

Research Article

Transition Period and Immunosuppression: Critical Period of Dairy Cattle Reproduction

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Abstract: This seminar study is prepared on the objectives of: revising important aspects of transition period of dairy cattle and highlighting some potential areas of research and challenges for the future. It has sufficiently been discussed that improved understanding of this frontier of the biology, immunology, nutrition and management of cows during the transition period will provide the largest gains in productivity and profitability of dairy farms. In the manuscript under each specific topic, transition cow program and reproductive performance, immunosuppressant effect of transition period, early predictors of disorders and major abnormalities are discussed in an informative way. Future potential areas of research and possible challenges are also indicated briefly. Finally, it is concluded that despite decades of research in the area of transition cow health and management the high incidence of health disorders around calving continues to negatively affect milk production and reproductive performance; and as recommendation, implementing a transition nutrition program with the help of nutritionists can help dairy herd avoid most of the costly problems and molecular level research studies should get due attention to further understand the situation and devise proper intervention techniques.

Keywords: Dairy cattle, immunosuppression, nutritional management, reproductive performance, transition period

INTRODUCTION

Dairy Cattle are an integral part of agriculture worldwide, providing many products in addition to milk for the human population. The income that a dairy cow generates comes from her milk production, sale of offspring and finally, her carcass value (Grohn *et al.*, 2003). The efficient production of these products is of utmost importance and high reproductive performance is absolutely crucial to this. Biological adjustments to support dramatic production increase have occurred within the cow. Obviously factors that influence each of these income streams (e.g., cattle genetics, nutrition, environmental management and herd health) can affect the overall profitability of a dairy herd.

Perhaps one of the most challenging areas for both producers and scientists has been addressing the issues of herd health at the transition from dry cow to fresh cow. Yet, a successful transition is needed to set the stage for a successful lactation and optimum production, reproduction, health and culling. Understanding important aspects of the biology of the transition cow helps guide feeding and management decisions. Although the length of time classified as the transition period has been defined differently by different authors, (Grummer, 1995; James, 1999) as the last 3 weeks before parturition to 3 weeks after parturition and this definition is referred in this text.

During this period the cows undergo extensive metabolic adaptation in glucose, fatty acids and mineral metabolism to support the fetus and lactation as well as to avoid metabolic dysfunction (Overton and Waldron, 2004). Metabolic disorders and health problems are common during this time and can easily erase the entire profit potential for dairy cow farms (Drackley, 1999). The practical goal of nutritional management within this timeframe is to support the respective metabolic adaptations.

Thus, the occurrence of health problems is centered disproportionately on this relatively short period, which certainly contributes to make this an “interesting” time for dairy producers and animal health scientists. The well-being and profitability of the cow could be greatly enhanced by understanding those factors that account for the high disease incidence in periparturient cows. Efficient milk production continues to require the dairy cow to experience gestation and parturition each year. The etiology of many of those metabolic diseases that are not clinically apparent during the first 2 weeks of lactation, such as laminitis, can be traced back to insults that occurred in early lactation. In addition to metabolic disease, the overwhelming majority of infectious diseases, especially mastitis, but also diseases such as Johne’s disease and Salmonellosis, become clinically apparent during the first 2 weeks of lactation (Smith *et al.*, 1985; Mallard *et al.*, 1998).

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Three basic physiologic functions must be maintained during the periparturient period if disease is to be avoided. These are:

- Adaptation of the rumen to high energy density lactation diets to reduce the degree of negative energy balance experienced by the cow
- Maintenance of normocalcemia
- Reducing the degree of immunosuppression that occurs around parturition

Both metabolic disease and infectious disease incidence are greatly increased whenever one or more of these physiological functions is impaired. The etiological role of each of these three physiological factors on the development of each of the common diseases encountered during the periparturient period should be clearly understood (Goff and Horst, 1997).

Improved understanding of this frontier of the biology, immunology, nutrition and management of cows during the transition period will provide the largest gains in productivity and profitability of dairy farms.

The objective of this seminar study is therefore:

- To review some of the important aspects of transition periods by focusing on its immunosuppressive effect
- To discuss some recent insights on metabolism during the transition period and past and present efforts in determining early indicators of disease in transition dairy cattle
- To highlight some potential challenges for the future to further our understanding of this critical period in the lactation cycle

PHYSIOLOGICAL AND NUTRITIONAL STATUS OF A TRANSITION COW

During the transition period there are many physiological, metabolic and endocrine challenges related to parturition and the onset of lactation. Nutrient demands of the dairy cow increase during this period to support the final stages of fetal development and, after calving, milk production. A further challenge is that Dry Matter Intake (DMI) during the transition period is generally insufficient to meet the energy requirements for lactation and maintenance (Drackley, 1999).

The dry period, in particular the transition period, is characterized by dramatic changes in the endocrine status (Hayirli *et al.*, 2002). Plasma insulin decreases and growth hormone increases as the cow progresses from the late gestation to the early lactation. Surges of both hormones in plasma concentrations are visible at the parturition (Kunz *et al.*, 1985). Plasma thyroxine (T4) gradually increases during late gestation, decreases about 50% at parturition and then begins to increase

again (Kunz *et al.*, 1985). Progesterone concentrations during the dry period are elevated for the sake of pregnancy maintenance, but decline rapidly two days before calving. Estrogen (primarily estrone of placental origin) increases in plasma during late gestation, but decreases immediately at calving (Chew *et al.*, 1979). Concentrations of glucocorticoid and prolactin increase on the day of calving and return to near prepartum concentrations the day after (Edgerton and Hafs, 1973). These changes prepare the cow for lactogenesis and parturition.

