TRIENNIAL LACTATION SYMPOSIUM:
Nutrient partitioning during intramammary inflammation:
A key to severity of mastitis and risk of subsequent diseases?¹

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ABSTRACT: In early lactation, susceptibility to disease is greatest, impacting cow health and productivity and leading to economic losses. Mastitis is the most economically costly disease to the dairy industry and is most frequent at this time. The objective of this paper is to discuss the energetic fuels used by leukocytes in the metabolic response during mastitis that may reveal potential mechanisms linking mastitis with the development of subsequent metabolic diseases for dairy cows during lactation. Glucose and glutamine are the primary fuels used by leukocytes and are essential substrates for optimal leukocyte function. Yet because these substrates are in high demand to support milk synthesis during early lactation, their supply to leukocytes may be compromised and may partly contribute to immunosuppression observed at this time. Production-related metabolic diseases during early lactation, such as ketosis and hepatic lipodisosis, can also adversely affect health and productivity. Risk of subsequent disease for cows during mastitis has not been fully elucidated. Regardless of stage of lactation and physiological state, increases in circulating NEFA and glucose and decreases in ketones during an intramammary inflammation in dairy cows have been reported. In addition, previous work indicates that hepatic metabolism may be impaired during inflammation. These results indicate a potential link between mastitis and the risk of subsequent metabolic disease for dairy cows during lactation. This paper will discuss the complex relationships between metabolism and immune function and how these immunometabolic interactions relate mastitis with increased risk of subsequent disease during early lactation.

Key words: cow, inflammation mammary gland, mastitis, metabolism, nutrient partitioning

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INTRODUCTION

Over the past few decades, the focus on maximizing milk output and lowering feed cost for farmers has been partly replaced with growing concern for animal health and well-being. Today, the dynamics of the dairy industry have substantially changed, which can be partly attributed to growing consumer concerns associated with improving animal health and well-being and reducing antibiotic usage (Bannantine et al., 2013).

Improvements in genetics and management practices have resulted in increased milk production per cow; however, disease incidence still remains substantial (Ingvartsen and Moyes, 2013). Development of disease, especially during early lactation, may negatively impact animal efficiency and profitability via reduced milk production and milk quality and increased veterinary costs associated with treatment (Ingvartsen and Moyes, 2013). Reducing risk of disease will lead to improvements in animal health and thereby improve economic outcome for the farmer.

Many diseases often occur as a complex where cows with one disease are at a greater risk of developing a subsequent disease (Dohoo and Martin, 1984; Grohn and Bruss, 1990; Goff and Horst, 1997). To the author’s knowledge, the risk of subsequent disease for cows with mastitis and inflammation of mammary

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Nutrient partitioning during mastitis

The transition from late gestation to early lactation is the most metabolically challenging stage in the life cycle of a dairy cow and, therefore, cows are at a greater risk of disease than at any other physiological state. Approximately 75% of disease incidences occur during this time for both infectious (e.g., mastitis and metritis) and noninfectious diseases (e.g., hepatic lipidosis or fatty liver and ketosis; Ingvartsen, 2006; Ingvartsen and Moyes, 2013). The transition period generally encompasses the last 2 to 3 wk of gestation to the first 2 to 3 wk of lactation (Drackley, 1999). During early lactation, the homeorhetic mechanisms associated with hormonal changes as well as changes in the digestive system, nervous system, and immune system shift the partitioning of nutrients from peripheral tissues toward the synthesis of milk and milk components (Bauman and Currie, 1980). During this time, dietary nutrient intake is insufficient to meet the nutrient demands for the synthesis of milk components and, therefore, most cows mobilize body tissues to compensate. In turn, most cows experience a period of negative energy balance. An adequate coordination in the massive repartitioning of nutrients has been identified as a major contributor to the high risk of developing disease during this period (Ingvartsen, 2006).

