Identification of potential markers in blood for the development of subclinical and clinical mastitis in dairy cattle at parturition and during early lactation

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ABSTRACT

Our objective was to identify specific blood markers as risk factors for the development of mastitis during early lactation. We used a subset of cows from a larger experiment that consisted of a total of 634 lactations from 317 cows. Cows were of 3 breeds and ranged from parity 1 to 4. Blood samples were collected weekly from 56 d before expected calving date through 90 d in milk (DIM). Blood was analyzed for several hormones, metabolites, and enzymes, and energy intake and energy balance were calculated. Veterinary treatment records and daily composite milk somatic cell counts were analyzed and used to determine incidence and severity of mastitis in early lactation. Cows were separated into 2 groups: 1) WK0, consisting of cows that developed clinical mastitis (CM), cows that developed subclinical mastitis (SM), or cows that were healthy (H) during the first 7 DIM; and 2) EL, consisting of CM, SM, or H cows during wk 2 through 13 of lactation. Data were adjusted for numerous fixed effects (e.g., parity, breed, season, and DIM) before statistical analysis. The time of mastitis (TOM) was recorded as the DIM in which the first rise in somatic cell count was observed and was recorded as TOM = 0. The time before and after TOM was distinguished as ± n wk relative to TOM = 0. Healthy cows were paired with either a SM or CM cow and the TOM for each H cow was equal to the TOM for its paired SM or CM cow. Data from wk −1 and −2 relative to TOM were analyzed for group WK0 and EL, respectively. For all parameters, SM cows did not differ from H cows from either group. The CM cows had higher nonesterified fatty acid levels and a tendency toward higher β-hydroxybutyrate levels than H cows before mastitis for both groups. For group WK0, glucose was higher −1 wk relative to calving in CM than H cows. For group EL, aspartate aminotransferase was higher −2 wk relative to mastitis in CM than H cows during 8 to 90 DIM. All other variables were similar among CM, SM, and H cows for both groups. Our results indicate that substances in blood, especially nonesterified fatty acids and aspartate aminotransferase, may be potential markers for the risk of mastitis in early lactation.

Key words: metabolite, mastitis, dairy cattle

INTRODUCTION

The transition from late gestation through early lactation is the most metabolically challenging period in the life cycle of dairy cows. During this period, cows are at high risk for the development of metabolic disorders such as ketosis and diseases such as mastitis (Chagunda et al., 2006). Mastitis is the most costly of all diseases in the dairy industry (Bradley, 2002). Milk SCC represents the primary immune defense mechanism of the mammary gland, which consists of neutrophils, monocytes, and lymphocytes (Sordillo et al., 1997). Mastitis can be classified as subclinical or clinical depending on the presence of clinical signs. During subclinical mastitis, SCC in milk are usually elevated, milk production is decreased, inflammation occurs with or without an intramammary pathogen present, and no abnormalities in the milk or gland are visible (Sordillo et al., 1997). Clinical mastitis is characterized by an elevated SCC in milk and visual signs of inflammation such as clumpy, watery, bloody, or yellowish milk, and an intramammary pathogen may be isolated. Clinical mastitis may cause a decrease in DMI, swelling of the udder, and, in extreme cases, septicemia or endotoxemia that can cause death (Bradley, 2002). Understanding the conditions underlying development of mastitis will improve disease prevention and thereby animal welfare, which may enable cows to reach their maximum genetic potential for milk yield and increase profitability to dairy farmers.

The immunosuppression normally observed during the transition period as a result of the change in physiological state is one of the primary factors associated with the increased incidence of mastitis during early lactation (Mehrzad et al., 2001). The adaptation to lactation involves numerous endocrine and metabolic changes that lead to a state of negative energy balance.
(NEB) in support of lactation (Mallard et al., 1997). The severity of NEB during the transition period, which is characterized by the degree of increased concentrations of circulating NEFA and BHBA and the degree of decrease in glucose, may contribute to the suppression of immune system function. Several in vitro studies have reported that culture media supplemented with BHBA concentrations representative of cows with ketosis impaired immune cell functions such as neutrophil chemotaxis (Suriyasathaporn et al., 1999), respiratory burst (Hoeben et al., 1997), and lymphocyte proliferation (Franklin et al., 1991) when compared with control media. Therefore, cows experiencing severe NEB during the transition period may be at greater risk for developing mastitis than cows experiencing a more moderate state of NEB. In vivo studies examining the relationship between metabolites and hormones associated with NEB and the risk of mastitis indicate potential for their use as markers for mastitis in early lactation (Jánosi et al., 2003; Nyman et al., 2008), but results were conflicting and warrant further investigation.

