

Nutritional Strategies to Enhance Immunity during the Transition Period of Dairy Cows

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Introduction

Infection occurs when a population of invading pathogens (i.e., bacteria, viruses, protozoa, etc.) becomes established within another living organism. Infections of the mammary gland (mastitis) or uterus (metritis) are common sources of inflammation in lactating dairy cows, particularly during the periparturient period. Other health disorders common during this period (e.g., milk fever and ketosis) do not arise from infectious organisms, but instead have metabolic origins. Although the etiologies of infectious and metabolic disorders differ, epidemiologists report a significant association between their occurrences. For example, Curtis et al. (1985) reported that cows with milk fever were 5.4 times more likely to contract clinical mastitis than animals without milk fever. These data do not imply cause and effect; however, they suggest an association between the occurrence of one disease with the occurrence of a second disorder. Recent research highlights the interplay between the metabolic and immune systems such that we should not be surprised at the association between clinical events. Researchers and dairy advisors need to begin to think about nutrition and immunity not as exclusive concepts, but rather as integrated systems whereby the activity or events in one system have direct effects on the other.

Immunophysiology

To understand how one health disorder might impact the occurrence of another, it becomes necessary to envision events of immunophysiology and metabolism in the animal afflicted with only one disorder. Gram negative bacteria are responsible for the pathology of many diseases in livestock production. In dairy cattle, these organisms are responsible for neonatal coliform septicemia, coliform mastitis, salmonellosis, certain pneumonias, brucellosis, metritis, and multiple other types of infections (Cullor, 1992). These organisms cause a series of predictable perturbations to the normal metabolic and physiologic processes of the host that result in morbidity, if not mortality. Using coliform mastitis as an example for infection, the pathogenic organism first gains entry to the mammary gland and eventually localizes to the mammary epithelium. En route to colonizing the mammary epithelium, the infectious organism may be detected by white blood cells (leukocytes) of the innate immune system such as monocytes, macrophages, or polymorphonuclear neutrophils (PMN) and be destroyed, thereby

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averting colonization and infection. When secreted in milk, leukocytes are known as somatic cells. Thus, the somatic cell count in milk increases at times when significant numbers of pathogens have gained access to the gland. However, when pathogens outnumber leukocytes, or when leukocyte function is impaired (such as during periparturient immunosuppression), replicating populations of pathogens may become established, resulting in infection of the tissue. When leukocytes engage pathogens, they secrete various signaling molecules such as cytokines and chemokines. These signaling molecules diffuse from the site of infection and result in the recruitment of more leukocytes out of the blood to the site of infection, in this case, the mammary gland.

Cytokines also enter the blood stream and can affect the metabolism of many other tissues including the endocrine glands and liver. Pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1), and interleukin-6 (IL-6) act on endocrine glands to affect the release of insulin, glucagon, glucocorticoids, growth hormone, thyroxine, (Klasing, 1988) and prolactin (Smith and Wagner, 1984). In turn, these hormones may feedback to affect cytokine production from monocytes and macrophages (Pettipher et al., 1996). Direct action of cytokines and changes in circulating hormone levels of the host result in altered patterns of energy and protein metabolism characteristic of the immune response (Fong and Lowry, 1990; Klasing, 1988).

Aspects of Periparturient Metabolism

The periparturient dairy cow transitioning from pregnancy into lactation, experiences a dramatic increase in nutrient requirements that cannot be met by feed intake alone (Bell, 1995). Thus, the animal experiences a period of negative energy balance and must mobilize body tissue lipid and protein in order to sustain productive function. Estimates of demand for glucose, amino acids, fatty acids, and net energy by the gravid uterus at 250 d of gestation in comparison with the lactating mammary gland at 4 d postpartum indicate approximately a tripling of demand for glucose, a doubling of demand for amino acids, and approximately a 5-fold increase in demand for fatty acids during this timeframe (Bell, 1995). Furthermore, the requirement for Ca increases approximately four-fold on the day of parturition (Horst et al., 1997). Delicate coordination of hepatic gluconeogenesis, fatty acid, and calcium metabolism are of paramount importance if the animal is to avoid metabolic dysfunction.