Different changes in endocrine status and a decrease in Dry Matter Intake (DMI) during late pregnancy influence metabolism and lead to fat mobilisation from the adipose tissue and glycogen from the liver (Holtenius *et al.*, 2003). Plasma Nonesterified Fatty Acids (NEFA) increase more than a two-fold between 2 to 3 weeks prepartum, at which time concentration increases dramatically until completion of parturition (Vasquez-Anon *et al.*, 1994; Grum *et al.*, 1996). Force feeding cows during the prefresh transition period reduced the magnitude of NEFA increase, but did not completely eliminate it (Bertics *et al.*, 1992). The rapid rise in NEFA on the calving day is due to the calving stress. Plasma NEFA concentration decrease rapidly after calving but concentration remains higher than they were before calving (Bacic *et al.*, 2006). Plasma glucose remain stable or increase slightly during the prefresh transition period, increase dramatically at calving and then decrease immediately after calving (Kunz *et al.*, 1985; Vasquez-Anon *et al.*, 1994; Bacic *et al.*, 2007). Blood calcium decreases during the last few days prior to calving due to the loss of calcium for the synthesis of colostrums (Goff and Horst, 1997) and usually do not return to a normal level until several days postpartum.

Dairy cows also must adapt to numerous management challenges during the transition period. On many North American dairy farms the transition from pregnancy to lactation is marked by several social regroupings and changes in diet. The first group change, occurring approximately 3 weeks before the cow's expected calving date, allows cows to be fed a diet with increased energy and nutrients to support the final stages of fetal development and prepare the cow for the physiological and metabolic adaptations necessary for parturition and the onset of lactation (Overton and Waldron, 2004). This regrouping also enables producers to closely monitor the cows as they approach their expected calving date. There is evidence, however, that regrouping has negative consequences on both behavior and production. Phillips and Rind (2001) reported that regrouped animals had shorter feeding times, longer standing times and decreased milk production relative to cows kept in a stable group. When new cows are introduced to a pen the group dynamics change and this can lead to increased levels

of aggression among individuals as social relationships become established (Von Keyserlingk *et al.*, 2008). As parturition approaches, cows commonly are moved again to a maternity pen where they are usually isolated from the herd. Social isolation in unfamiliar surroundings has been shown to elicit stress responses in dairy cows in the form of increased heart rate, high cortisol concentrations and increased vocalizations (Rushen *et al.*, 1999). After calving the calf is removed and the cow is moved again. This time she joins the lactating herd and is fed a new diet formulated to provide more energy to support the increased nutrient demands for lactation.

As cows begin and terminate the dry period, there are changes in rumen dynamics. These alterations are nutritionally rather than physiologically induced. Changing from the high concentrate to a high fiber diet causes alterations in the microbial population and in the characteristics of the rumen epithelium and papillae. High concentrate diets favor starch utilizing bacteria that enhance production of lactate and propionate (Bacic, 2006). High fiber diets favor cellulolytic bacteria and methane production and discriminate against bacteria that produce propionate and utilize lactate. End products of fermentation influence papillae growth in the rumen (Dirksen *et al.*, 1985). They are responsible for the absorption of volatile fatty acids. High fiber diet causes the papillae to shorten, while increasing the grain in the diet and propionate concentration in the rumen favors elongation of papillae. As much as 50% of the absorptive area in the rumen may be lost during the first 7 weeks of the dry period and elongation of papillae after reintroduction of concentrate takes several weeks (Mashek and Beede, 2001). Consequently, sudden introduction of grain immediately post calving has several negative effects. Lactate production increases prior to the re-establishment of lactate utilizing bacteria. Lactate is very potent in reducing ruminal pH and volatile fatty acids are absorbed at a faster rate when pH is low (Goff and Horst, 1997). Rumen papillae will not have enough time to elongate. Therefore volatile fatty acid absorption is limited.

Transition nutrition and reproductive performance:

From a reproductive performance perspective, critical control points to consider are uterine involution and recovery time to first ovulation, estrus activity and conception. Cows with severe negative energy balance in early lactation, evidenced by excessive body condition score losses (>1 score), had longer times to first estrus and ovulation (Butler and Smith, 1989; Britt, 1991; Canfield and Butler, 1991; Ferguson, 2001). Prepartum malnutrition resulting in increased liver fat may induce greater negative energy balance via a reduction in dry matter intake postpartum. Nutritional insults during the peripartum period may adversely

affect maturation of the growing follicle, thus resulting in reduced reproductive performance (Britt, 1991). Cow's metabolic status in early lactation had significant effects on later reproductive performance (Pushpakumara *et al.*, 2003).