During the transition period, large variations in metabolites and hormones are observed at both the herd and cow level and are associated with several fixed parameters such as genetic line, breed, parity, diet, and DIM (Drackley, 1999; Ingvartsen and Andersen, 2000). Previous work in our laboratory examined the between-animal variation for traits such as NEFA, BHBA, and glucose in order to establish future management and feeding strategies that will help prevent the incidence of diseases (Ingvartsen and Friggens, 2005). Ingvartsen and Friggens (2005) generated the individual between-cow variation after adjustments for fixed effects such as breed, parity, diet, genetic line, season, year, DIM, and their interactions. Interestingly, after adjustments for the systematic effects, the remaining individual between-cow variation was substantial. This raised 2 important questions: 1) what is the source of this variation, and 2) how does this variation relate to the risk of diseases (i.e., mastitis), reproduction, and production? The current study focused on question 2 above. Using a subset of cows from the adjusted data set provided by Ingvartsen and Friggens (2005), our objective was to use a case-control epidemiological study approach to identify specific blood markers, using several energy metabolites, minerals, enzymes, and hormones, as risk factors for the development of naturally occurring mastitis during early lactation. This case-control model was similar to previous work in our laboratory (Friggens et al., 2007b). For this study, we define case as cows that developed mastitis and control as cows that did not develop mastitis during early lactation.

Materials and Methods

Experimental Design and Animals

Data for Ingvartsen and Friggens (2005) and this study originated from a larger experiment that totaled 634 lactations within 317 cows ranging from parity 1 through 4 at the farm Ammitsbøl Skovgaard (Denmark). Descriptions of experimental design and data collection were previously provided by Nielsen et al. (2003). Briefly, data were collected from January 1996 to October 2001 from cows of 3 breeds: Danish Red (n = 104), Danish Holstein (n = 130), and Jersey (n = 83). At dry-off, 56 d before expected calving date, cows were housed in group facilities and fed a TMR consisting of low energy density [12.9 MJ of digestible energy (DE)/kg of DM] throughout the dry period. Four weeks before expected calving date, cows were moved into individual tie stalls and were placed in box stalls, if available, around the time of parturition. During lactation, cows remained in tie stalls and were randomly assigned to either the low-density TMR or a TMR consisting of normal energy density (13.6 MJ of DE/kg of DM). The TMR was based on whole-crop wheat silage and energy density was varied by substituting whole-crop wheat silage for wheat straw. Concentrates were the same for both rations. Cows were fed ad libitum to meet 5% refusals, and all cows remained on their dietary treatments until the end of their productive lives.

Production Parameters

Milk Yield and Milk Composition. Cows were milked twice daily, between 0600 and 0800 h and then again between 1600 and 1800 h. At each milking, composite milk samples were collected (Milk Meter, S.A. Christensen, Kolding, Denmark), milk yield was measured (Milk Meter, S.A. Christensen), and fat, protein, lactose, and SCC (1,000 cells/mL) were analyzed (Milk Meter, S.A. Christensen) and milk composition.

Blood Collection. Blood samples were collected via puncture of the jugular vein weekly between 1030 and 1200 h throughout the experiment at approximately −28, −21, −14, −7, 1, 3, 7, 11, 18, 25, 32, 39, 46, 53, 60, 74, 88, 130, 172, 214, 256, and 298 d relative to parturition. In addition, blood samples were collected at d 1 and 3 after parturition. For further details on blood sampling see Ingvartsen and Friggens (2005). Samples were analyzed for insulin, growth hormone (GH), triiodothyronine (T3), NEFA, BUN, aspartate aminotransferase (ASAT), cholesterol, glucose, albu-
min, and BHBA according to methods described by Mashek et al. (2001).