Infectious Diseases: Mastitis

Mastitis is defined as an inflammation of the mammary gland usually associated with the invasion of a pathogen. Mastitis is the most costly disease to the dairy industry, exceeding US$2 billion annually in the United States alone (Bar et al., 2008) and, therefore, the prevention of mastitis is critical to maintain overall animal health and profitability for dairy farmers. Mastitis is characterized by an increase in milk somatic cell count (SCC) and may be accompanied by the presence of an intramammary pathogen (Paape et al., 2003). In healthy mammary quarters, the SCC is primarily composed of resident macrophages and lymphocytes and composite milk SCC are <200,000 cells/mL. During mastitis, there is a shift in the milk SCC population where approximately 95% of the milk SCC is composed of infiltrating polymorphonuclear leukocytes (PMNL). These phagocytic cells migrate from circulation to the site of infection via a process called chemotaxis. Once at the site of infection, the main function of PMNL is to engulf (phagocytosis) and then kill invading organisms via respiratory burst. The natural immunosuppression observed for cows during the transition period is considered a high-risk period for the development of mastitis. Hence, improving immune function, especially PMNL function, is vital for controlling mastitis and improving economic outcome to the farmer.

Metabolic Diseases

The transition from late gestation to early lactation is a critical time period associated with major changes in several hormonal, digestive, neurological, and immune metabolic systems, and ultimately cows are at high risk for the development of infectious and metabolic diseases during the transition period. Hence, disease incidence relating to retained placenta, displaced abomasums, and milk fever are greatest at this time. However, the discussion of these diseases is beyond the scope of this paper, which will focus on metabolic diseases such as ketosis and hepatic lipidosis.

Cows experiencing deviations from normal changes in metabolism during the transition period may undergo in a period of severe negative energy balance, as reflected by the degree of increase in circulating NEFA and ketone bodies (e.g., β-hydroxybutyrate [BHB]) and the degree of decrease in blood glucose (Drackley, 1999). As a consequence, cows are at a greater risk of developing production diseases (such as ketosis and hepatic lipidosis) at this time that can adversely affect the health and productivity of the cow, resulting in culling or even death.

The liver’s ability to utilize NEFA and secrete triacylglycerol decreases as the severity of negative energy balance increases (Morrow, 1976). Rapid mobilization of adipose tissue resulting in increased infiltration of circulating NEFA in the liver can lead to a buildup and accumulation of triacylglycerol in the liver that may result in hepatic lipidosis. Hepatic lipidosis, or fatty liver disease, is a major problem most likely associated with cows that are overconditioned at parturition and results in dramatic decreases in feed intake, a greater incidence of downer cows, and negative health effects that may be irreversible (Bobe et al., 2004). Ketosis is primarily characterized by high concentrations of circulating BHB (>1.2 mM) along with physical signs, such as loss
of appetite, a decrease in blood pH, and decreased BW
and milk production, and it is usually associated with
excessive body condition prepartum (overconditioned)
and an increase in susceptibility to infectious diseases
(Gustafsson et al., 1993; Kremer et al., 1993; Geishauser
et al., 1998). The rate and extent of tissue mobiliza-
tion may also lead to a period of physiological imbal-
ance (PI) for individual cows. Physiological imbalance
is defined as cows whose physiological characteristics
deviate from the normal at a given physiological state
and stage of lactation (Ingvarsten and Moyes, 2013) and
that consequently have an increased risk of developing
production diseases (Moyes et al., 2013b). An index
for PI has been generated from changes in circulating
NEFA, BHB, and glucose (Moyes et al., 2013a,b) that
better reflects the mechanisms directly associated with
disease development. This index has proven to be a bet-
ter predictor of disease than calculated energy balance
or individual metabolites alone (Moyes et al., 2013b).
Identifying potential biomarkers in milk for degree of PI
is needed for early detection of “risk animals” on farm.
This paper will not focus on this topic and reviews ex-
amining the relationships between PI and disease can be
found elsewhere (Ingvarsten and Moyes, 2015).

