METABOLIC CONSIDERATIONS FOR IMMUNITY

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ABSTRACT

Dairy cows experience reduced immune function from about 3 wk before calving until about 3 wk after calving. This immunosuppression results in an increased incidence and severity of infections around the time of calving. The cause of periparturient immunosuppression is unknown, but many factors seem to be involved. Among the factors studied, aspects of metabolism including negative energy balance, nonesterified fatty acids, ketones, and Ca appear to play some role in the development of immunosuppression. However, contradictory results from animal models meant to mimic periparturient negative energy and nutrient balance are perplexing. Careful nutritional management to provide high quality nutritional profiles and to maximize metabolic health is currently our best strategy to maximize periparturient immune function. Once immune activation is triggered, inflammation results in decreased production efficiency that is mostly due to coordinated changes in metabolism, not just a pilfering of nutrients by the immune system. In addition to sound nutritional management, best management practices to maximize hygiene and minimize stressors are crucial to helping prevent infection.

PERIPARTURIENT HEALTH

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 more than 5 times as likely to contract clinical mastitis as animals without milk fever. These results do not imply cause and effect; however, they suggest an association between the occurrences of one disease with that of a second disorder. Potential causal relationships between periparturient metabolism and immune function have been investigated for about the last 20 yr, but this research has intensified recently.

IMMUNOSUPPRESSION: AN INTERACTION BETWEEN METABOLISM AND IMMUNOPHYSIOLOGY?

Part of the reason for the increased number and severity of infections around the time of calving is due to a weakened immune system, often termed as immunosuppression. This immune dysfunction is not limited to isolated immune variables; rather it is broad in scope and affects multiple functions of various immune cell types (Sordillo and Streicher, 2002). 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 periparturient period (Mallard et al., 1998). Grommers et al. (1989) reported that fewer mammary quarters responded to low-dose *E. coli* endotoxin, and maximum somatic 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).

Research results from our laboratory are in agreement with this decreased immune function around the time of calving and perhaps give some insights into which mechanisms may be impaired. Neutrophils (**PMN**) are recognized as being one of the most important cell types in protecting the mammary gland and uterus from infection (Paape et al., 2002). We isolated PMN from midlactation (220-350 DIM and 100-200 d of gestation, n = 9), prepartum (12 d prior to calving, n = 8), and postpartum (7 DIM, n = 8) cows and studied various functional activities of these cells. The PMN from postpartum cows produced fewer intracellular (data not shown), extracellular (data not shown), and total **(**Figure 1**)** reactive oxygen species (**ROS**). These ROS are compounds, such as hydrogen peroxide, that kill bacteria upon contact. Production of these ROS is part of how the immune system works to fight infection. This postpartum decrease in ROS expression is in agreement with other reports (Mehrzad et al., 2001) and could contribute to the

attenuated pathogen killing capacity that has been reported after calving (Dosogne et al., 2001).

A novel finding from our lab relates to the ability of PMN to produce neutrophil extracellular traps (**NET**). These bacteriocidal structures were first reported by Brinkmann et al. (2004) and were subsequently reported to be expressed at similar levels in milk and blood (Lippolis et al., 2006), contrary to other antimicrobial mechanisms. Using the same experimental design as above for ROS production, we report that PMN NET expression is increased in PMN incubations isolated from cows 12 d prepartum, compared to PMN from postpartum or midlactation cows (Figure 2). This finding, along with the expression of NET in milk (Lippolis et al., 2006), suggests that NET expression by PMN is an important protective mechanism for the mammary gland of transition cows.

EFFECTS OF METABOLISM ON IMMUNOCOMPETENCE

The cause of periparturient immunosuppression is not known, but is the subject of much research. Research to date suggests that this immune dysfunction appears to be due to a combination of endocrine and metabolic factors. Glucocorticoids (e.g. cortisol), known endocrine 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). However, changes in any of these steroid hormones do not overlap with the entire period of immunosuppression, suggesting that other causes are at least partially responsible for immune dysfunction.