**Calculation of Energy Balance.** Energy balance (EBAL) was calculated based on the following equation for body energy change (EBody) from feed energy input (EFeed) and the energy requirements for milk (EMilk), lean tissue growth (EGrowth), conceptus growth (EConceptus), maintenance (EMaintenance), and activity (EActivity):

\[
E\text{Body} = E\text{Feed} - (EMilk + EGrowth
+ E\text{Conceptus} + EMaintenance + E\text{Activity}).
\]

Details of the individual calculations for the equation components are described elsewhere (Friggens et al., 2007a).

**Definition of Variables**

**Udder Health Status.** Udder health status (UH) for each cow was defined by daily composite SCC and veterinary treatment records obtained throughout the first 90 DIM. The identification of UH followed definitions according to Chagunda et al. (2006), based on International Dairy Federation (1997) guidelines, with minor modifications. Cows were classified into 2 groups: group WK0 included cows that developed mastitis ≤7 DIM, and group EL included cows that developed mastitis during early lactation (between 8 and 90 DIM).

For both groups WK0 and EL, the UH for each cow was classified as being healthy (H) or having subclinical mastitis (SM) or clinical mastitis (CM). Because of natural increases in SCC normally observed during the colostrum period, cows in group WK0 with daily composite SCC <500,000 cells/mL that were not treated for clinical mastitis within the first week of lactation were classified as H. Madsen et al. (2004) reported that quarter milk samples from H cows during the first 2 milkings after parturition did not increase above 1,000,000 cells/mL. Therefore, to account for the dilution effect observed between quarter and composite milk SCC samples (Thurmond, 1990), a threshold of 500,000 cells/mL was used for H cows within group WK0 only because composite rather than quarter milk samples were collected for this study. Within group WK0, cows with composite SCC ≥800,000 cells/mL for 3 consecutive days and not treated for clinical mastitis were classified as SM; cows that were treated for clinical signs of mastitis and had composite SCC >800,000 cells/mL for at least 3 consecutive days were classified as CM. Clinical signs, such as udder inflammation and abnormal milk (International Dairy Federation, 1997; Chagunda et al., 2006), were first identified by farm staff and then confirmed by a veterinarian. The same protocol was used to treat clinical cases of mastitis throughout the experimental period. A threshold of 800,000 cells/mL for at least 3 d (Sloth et al., 2003; Chagunda et al., 2006) was used 1) to distinguish outliers from actual increases in SCC; 2) to reduce sampling error (i.e., on-farm, laboratory, and daily individual cow variations in SCC); 3) to allow us to be confident that no overlaps existed between cows that did and did not develop mastitis; 4) to avoid using data that may have been misdiagnosed by a veterinarian; and 5) to improve prediction of mastitis (Sloth et al., 2003).

For group EL, if daily SCC were less than 100,000 cells/mL for the duration of the study period and clinical veterinary treatment for mastitis was not administered, then cows were classified as H. A threshold of 100,000 cells/mL was used for this analysis because composite rather than quarter milk samples were collected (Chagunda et al., 2006). Cows were defined as SM if daily SCC were ≥800,000 cells/mL for at least 3 consecutive days with no clinical veterinary treatment administered. Cows with SCC ≥800,000 cells/mL for at least 3 consecutive days and that were treated for clinical signs of mastitis were classified as CM. All cows within group EL did not develop subclinical or clinical mastitis during the first 7 DIM based on the criteria for WK0.

**Time of Mastitis.** The time of mastitis (TOM) was recorded based on the DIM in which the first initial increase (i.e., >100,000 cells/mL) in daily composite SCC was observed, and was recorded as TOM = 0. To clarify, for SM and CM cows in both groups WK0 and EL, composite milk SCC must have been ≥800,000 cells/mL within the first 3 d of the initial increase in SCC and remained ≥800,000 cells/mL for 3 consecutive days to be eligible for this study. Because blood markers for mastitis were collected on a weekly basis, time before and after TOM was distinguished as ±n weeks relative to TOM = 0. During wk 1 of lactation, blood samples were collected on d 1, 3, and 7, and a weekly average concentration for each blood marker was calculated. Within group EL, H cows were paired with an SM or CM cow that developed clinical or subclinical mastitis based on breed, parity, and TOM. Because H cows did not develop mastitis, the TOM for each H cow was equal to the TOM (i.e., DIM) for its respective pair that developed mastitis ≥8 DIM.