The cow is mostly dependent on gluconeogenesis from propionate, glucogenic amino acids, lactate, and glycerol in the liver and kidney to meet her glucose needs (Reynolds et al., 2003). During the period of periparturient negative energy balance, body fat is mobilized into the bloodstream in the form of nonesterified fatty acids (NEFA). These NEFA cannot be used to make glucose but are an important fuel source for body tissues such as muscle and are incorporated into milk fat; however, excessive mobilization of NEFA results in uptake of NEFA by the liver beyond that required to support hepatic oxidative processes. Impairment of liver function can occur as excess

NEFA accumulate as triglycerides in the liver (Overton et al., 2000; Overton and Waldron, 2004).

With the onset of copious milk secretion and its attendant drain on plasma calcium concentrations, calcium homeostasis is maintained by the actions of parathyroid hormone and 1,25-dihydroxy vitamin D₃ via increased bone resorption and intestinal absorption. During early lactation, these processes cannot fully meet the calcium needs secreted in milk and most cows experience negative calcium balance resulting in at least some degree of hypocalcemia (Horst et al., 1994). Intracellular calcium is important in many signaling pathways and is obligate for normal functioning of neural and muscular tissue. When plasma calcium becomes too low, nerve and muscle function is impaired, resulting in parturient paresis, or milk fever (Goff and Horst, 1997). Animals that do not develop milk fever efficiently mobilize bone tissue to contribute to the circulating pools of calcium and phosphorus. Animals that develop milk fever do not have efficient bone resorption and therefore become hypocalcemic and hypophosphatemic.

Effects of Inflammation on Metabolism in Periparturient Cows

We were concerned about the metabolic impact of an infection at a time when metabolic health is so tenuous, such as in the periparturient dairy cow. Although there is a correlation between infectious disease and metabolic disorders, it is not known if the onset of infection might actually cause a metabolic disturbance that would result in a disorder such as ketosis or milk fever. We created experimental mastitis in dairy cows on day 7 after calving and then studied the effect on metabolism during the early stages (8 hours) of inflammation (Waldron et al., 2005; 2006). We would expect that if mastitis somehow caused ketosis, glucose production by the liver would be decreased and there would have been an increase in plasma NEFA and the ketone, BHBA. If mastitis was causal toward milk fever, then we would expect a decrease in plasma concentrations of calcium. Acute mastitis does not appear to cause ketosis because glucose production is increased (Figure 1) and plasma levels of NEFA and BHBA are decreased (Figures 2 and 3, respectively) relative to pair-fed healthy control cows. Further research is warranted and ongoing regarding the potential causal effect of mastitis on milk fever because acute mastitis dramatically decreased plasma calcium concentration (Figure 4) relative to control cows.

Immunosuppression: An Interaction between Metabolism and Immunophysiology

In addition to the potential hepatic metabolic disease associated with negative energy balance, periparturient dairy cows also undergo a period of reduced immunological capacity during the weeks around calving. This immune dysfunction is not limited to isolated immune parameters; rather it is broad in scope and affects multiple functions of various immune cell types. The combined results of these dysfunctions are that dairy cows may be hyposensitive and hyporesponsive to antigens, and therefore more susceptible to infectious disease such as mastitis during the

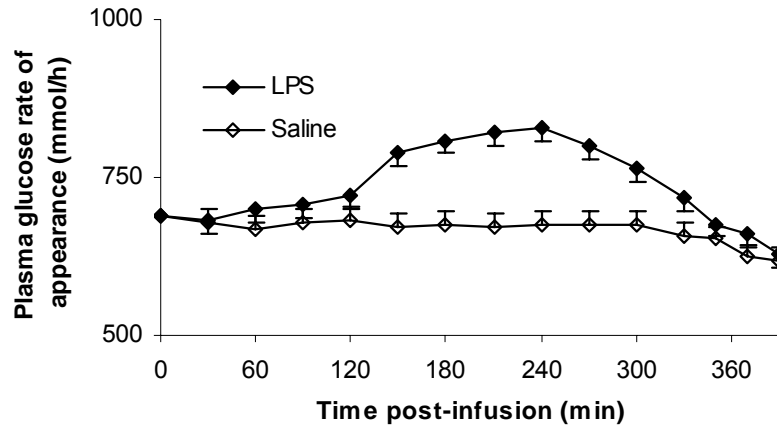


Figure 1. Plasma glucose rate of appearance of early-lactation dairy cows administered intramammary lipopolysaccharide (to cause mastitis) or saline at 0 minutes^a. Data were covariately adjusted using the mean rate of appearance during the steady state period from -90 through 0 minutes.