**METABOLIC FATES
OF NUTRIENTS BY IMMUNE CELLS**

Innate or nonspecific immunity is rapidly activated
and is the primary immune defense in the initial stages
of an infection. Innate immunity includes phagocytic
cells such as PMNL and macrophages (Paape et al.,
1991). The metabolism and fates of energetic fuels by
phagocytic cells have been studied primarily using hu-
man or rodent models (Fig. 1; Newsholme et al., 1986,
1987; Calder et al., 1990). Neutrophils are the primary
phagocytic cell compromising the innate immune re-
response. These cells have a short half-life (approximately
9 h), contain few mitochondria, and utilize little oxygen.
Using radiolabeled isotopes in leukocytes (Newsholme
et al., 1986, 1987; Newsholme and Newsholme, 1989),
it was determined that very little glucose (approxima-
tely 5%) enters the tricarboxylic acid (TCA) cycle
(Fig. 1). The majority of glucose is directed toward the
pentose phosphate pathway for the generation of reduc-
ing equivalents (i.e., nicotinamide adenine dinucleotide
phosphate) required for phagocytosis or is converted
to lactate (anaerobic glycolysis). Hence, the phagocy-
tic cells primarily utilize the AA glutamine for energy
where the majority of glutamine is converted to glut-
ate and can then enter the TCA for oxidation. In con-
trast, fatty acids, such as oleate, are primarily incorpo-
rated into cellular lipids rather than oxidized for energy.

![Figure 1: Fates of 14C-labeled glucose, fatty acids (AA), and ketones by leukocytes. 1) Approximately 5% of glucose is completely oxidized via the Krebs cycle and the rest is either directed toward the pentose phosphate pathway (PPP) for the generation of nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor during respiratory burst or is converted to lactate. 2) Glutamine is the preferred AA utilized by phagocytes, with approximately 74% of glutamine being completely oxidized. 3) Fatty acids are primarily incorporated into cellular lipids, including triacylglycerols (TAG; 79%), phospholipids (18%), and cholesterol (3%) are not utilized as an energy source by phagocytes (adapted from Calder et al., 1990; Newsholme et al., 1986, 1987).](image-url)
Ketones, such as acetoacetate and BHB, are not utilized as energy sources by phagocytic cells (Newsholme et al., 1987). Using $^{13}C_6$ glucose followed by monitoring of nonessential AA, TCA cycle intermediates, and lactate in vitro, bovine blood PMNL revealed patterns similar to that observed in other studies where glucose was not the main substrate oxidized for energy in the TCA cycle and was not altered by stage of lactation (Qu et al., 2014). For cows experiencing severe negative energy balance during early lactation, nutrient availability is low and may compromise PMNL function. Characterizing the partitioning of nutrients by bovine phagocytes may lead to new management strategies to improve immune response and reduce risk of disease during early lactation.

**METABOLIC RESPONSE DURING INFLAMMATION**

Most studies have focused on how development of disease, plane of nutrition, or metabolic status alters the immune response and risk of mastitis (Curtis et al., 1985; Moyes et al., 2009b; Graugnard et al., 2012). Although the mechanistic links between metabolism and mastitis are unclear, the metabolites that characterize energy balance and PI have been directly linked to impaired immune function especially for cows during early lactation (Suriyasathaporn et al., 1999; Lacetera et al., 2002). Most studies examining the host response to inflammation mainly focus on the immune response. Yet few studies have focused on the metabolic response during inflammation and unraveling the metabolic changes during inflammation may lead to new management strategies that prevent or control mastitis and reduce risk of subsequent diseases of dairy cows during lactation. This section will review some of the current literature regarding the metabolic response in blood and tissue of dairy cows during inflammation.

**Circulating Metabolites**

The immune response to major mastitis-causing pathogens (*Escherichia coli*, *Streptococcus uberis*, and *Staphylococcus aureus*) have been well documented (Bannerman et al., 2004; Schukken et al., 2011; Ballou, 2012) but the characterization of the metabolic responses in dairy cows during an intramammary infection (IMI) are lacking. Few studies have reported changes in the metabolic response during inflammation using intramammary challenge models (Steiger et al., 1999; Graugnard et al., 2013; Moyes et al., 2014). There is evidence for increased circulating NEFA and glucose (Steiger et al., 1999; Moyes et al., 2009a) and reduced concentrations of circulating BHB during the inflammatory response. Furthermore, this response has been shown to be independent of stage of lactation (Graugnard et al., 2013; Moyes et al., 2014), metabolic status (Moyes et al., 2009a), or the inflammatory challenge model used (Steiger et al., 1999; Moyes et al., 2014). Some of the potential mechanisms that explain the changes in metabolites during inflammation are shown in Fig. 2.