**Criteria for Data Subset**

All cows within groups WK0 and EL were included in the respective analysis based on the following criteria: 1) cows must have complete data sets from −28 to 90 DIM for production parameters such as milk SCC and
Table 1. Frequency distributions and the classification of udder health status for cows that did or did not develop mastitis during wk 1 (group WK0) or during wk 2 through 13 (group EL) of lactation

<table>
<thead>
<tr>
<th>Udder health status</th>
<th>WK0</th>
<th>EL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical mastitis (CM)</td>
<td>58 (58)</td>
<td>19 (20)</td>
</tr>
<tr>
<td>Subclinical mastitis (SM)</td>
<td>97 (97)</td>
<td>14 (14)</td>
</tr>
<tr>
<td>Healthy (H)</td>
<td>97 (110)</td>
<td>57 (62)</td>
</tr>
<tr>
<td>Total</td>
<td>199 (265)</td>
<td>85 (96)</td>
</tr>
</tbody>
</table>

1CM = for both groups WK0 and EL, cows treated for clinical mastitis and had daily composite milk SCC ≥800,000 cells/mL.
2Cows, n (lactations, n).
3SM = for both groups WK0 and EL, cows not treated for clinical mastitis and had daily composite milk SCC ≥800,000 cells/mL.
4H = for WK0, cows that were not treated for clinical mastitis and had daily composite milk SCC ≤100,000 cells/mL within wk 1 of lactation. For EL, cows that were not treated for clinical mastitis, had daily composite milk SCC ≤100,000 cells/mL, and SCC did not increase above 100,000 cells/mL for ≥3 consecutive days during wk 2 through 13 of lactation.
5Total number of cows that were classified into WK0 and EL. Total does not reflect the sum of individual udder health status categories because several cows may have been classified as H in one lactation and CM or SM in a subsequent lactation.

Statistical Analysis

Adjustments for Random and Individual Cow Variation. The first step in the preliminary analysis was to correct for variation in parameters other than UH. In addition, raw data for components in blood (except glucose and cholesterol) was log-transformed before statistical analysis. Before statistical analysis for this study, Ingvartsen and Friggens (2005) adjusted the data set for systemic variation in the parameters measured. The univariate REML procedure of SAS (2003) was used to correct for the systematic effects of breed, parity, cow, genetic selection line within breed, interassay variation, diet, calving date, days dry, DIM, year, and season, as well as the relevant interactions (e.g., breed × parity + parity × genetic line + diet × parity + breed × DIM + diet × DIM + parity × DIM + breed × parity × DIM + diet × parity × DIM + season × year) between these effects as described by Ingvartsen and Friggens (2005). This model quantified the variation between individual cows and the residual. The individual variance (i.e., cow variation) was then used for our analysis of how individual cow variation influences the risk of mastitis.

Final Analysis. Data were analyzed using the mixed linear model (i.e., PROC MIXED) of SAS (2003) with a random effect of cow within UH. Class variables included cow and UH and the model included UH. The model was used to determine potential differences in hormones, metabolites, minerals, enzymes, energy intake, and EBAL before the development of mastitis between healthy cows and cows that developed clinical or subclinical mastitis in early lactation. Statistical differences were declared as significant and highly significant at P < 0.05 and P < 0.01, respectively. Data were analyzed at wk −1 or −2 relative to the development of...
mastitis (i.e., TOM = −1 or −2) for groups WK0 and EL, respectively. For group EL, parameters −1 wk before TOM were not evaluated for this data set because of the close proximity of the sampling time (i.e., −1 to −7 d before TOM = 0) relative to mastitis, which may have affected the variation in concentrations of the parameters analyzed. The equation is as follows:

\[ Y_{ij} = \mu + UH_i + A_j + \varepsilon_{ij} \]

where \( Y_{ij} \) = dependent variable at TOM (−1 or −2 for groups WK0 and EL, respectively), for the \( j \)th cow within the \( i \)th UH (i.e., CM, SM, or H); \( \mu \) = overall mean; \( A_j \) = random effect of cow \( j \); and \( \varepsilon_{ij} \) = residual error.