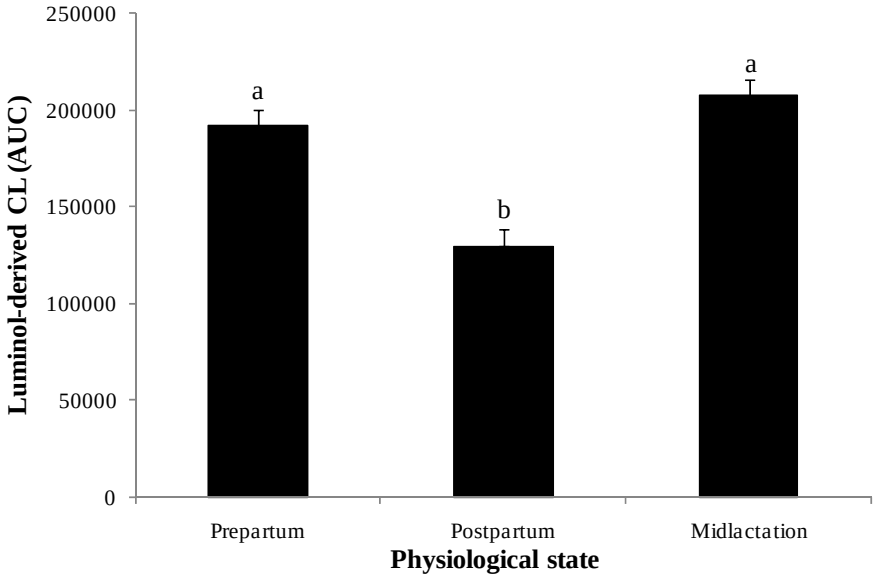

Figure 1. Effect of stage of lactation[1] on bovine neutrophil total reactive oxygen species production measured by luminol-dependant chemiluminescence (CL).*

[1] Neutrophils were collected from midlactation (100-200 d pregnant; n = 9), prepartum (-12 d; n = 8) and postpartum (7 DIM; n = 8) cows.
* Day of lactation effect, $P < 0.01$.
[a,b] Bars with different letters differ ($P < 0.01$).

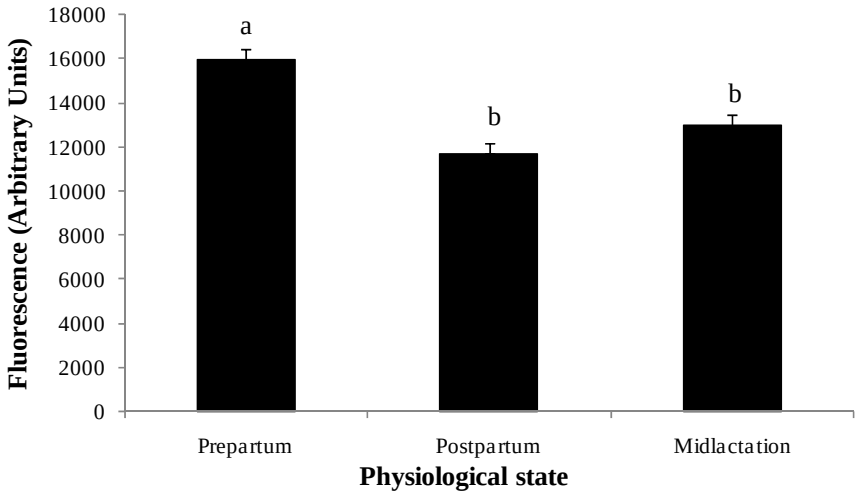

Figure 2. Effect of stage of lactation[1] on bovine neutrophil extracellular trap formation.*

[1] Neutrophils were collected from midlactation (100-200 days pregnant; n = 9), prepartum (-12 d; n = 8) and postpartum (7 DIM; n = 8) cows.
* Day of lactation effect, $P < 0.01$.
[a,b] Bars with different letters differ ($P < 0.01$).