One of the major challenges that occur when characterizing the metabolic and hormonal changes after an inflammatory challenge is accounting for decreases in feed intake and milk yield normally observed during the inflammatory response. Identifying the metabolic changes as well as the hormonal changes that occur during the early response, before changes in feed intake and milk production, to an invading microorganism may lead to new management strategies to improve host response and thereby control mastitis and the risk of subsequent disease. Elevated circulating NEFA are shown after inflammatory challenge of dairy cows (Steiger et al., 1999; Waldron et al., 2003; Graugnard et al., 2013), reflecting increased adipose tissue lipolysis and release of NEFA into the bloodstream during inflammation (Zu et al., 2009; Fig. 2). Although NEFA are not utilized as a primary fuel source by phagocytic cells (Fig. 1), the effect of NEFA (e.g., saturated and unsaturated) on immune response is inconclusive and poorly understood. This topic is not the focus of this paper and is discussed in other reviews (Sordillo et al., 2009; Ingvartsen and Moyes, 2013). Regardless, increased rates of adipose tissue lipolysis and circulating NEFA associated with the inflammatory response may increase risk of subsequent metabolic disease, such as hepatic lipidosis, especially during early lactation.

Most studies have reported declines in circulating concentrations of BHB during an intramammary challenge for lactating dairy cows (Steiger et al., 1999; Waldron et al., 2006; Graugnard et al., 2013). During inflammation, many factors may play a role in decreases in BHB in blood such as increased transfer into the mammary gland, increased blood glucose, reduced rumen motility, and/or impaired synthesis of BHB in the liver (Moyes et al., 2014; Fig. 2). Studies have shown that BHB is not utilized by phagocytic cells as a fuel source (Newsholme et al., 1986) and may have inhibitory impacts on the immune response, specifically including reduced trap formation, chemotaxis and phagocytosis of PMNL, and impaired lymphocyte blastogenesis (Grinberg et al., 2008; Ingvartsen and Moyes, 2013). Nevertheless, reduced circulating BHB during inflammation may be beneficial for the immune response via reducing its inhibitory effects as well as reducing risk for ketosis. Therefore, the major metabolic disease that may develop as a consequence of mastitis may be fatty liver disease rather than ketosis.
Glucose is the preferred fuel source for the generation of reducing equivalents for phagocytosis (Ingvartsen and Moyes, 2013, 2015). Elevated glucose has been observed after inflammation (Steiger et al., 1999; Moyes et al., 2009a) but it is difficult to determine whether changes in plasma glucose were associated with changes in feed intake, reduced demand for lactose synthesis in mammary gland, or a reduced uptake in peripheral tissues (skeletal muscle; Fig. 2) and warrants further investigation. Gross et al. (2014) proposed that when glucose availability is low, the mammary gland may prioritize glucose for the immune response rather than the synthesis of milk components during mastitis challenge. The mechanisms regulating glucose homeostasis during mastitis are unclear and several theories are discussed elsewhere (Moyes et al., 2014). Regardless of changes in feed intake, elevated circulating glucose and NEFA and decreases in BHB were observed for cows subjected to either a dietary-induced negative energy balance model or fed ad libitum during mid lactation (Moyes et al., 2009a). In addition, Steiger et al. (1999) observed increases in plasma glucose after intravenous infusion of lipopolysaccharide (LPS) in nonlactating heifers. Growing evidence indicates that hyperglycemia observed during inflammation associated with invasion of an intramammary pathogen may be independent of changes in feed intake and milk production in dairy cows during lactation.