The role of protein in reproductive function has not been completely defined. Many, but not all, studies have shown a negative effect on conception rate by the overfeeding of protein or imbalanced dietary protein fractions resulting in reduced conception rate (Butler, 2000). There has been interest in the role of protein feeding during the transition period and its effect on reproductive performance. Some studies using dairy and beef heifers showed improved reproductive performance when fed additional proteins over the transition period (Strauch *et al.*, 2001). This effect may be a direct effect on oocyte development or indirect through protein's effects on metabolic status. Feeding additional proteins prepartum to mature dairy cows resulted in reduced days open and improved conception rate (Van Saun and Sniffen, 1995). The association between postpartum negative energy balance and days to first ovulation previously documented (Canfield and Butler, 1991), was not present for cows fed the additional prepartum protein (Van Saun and Sniffen, 1995). This data might suggest that metabolic inhibition of postpartum reproduction may be minimized via prepartum protein feeding.

Immune mechanisms may be suppressed sufficiently by a number of nutritional deficiencies to prevent the uterus and mammary gland's ability to clear pathogens. Prepartum mineral and vitamin nutrition can also influence postpartum reproductive performance through their influence on incidence of milk fever and retained placenta. Both diseases lead to a greater incidence of metritis and mastitis (Lewis, 1997). Cows with mastitis prior to first service have been shown to have more days to first service and lower conception rate, all resulting in more days from calving to conception (Barker *et al.*, 1998; Schrick *et al.*, 2001). The benefit of supplemental vitamin E on decreasing mastitis incidence and clinical severity has been well documented (Weiss *et al.*, 1990). Other factors are also incriminated as causes of immunosuppression in dairy cows at transition periods.

WHY ARE DAIRY COWS SO IMMUNOSUPPRESSED DURING TRANSITION PERIOD?

During the transition period, the immunologic status of the cow is compromised and it may be related to nutritional and physiological status of the cow. Estrogen and glucocorticoids are immunosuppressive agents and they increase in plasma near the calving time (Goff and Horst, 1997). Intake of vitamin A and E and other nutrients essential for the immune system

may be insufficient as Dry Matter Intake (DMI) is reduced during the periparturient period.

Endocrine and metabolic stresses play the central roles in immunosuppression of dairy cows during transition period. Estrogens, which increase dramatically at the end of gestation, have been found in some experiments to stimulate the humoral immune response, but most workers agree they have a strong suppressive effect on cell-mediated immunity (Wyle and Kent, 1977). Glucocorticoids have long been used as powerful immunosuppressive agents. Plasma cortisol concentrations (primarily of maternal adrenal origin) increase from 4 to 8 ng/mL three days before calving to 15 to 30 ng/mL at parturition and the day after calving. The cortisol secretion response is even more pronounced in those cows that develop milk fever (Goff, 2000). Thus, the immunosuppressive effects of the plasma estrogen and cortisol increases observed in the periparturient period would be likely suspects as causative agents of the immunosuppression observed at calving.

There are significant interactions between the immune system and cells and tissues of the reproductive system that are critical for the maintenance of pregnancy, but are responsible for immune suppression that is associated with increased risk of disease. Numerous studies have demonstrated reduced immune competence in the dairy cow around the time of calving. Facets of both the innate and acquired immune response are compromised, beginning about 1-2 weeks before calving and recovering between 2 and 4 weeks after calving (Kashiwazaki *et al.*, 1985; Kehrlı and Goff, 1989; Kehrlı *et al.*, 1989; Kehrlı *et al.*, 1990). Impaired neutrophil and lymphocyte functions during this periparturient period contribute to new infections leading to such diseases as mastitis and metritis (Oliver and Sordillo, 1988). Cows that have silently carried pathogenic bacteria, such as *Mycobacterium avium* subspecies paratuberculosis and *Salmonellae* for many years will suddenly break with clinical disease within a short time after calving (Radostits *et al.*, 2000), attributable to decreased integrity of innate and/or acquired immune cell functions that had kept these bacteria in check. Here under are the different causes of immunosuppression in dairy cows during the transition period.

The effect of pregnancy on immune cells functions:

Pregnancy is a nexus of physiological events that combine to have a profound effect on the immune system. Immunosuppression is manifested in a wide range of immunological dysfunctions, including impaired neutrophil and lymphocyte functions (Kehrlı *et al.*, 1989; Mehrzad *et al.*, 2001). As part of the innate immune system, the neutrophil is an essential first responder to infection and is considered vital to effective clearance of bacteria from the mammary gland

of the dairy cow (Smith, 2000; Zychlinsky *et al.*, 2003; Paape *et al.*, 2003). Neutrophils have various killing mechanisms to destroy pathogens (Smith, 2000; Segal, 2005). Upon encountering invading bacteria neutrophils will ingest the bacteria into phagosomes that are fused with lysosomes. This process stimulates neutrophils to produce large amounts of oxidizing agents in a process referred to as the respiratory burst, in which oxygen radicals are generated that serve as precursors to various antimicrobial oxidants. In addition to oxidizing agents neutrophils contain numerous antimicrobial proteins, such as cathelicidins, hydrolases, proteases, lactoferrin and lysozyme within granules. These proteins are either released into phagosomes to destroy ingested pathogens, or the granule contents are released out of the cell. These neutrophil functions are suppressed at and around the time of parturition (Kehrlı *et al.*, 1989; Mehrzad *et al.*, 2001). The molecular causes of periparturient neutrophil functional suppression are an area of intense research.

