.

### Conclusions

Mastitis outcomes are the interplay between bacterial invasion and pathogenicity and a cow’s immune responsiveness. Over the past 15 years, the dry cow and especially the transition period have been recognized as critical elements to improving dairy productive efficiency. Numerous research studies completed over this time period have better explained dynamic metabolic and physiologic changes taking place as a cow transitions from pregnancy into lactation and their potential role in health-related problems. Role of immune dysfunction during the transition period has been highlighted. Improved characterization of nutrient requirements and management practices has allowed for feeding recommendations that can help to reduce the negative impact of transitional immune dysfunction. Transition nutrition is not a magic bullet; it must be coordinated with good cow comfort, feeding management and methods to reduce behavioral and environmental stressors.
References and Selected Readings


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Penn State College of Agricultural Sciences research and extension programs are funded in part by Pennsylvania counties, the Commonwealth of Pennsylvania, and the U.S. Department of Agriculture.
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