**RESULTS**

**Results for Group WK0**

Table 2 shows the least squares means and standard error of the means for estimated between-cow differences for plasma components 1 wk before parturition (i.e., TOM = −1) among SM, CM, and H cows during the first 7 DIM (i.e., group WK0). Prior to parturition, plasma NEFA levels were greater in CM cows (\( P < 0.01 \)) and tended to be greater in SM cows (\( P = 0.06 \)) when compared with their healthy pairmates (i.e., H cows). Overall estimated between-cow differences in BHBA concentrations were not significant (\( P = 0.10 \)) among UH categories. However, within UH categories, between-cow differences for CM versus H indicated that CM cows had higher BHBA concentrations (\( P = 0.04 \)) than H cows before parturition. Interestingly, glucose concentrations were higher in CM cows than in H cows −1 wk relative to calving (\( P = 0.01 \)), whereas between-cow differences in glucose were not significant between SM and H cows (\( P = 0.12 \)). Despite the higher glucose concentration in CM cows compared with H cows, plasma insulin was not significantly different (\( P = 0.19 \); Table 3) between CM and H cows. Other metabolites (cholesterol, albumin, and BUN), enzymes (ASAT), hormones (insulin, T3, and GH), minerals (Ca, P, and Mg), EBAL, and energy intake were not significantly different (\( P \geq 0.14 \)) among CM, SM, and H cows within group WK0 (Tables 2 and 3).

**Results for Group EL**

To evaluate the use of components in blood as markers for the risk of mastitis during early lactation, data were analyzed −2 wk relative to TOM. Table 4 shows the between-cow differences in energy metabolites −2 wk relative to TOM among CM, SM, and H cows. Similar to group WK0 results, NEFA concentrations were higher in CM cows (\( P < 0.01 \)) than H cows 2 wk before the development of mastitis. No differences in NEFA concentrations were observed between SM and H cows (\( P = 0.41 \)) estimated between-cow differences in BHBA concentrations tended to differ among UH categories (\( P = 0.08 \)). Within UH, CM cows had higher BHBA concentrations than H cows 2 wk before TOM (\( P = 0.03 \)), but this difference was not observed between SM and H cows (\( P = 0.98 \)). When evaluating between-cow variations in BHBA concentrations, a tendency (\( P = 0.09 \)) toward higher BHBA was observed in CM cows but not in SM cows (data not shown). In contrast to results from group WK0, glucose concentrations were not significantly different among UH categories (\( P = 0.63 \)), whereas higher activities of ASAT were observed in CM cows than in H cows (\( P < 0.01 \)), but no differences were observed between SM and H cows (\( P = 0.45 \)). No differences in ASAT were observed between CM and
Our results clearly indicate that increased NEFA concentrations are related to the development of mastitis at calving (i.e., group WK0) and during early lactation (i.e., group EL). Increased NEFA concentrations are an indicator of increased lipolysis and therefore cows that developed clinical mastitis and subclinical mastitis during the first week of lactation had mobilized more body tissue energy reserves than cows that did not develop mastitis. However, the mechanisms involved in the relationships among NEFA, immune function, and susceptibility to mastitis are not well understood. Several researchers have examined the effect of high NEFA concentrations, similar to that of cows during postpartal NEB, on immune cell function in vitro. Scalia et al. (2006) observed that bovine blood neutrophils incubated with 2 mEq/L of NEFA did not have inhibited phagocytosis capabilities but that cell viability was reduced and cell necrosis and oxidative burst activity were increased when compared with cells incubated in control media without NEFA. In murine macrophages, Calder et al. (1990) showed that NEFA decreased macrophage phagocytosis capabilities by 7% in vitro. In

Table 3. Least squares means (SEM in parentheses) for estimated between-cow differences in energy balance (EBAL),1 energy intake (EI),2 and various components in blood −1 wk relative to the time of mastitis for dairy cattle that did or did not develop mastitis during wk 1 of lactation (group WK0)