Stimulated neutrophils from pregnant women showed significantly less respiratory burst activity compared to a control group (Crouch *et al.*, 1995). Similarly, two enzymes in the hexosemonophosphate shunt that is part of the pathway that produces reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) required for respiratory burst activity are localized to different subcellular areas in neutrophils from pregnant versus non-pregnant women (Kindzelskii *et al.*, 2004). Finally, subcellular location of myeloperoxidase, an enzyme critical to oxidative burst, is altered in non-pregnant women (cytosol) compared to pregnant women (external to the cell and associated with the cell membrane) (Kindzelskii *et al.*, 2006). These alterations in neutrophil functions associated with antimicrobial activities indicate significant perturbation of the neutrophil cellular functions as a result of pregnancy. These observations support the long held idea that immune suppression is an important mechanism in the maintenance of pregnancy and a breakdown of the suppression is a factor in spontaneous abortions (Vince *et al.*, 2001).

One other example of the immune system's importance to reproduction particularly during pregnancy is illustrated by the interaction between leukocytes and the corpus luteum (Pate and Keyes, 2001). The corpus luteum is the remnant of the ovulatory follicle. Its function is to produce progesterone, which is essential for the maintenance of pregnancy. In the absence of an embryo, the corpus luteum regresses and this regression is initiated by uterine release of prostaglandin F2 alpha. Regression of the corpus luteum will allow a new follicle to ovulate. Interestingly, both macrophages and T-cells are found in the corpus luteum. During luteal regression, the number of lymphocytes and macrophages in the tissues increases by both recruitment of cells and proliferation

of resident cells (Bauer *et al.*, 2001). Cytokines thought to be expressed by luteal immune cells have the ability to inhibit progesterone synthesis by the bovine luteal cells and cause apoptosis of these cells and thus regression of the corpus luteum (Pate and Keyes, 2001). The exact mechanism by which the immune cells are signaled to actively work toward regression of the corpus luteum is the subject of much research. Understanding this mechanism may help in the generation of new methods to increase fertility in domestic animals.

Effect of lactation on immune status of dairy cattle:

Neutrophil and lymphocyte function is diminished in the periparturient period, especially in the dairy cow (Kehrli *et al.*, 1989). The onset of milk production imposes tremendous challenges to the mechanisms responsible for energy, protein and mineral homeostasis in the cow. Negative energy, protein and/or mineral balance associated with the onset of lactation may be partially responsible for the immunosuppression observed in periparturient dairy cattle. Mastectomy of pregnant dairy cows removes the impact of milk production, while presumably maintaining endocrine and other changes associated with late pregnancy and parturition. Mastectomy would be expected to improve immune function in the periparturient dairy cow, if milk production is an immunosuppressive factor.

To study the affect of lactation on the immune system, normal dairy cows were compared to cows that had undergone a mastectomy. Specific immune cell (lymphocytes or neutrophil) function was assessed in the mastectomized animals and compared to normal animals. Studies showed that lymphocyte function was significantly different in mastectomized animals compared to normal animals during transition. This demonstrated that the depression of lymphocyte function during the periparturient period could largely be attributed to the metabolic demands of milk production. In contrast to the lymphocytes, neutrophils showed a decrease in function starting about two week prior to calving and reaching the low point at the time of calving in both mastectomized and normal cows. However, mastectomized cows quickly recovered neutrophil function (7 days), whereas, normal animals had not recovered neutrophil function after 20 days (Kimura *et al.*, 1999). These data suggest that lactation plays a significant role in the recovery phase of periparturient immunosuppression. Importantly, the absence of the mammary gland did not affect the manifestation of periparturient immunosuppression but only affected the duration of the suppression after calving

In intact cows, all T cell subset populations (i.e., CD3, CD4, CD8 and gamma-delta positive cells) decreased at the time of parturition, while the percentage of monocytes increased. These population

changes have previously been shown to be associated with the immune suppression commonly observed in periparturient cows. Mastectomy eliminated these changes in leukocyte subsets (Kimura *et al.*, 2002). These results suggest:

- The mammary gland may produce substances which directly affect immune cell populations
- Metabolic demands associated with the onset of lactation negatively impact the composition of circulating Peripheral Blood Mononuclear Cells (PBMC) populations

It can be made the assumption that the second possibility is the more likely. Two metabolic factors were greatly impacted by mastectomy. The first one is mastectomy eliminated hypocalcemia at parturition. The second one is, plasma Non-Esterified Fatty Acid (NEFA) concentration rose dramatically in intact cows at calving and did not return to baseline level for >10 days. In contrast NEFA concentration in mastectomized cow plasma rose only slightly at calving and returned to baseline level 1-2 days after calving. It is clear that the intact cow mobilizes a much larger amount of body fat than does the mastectomized cow, suggestive of a severe negative energy balance at the onset of lactation.

Stress and the immune system: The causes of stress in animals are as varied as its manifestation. Types of stress include heat, negative energy balance, transportation, pregnancy and mixing of unfamiliar animals. Some ways that an animal will manifest stress is in the form of sickness and failure to thrive. Recently, these very general manifestations have begun to be defined on a cellular and molecular level. Various immune cells, such as neutrophils, T-cells and dendritic cells, are affected when an animal is stressed and expression of specific molecules, such as CD62L (L-selectin), is affected during stress (Burton and Kehrli, 1995; Burton *et al.*, 1995; Burton *et al.*, 2005).