^a treatment by time effect, $P < 0.01$

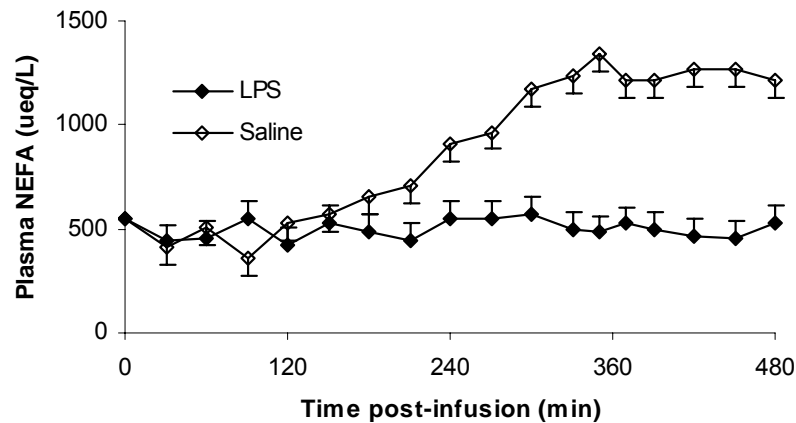


Figure 2. Plasma nonesterified fatty acid (NEFA) concentration following intramammary lipopolysaccharide (to cause mastitis) or saline infusion into early-lactation dairy cows^a. Means were adjusted by analysis of covariance using the mean NEFA concentration for each treatment group from -240 through 0 minutes relative to intramammary infusion.

^a treatment by time effect, $P < 0.01$

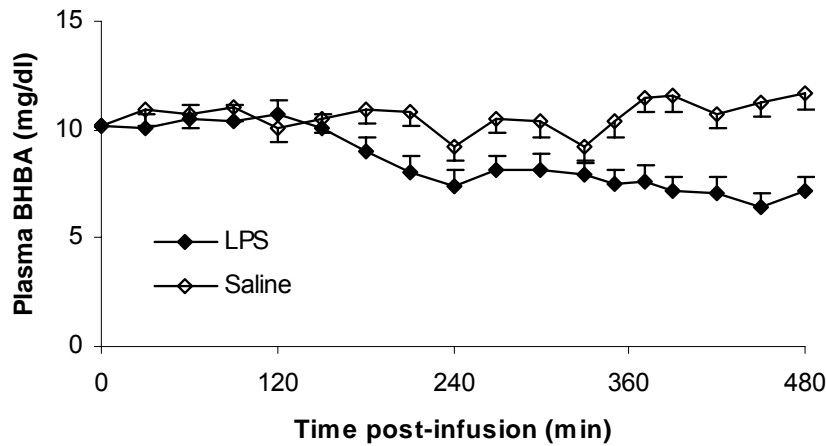


Figure 3. Plasma β -hydroxybutyrate (BHBA) concentration following intramammary lipopolysaccharide (to cause mastitis) or saline infusion into early-lactation dairy cows^a. Means were adjusted by analysis of covariance using the mean BHBA concentration for each treatment group from -240 through 0 minutes relative to intramammary infusion.

^b treatment by time effect, $P < 0.01$

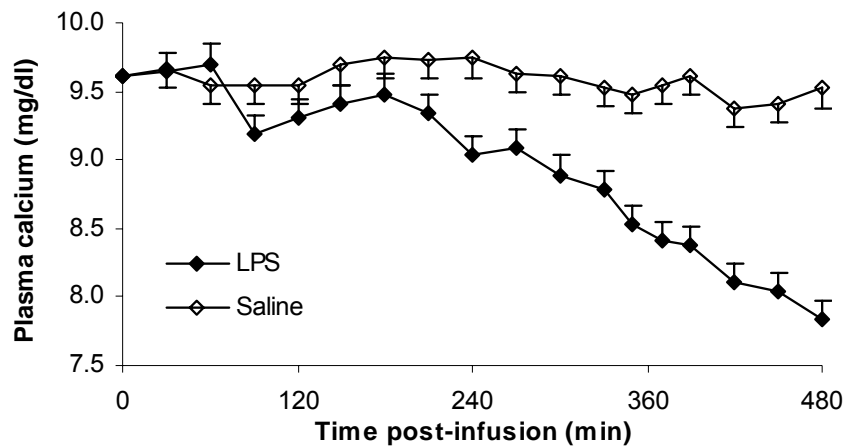


Figure 4. Plasma calcium concentration following intramammary lipopolysaccharide (to cause mastitis) or saline infusion into early-lactation dairy cows^a. Means were adjusted by analysis of covariance using the mean calcium concentration for each treatment group from -240 through 0 minutes relative to intramammary infusion.

