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Factors contributing to immunosuppression in the dairy cow during the periparturient period

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Abstract

The transition from late gestation to early lactation results in dramatic physiological changes including metabolic changes and immunosuppression in the dairy cow. As a result, cows are at a high risk for disease during this time. Evidence supporting a link between metabolic status and naturally occurring immunosuppression is growing. This review focuses on the impacts of metabolic status, and the metabolites that characterize it, on the immune response of cows during the transition period. Glucose is the preferred fuel for immune cells and its low concentration during the transition period may partly explain the naturally occurring immunosuppression at this time. To our knowledge, ketones are not utilized by immune cells and primarily have been shown to inhibit the immune response when concentration is relatively high. The effect of fatty acids on the immune system response remains unclear. Evidence suggests that the type of fatty acid can either stimulate (*i.e.* saturated fatty acids) or inhibit (*i.e.* unsaturated fatty acids) the immune response. We have suggested that an index for physiological imbalance (PI), based on circulating metabolites that characterize metabolic status, directly relates to mechanisms associated with the development of disease and is superior to calculated energy balance and therefore is a better predictor of risk of disease. The usefulness of the PI index as a predictor of risk of disease and the mechanisms associated with the links between degree of PI and immunosuppression for dairy cows during the transition period warrants further investigation.

Key Words: cow, immunosuppression, nutrition

Introduction

Large improvements have occurred in the

production and efficiency of the dairy cow during the last 3 decades worldwide. In Denmark, the milk yield has increased by ~1.5% annually

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reflecting improvements in genetics, feeding, management and housing. However, based on veterinary records, these improvements have unfortunately not been followed by simultaneous improvements in the disease incidence. Therefore, it is highly relevant to focus on how we may monitor individual cows to improve animal health.

During the transition from late gestation through early lactation, most dairy cows undergo major changes in *e.g.* endocrine regulation that cause extensive mobilization of body tissue, primarily adipose tissue, in order to meet the nutrient demands for maintenance and milk production are characterized by increased circulating concentrations of *e.g.* non-esterified fatty acids (NEFA) released primarily from adipose tissue, increased circulating concentrations of beta-hydroxybutyrate (BHB) reflecting incomplete fatty acid oxidation in the liver, and decreased concentrations of glucose in the bloodstream (Drackley, 1999). Due to recent advances in genetics and management, modern dairy cows produce more milk resulting in a greater mobilization of body tissue nutrients in order to meet the increasing demands for lactation (Chapinal *et al.*, 2011; Drackley *et al.*, 2006). During lipolysis of adipose tissue, circulating NEFA released from adipose tissue enter the liver and have three fates: 1) they can be completely oxidized for energy via the Krebs cycle, 2) converted to BHB, or 3) they can be re-synthesized to triglycerides (TG) where they can either be exported via very low density lipoproteins (VLDL), or they can be stored in the liver (Ingvarsten and Moyes, 2011; Ingvarsten, 2006). The liver has a very limited capacity for export of TG via VLDL, and therefore, especially during early lactation, the rapid and extensive influx of circulating NEFA into the liver can result in a buildup of TG as well as an increased export of BHB into circulation. Nearly 50% of all cows experience an accumulation of liver TG at this time (Bobe *et al.*, 2004). However, some cows experience greater deviations in circulating

NEFA and BHB and liver TG which can increase the risk of metabolic diseases such as ketosis, usually defined as > 1.2 or > 1.4 mM of BHB, and hepatic lipidosis, severe or clinical fatty liver $> 10\%$ liver TG on a wet weight basis (Bobe *et al.*, 2004; Drackley, 1999).

The rate and extent of tissue mobilization has been linked to immunosuppression and risk of diseases during the transition period (Cai *et al.*, 1994; Contreras and Sordillo, 2011; LeBlanc, 2012). Other factors have been associated with increased rates of tissue energy mobilization such as dry matter intake and prepartum body condition score (Gearhart *et al.*, 1990; Ingvarsten and Andersen, 2000; Leblanc, 2010; Nielsen *et al.*, 2003). However, these factors will not be discussed in this review.