<table>
<thead>
<tr>
<th>Variable</th>
<th>UH³</th>
<th></th>
<th></th>
<th>P-value</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CM</td>
<td>H</td>
<td>SM</td>
<td>Overall</td>
<td>H vs. CM</td>
<td>H vs. SM</td>
</tr>
<tr>
<td>Insulin, ln (pM)</td>
<td>−0.05 (0.04)</td>
<td>0.03 (0.03)</td>
<td>−0.03 (0.03)</td>
<td>0.19</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>GH, ln (ng/mL)</td>
<td>0.07 (0.08)</td>
<td>−0.03 (0.06)</td>
<td>−0.004 (0.07)</td>
<td>0.38</td>
<td>0.18</td>
<td>0.46</td>
</tr>
<tr>
<td>T³, pM</td>
<td>−0.02 (0.03)</td>
<td>−0.04 (0.02)</td>
<td>−0.02 (0.02)</td>
<td>0.88</td>
<td>0.70</td>
<td>0.66</td>
</tr>
<tr>
<td>EL, MJ/kg of DM</td>
<td>0.83 (3.0)</td>
<td>0.92 (2.2)</td>
<td>2.4 (2.4)</td>
<td>0.88</td>
<td>0.98</td>
<td>0.65</td>
</tr>
<tr>
<td>EBAL, MJ of EE/d</td>
<td>5.3 (3.7)</td>
<td>−0.57 (2.5)</td>
<td>4.3 (2.8)</td>
<td>0.29</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Ca, mM</td>
<td>−0.003 (0.01)</td>
<td>−0.005 (0.003)</td>
<td>0.003 (0.003)</td>
<td>0.21</td>
<td>0.71</td>
<td>0.08</td>
</tr>
<tr>
<td>Mg, mM</td>
<td>0.004 (0.003)</td>
<td>0.003 (0.003)</td>
<td>0.008 (0.003)</td>
<td>0.35</td>
<td>0.75</td>
<td>0.16</td>
</tr>
<tr>
<td>P, mM</td>
<td>−0.0002 (0.01)</td>
<td>−0.003 (0.01)</td>
<td>−0.02 (0.01)</td>
<td>0.26</td>
<td>0.89</td>
<td>0.14</td>
</tr>
</tbody>
</table>

1The calculation for EBAL was equated to body energy change based on the effective energy (EE) system (Emmans, 1994).
2EI was calculated based on individual cow DMI and the energy content of the feed components.
3UH = classification of udder health status for cows that did not develop mastitis (i.e., healthy; H) or cows that developed either subclinical or clinical mastitis during the first week of lactation; H = daily composite milk SCC ≤500,000 cells/mL and were not treated for clinical mastitis; SM = cows not treated for clinical mastitis and had daily composite milk SCC ≥800,000 cells/mL; CM = cows treated for clinical mastitis and had daily composite milk SCC ≥800,000 cells/mL.
4Growth hormone.
5Triiodothyronine.

Table 4. Least squares means (SEM in parentheses) for estimated between-cow differences in plasma components 2 wk before the time of mastitis for dairy cattle that did or did not develop mastitis during wk 2 through 13 of lactation (group EL)

<table>
<thead>
<tr>
<th>Variable</th>
<th>UH¹</th>
<th></th>
<th></th>
<th>P-value</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CM</td>
<td>H</td>
<td>SM</td>
<td>Overall</td>
<td>H vs. CM</td>
<td>H vs. SM</td>
</tr>
<tr>
<td>NEFA, ln (mEq/L)</td>
<td>0.06 (0.04)</td>
<td>−0.06 (0.02)</td>
<td>0.01 (0.04)</td>
<td>0.02</td>
<td>0.008</td>
<td>0.16</td>
</tr>
<tr>
<td>BHBA, ln (mM)</td>
<td>0.09 (0.04)</td>
<td>−0.02 (0.02)</td>
<td>−0.02 (0.05)</td>
<td>0.08</td>
<td>0.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>−0.07 (0.05)</td>
<td>−0.04 (0.03)</td>
<td>−0.001 (0.05)</td>
<td>0.63</td>
<td>0.59</td>
<td>0.51</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>−0.01 (0.14)</td>
<td>0.20 (0.08)</td>
<td>0.19 (0.17)</td>
<td>0.44</td>
<td>0.87</td>
<td>0.52</td>
</tr>
<tr>
<td>ASAT,² ln (μkat/L)</td>
<td>0.28 (0.11)</td>
<td>−0.10 (0.06)</td>
<td>0.008 (0.13)</td>
<td>0.02</td>
<td>0.005</td>
<td>0.45</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>0.39 (0.32)</td>
<td>−0.30 (0.18)</td>
<td>−0.32 (0.35)</td>
<td>0.16</td>
<td>0.06</td>
<td>0.96</td>
</tr>
<tr>
<td>BUN, ln (mM)</td>
<td>0.02 (0.02)</td>
<td>−0.12 (0.01)</td>
<td>0.002 (0.02)</td>
<td>0.34</td>
<td>0.15</td>
<td>0.57</td>
</tr>
</tbody>
</table>