^btreatment by time effect, $P < 0.01$

periparturient period (Mallard et al., 1998). Grommers et al. (1989) reported that fewer mammary quarters responded to low-dose *E. coli* endotoxin, and maximum cell count also was somewhat later and less pronounced during early lactation than during mid-lactation. Furthermore, when live *E. coli* were administered into the mammary gland, periparturient cows experienced more rapid bacterial growth, higher peak bacterial concentration, higher fever, and equal or greater proinflammatory cytokine concentrations in foremilk than did midlactation cows (Shuster et al., 1996). The cause of this immunosuppression is not known, but is the subject of much research. Research to date suggests that periparturient immunosuppression appears to be due to a combination of endocrine and metabolic factors. Glucocorticoids (e.g. cortisol), known immunosuppressants, are elevated around the time of calving, and have been postulated to be at least partly responsible for periparturient immunosuppression (Burton et al., 1995). Furthermore, changes in estradiol and progesterone just prior to calving may directly or indirectly affect immunocompetence (Weber et al., 2001).

Effects of Metabolism on Immunocompetence

Periparturient negative energy balance has also been implicated in contributing to immunosuppression. However, negative energy balance alone had little effect on the expression of adhesion molecules on the surface of bovine leukocytes (Perkins et al., 2001). Furthermore, negative energy balance in midlactation cows did not affect the clinical symptoms associated with an intramammary endotoxin infusion (Perkins et al., 2002). These results are contrary to work in periparturient cows where the presence of a mammary gland (vs. mastectomized cows) and its attendant metabolic demands slowed recovery of neutrophil function, suggesting that the metabolic stress of lactation exacerbated periparturient immunosuppression (Kimura et al., 1999). Other work has investigated individual metabolic components associated with negative energy balance, and has concluded that although hypoglycemia alone is not likely to exacerbate periparturient immunosuppression (Nonnecke et al., 1992), hyperketonemia appears to have multiple negative effects on aspects of immune function (Suriyasathaporn et al., 2000). Ketosis may increase the risk of mastitis in periparturient immunosuppressed cattle because many immune cell types are negatively affected by metabolite levels typical of a ketotic environment (i.e., low concentrations of glucose and high concentrations of ketone bodies and NEFA). A ketotic environment suppressed bovine lymphocyte blastogenesis (Sato et al., 1995), decreased the respiratory burst activity of PMN (Hoeben et al., 1997), lowered the chemotactic capacity of leukocytes (Suriyasathaporn et al., 1999), decreased interferon- γ and tumor necrosis factor- α titers from bovine aorta endothelial cells (Zdzisinska et al., 2000), decreased the bactericidal activity of ovine neutrophils (Sartorelli et al., 2000), and inhibited human T-cell proliferation in vitro (Gregory et al., 1993). Furthermore, experimental mastitis in ketonemic cows was more severe than mastitis in non-ketonemic cows regardless of preinfection chemotactic response (Kremer et al., 1993). As reviewed by Suriyasathaporn et al. (2000), impairment of the udder defense mechanism in cows experiencing negative energy balance seems to be related to hyperketonemia.

Reports of the negative effects of ketosis on immune function may be related to or compounded by the impact of fatty liver on immune function. Triglyceride accumulation in the liver is reported to have metabolic effects whereby hepatic ureagenic and perhaps gluconeogenic capacity is reduced, but fatty liver also affects immune function. Andersen et al. (1996) reported that cows without fatty liver cleared bacterial endotoxin from circulation within 30 min of intravenous endotoxin administration, whereas cows with fatty liver were unable to clear the administered endotoxin even after 6 h. Furthermore, these authors reported that whereas zero of 18 healthy cows had severe reactions to endotoxin administration, one of four cows with fatty liver died following endotoxin administration. Zerbe et al. (2000) reported that PMN harvested from the blood or uterus of periparturient cows with liver triglyceride greater than 40 mg/g had decreased expression of functional surface molecules and had decreased in vitro functional capacity (cytotoxicity and reactive oxygen species generation). Reid and Roberts (1983) reported preliminary results whereby cows with fatty liver displayed decreased neutrophil extravasation in vitro and Hill et al. (1985) reported that cows with fatty liver took significantly longer to resolve intramammary infection than did cows without hepatic lipidosis. Other aspects of metabolic status were not reported in these studies, so it is unknown to what extent fatty liver was solely responsible for the reported results; however, it seems clear that indeed fatty liver and attendant metabolic perturbations negatively impact immune function in dairy cattle.