Natural immunosuppression occurs in most cows during very late pregnancy and early lactation. Immune cells involved in both the innate and adaptive immune response are altered during the transition period. Extensive investigations of the natural immunosuppression around parturition have been carried out (Ingvarsten and Moyes, 2013; Madsen *et al.*, 2002; Sordillo *et al.*, 2009). The function of neutrophils, that are part of the first line of defense against invading microorganisms, is reduced at this time including chemotaxis, *i.e.* migration to the site of infection, phagocytosis, *i.e.* engulfment of the invading microorganism, and oxidative burst, *i.e.* killing of the invading microorganism during phagocytosis (Paape *et al.*, 2002; Schukken *et al.*, 2011). Further, the number and proliferation of lymphocytes is reduced during the transition period (Kehrli, Jr. *et al.*, 2009). The changing hormonal environment, especially increases in circulating glucocorticoids at parturition, has been associated with the naturally occurring immunosuppression observed at this time (Burton *et al.*, 2005; Weber *et al.*, 2006). However, glucocorticoids are elevated for only ~ 24 hour around parturition and immunosuppression has been observed during the first three weeks after parturition (Graugnard *et*

al., 2012a; Moyes *et al.*, 2014) and therefore, changes in glucocorticoids cannot be a major contributor to the immunosuppression observed during the transition period.

In addition to the increased risk of metabolic diseases, the changes in the hormonal, metabolic, digestive, immune and neurological systems increase the risk of not only metabolic disorders but also infectious diseases, primarily metritis and mastitis during the transition period (Cai *et al.*, 1994; Grohn and Rajala-Schultz, 2000). This review will focus on the increased risk of mastitis during the transition period. Mastitis is defined as an inflammation of the mammary gland usually as a consequence of a pathogenic invasion. It is the most common and costly of all diseases in the dairy industry worldwide, exceeding \$2 billion annually in the US alone (Cha *et al.*, 2011); hence, its prevention is critical for animal welfare as well as economic outcome for the farmer.

In healthy cows, the milk somatic cells (*i.e.* immune cells) primarily include resident macrophages and lymphocytes. During mastitis, the somatic cell population shifts to a large proportion of neutrophils (~90%). Neutrophils are the main immune cell responsible for killing invading microorganisms during mastitis. Due to the naturally occurring immunosuppression around parturition, this period is considered a high risk period for the development of mastitis (Green *et al.*, 2002).

Individual Animal Variations

In studies of physiology, nutrition, and other disciplines, different designs are used where animal differences, *e.g.* within a group or treatment, are considered a part of the residual. The residual is the variation that is not explained by the model used and is generally considered to be noise and therefore of little interest. The size of the between-animal variation may vary depending on the physiological stage of

the animals and is large in early lactation cows for traits such as plasma NEFA, BHB and glucose. We have stated that it is important to understand the nature of such variation and to take it into account in future management and feeding strategies in order to prevent and reduce the incidence of production diseases and reproduction problems and simultaneously to optimise milk production and efficiency at the level of individual animals (Ingvarlsen *et al.*, 2003; Ingvarlsen, 2006; Ingvarlsen and Friggens, 2005). It is well known from the vast amount of literature available that there is a large variation in milk yield and health between herds. It has also become clear that there is an even larger variation between animals within the herd in production parameters and important metabolic parameters (Ingvarlsen *et al.*, 2003; Ingvarlsen, 2006). We believe it is important to use knowledge on this variation in *e.g.* physiological parameters to prevent diseases and avoid suboptimal performance.

Ingvarlsen and Friggens (2005) showed that a large proportion of the individual variability in milk yield can be explained by the variability in selected plasma hormones, metabolites, and energy intake. Results from 317 cows and 634 lactations were used (Ingvarlsen & Jensen, 2001; Nielsen *et al.*, 2003). Cows entering the experiment 8 weeks prior to primiparous calving were randomly (within breed and genetic line) assigned to one of two feeding treatments, a total mixed ration with either low or normal energy density, on which they remained until they left the experiment at the end of their productive lives (Nielsen *et al.*, 2003). Detailed registration of performance was carried out on these cows and 10,809 plasma samples were analysed for selected hormones and metabolites. Univariate analysis was carried out on energy corrected milk yield and concentrations of selected plasma hormones (insulin, growth hormone (GH) and triiodothyronine (T3)), metabolites (NEFA, glucose, BHB and urea nitrogen), and digestible energy intake (DE intake) to estimate

between-cow variation through lactations. Partial least square models were subsequently run to estimate the extent to which between-cow differences in energy corrected milk yield could be explained by between-cow differences in hormone concentrations, metabolite concentration, or DE intake. For details on the methods used the reader is referred to Ingvarlsen & Friggens (2004).