Periparturient negative energy balance has been implicated in contributing to immunosuppression. However, experimentally-induced negative energy balance alone had little effect on the expression of adhesion molecules on the surface of bovine leukocytes (Perkins et al., 2001). Furthermore, experimental negative energy balance in midlactation cows did not affect the clinical symptoms associated with an intramammary endotoxin infusion (Perkins et al., 2002). Similarly, Moyes et al. (2009) reported only minor differences in immunocompetence of post-peak cows subjected to nutrient restriction for 5 d prior to intramammary experimental mastitis. 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). The disagreement between experimental models of nutrient restriction and periparturient dairy cows suggests that other variables during the periparturient period are more likely responsible for immunosuppression than nutrient balance or transient changes in circulating metabolites. 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). Furthermore, experimental mastitis in ketonemic cows was more severe than mastitis in non-ketonemic cows (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.

Another aspect of periparturient metabolism that has the potential to impact immune competence is Ca metabolism. Significant quantities of Ca are required for milk synthesis and an inadequate adaptation to this Ca sink at the onset of lactation results in hypocalcemia (milk fever). Although it is important for milk synthesis, Ca is also important for intracellular metabolism and signaling in most cell types, including the leukocytes of the immune system. Realizing the importance of Ca in leukocyte activation, Kehrli and Goff (1989) hypothesized that low blood Ca 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 Ca concentration. This revelation rekindled interest in the potential role for Ca metabolism to be causal toward impaired immunity. Recently, Kimura et al. (2006) reported that Ca stores in mononuclear leukocytes are depleted prior to the development of hypocalcemia in the blood, and that this depletion of intracellular Ca does potentially contribute to immunosuppression. Interestingly, it appears that intracellular Ca stores are a more sensitive measure of Ca stress than is blood Ca concentration.

SPECIFIC NUTRIENTS AND IMMUNITY

Completing the relationship between immune function and metabolism, it has also been reported that multiple nutrients and metabolites influence immunity. The role of dietary nutrients in supporting immune function has received significant research attention. Vitamins (e.g., vitamins C, D, and E) and trace minerals (e.g., Zn or Se) are all familiar to us from advertisements touting the role of these nutrients in human health and disease. Furthermore, at least basal levels, and in some cases supranutritional levels, of these nutrients have been shown to be supportive for animal health in livestock production systems (Spears and Weiss, 2008; Spears, 2000; Weiss, 1998). Other nutrients such as specific fatty acids have been studied for their ability to influence immune function (Calder, 2006) and hold promise for future use in livestock species.

PRACTICAL CONSIDERATIONS WHEN FEEDING FOR IMMUNITY

Feeding Management

No matter how good the diet is on paper, the nutrients that make it into the blood of the cow are what counts. There is no replacement for watching the cows to truly tell you how good your nutrition program is. Any significant imbalances have the potential to alter immunity. Unfortunately, we don't know all of the imbalances that tip the scale or know how severe the imbalances must be in order to negatively affect immunity.

Stay Ahead of Problems

It's much easier to prevent or catch problems early than to have the proverbial *train wreck.*

Avoid Stressors

Stress can be a potent immunosuppressant and the effects of an excellent nutritional program can be negated if the cows are stressed.

Manage for Metabolic Health

At this time, some of the best strategies for us to avoid losses due to infectious disease are to pay strict attention to the details of close-up and fresh cow management, such that metabolic disorders are also avoided. Strategies to minimize negative energy balance, and the accompanying fat mobilization and ketone body production, are keys to minimizing immunosuppression. Likewise, management of Ca metabolism to prevent hypocalcemia may have benefits beyond just the avoidance of metabolic disorders. These strategies will minimize nutrient deficiencies and negative metabolic impacts on immune function; thereby maximizing the health of the periparturient cow.

SOME THOUGHTS ON PRODUCTION EFFICIENCY

The relationship between immunity and metabolism has been realized as very complex and interconnected. Traditional thinking is that activation of the immune system represents a significant nutritional demand that competes with productive processes, such as protein and milk synthesis. Dogma states that this drain of nutrients is the cause for decreased productive efficiency of livestock animals during sickness. Although there is no doubt that productive efficiency is decreased during morbidity, the reason behind this decrease in efficiency is likely much more of a coordinated response rather than a competition for substrates. Many underlying metabolic adaptations occur to support immune function during periods of sickness such that variables important to livestock production systems (i.e., growth, reproduction, lactation, or metabolic health) are compromised despite the presence of seemingly sound dietary formulation.