¹UH = classification of udder health status for cows that did not develop mastitis (i.e., healthy; H) or cows that developed either subclinical or clinical mastitis during the first week of lactation; H = daily composite milk SCC ≤500,000 cells/mL and were not treated for clinical mastitis; SM = cows not treated for clinical mastitis and had daily composite milk SCC ≥800,000 cells/mL; CM = cows treated for clinical mastitis and had daily composite milk SCC ≥800,000 cells/mL.
²Aspartate aminotransferase; 1 katal (kat) = 1 mol/s.
addition, incubation of bovine mononuclear leukocytes (i.e., monocytes and lymphocytes) with 2.0, 1.0, or 0.5 mM of NEFA inhibited IgM secretion and DNA synthesis and decreased interferon gamma IFN-γ production (Lacetera et al., 2004). In vitro data indicate that higher circulating NEFA may inhibit immune response, leading to increased susceptibility to mastitis.

To examine this relationship in vivo, Lacetera et al. (2005) examined the association between BCS and lymphocyte function during the periparturient period. They observed that overconditioned cows (BCS >3.5 on a 5-point scale) during the prepartum period lost more BCS than thin cows (BCS <2.5) and had higher plasma NEFA concentrations during early lactation than thin or moderate-conditioned cows (2.5 ≤ BCS ≤ 3.5). Blood monocellular cells isolated from overconditioned cows at 14 and 35 DIM had lower IgM secretion than cells from thin cows and had lower IFN-γ production 7 d before parturition than thin and moderate cows. Results from our study also support the findings of Nyman et al. (2008), who evaluated the association between metabolites and Box-Cox transformed SCC in primiparous cows during the transition period. Their results showed that primiparous cows with higher NEFA concentrations prepartum as well as cows with greater changes in NEFA concentrations during the transition period had higher Box-Cox transformed SCC at first test-day milking. In another large-scale study during the transition period, Jánosi et al. (2003) observed a tendency ($P = 0.08$) toward a positive correlation between elevated NEFA concentrations at calving (1–3 DIM) and incidence of mastitis within the first 28 DIM in Holstein cows. Our results along with these from other researchers provide evidence that higher NEFA concentrations prepartum may exacerbate the immunosuppression observed during the transition period, thereby increasing the risk of mastitis in early lactation.

Results from our study indicate that higher BHBA is also associated with the development of clinical mastitis at calving as well as during early lactation. The overall effect of UH tended to be significant ($P ≤ 0.10$) within both the WK0 and EL groups. When comparing CM and H cows, this effect became significant; CM cows had higher BHBA $−1$ wk relative to parturition (i.e., group WK0) and higher BHBA $−2$ wk relative to the development of clinical mastitis between 8 and 90 DIM (i.e., group EL) than H cows. Plasma BHBA is an indicator of extended and more severe NEB (Drackley, 1999). Several studies have shown an impairment of cellular immunity with increased concentrations of ketone bodies (Franklin et al., 1991; Suriyasathaporn et al., 1999). Suriyasathaporn et al. (1999) examined the effect of physiological BHBA concentrations on the in vitro chemotaxis differential of bovine leukocytes. They reported that leukocytes incubated in culture media supplemented with high BHBA concentrations (1.6 mM), representative of cows during the postpartal period, had a reduced chemotactic differential (2.1 mm) when compared with cells incubated in control medium without BHBA (5.7 mm). Researchers have also investigated the relationship between circulating BHBA concentrations and the respiratory burst activity of bovine neutrophils (Hoeben et al., 1997). Franklin et al. (1991) studied the effects of ketone bodies, acetate,