The between-cow variability in energy corrected milk yield and the hormones and metabolites were generally found to be considerable and total variance changed through lactation, particularly for GH, T3, NEFA, and BHB (Ingvarlsen & Friggens, 2004). When analysed separately using partial least square models, hormones, metabolites, and DE intake accounted for 24%, 25% and 26% of the variability in energy corrected milk (ECM), respectively. When including both the hormones and metabolites, the model explained 36% of the between-cow variability in ECM, and this figure was increased to 53% if DE intake was also included (Ingvarlsen & Friggens, 2004). The lack of additivity in the variability explained shows that hormones, metabolites, and DE intake were correlated illustrating the integration and orchestration of metabolism and intake as discussed in several reviews (Bauman, 2000; Drackley, 1999; Ingvarlsen and Andersen, 2000).

The results from this study clearly show that there are very large differences in endocrine and metabolic parameters between cows that would traditionally be assumed to be alike, *i.e.* same breed, parity, and same rearing and nutritional history. Despite this, and even though these cows were kept in as constant an environment as possible (nutrition, management, housing), there was considerable between-cow variation in the ECM, hormones, metabolites and energy intake measured. These between-cow differences are all the more noteworthy given that they are differences that remain after the effects of treatment, stage of lactation etc. have been accounted for. The results of this study raise two

issues: What is the source of this variation; and how can this variation be exploited in practice?

It is reasonable to assume that the between-cow differences observed reflect, at least partly, genetic differences between cows. Another source of variation may be differences in the degree of physiological imbalance experienced by these cows. Physiological imbalance arises when an animal is failing to cope (homeorhetic and homeostatic regulation) with external constraints or stressors, and consequently cannot maintain optimal performance and function. Physiological imbalance has been defined as cows whose physiological parameters deviate from the normal and who consequently have increased risk of developing production diseases (clinical or subclinical) and reduced production and/or reproduction (Ingvarlsen, 2006). Given that there are differences between cows in genetic potential for milk yield, then different cows will have experienced the nutritional environment as more or less constraining resulting in various degrees of physiological imbalance. Regardless of its source, this between-cow variation strongly suggests that there could be substantial benefits of individual cow management if it can be exploited in practice to prevent disease and suboptimal performance.

Metabolites and Immunosuppression

Our previous review discussed in detail the relationship between metabolites and several aspects of the immune response during the transition period for dairy cows (Ingvarlsen and Moyes, 2013). For this review, we will summarize those studies and provide any new information regarding the link between variations in individual metabolites and the immune response for transition dairy cows. This section will focus specifically on circulating metabolites including NEFA, BHB and glucose.

It is well established that glucose is the preferred nutrient of immune cells for fuel rather

than fatty acids (Newsholme *et al.*, 1986; Newsholme and Newsholme, 1989). Previous studies have shown that glucose has no inhibitory effects but rather stimulatory effects on the immune response, which include increased proliferation and differentiation of leukocytes and improved neutrophil chemotaxis and phagocytosis (Barghouthi *et al.*, 1995; Gamelli *et al.*, 1996; Pithon-Curi *et al.*, 2004). Most research evaluating the impact of circulating glucose concentrations on the inflammatory response has focused on human type II diabetes mellitus (T2D), which reflects a hyperglycemic state associated with insulin resistance. De Vries *et al.* (2015) reported increased leukocyte activation in T2D patients via a 39% higher expression of neutrophil CD66b, a marker of neutrophil degranulation, and monocyte CD11b, involved in adhesion, when compared with controls. It should be noted that other mechanisms associated with T2D *e.g.* endocrine changes may play a role regarding alterations in the inflammatory response, however, hyperglycemia is considered a major contributor to heightened inflammation in patients with T2D (Bastard *et al.*, 2006; Tilg and Moschen, 2008). Glucose concentration is low during the early postpartum period and this may partly explain the naturally occurring immunosuppression in dairy cows.

Ketone bodies, such as BHB, have been shown to negatively impact the immune response including reduced trap formation, chemotaxis and phagocytosis of neutrophils and reduced lymphocyte blastogenesis (Grinberg *et al.*, 2008; Ingvarlsen and Moyes, 2013). To our knowledge, immune cells do not use ketone bodies as a fuel cell source (Newsholme *et al.*, 1987) nor ketones enhance immunity *in vitro* (Ingvarlsen and Moyes, 2013). More recent data have involved *in vivo* differences in various metabolic conditions for dairy cows as explained in the next section.