INTEGRATION OF IMMUNE FUNCTION AND METABOLISM

During infection, a pathogen gains entry through the physical or mucosal barriers of the animal and becomes established within the tissue. Certain white blood cells, or leukocytes, of the immune system [including macrophages, monocytes, and polymorphonuclear neutrophils (**PMN**)] serve as sentinels for the animal and become activated when they come in contact with these *non-self* pathogens. Upon activation, leukocytes secrete signaling molecules that support the immune response called proinflammatory cytokines. The proinflammatory cytokines include tumor necrosis factor-α (**TNF-α**), interleukin-1β (**IL-1**), and interleukin-6 (**IL-6**); however, numerous other cytokines exist to support the immune response as well. These cytokines are initially secreted by leukocytes at the site of infection where they act locally to activate other immune cells.

In addition to their effects on leukocytes, cytokines also have effects outside of the *traditional* immune system because metabolic tissues have functional receptors for these signaling molecules of the immune system. For example, the proinflammatory cytokines have direct effects on such metabolic tissues as the brain, skeletal muscle, adipose tissue (fat), liver, and endocrine glands (Johnson, 1997). Klasing (1988) reviewed the impacts of cytokines on metabolism and reported that feed intake, protein metabolism, fat metabolism, carbohydrate metabolism, mineral metabolism, and endocrine secretions were all affected by inflammation. Furthermore, secondary effects of immune activation also occur via classical metabolic endocrine regulation due to changes in endocrine gland secretions (Waldron et al., 2003; Waldron et al., 2006).

Further complicating the relationship between immune function and metabolism, it is now clear that not only do metabolic tissues respond to signals from the immune system, in many cases *metabolic* tissues actually produce and secrete immune-related molecules as well. Far from an exclusive list, it has now been shown that mammary epithelial cells produce several acute-phase proteins and cytokines including TNF-α and interleukin-8 (Wellnitz and Kerr, 2004); the liver produces antimicrobial peptides (Sang et al., 2006) and acute-phase proteins and cytokines (Loor et al., 2005), the anterior pituitary gland produces among others, IL-6 and prostaglandins (Abraham et al., 1998); and fat cells (adipocytes) produce such a wide range of immune-related molecules (including membrane-bound TNF-α, IL-6, resistin, etc.) that these molecules have been termed adipocytokines or adipokines (Hutley and Prins, 2005).

EFFECTS OF IMMUNE ACTIVATION ON LACTATION

Immune activation results in dramatic changes in circulating concentrations of cytokines and hormones in the blood. These alterations in endocrine profile, and cytokines themselves, can cause markedly decreased milk production in lactating cows (Rajala-Schultz et al., 1999; Shuster and Harmon, 1992; Shuster et al., 1991a). Decreased milk synthesis is not due simply to decreased feed intake associated with sickness because healthy cows that were pair-fed to acutely mastitic cows displayed normal milk production, while their mastitic counterparts decreased milk production by up to 70 % (Waldron et al., 2006). Lohuis et al. (1990) reported the loss of total daily milk production of cows was related positively with areas under the curves of heart rate, rumen amplitude, and counts of *E. coli* in secreta from inoculated quarters. The decreased milk production due to mastitis is mediated by multiple pathophysiological events and is not solely due to inflammatory damage in the mammary epithelium. Part of the reduced lactational performance may result from escape of milk components from the udder into the circulation (Shuster et al., 1991b). Reduced lactational performance is not mediated by the acute cortisol increase associated with inflammation (Shuster and Harmon, 1992) or by reduced concentrations of growth hormone or IGF-1 (Shuster et al., 1995). These authors also noted that inflammatory cytokines are produced at a time consistent with a possible role in the inhibition of milk synthesis (Shuster et al., 1995). The positive effects of growth hormone on milk production and recovery from coliform mastitis may be due to the enhanced function of neutrophils resulting in a better defense of the mammary gland (Burvenich et al., 1999).

