Physiological Imbalance and Risk of Disease During Early Lactation

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ABSTRACT

Physiological imbalance (PI), which occurs particularly during early lactation, is defined as cows whose physiological parameters deviate from the normal and who consequently have an increased risk of developing diseases and reduced production and/or reproduction. Based on previous research, an index for PI has been developed based on plasma concentrations of free fatty acids (NEFA), glucose and beta-hydroxybutyrate (BHBA). The index for PI has recently been shown to be a better predictor of certain diseases, i.e. metritis, mastitis, retained placenta and milk fever, than standard methods (calculated energy balance; EBAL) are individual metabolites alone (e.g. BHBA and glucose). Recent data involving gene transcripts suggests that cows experiencing higher degrees of PI undergo more rapid adipose tissue lipolysis and metabolic overload in the liver that predisposes these 'risk' cows to metabolic disorders, i.e. ketosis and hepatic lipidosis, during early lactation. Degree of PI is also linked to immunosuppression thereby increasing susceptibility to infectious diseases such as mastitis and metritis. Evidence also shows that cows with a high degree of PI also experience reduced reproductive performance. Future studies that identify the links between degree of PI and risk of disease are needed for the development of automated in-line and real-time surveillance systems for early detection of 'at risk' animals on-farm. An automated system 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.

INTRODUCTION

Genetic selection programs designed to increase milk yield coupled with improvements in feeding, housing and management of dairy cows have led to dramatic increases in production and efficiency; however, reproductive performance has declined (Berry et al., 2003; Royal et al., 2002) and the incidence of disease is still substantial (Ingvartsen and Moyes, 2012). The majority of health problems in the dairy cow occurs during the periparturient period, defined as ± 3 weeks relative to parturition, and relate to cows having difficulty adapting to the nutrient needs for lactation. This paper will discuss the complex transcriptomic relationships between metabolic status and increased risk of disease for dairy cows with a special focus on how degree of PI reduces reproductive performance and increases risk of disease at the transcriptomic-level.

WHAT IS PHYSIOLOGICAL IMBALANCE?

The periparturient period is a critical time in the life cycle of the dairy cow, characterized by major changes in the e.g. endocrine, immune and digestive systems, and cows are at a greater risk for disease than any other time period (Ingvartsen, 2006). Reducing degree of PI in individual cows, 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 (Ingvartsen, 2006), will reduce the risk of disease thereby improve production and reproductive performance (Ingvartsen, 2006; Moyes et al., 2010c). For decades, researchers have used calculated energy balance (EBAL), primarily based on dry matter intake, milk yield and milk components, as the 'gold standard' to reflect the degree of change in energy mobilization during early lactation in relation to risk of disease. Studies have shown that calculated negative EBAL was associated with reduced fertility (Walsh et al., 2011) and immune function and increased risk of some infectious (Ingvartsen et al., 2003; Ingvartsen and Moyes, 2012) and non-infectious diseases (Huzzey et al., 2011; Ingvartsen, 2006).

A number of reviews (Ingvartsen, 2006; Ingvartsen and Moyes, 2012) identified plasma non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA) and glucose as the major metabolites that relate to degree of PI. Recent investigations showed that an index for PI, based on plasma NEFA, BHBA and glucose, was a better predictor of risk of diseases than calculated EBAL (Friggens et al., 2007; National Research Council, 2001) and, therefore, more directly relates to mechanisms associated with the development of certain diseases during early lactation (Moyes et al., 2012). This paper will discuss the potential mechanistic links between degree of PI and the development of disease at the transcriptomic-level that may help unravel the biological understanding of PI in relation to reproductive performance and risk of disease for dairy cows during lactation. These findings will serve as the basis for the development of new management strategies to detect and combat PI thereby improve reproductive performance and reduce risk of disease during lactation.

PHISIOLOGICAL IMBALANCE AND RISK OF DISEASE

Non-infectious diseases. Recent evidence suggests that individual concentrations of circulating metabolites, such as NEFA, BHBA and glucose, are associated with multiple diseases such as ketosis and displacement of the abomasum (Li et al., 2012; Seifi et al., 2010). Since most diseases in the dairy industry are multi-faceted (Ingvartsen, 2006; Ingvartsen and Moyes, 2012), the use of individual metabolites (e.g. urine/milk BHBA for ketosis and liver triacylglycerol (TAG) content for hepatic lipidosis) may not be the most optimal method to predict risk of clinical disease for lactating dairy cows. In a recent study, degree of PI during the prepartal period was a better predictor of milk fever and lameness after calving than calculated EBAL, plasma BHBA and glucose (Moyes et al., 2012). In the same study, degree of PI was positively correlated with total lipid and TAG and negatively correlated with glycogen content in liver during the postpartal period. This suggests that PI may relate to enhanced lipolysis of adipose tissue after parturition and provides support for the use of an index for PI as a predictor of metabolic disease, e.g. ketosis and hepatic lipidosis, during early lactation.