The effect of NEFA on the immune response is inconclusive and poorly understood. The majority of the present studies that have evaluated the effect of circulating NEFA on the

immune response in dairy cows have focused on the metabolic state and disease as well as individual NEFA (LeBlanc, 2012; Sordillo *et al.*, 2009). Some of these findings will be discussed in the section below. Studies have shown that leukocytes are activated by lipids (Alipour *et al.*, 2008; de Vries *et al.*, 2015). In humans, De Vries *et al.* (2015) examined leukocyte activation between T2D and hyperlipidemia (FHC) patients where FHC patients experienced elevated leukocyte activation when compared with controls. Although the circulating fatty acid profiles were not reported, FHC patients were defined as those who met the diagnostic criteria set by the World Health Organization for FHC including elevated plasma apolipoprotein B and triglyceride levels (> 1.7 mM). We recently reported that circulating NEFA prepartum were considered a better risk factor for the development of metritis, milk fever and retained placenta than plasma BHB and glucose or calculated energy balance (Moyes *et al.*, 2013). These studies provide further evidence that circulating NEFA can alter the immune response, but the mechanisms remain unclear.

In human medicine using patients or rodent models, individual fatty acids have been shown to have both inhibitory and stimulatory effects on the immune response. In general, unsaturated fatty acids (*e.g.* C18:1, C18:2 and C20:4) impair the immune response whereas saturated fatty acids (*e.g.* C12:0, C14:0 and C16:0) improve the immune response (Lee *et al.*, 2001; Lee *et al.*, 2004; Lee and Hwang, 2006). Saturated fatty acids may also be responsible for stimulation of toll-like receptor (TLR) signaling (Moyes *et al.*, 2010; Sordillo *et al.*, 2009). Using a dietary-induced negative energy balance for cows in mid-lactation, an up-regulation of genes encoding for TLR-4 and TLR-2 in bovine blood neutrophils was observed for cows in negative energy balance, but not for cows in positive energy balance (Moyes *et al.*, 2010). During the normal course of lipolysis after parturition, the circulating NEFA pool becomes enriched with the major fatty acids of adipose tissue, such as 16:0

(Douglas *et al.*, 2007). The TLR recognize pathogen-associated molecular pattern motifs, among which are lipids including lauric acid (12:0), myristic acid (14:0), and 16:0 (Lee and Hwang, 2006). Although circulating fatty acid profiles were not measured, dietary-induced negative energy balance most likely resulted in increased fatty acids released from adipose tissue into circulation, especially 16:0 that may have stimulated TLR on blood neutrophils. It should be noted that these relationships were observed at the gene level and post-translational modifications may not result in increased expression at this time. Furthermore, these results contradict those of others that identified negative relationships between elevated circulating NEFA and immunosuppression (Brassard *et al.*, 2007; Lacetera *et al.*, 2004). Using *in vitro* models, Scalia *et al.* (2006) reported increased phagocytosis-associated oxidative burst activity whereas viability was reduced in bovine blood neutrophils. Therefore, more research is needed regarding the relationships between types of NEFA and their effects on the immune response in dairy cows during the transition period.

Physiological Imbalance and Disease

Most research regarding the relationship between metabolic status and immunosuppression and risk of disease has involved calculated negative energy balance. This calculation has primarily been based on recommendations set by the National Research Council (2001) that take into account daily milk yield, milk fat and protein content, the net energy for lactation in the ration and body weight. Other calculations for energy balance have primarily been based on an equation including body energy change from feed energy input and the energy requirements for milk, lean tissue growth, conceptus growth, maintenance, and activity (Friggens *et al.*, 2007). Studies have shown that severity of energy balance is associated with reproductive problems

and increased risk of disease (van Knegsel *et al.*, 2005). In addition to energy balance, other diseases that are commonly observed during the transition period, *i.e.* hypocalcemia, have also been linked to immunosuppression (Martinez *et al.*, 2014), but these relationships will not be discussed in this review.