The molecular mechanisms that explain the effects of stress are a subject of current research. Several groups have used gene expression micro array analysis to determine the genes affected by stresses, such as thermal stress (Collier *et al.*, 2006), fodder privation (Oilier *et al.*, 2007) and treatment with stress hormone, such as cortisol (Burton and Kehrli, 1995; Burton *et al.*, 2005). One of the most well studied molecular effects of stress on the immune system is the effect of cortisol on the expression of the protein CD62L, which is expressed on the surface of immune cells, such as neutrophils and is necessary for the transmigration of the cell from the vasculature into the tissue at the site of an infection. Cortisol causes the loss of CD62L expression on neutrophils and, thus, the loss of the ability to migrate through the vascular endothelium. This loss of neutrophil response is correlative with

increased susceptibility of the animal to mastitis (Burton *et al.*, 1995).

The initiation of a stress response involves the activation of the hypothalamus, pituitary gland and the adrenal gland to release hormones such as cortisol, epinephrine and norepinephrine. This response is known to have a dramatic effect on the immune system. For example, chronic stress in pigs caused by mixing unfamiliar animals resulted in subordinate pigs having significantly fewer white blood cells compared to the dominant animals (Sutherland *et al.*, 2006). Furthermore, it has been established that animals subjected to restraint stress fail to mount a normal immune response that can result in failure to mount a protective immune response subsequent to pathogen challenge (Anglen *et al.*, 2003).

Metabolic adaptations and the immune system during the transition period:

An emerging area within transition cow metabolism and management is the consideration of interrelationships with the immune system (Drackley, 1999; Drackley *et al.*, 2001). In addition to the adaptations in classical metabolism, transition dairy cows also undergo a period of reduced immunological capacity during the periparturient period. As reviewed by Mallard *et al.* (1998), this immune dysfunction is not limited to isolated immune parameters; instead it is broad in scope, affects multiple functions of various cell types and lasts from about 3 week prior to calving until about 3 week after calving. The consequence of immunosuppression is that cows may be hyposensitive to invading pathogens and therefore more susceptible to disease, particularly mastitis, during the periparturient period. Paradoxically, although leukocytes from immunosuppressed cows are functionally compromised and hyposensitive to pathogens, they are also hyper responsive once activated and produce more proinflammatory cytokines (Sordillo *et al.*, 1995).

Due in part to immunosuppression, it was resulting in decreased milk production, altered milk composition and impaired mammary function. Virulence factors produced by mastitis pathogens may influence mammary epithelial proliferation *in vivo*, which could be important during the periparturient period, when mammary tissue undergoes rapid differentiation and growth (Matthews *et al.*, 1994). Perhaps slightly more obscure are the concerns that either a mammary infection during immunosuppression will predispose the animal to a greater risk of other pathologies or that other pathologies will increase the risk of mastitis at a time when the immune system is compromised. Indeed, Dosogne *et al.* (1999) reported that circulating percentages of polymorphonuclear neutrophils (leukocytes) also important in defense of the mammary gland against mastitic pathogens; were lower in cows with retained fetal membranes and that a greater

percentage of neutrophils in these cows were immature- perhaps leading to impaired function. Kimura *et al.* (2002) utilized neutrophils isolated from cows with or without retained placenta in an *in vitro* system to evaluate neutrophil function and reported that neutrophils from cows with retained placenta had impaired cellular killing capacity and cotyledonary chemotactic migration activity.

In addition to interactions of immunity with metabolism, clinical mastitis has also been shown to reduce reproductive performance in lactating dairy cows (Barker *et al.*, 1998). Immune activation via experimental means or natural infection of the mammary gland has been shown to affect multiple reproductive tissues at various times in the estrous cycle. Huszenicza *et al.* (1998) reported that mastitis infection occurring in the first 14 days after calving did not affect ovarian cyclicity, but that mastitis between days 15 through 28 delayed the time to first ovulation and first estrus. The authors also reported that gram-negative mastitis in already cycling cows during the luteal phase resulted in luteolysis, whereas mastitis during the follicular phase increased period of low progesterone, perhaps resulting in degeneration of the dominant follicle. During clinical gram-positive mastitis of cows in the luteal phase of their cycle, Hockett *et al.* (2000) reported elevated circulating cortisol concentrations and, following oxytocin administration, greater circulating prostaglandin F_{2α} concentrations. Such endocrine changes could result in luteal regression and decreased embryo viability.

The etiology of periparturient immunosuppression is multifactorial and not well understood, but seems to be due to physiologic changes associated with parturition and the initiation of lactation and to metabolic factors related to these events. Glucocorticoids are known immunosuppressants (Roth and Kaeberle, 1982) are elevated at parturition and have therefore been postulated to play a role in periparturient immunosuppression. However, cortisol is elevated for only hours around calving and therefore its role in prolonged immunosuppression around the time of calving has been questioned. Although cortisol concentrations are only transiently elevated, changes in glucocorticoid receptor expression driven by changes in estrogen and progesterone at the time of parturition might contribute to immunosuppression for at least several days around calving (Preisler *et al.*, 2000).

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).