To the author's knowledge, no studies have evaluated the mechanistic link between degree of PI and risk of non-infectious diseases at the transcriptomic-level. Physiological imbalance occurs before clinical signs of disease and, therefore, relates to early warning signs for disease at the sub-clinical level. Developing new management strategies to detect and combat PI will thereby reduce the incidence of several diseases of dairy cows during lactation. Several studies have begun to identify the mechanisms associated with the development of certain metabolic disease, i.e. ketosis and hepatic lipidosis, with a special focus on hepatic mRNA expression of dairy cows during the periparturient period (Li et al., 2012; Loor et al., 2007; van Dorland et al., 2009). Recent findings may unravel the links between degree of PI and risk of metabolic disease of dairy cows during early lactation. For instance, Li et al. (2012) reported a significant increase in hepatic mRNA expression of acyl-CoA synthetase (*ACSL*), an activator of long-chain fatty acid oxidation, and decrease in carnitine-acylcarnitine translocase I (*CPT-I*) and II (*CPT-II*) in ketotic cows (BHBA > 1.2 mmol/L) 4 weeks postpartum and concluded that ketotic cows experience enhanced oxidation of long-chain fatty acids, derived primarily from adipose tissue, in order to meet the energy demands for milk production at this time. This is further supported by Xu et al. (2011) where *CPT-I* was lower in cultured bovine hepatocytes when supplemented with > 1.2 mmol/L of NEFA whereas cows experiencing normal changes in plasma NEFA, BHBA and glucose during the periparturient period, i.e. a lower degree of PI, mRNA expression of *CPT-1* was higher (Loor et al., 2005). McCarthy et al. (2010) observed widespread changes in the expression of genes associated with lipid metabolism, cell cycle and metabolic disease in liver tissue from cows experiencing severe negative EBAL during early lactation. These results suggest increased risk of metabolic overload in the liver for cows experiencing a greater degree of PI during in early lactation providing potential links between degree of PI and risk of metabolic disease, i.e. ketosis and hepatic lipidosis, during early lactation.

Infectious diseases. This section discusses the complex relationships between metabolic status and immune function and how degree of PI, and the metabolites that characterize PI (primarily NEFA) may contribute to immunosuppression during the periparturient period. During this time, cows are at high

risk for developing new intramammary infections (IMI) associated with the dramatic changes in hormones and metabolism at parturition, which alter the immune system function (Godden et al., 2003; Grinberg et al., 2008). The rate and extent of NEFA oxidation in the liver, reflecting degree of PI, can lead to higher rates of oxidative stress in the liver that may alter normal hepatic metabolism thereby increasing risk of disease (Bossaert et al., 2012; Drackley, 1999). The relationship between NEFA, and the type of NEFA, and the immune response is complex and poorly understood. Higher circulating NEFA concentrations for cows during the periparturient period have been associated with increased risk of infectious disease such as mastitis (Moyes et al., 2009) and metritis (Chapinal et al., 2011). In bovine mammary epithelial cells (MEC), Rezamand and McGuire (2011) observed a down-regulation of mRNA expression of major pro-inflammatory cytokines, interleukin-6 (*IL6)* and *IL8*, for MEC sub-cultured with NEFA when compared to controls (i.e. no NEFA). In contrast, immunostimulatory effects of NEFA on immune function have also been reported. Rezamand and McGuire (2011) also reported an up-regulation of *IL1B* and *ICAM1* for bovine MEC incubated in NEFA when compared to controls. Future studies examining the effect of shifts in blood NEFA profiles in cows during the periparturient period on suppression and stimulation of immune cells are warranted to potentially uncover new linkages between degree of PI and risk of disease during early lactation.

The impact of calculated negative EBAL on immune response of dairy cows has been described in more detail. The use of potent research tools in genomics has provided information into the mechanistic links between metabolic status the risk of infectious diseases, i.e. mastitis and metritis. Dietary-induced negative EBAL for cows in mid-lactation (> 90 days in milk) has been used as a model to identify the mechanisms that link energy status with immune system function during early lactation without the confounding effects of glucocorticoids that are known to cause immunosuppression during the periparturient period. Moyes et al. (2010b) evaluated the transcriptomic-level profiles of mammary tissue from cows in negative EBAL and positive EBAL after IMI with *Streptococcus uberis* (strain 0140J) in mid-lactation. Microarray analysis revealed *IL-8 Signaling* as one of the most affected pathways by negative EBAL. Interleukin-8 is a cytokine that primarily stimulates neutrophil recruitment and enhances neutrophil function via its effects on neutrophil degranulation and respiratory burst activity. Within *IL-8 Signaling*, 80% of the genes were down-regulated for cows experiencing negative EBAL during IMI and may suggest a suppression of the pro-inflammatory response and neutrophil recruitment. With regards to individual genes, a down-regulation of several genes involved in the immune response was observed (e.g. *AKT1, IRAK1, MAPK9,* and *TRAF6*). During naturally occurring EBAL after parturition, microarray technology revealed dramatic changes in cows experiencing severe negative EBAL that altered genes associated with oxidative stress, natural killer cell signaling and a down-regulation of *IL15*, *BCL2* and interferon-gamma (*IFNG*) in the spleen of dairy cows. Studies involving both dietary-induced and naturally occurring EBAL reveal negative effects of metabolism on the immune response that may potentially link degree of PI with risk of infectious disease during early lactation.