Nutritional strategies to reduce the severity of energy balance and combat immunosuppression during the transition period have been examined (Graugnard *et al.*, 2012b; Moyes *et al.*, 2014). Controlling energy intake is one management strategy that has been studied. Feeding to meet energy demands during the dry period has been shown to prevent large changes in calculated energy balance and reduced plasma NEFA and BHBA and liver lipid and triacylglycerol accumulation postpartum (Graugnard *et al.*, 2013; Janovick *et al.*, 2011). With regard to the immune response, recent work has shown that over-feeding (*i.e.* ad libitum intake) during the dry period reduced serum haptoglobin, an acute phase protein, and reactive oxygen metabolites (Graugnard *et al.*, 2013) and up-regulated the transcription of key genes associated with extracellular trap formation (*i.e.* *HIF1A*), oxidative metabolism (*i.e.* *S100A9*) and TLR signaling (*i.e.* *TLR2*) in bovine blood neutrophils (Moyes *et al.*, 2014) after intramammary endotoxin challenge throughout the early postpartal period when compared with cows fed controlled energy (*i.e.* feeding to meet energy requirements). These provide a promising nutritional strategy to not only control energy balance, but also improve immunity during the transition period.

Although calculated energy balance is generally accepted as the 'gold standard' for measuring metabolic status for individual cows at certain physiological states, our recent work has shown that physiological imbalance (PI) was a better predictor of risk of disease than calculated energy balance (Moyes *et al.*, 2013). We have previously calculated a PI index based on the following formula: $PI = (x1 \times [NEFA] + x2 \times [BHBA] - x3 \times [glucose]) / 3$; where $x1$, $x2$

and x_3 represent the adjusted weights for NEFA, BHBA and glucose estimated from regression coefficients within a week of lactation (Moyes *et al.*, 2013). Using this formula and the individual variations in circulating concentrations of NEFA, BHB and to calculate the PI index, we believe to have a biomarker that is more directly related to the development of disease than calculated energy balance (Ingvarlsen and Moyes, 2013; Moyes *et al.*, 2013). Our results showed that cows with higher PI prepartum were at a greater risk of developing infectious diseases *i.e.* mastitis and metritis, as well as non-infectious diseases, *i.e.* retained placenta, milk fever and lameness, after calving when compared with calculated energy balance. These results indicate that the effects of circulating NEFA, BHB and glucose in combination may play a major role regarding the severity of immunosuppression during early lactation. Understanding the relationship between degree of PI and risk of disease may lead to new management strategies that prevent and reduce the incidence of diseases and reproduction/production problems at the level of individual animals. However, the mechanisms associated with degree of PI and immunosuppression have not been elucidated and warrants further investigation.

Over the past few decades in the dairy industry, the number of cows per farm has increased thereby increasing the number of cows being managed per herd personnel. The need for automated surveillance systems for in-line and real-time measurements for early detection of 'at risk' animals on-farm is vital to improve animal welfare and sustainability of farms in the dairy industry worldwide.

Identifying biomarkers in milk that reflect degree of PI is one strategy to meet the growing demands for individual animal surveillance and their risk of disease on farm. Future testing of milk components and their use as biomarkers for degree of PI for automated systems on-farm will improve surveillance and help farmers carry out proactive risk management to prevent disease

and improve dairy cow welfare, reproduction, productivity, and economic outcome.

Conclusions

Immune suppression occurs in dairy cows particularly in the transition period and many factors are potentially involved, including metabolites reflecting the physiological status of the cow. Glucose is the preferred nutrient of immune cells for fuel rather than fatty acids or ketones and glucose has stimulatory immune response effects, which include increased proliferation and differentiation of leukocytes and improved neutrophil chemotaxis and phagocytosis. The low glucose availability made partly explain reduced immune cell function in early lactation. BHB may, at higher concentrations, have negative impacts on the immune response including reduced trap formation, chemotaxis and phagocytosis of neutrophils and reduced lymphocyte blastogenesis. The effect of NEFA on the immune response is inconclusive and poorly understood. However, circulating NEFA prepartum is considered a better risk factor for the development of *e.g.* metritis, milk fever and retained placenta than plasma BHB and glucose or calculated energy balance. A developed index of physiological imbalance indicate that the effects of circulating NEFA, BHB and glucose in combination may play a major role regarding the severity of immunosuppression during early lactation and that this PI-index is a better biomarker than the individual metabolites and calculated energy balance. Growing evidence supports the relationship between metabolic status, and the metabolites that characterize metabolic status, and immunosuppression during the transition period. The mechanisms associated with degree of PI and immunosuppression warrant further investigation.

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