In addition to effects of metabolic dysfunction on immunological capacity, it is possible that perturbations of the immune system also may impact the normal adaptations of other aspects of metabolism during the transition period. In experiments conducted by Waldron *et al.* (2003a) reported that lactating cows subjected to activation of the immune system via endotoxin administration responded with dramatic changes in circulating concentrations of cortisol, glucagon and insulin in order to maintain glucose homeostasis. Furthermore, immune system activation resulted in decreased concentrations of circulating Ca and P (Waldron *et al.*, 2003b). In light of these results, it is conceivable that a vigorous immune response during the periparturient period may also predispose cows to the development of secondary metabolic disorder. Unfortunately, this potentially beneficial adaptation also allows for the establishment of more severe infections and greater inflammatory reactions when immune challenges do occur. These concepts must be evaluated in periparturient cows and the biological magnitude of the potential interface of nutrient metabolism and immune function determined so that researchers can focus on methods to determine whether these interactions account for variation in response to nutritional management strategies across commercial dairy farms.

Nutrients and the immune system: The impact of nutrition on health is the subject of a significant body of research. Many researches have shown that nutrition can affect the ability of an animal's immune system to fight a disease. This connection between nutrition and immune function has been described at the cellular and even the molecular levels. A significant amount of research is being focused on the cellular and molecular processes affected by calcium and vitamins A and D. Calcium and vitamins A and D have been shown to have a significant effect on the functionality of immune cells.

Hypocalcaemia: Several epidemiological studies have shown an association between a diagnosed metabolic disease and subsequent development of mastitis. One metabolic disease that has been associated with immune system disorder is hypocalcaemia or milk fever. A study of over 2000 cows showed that cows with hypocalcaemia were 8 times more likely to develop mastitis than cows with normal blood calcium levels (Curtis *et al.*, 1983). Severe hypocalcaemia leads to the loss of proper skeletal muscle control. Clinical hypocalcaemia occurs in 5-7% of transition dairy cows. Contraction rate and strength of smooth muscle tissue have been shown to be directly related to the level calcium in the blood (Daniel, 1983). A current hypothesis is that even a sub-clinical hypocalcaemia cow would have decreased muscle tone in the smooth

muscle that makes up the teat sphincter and that this loss of muscle tone would cause the teat canal to remain partially open thus exposing the mammary gland to environmental pathogens.

In addition to calcium's critical role in muscle function, it also plays an essential role in intracellular signaling. In immune cells, intracellular calcium regulates many cellular functions including cytokine production, cytokine receptor expression and cell proliferation. Recently it has been shown that stimulated peripheral mononuclear cells from hypocalcemic cows have a muted intracellular calcium response compared to cows with normal blood calcium levels. Furthermore, when stimulated peripheral mononuclear cells from hypocalcemic cows were compared with stimulated peripheral mononuclear cells obtained from the same cows after intravenous treatment with a calcium solution, a muted intracellular calcium response was demonstrated only when the animals were hypocalcemic (Kimura *et al.*, 2006). A muted intracellular calcium response would have a significant effect on the functional capacity of the cells of the immune system.

Maintenance of proper blood calcium levels is critical for an animal's health. There are two effective means of preventing periparturient hypocalcaemia, both of which are diets used in the weeks prior to calving. The first diet reduces calcium intake prior to calving. The theory to this approach is that a mild hypocalcaemia prior to lactation will cause the animal's calcium transport machineries to up regulate, making the absorption of calcium more efficient. Then as lactation begins and the demand for calcium rapidly increases, the capacity for calcium transport has already been increased. The second means of preventing periparturient hypocalcaemia is the use of the Dietary Cation-Anion Difference (DCAD) diet. Researchers in the early 1970's showed that the use of anionic salts in the diet could prevent hypocalcaemia in dairy cows (Ender *et al.*, 1971; Dishington, 1975). Since that time numerous studies have shown that adjustment of the cation-anion balance can reduce the incidence of hypocalcaemia seen in periparturient cows (Block, 1984; Goff, 2000).

Vitamins: T cells are generally divided into two general categories, cytotoxic and helper. In Turn Helper T cells (TH) are subcategorized by the different cytokines they express. Each of the TH cell types focuses the immune response towards a specific type of pathogenic challenge (Reiner, 2007). A recent study has shown that retinoic acid can affect which TH cell types are generated. In addition to responding to different types of pathogens, the various TH cell types are also associated with pathologies such as autoimmune and allergy responses. The bacterial flora of the gastrointestinal tract provides a unique challenge to the

immune system to not react against normal gut bacteria (Reiner, 2007).

Vitamin D affects the immune system through two pathways. First, the endocrine pathway affects serum calcium homeostasis. Cows generally suffer a decline in plasma 25-hydroxyvitamin D3 [25 (OH) D3] around the time of calving as the calcium needs of the cow are in flux due to the demands of milk production (Horst *et al.*, 2005). This periparturient period has been shown to be a time of general immunosuppression and leaves the animals susceptible to various diseases (Kashiwazaki *et al.*, 1985; Oliver and Sordillo, 1988; Kehrl *et al.*, 1989; Kehrl *et al.*, 1990). Through an autocrine pathway, vitamin D analogs directly affect DNA gene expression of immune cells. This is accomplished when the immune cells take up serum 25 (OH) D3 and convert it to 1, 25-dihydroxyvitamin D3 [1, 25 (OH) 2D3], which in combination with a nuclear transcription factor (vitamin D Receptor), can bind to specific DNA sequences and affects expression of multiple genes.