EFFECTS OF IMMUNE ACTIVATION ON PERIPARTURIENT METABOLIC HEALTH

Periods of negative energy balance in livestock species, including the early-lactation period of dairy cows, are marked by the mobilization of lipid stores and increased circulating concentrations of nonesterified fatty acids (**NEFA**) in blood. As such, the plasma NEFA concentration is a good indicator of energy balance in healthy, un-stressed animals. Although dry matter intake is significantly reduced

during periods of inflammation, the activity of pro-inflammatory cytokines in dampening synthesis in productive tissues, allows for the animal to remain in apparent positive energy balance during short periods of inflammation (Figures 3-5; Waldron et al., 2006). Furthermore, despite the requisite use of glucose by leukocytes of the innate immune system, plasma glucose concentration was maintained during experimental mastitis in early-lactation dairy cows (Waldron et al., 2006). Estimates in poultry suggest that immune activation results in increased total energy and protein utilization of 4-5 % (Klasing, 2004). However, apparently the decrease in productive tissue synthesis offsets this increased demand (Table 1) to allow the animal to effectively fight infection and maintain metabolic health. This balancing of energy metabolism clearly represents coordination of the immune and metabolic systems with no evidence of competition, at least during the short-term. Although energy metabolism appeared to be well-managed in these animals, further quantitative study during longer periods of inflammation is warranted.

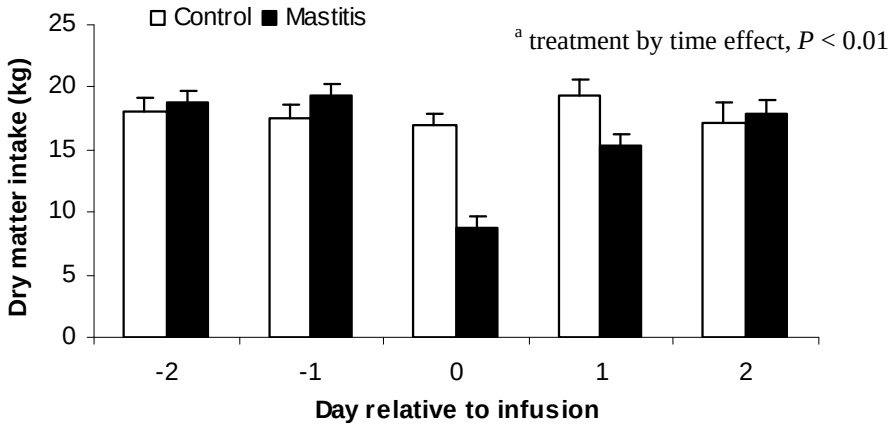

Figure 3. Daily dry matter intake following intramammary lipopolysaccharide (to cause mastitis) or saline infusion into early-lactation dairy cows[a]. Experimental mastitis was induced approximately 4 hr after morning feeding on d 0 relative to infusion.