Hormonal changes that occur during inflammation play pivotal roles with regards to changes in circulating metabolites. However, the hormonal response during mastitis in dairy cows should not be disregarded but is beyond the scope of this paper. Briefly, most studies examining hormonal changes during inflammation have focused on elevated plasma cortisol during inflammation and its negative impacts on the immune response, especially around parturition (Weber et al., 2006). There is growing evidence of potential insulin resistance that may occur during inflammation (Waldron et al., 2006; Moyes et al., 2009a) and may partly explain the changes in glucose concentration observed during inflammation. Elevated proinflammatory cytokines in circulation, such as tumor necrosis factor-α, after inflammatory response.
Nutrient partitioning during mastitis

The relative contribution of visceral adipose and liver tissue to alterations in metabolic and immune function during mastitis in dairy cows is complex and not fully understood and warrants further investigation.

**Immunometabolic Response of Tissue during Inflammation**

Liver and adipose are key tissues involved in dairy cattle metabolism. However, their contribution to the inflammatory response and increased risk of metabolic disease during an IMI are only partially known. Most studies investigating the host response to mastitis have focused on the mammary gland and, specifically, mammary epithelial cell response during mastitis, with very little focus on the response of peripheral tissues, especially the liver and adipose tissue. Researchers have examined the immunometabolic responses of liver and adipose tissue during inflammation at the transcriptional level (Vels et al., 2009; Mukesh et al., 2010; Jorgensen et al., 2012). During the inflammatory response, the liver secretes inflammatory mediators and acute phase proteins, and its central role in metabolism of carbohydrates, lipids (primarily NEFA from adipose tissue), AA, minerals, and vitamins (Drackley, 1999) may be compromised. Data show that key genes associated with hepatic gluconeogenesis including phosphoenolpyruvate carboxykinase 1 (PCK1; i.e., PEPCK) and glucose-6-phosphatase (G6PC) were downregulated after intramammary challenge with LPS (Vels et al., 2009) or E. coli (Jorgensen et al., 2012). Other research indicates that metabolic capacity of the liver is compromised during IMI challenge with E. coli via a downregulation of key pathways involved in the production and utilization of glucose for energy and lipid metabolism (Bionaz et al., 2014).

Visceral adipose tissue is well documented as being related to adverse lipid profiles (Ribeiro-Filho et al., 2003), insulin resistance (Carey et al., 1996), impaired glucose tolerance (Hayashi et al., 2003), and metabolic syndrome in humans (Liu et al., 2006). Without sacrificing the animal or animals, collection of different adipose tissue depots is difficult in most dairy cow experimental models; therefore, few studies have examined the effect of information on adipose response in dairy cows. Mukesh et al. (2010) observed a greater proinflammatory response to LPS by mesenteric adipose compared with subcutaneous adipose tissue from dairy cows in vitro. The relative contribution of visceral adipose and liver tissue to the metabolic and inflammatory response during an IMI and increased risk of metabolic disease during early lactation remains uncertain. Data indicate that the host immune–metabolic response during mastitis may alter peripheral tissue function and increase risk of subsequent metabolic disease for dairy cows during lactation.

**SUMMARY AND CONCLUSIONS**

Changes in the hormonal, metabolic, and immune systems that occur from the transition from late gestation to early lactation are complex. Furthermore, the metabolic response during inflammation, such as during mastitis, is poorly understood. Immune cells primarily use glucose for the generation of reducing equivalents for phagocytosis or convert glucose to lactate during anaerobic glycolysis. Immune cells primarily utilize glucose for energy whereas fatty acids are converted to cellular lipids and ketones are not utilized as an energy source. Metabolic changes that occur during the inflammatory response include increased circulating NEFA and glucose, reduced circulating BHB, and impaired hepatic function. These responses are similar regardless of stage of lactation and different physiological states. A better understanding of the complex interactions between metabolism and inflammation in blood and peripheral tissue may lead to new strategies to prevent or control mastitis and potentially reduce the risk of subsequent metabolic disease for dairy cows during lactation.

**LITERATURE CITED**


Nutrient partitioning during mastitis