The autocrine pathway for immune cell regulation requires sufficient circulating 25 (OH) D3 such that activated immune cells can produce their own 1,25 (OH) 2D3 in their local environment at cell concentrations that activate key pathways that would not be activated by circulating endocrine produced 1, 25 (OH) 2D3. Screening of human and mouse genomes revealed over 3,000 genes with a vitamin D response element to which 1, 25 (OH) 2D3, in combination with the vitamin D binding protein, affects gene expression (Wang *et al.*, 2005), some of which are involved in immune cell regulation.

EARLY PREDICTORS OF TRANSITION COW DISORDERS

The ability to identify the first signs of herd distress/disease could lead to prompt intervention and thus benefit future herd health and performance. Knowing how a facility and/or management system is influencing overall transition cow success would also help a dairy producer decide whether management changes or capital investment would lead to better long-term success. For early prediction of transition cow disorders metritis will be considered as typical example how different feeding and behavioral acts are used.

Behavior as predictor of disease: Animals that are acutely sick from a systemic infection commonly display a variety of symptoms including changes in body temperature, lethargy and decreased appetite (Hart, 1988). For veterinarians these symptoms, particularly behaviors, have been considered to be a consequence of a debilitated physiological state that prevents the animal from engaging in normal behavior (Aubert, 1999). However, Hart (1988) argued that

sickness behavior is a coordinated set of physiological and behavioral changes that develop in response to an infection. He proposed that fever and the corresponding depressions in appetite and activity are the body's adaptive responses for fighting the invading pathogen. For example, inactivity results in reduced muscular activity consequently causing the body to conserve energy for the increased metabolic costs of mounting a fever and immune response. Additionally, the act of feeding can require movement to the feeding area, competition with others for access to feed and the ingestion and digestion of feed, all of which increase energy expenditure.

In the studies by Quimby *et al.* (2001) and Urton *et al.* (2005) it is unclear whether the reduction in feeding activity in cattle that were later identified as sick was due to an expression of sickness behavior or whether the difference in behavior contributed to the development of the disease. Changes in feeding behavior may influence changes in intake, but how this occurs is not well understood. As discussed previously inadequate nutrients (Weiss *et al.*, 1990; Huzzey *et al.*, 2006) and intake (Hammon *et al.*, 2006) before calving have been associated with an increased incidence of disease after calving.

Feeding behavior and intake predict metritis: At the University of British Columbia's Dairy Education and Research Center individual feeding and drinking times as well as intakes on dairy cows housed in a free-stall barn can be monitored using an electronic monitoring system (INSENTEC, Marknesse, Holland). This system was used to test the prediction that cows with reduced feeding and drinking activity (depressions in intake and duration of feeding/drinking) during the weeks before calving would be more likely to be diagnosed with metritis after calving. In addition, it was predicted that these animals would be less successful at competitive interactions at the feed bunk during the period before calving relative to cows that remained healthy (Bacic *et al.*, 2007).

In this study a total of 101 Holstein dairy cows were monitored from 2 week before until 3 week after calving. Feeding behavior, drinking behavior and dry matter intake were continuously monitored using the INSENTEC feed intake system. Social behavior at the feed bunk was assessed from video recordings and daily milk yields were recorded for 21 days after calving. Metritis severity was diagnosed based on daily rectal body temperature as well as condition of Vaginal Discharge (VD) that was assessed every 3 days after calving until days +21. Cows were classified as having severe metritis if they had at least one VD score of 4 and one recording of fever ($\geq 39.5^{\circ}\text{C}$). Cows were classified as having mild metritis if they had at least one VD score of 2 or 3 and no VD score of 4. Mildly metritic cows may or may not have had a fever. Cows

were categorized as healthy if they had a maximum VD score of 1 and no fever after calving. All animals that did not meet these classification criteria nor had clinical symptoms of other transition related disorders (i.e., milk fever, ketosis or mastitis) were dropped from the analysis.

Cows with severe or mild metritis consumed less water than healthy cows before and after calving, although the difference between healthy and severely metritic cows before calving only bordered on statistical significance. Water intake during the week before calving showed a tendency to be a risk factor for severe metritis. For every 1 kg decrease in water intake during this period the odds of a diagnosis of severe metritis increased by 1.21. After calving water intake for both the mildly and severely metritic cows was lower throughout the 3 weeks following calving compared to the healthy animals; this reduction in water intake likely contributed to the reduction in milk yield observed in the metritic cows. The average daily milk production was 8.3 kg/days less for the severely metritic and 5.7 kg/days less for the mildly metritic cows, compared to the cows that remained healthy throughout the 21 days after calving.

Cows with severe metritis consumed less feed and spent less time at the feed bunk during the 2 week period prior to calving and for nearly 3 weeks prior to the observation of clinical signs of infection. The average number of days from calving to the first signs of pathological discharge (VD ≥ 2) was 5.3 ± 1.9 days (mean \pm S.D.) after calving for cows with severe metritis and 9.1 ± 3.9 days for cows with mild metritis. Cows with mild metritis also consumed less and tended to spend less time at the feed bunk during the week before calving. During the week before calving cows were 1.72 times more likely to be diagnosed with severe metritis for every 10 min decrease in feeding time and for every 1 kg decrease in DMI during this period, cows were nearly 3 times more likely to be diagnosed with severe metritis.