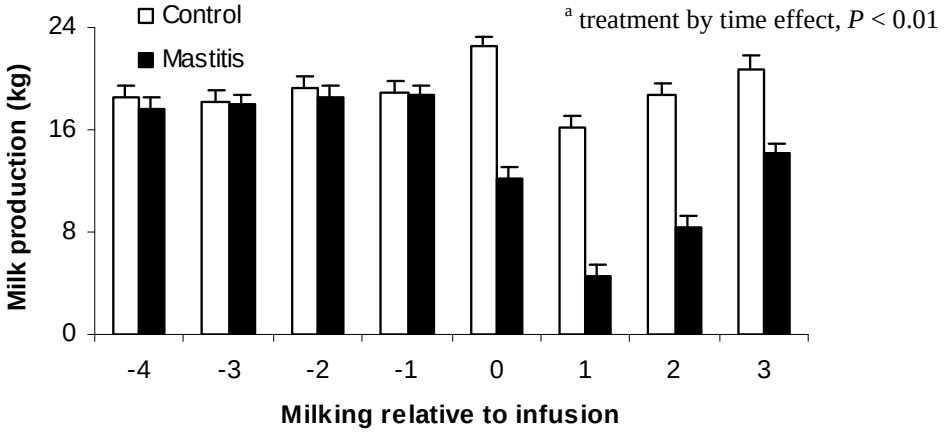

Figure 4. Milk production at each milking following intramammary lipopolysaccharide (to cause mastitis) or saline infusion into early-lactation dairy cows[a]. Experimental mastitis was induced approximately 4 hr after morning milking and 10 hr before evening milking on d 0 relative to infusion.

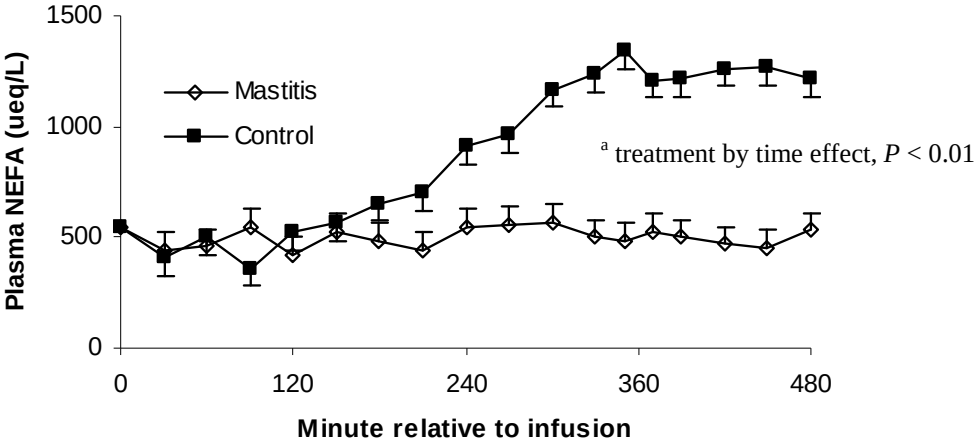

Figure 5. Plasma nonesterified fatty acid (NEFA) concentration following intramammary lipopolysaccharide (to cause mastitis) or saline infusion into early-lactation dairy cows[a]. Means were adjusted by analysis of covariance using the mean NEFA concentration for each treatment group from –240 through 0 min relative to intramammary infusion.

Table 1. Calculated metabolizable energy (ME, Mcal) requirements following intramammary lipopolysaccharide (to cause mastitis) or saline infusion into early-lactation dairy cows.

	Mastitis Cows (ME, Mcal)	**Control Cows** (ME, Mcal)
Maintenance	8.9	9.0
Milk Synthesis	14.6	31.0
Fever	1.1	---
Estimated Immune Costs	1.2	---
TOTAL	**25.8**	**40.0**

OVERALL SUMMARY

The periparturient dairy cow experiences immune dysfunction around the time of calving. To date, no single factor has been reported to be responsible for this immune dysfunction. Experimental models of under-nutrition have generally failed to reproduce the typical periparturient problem. Aspects of energy metabolism, especially ketones, have been reported to negatively impact immune function. Although not as well understood, high-levels of circulating NEFA and Ca metabolism may also contribute to periparturient immunosuppression. Given the interplay between metabolism and immunity, strategies to carefully manage metabolic health are also our best recommendation to maximize periparturient immune function of the dairy cow.

Immune activation impacts nutrition, metabolism, and production efficiency. Pro-inflammatory cytokines secreted during an immune response directly act on metabolic tissues and endocrine glands to affect metabolism and hormone action. Synthesis in productive tissues is thus directly attenuated. Indeed, the effects of immune activation on metabolism are a coordinated response, not simply competition for substrates. During a

short-term immune response, the animal appears to effectively manage energy metabolism. However, mineral and vitamin metabolism may frequently be suboptimal, even during short-term inflammation. Physiological state and nutrient status prior to immune activation appear to be important for effective immunity.