Another aspect of periparturient metabolism that has the potential to impact immune competence is calcium metabolism. Significant quantities of calcium are required for milk synthesis and an inadequate adaptation to this calcium sink at the onset of lactation results in hypocalcemia (milk fever). Although it is important for milk synthesis, calcium is also important for intracellular metabolism and signaling in most cell types, including the leukocytes of the immune system. Realizing the importance of calcium in leukocyte activation, Kehrl and Goff (1989) hypothesized that low blood calcium around the time of calving could contribute to periparturient immunosuppression. However, they were unable to substantiate this hypothesis when they compared the functional capacity of leukocytes from hypocalcemic cows and cows that were made normocalcemic through the administration of intramuscular parathyroid hormone. This study squelched the theory of a hypocalcemic contribution to immunosuppression for a number of years, until the same group revealed that mastectomized cows were less immunosuppressed than were animals with an intact mammary gland (Kimura et al., 1999). One of the key variables that was different between mastectomized and intact cows was plasma calcium concentration. This revelation rekindled interest in the potential role for calcium metabolism to be causal toward impaired immunity. Recently, Kimura et al. (2006) reported that calcium stores in mononuclear leukocytes are depleted prior to the development of hypocalcemia in the blood, and that this depletion of intracellular calcium does potentially contribute to immunosuppression. Interestingly, it appears that intracellular calcium stores are a more sensitive measure of calcium stress than is blood calcium concentration.

Practical Recommendations

It is evident that the effects of nutrition and immunity are interrelated. Nutritional physiology impacts immune function and immune activity alters metabolism. At this time, specific recommendations to optimize nutritional immunology are difficult because research is lacking. Our current nutritional recommendations are based on the amount of a given nutrient that will result in no deficiency symptoms for the nutrient and maximizes productive processes (e.g., growth, milk production) - the amount of that same nutrient that maximizes immunity may be very different. Obviously, the best way to minimize the negative effects of immune activity on nutrition and metabolism is to minimize the occurrence and severity of infection and inflammation in livestock production systems. Unfortunately, we have little peer-reviewed research to support specific nutritional recommendations to maximize immunity, and most of our efforts to minimize immune activation will be management oriented. At this time, our best recommendations in feeding for optimal nutritional immunity are concept-based. That is, we don't know the specific requirements yet, but we are beginning to understand some of the nutrients that impact immune function, and we can therefore nutritionally manage the animal to optimize those nutrients and/or metabolites. For example, we know that many trace minerals and vitamins are important in immune function. We don't know what level of these nutrients maximizes immunity, but we do know that deficiencies impair immunity. Therefore, we strive to meet or exceed National Research Council requirements with these nutrients coming from high quality sources. Another example of concept-based feeding regards managing the periparturient dairy cow to minimize negative energy balance. We know that excessive plasma NEFA mobilization and ketone-body production can impair immunity, therefore we must attempt to manage the animal such that plasma NEFA and BHBA concentrations remain at moderate levels. To accomplish this we can incorporate the same strategies as those to maximize metabolic health in fresh cows – namely, balanced pre- and post-calving diets, watching for changes in the forage base that will result in nutritional imbalances, excellent feeding management, monitoring fresh cows to identify potential problems quickly, and minimizing stress on these animals.

Implications

Recent research highlights the interplay between the metabolic and immune systems such that we should not be surprised at the association between clinical events of the immune and metabolic systems. Researchers and farm advisors need to begin to think about nutrition and immunity not as exclusive concepts, but rather as integrated systems whereby the activity or events in one system have direct effects on the other. As such, the barriers between immunological and metabolic knowledge must be removed. Nutritionists need to make a concerted effort to understand at least the basics of immunology, and immunologists need to become comfortable with nutritionists delving into the subjects related to immunology. Growth, reproduction, milk synthesis, and metabolic health are all negatively impacted by immune activity; therefore, farm managers must strive to minimize both chronic and acute infectious or inflammatory insults in order to maximize the potential of dietary formulations.

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