The results of studies provided clear evidence that reduced feeding time and DMI increases the risk of cows developing infectious disease after calving. However, whether a reduction in intake and feeding time before calving is a cause of post-partum infectious disease or an effect of a pre-existing condition is not known. Ketotic environments (i.e., low concentrations of plasma glucose and high concentrations of NEFA and Beta hydroxyl Butyric Acid) have been shown to impair immune function through a variety of pathways (Lacetera *et al.*, 2004). Hammon *et al.* (2006) reported that cows that went on to develop puerperal metritis and sub-clinical endometritis, had a greater degree of immunosuppression relative to healthy animals as measured by neutrophil function.

Social behavior predicts metritis: In the study discussed above the first evidence that social behavior

before calving may be related to the incidence of metritis after calving was demonstrated. During the week before calving cows that were later diagnosed with severe metritis engaged in fewer aggressive interactions at the feed bunk (i.e., displaced others from the feed bunk less often) and had reduced feeding times as well as intakes during the periods following fresh feed delivery, a time when cows are highly motivated to eat. Cows that were diagnosed with severe metritis after calving appeared to be less motivated to compete for access to the feed before calving. This lack of motivation may indicate that these cows are socially subordinate and unwilling to engage in interactions with more dominant individuals (Huzzey *et al.*, 2007).

During the transition periods numerous changes occur including frequent mixing and regrouping of animals. Socially subordinate cows may be unable to adapt to these frequent social restructurings and consequently these cows may respond by reducing their feeding time and DMI and increasing their avoidance behavior in response to social confrontations. These behavioral strategies may put these cows at greater risk for nutritional deficiencies that impair immune function and increase susceptibility to disease (Bacic *et al.*, 2007).

As future directions, dairy cows can face numerous stressors and it has often been suggested that animals suffering from chronic stressors are most at risk for future health complications. During the transition period situations such as frequent regroupings, mixing heifers and cows, or overcrowding test the dairy cows' ability to adapt to varied situations with potential effects on physiology, feeding behavior, or both. Knowing how a facility and/or management system is influencing overall transition cow success would also help a dairy producer decide whether management changes or capital investment would lead to better long-term success.

Presently, dairy producers lack objective tools to measure how much of an impact these management systems and corresponding stressors are having on overall health and performance. Current research are evaluating key physiological markers that relate to stress, immune function, inflammation and energy status before calving to determine if these markers can identify or predict those cows at risk for health disorders or poorer production and reproductive performance after calving. These markers include plasma cortisol, tumor necrosis factor-alpha, haptoglobin, nonesterified fatty acids and fecal cortisol metabolites (Bacic *et al.*, 2007).

The next step for future research in this area will be to identify how stress responses (i.e., behavioral, physiological and immunological) change under different management regimes (e.g., various levels of feeding/resting space availability or social instability) and then evaluate whether animals identified as being at a high risk for health complications after calving benefit

from those management strategies identified as reducing stress and promoting animal well-being.

MAJOR CHALLENGES OF TRANSITION COWS

Several complex problems are common during the transition period as discussed earlier in the whole body of this manuscript. However, some of the major challenges that occur during transition period and severely influence transition program include; metabolic disorders like milk fever, fatty liver and ketosis; reproductive disorders including retained placenta and metritis; digestive disorders like subclinical rumen acidosis and Displaced Abomasums (DA); rapid loss of body condition in early lactation; lower peak milk yield; poor fertility; increased veterinary cost and increased involuntary cull rates.

Health disorders can generate both direct disease related costs (drug treatment, veterinary costs and death of animals) as well as indirect costs. Diseases can influence production efficiency by decreasing milk production (Rajala and Grohn, 1998), reducing reproductive efficiency and increasing the risk of involuntary culling (this occurs when the farmer is forced to remove a productive, profitable cow due to illness, injury, infertility, or death) (Grohn *et al.*, 2003). An example is provided by LeBlanc *et al.* (2002) who reported that cows with clinical endometritis took 27% longer to become pregnant and were 1.7 times more likely to be culled for reproductive failure than cows without endometritis.

CONCLUSION AND RECOMMENDATIONS

The ability to identify the first signs of herd distress could lead to prompt intervention and consequently disease prevention; this would greatly improve farm profitability. Presently, dairy producers lack objective tools to measure how management, dietary and environmental stressors around the transition period impact health and performance. Future progress in this area must combine our understanding of nutrition, metabolism, physiology, immunology and behavior to determine the relationships between these stressors and disease and to develop management strategies that will reduce the incidence of disease after calving.

A solid transition program strengthens the immune system, encourages dry matter intake and provides the proper balance of trace minerals, proteins and carbohydrates. This will help to get cows off to a good start and reduce stress at the time of calving as well as reduce metabolic disorders. Metabolic resources would not be diverted from lactation until a cow became severely metabolically stressed. Several studies

indicated that changes in feeding behavior and dry matter intake in the weeks prior to calving may be used to identify cows at risk for metritis post partum but more work is required to validate this approach. At calving all cows' white blood cells show a decreased ability to fight off infections which increase the susceptibility to mastitis and uterine infections. In part the immunosuppression is thought to be due to changes in hormones at calving. However, better nutrition can also strengthen the immune system at that time. Implementing a transition nutrition program with the help of nutritionists can help dairy herd avoid many of the costly problems mentioned. It is recommended to animal science and health researchers that, due attention to this critical period for improved productivity in our local situation in addition to other priority areas.

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