LITERATURE CITED

Abraham, E. J., J. N. Morris-Hardeman, L. M. Swenson, E. L. Knoppel, B. Ramanathan, K. J. Wright, D. M. Grieger, and J. E. Minton. 1998. Pituitary function in the acute phase response in domestic farm animals: cytokines, prostaglandins, and secretion of ACTH. Domest. Anim. Endocrinol. 15:389-396.

Brinkmann, V., U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch, and A. Zychlinsky. 2004. Neutrophil extracellular traps kill bacteria. Science 303:1532-1535.

Burton, J. L., M. E. Kehrli, Jr., S. Kapil., and R. L. Horst. 1995. Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasone. J. Leukoc. Biol. 57:317-325.

Burvenich, C., M. J. Paape, D. Hoeben, H. Dosogne, A. M. Massart-Leen, and J. Blum. 1999. Modulation of the inflammatory reaction and neutrophil defense of the bovine lactating mammary gland by growth hormone. Domest. Anim. Endocrinol. 17:149-159.

Calder, P. C. 2006. Polyunsaturated fatty acids and inflammation. Prostaglandins Leukot. Essent. Fatty Acids. 75:197-202.

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.

Dosogne, H., F. Vangroenweghe, B. Barrio, P. Rainard, and C. Burvenich. 2001. Decreased number and bactericidal activity against Staphylococcus aureus of the resident cells in milk of dairy cows during early lactation. J. Dairy Res. 68:539-549.

Grommers, F. J., D. Van De Geer, H. Van Der Vliet, P. A. J. Henricks, and F. P. Nijkamp. 1989. Polymorphonuclear leukocyte function: relationship between induced migration into the bovine mammary gland and in vitro cell activity. Vet. Immunol. Immunopath. 23:75-83.

Hutley, L., and J. B. Prins. 2005. Fat as an endocrine organ: relationship to the metabolic syndrome. Am. J. Med. Sci. 330:280-289.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: an integrated view. J. Anim. Sci. 75:1244-1255.

Kehrli, M. E. Jr, and J. P. Goff. 1989. Periparturient hypocalcemia in cows: effects on peripheral blood neutrophil and lymphocyte function. J. Dairy Sci. 72:1188-1196.

Kimura, K., J. P. Goff, and M. E. Kehrli, Jr. 1999. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. J. Dairy Sci. 82:2385-2392.

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

Klasing, K. C. 2004. The costs of immunity. Acta Zoo. Sinica. 50:961-969.

Klasing, K. C. 1988. Nutritional aspects of leukocytic cytokines. J. Nutr. 118:1436-1446.

Kremer, W. D., E. N. Noordhuizen-Stassen, F. J. Grommers, Y. H. Schukken, R. Heeringa, A. Brand, and C. Burvenich. 1993. Severity of experimental Escherichia coli mastitis in ketonemic and nonketonemic dairy cows. J. Dairy Sci. 76:3428-3436.

Lippolis, J. D., T. A. Reinhardt, J. P. Goff, and R. L. Horst. 2006. Neutrophil extracellular trap formation by bovine neutrophils is not inhibited by milk. Vet. Immunol. Immunopathol. 113:248-255.

Lohuis, J. A., Y. H. Schukken, J. H. Verheijden, A. Brand, and A. S. Van Miert. 1990. Effect of severity of systemic signs during the acute phase of experimentally induced Escherichia coli mastitis on milk production losses. J. Dairy Sci. 73:333-341.

Loor, J. J., H. M. Dann, R. E. Everts, R. Oliveira, C. A. Green, N. A. Guretzky, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23:217-226.

Mallard, B. A., J. C. Dekkers, M. J. Ireland, K. E. Leslie, S. Sharif, C. L. Vankampen, L. Wagter, and B. N. Wilkie. 1998. Alteration in immune responsiveness during the periparturient period and its ramification on dairy cow and calf health. J. Dairy Sci. 81:585-595.

Mehrzad, J., H. Dosogne, E. Meyer, R. Heyneman, and C. Burvenich. 2001. Respiratory burst activity of blood and milk neutrophils in dairy cows during different stages of lactation. J. Dairy Res. 68:399-415.