With regards to metabolic effects of immune cells themselves, i.e. neutrophils, dietary-induced negative EBAL for cows in mid-lactation resulted in a down-regulation of neutrophil expression by negative EBAL including genes involved with antigen presentation (*HLADRA* and *HLAA*), respiratory burst (*SOD1*), and the pro-inflammatory response (*TNFA*) (Moyes et al., 2010a). However, certain genes up-regulated by negative EBAL were also associated with immune response, i.e. *IL1R2, IL6, TLR2* and *TLR4*. Increased NEFA has been linked to activate activation of certain genes associated with the immune reponse but this relationship is complex and depends heavily on the profile of NEFA exposed to neutrophils (Lee et al., 2003; Lee et al., 2004). In addition to the role of EBAL and NEFA on immune cells, low glucose availability during the periparturient period may be one mechanisms that links degree of PI with increased risk of infectious diseases since glucose has been shown to be the preferred metabolic fuel during inflammation for activated neutrophils, macrophages, and lymphocytes rather than fatty acids, amino acids, or ketone bodies (Gamelli et al., 1996; Newsholme et al., 1986). More research is needed in order to determine whether low glucose impacts susceptibility to infection during early lactation.

The mechanistic links between metabolism and reproductive disease and fertility in dairy cows during early lactation are not clear. The partitioning of nutrients for lactation involves numerous endocrine and metabolic changes that lead to a state of negative EBAL in support of lactation that subsequently increases risk of disease (Bauman and Currie, 1980; van Knegsel et al., 2007). Degree of PI during the prepartal period has been recently shown to be a better predictor of retained placenta after calving than calculated EBAL, plasma BHBA and glucose. It has been well documented that cows with milk fever and ketosis have an increased risk for development of retained placenta and dystocia via delayed uterine muscle contractions around parturition (Roche, 2006) providing potential links between degree of PI prepartum and reproductive disease. No studies have assessed the association between degree of PI and reproductive performance and future studies are needed that elucidate this potential relationship. With regards to studies involving individual metabolites that characterize PI, higher plasma NEFA are associated with reduced pregnancy at first artificial insemination (Chapinal et al., 2012), late embryonic mortality (Senosy et al., 2012) and increased risk of metritis (Chapinal et al., 2011). At the transcriptomic-level, NEFA concentrations alter follicular development and quality, viability and developmental capacity of the resultant embryo in vitro via increases in mRNA abundance of genes associated with glucose transport (*SLC2A1*), growth factors (*IGF2R*) and fatty acid synthesis (*ACSL1* and *ACACA*) (van Hoeck et al., 2010). It has also been documented that higher glucose metabolism of bovine blastocysts is correlated to improvements in embryonic development (De La Torre Sanchez et al., 2004) and low glucose availability reduces maturation in bovine oocytes (Leroy et al., 2006). However, hyperglycemic conditions reflecting high serum glucose concentrations similar to diabetic serum altered gene expression pathways associated with the disruption of preattachment development of bovine blastocysts *in vitro* (Cagnone et al., 2012). For cows experiencing severe negative EBAL 2 weeks postpartum, gene expression of chemokines cytokines in uterine tissue was up-regulated that may inhibit an effective immune response to invading microorganisms and reduce uterine recovery and fertility when compared to cows experiencing normal EBAL after parturition (Wathes et al., 2009). These data provide potential mechanistic links between degree of PI and reduced reproductive performance and increased risk of reproductive diseases during early lactation. Elucidating the etiology of the complex relationship between circulating metabolites, degree of PI and reproductive disease and performance is warranted.

CONCLUSION

The knowledge gained from previous studies has just begun to unravel the mechanistic linkages between degree of PI and risk of disease during the periparturient period. Early detection of PI via an automated in-line and real-time surveillance system for early detection of 'risk animals' on-farm will lead to proactive management to combat PI thereby improving animal health and welfare and economic outcome for the farmer. Therefore, identifying potential biomarkers in milk that relate to degree of PI as an early warning system for risk of disease on-farm will led to new management strategies to combat PI and reduce risk of disease especially in early lactation. Future research is needed for a better biological understanding associated with the links between degree of PI and risk of disease. Using recent advances in functional genomics, proteomics and metabolomics, we can begin to unravel the complex relationships between metabolic status, especially relating to degree of PI, and risk of disease.

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