Moyes, K. M., J. K. Drackley, J. L. Salak-Johnson, D. E. Morin, J. C. Hope, and J. J. Loor. 2009. Dietary-induced negative energy balance has minimal effects on innate immunity during a Streptococcus uberis mastitis challenge in dairy cows during midlactation. J. Dairy Sci. 92:4301-4316.

Nonnecke, B. J., S. T. Franklin, and J. W. Young. 1992. Effects of ketones, acetate, and glucose on in vitro immunoglobulin secretion by bovine lymphocytes. J. Dairy Sci. 75:982-990.

Paape, M., J. Mehrzad, X. Zhao, J. Detilleux, and C. Burvenich. 2002. Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J. Mammary Gland Biol. Neoplasia. 7:109-121.

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.

Perkins, K. H., M. J. VandeHaar, J. L. Burton, J. S. Liesman, R. J. Erskine, and T. H. Elsasser. 2002. Clinical responses to intramammary endotoxin infusion in dairy cows subjected to feed restriction. J. Dairy Sci. 85:1724-1731.

Rajala-Schultz, P. J., Y. T. Grohn, C. E. McCulloch, and C. L. Guard. 1999. Effects of clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 82:1213-1220.

Sang, Y., B. Ramanathan, J. E. Minton, C. R. Ross, and F. Blecha. 2006. Porcine liver-expressed antimicrobial peptides, hepcidin and LEAP-2: cloning and induction by bacterial infection. Dev. Comp. Immunol. 30:357-366.

Shuster, D. E., and R. J. Harmon. 1992. High cortisol concentrations and mediation of the hypogalactia during endotoxin-induced mastitis. J. Dairy Sci. 75:739-746.

Shuster, D. E., M. E. Kehrli, Jr, C. R. Baumrucker. 1995. Relationship of inflammatory cytokines, growth hormone, and insulin-like growth factor-I to reduced performance during infectious disease. Proc. Soc. Exp. Biol. Med. 210:140-149.

Shuster, D. E., R. J. Harmon, J. A. Jackson, and R. W. Hemken. 1991a. Reduced lactational performance following intravenous endotoxin administration to dairy cows. J. Dairy Sci. 74:3407-3411.

Shuster, D. E., R. J. Harmon, J. A. Jackson, and R. W. Hemken. 1991b. Suppression of milk production during endotoxin-induced mastitis. J. Dairy Sci. 74:3763-3774.

Shuster, D. E., E. K. Lee, and M. E., Kehrli, Jr. 1996. Bacterial growth, inflammatory cytokine production, and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows at midlactation. Am. J. Vet. Res. 57:1569-1575.

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

Spears, J. W. 2000. Micronutrients and immune function in cattle. Proc. Nutr. Soc. 59:587-594.

Spears, J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176:70-76.

Suriyasathaporn, W., C. Heuer, E. N. Noordhuizen-Stassen, and Y. H. Schukken. 2000. Hyperketonemia and the impairment of udder defense: a review. Vet. Res. 31:397-412.

Waldron, M. R., A. E. Kulick, A. W. Bell, and T. R. Overton. 2006. Acute experimental mastitis is not causal toward the development of energy-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 89:596-610.

Waldron, M. R., T. Nishida, B. J. Nonnecke, and T. R. Overton. 2003. Effect of lipopolysaccharide on indices of peripheral and hepatic metabolism in lactating cows. J. Dairy Sci. 86:3447-3459.

Weber, P. S., S. A. Madsen, G. W. Smith, J. J. Ireland, and J. L. Burton. 2001. Pre-translational regulation of neutrophil L-selectin in glucocorticoid-challenged cattle. Vet. Immunol. Immunopathol. 83:213-240.

Weiss, W. P. 1998. Requirements of fat-soluble vitamins for dairy cows: a review. J. Dairy Sci. 81:2493-2501.

Wellnitz, O., and D. E. Kerr. 2004. Cryopreserved bovine mammary cells to model epithelial response to infection. Vet. Immunol. Immunopathol. 101:191-202.