### Table 5

Least squares means (SEM in parentheses) for estimated between-cow differences in energy balance (EBAL), energy intake (EI), and various plasma components 2 wk before the time of mastitis for dairy cattle that did or did not develop mastitis during wk 2 through 13 of lactation (group EL)

<table>
<thead>
<tr>
<th>Variable</th>
<th>CM</th>
<th>H</th>
<th>SM</th>
<th>Overall</th>
<th>H vs. CM</th>
<th>H vs. SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, ln (pM)</td>
<td>−0.03 (0.06)</td>
<td>−0.07 (0.04)</td>
<td>−0.02 (0.07)</td>
<td>0.81</td>
<td>0.65</td>
<td>0.57</td>
</tr>
<tr>
<td>GH, ln (ng/mL)</td>
<td>−0.12 (0.15)</td>
<td>−0.03 (0.09)</td>
<td>−0.10 (0.17)</td>
<td>0.85</td>
<td>0.60</td>
<td>0.72</td>
</tr>
<tr>
<td>T₃, pM</td>
<td>−0.04 (0.05)</td>
<td>0.02 (0.03)</td>
<td>−0.02 (0.06)</td>
<td>0.54</td>
<td>0.30</td>
<td>0.55</td>
</tr>
<tr>
<td>ca, mM</td>
<td>2.9 (3.6)</td>
<td>0.37 (2.0)</td>
<td>−6.1 (4.2)</td>
<td>0.25</td>
<td>0.53</td>
<td>0.17</td>
</tr>
<tr>
<td>EBAL, MJ EE/d</td>
<td>−3.8 (4.3)</td>
<td>−2.7 (2.4)</td>
<td>−5.2 (5.2)</td>
<td>0.89</td>
<td>0.38</td>
<td>0.32</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>0.99 (0.94)</td>
<td>0.63 (0.53)</td>
<td>−0.51 (1.1)</td>
<td>0.57</td>
<td>0.74</td>
<td>0.36</td>
</tr>
<tr>
<td>Ca, M</td>
<td>0.01 (0.01)</td>
<td>−0.01 (0.006)</td>
<td>−0.001 (0.01)</td>
<td>0.25</td>
<td>0.11</td>
<td>0.53</td>
</tr>
<tr>
<td>Mg, M</td>
<td>0.005 (0.01)</td>
<td>0.01 (0.006)</td>
<td>−0.0002 (0.01)</td>
<td>0.88</td>
<td>0.90</td>
<td>0.63</td>
</tr>
<tr>
<td>P₃, mM</td>
<td>−0.01 (0.02)</td>
<td>0.012 (0.01)</td>
<td>−0.015 (0.03)</td>
<td>0.57</td>
<td>0.45</td>
<td>0.38</td>
</tr>
</tbody>
</table>

1The calculation for EBAL was equated to body energy change based on the effective energy (EE) system (Emmans, 1994).
2EI was calculated based on individual cow DMI and the energy content of the feed components.
3UH = classification of udder health status for cows that did not develop mastitis (i.e., healthy; H) or cows that developed either subclinical or clinical mastitis during the first week of lactation; H = daily composite milk SCC ≤ 500,000 cells/mL and were not treated for clinical mastitis; SM = cows not treated for clinical mastitis and had daily composite milk SCC ≥ 800,000 cells/mL; CM = cows treated for clinical mastitis and had daily composite milk SCC ≥ 800,000 cells/mL.
4Growth hormone.
5Triiodothyronine.
butyrate, and glucose on in vitro bovine lymphocyte proliferation. They observed that high-serum BHBA (>6.25 mM) inhibited lymphocyte proliferation by 60% when compared with control culture media. A concentration of 6.25 mM tends to be higher than physiological circulating concentrations of BHBA observed in cows with clinical ketosis (>2.5 mM), but these results indicate that lymphocyte proliferation decreases with high concentrations of BHBA.

Recently, researchers have examined the association between elevated BHBA concentrations during the transition period and the development of mastitis during early lactation. Data from a large-scale epidemiological study indicated that cows with serum BHBA ≥1.0 mM were predisposed to mastitis within the next 4 wk of lactation (Jánosi et al., 2003). These data support our results showing positive associations between BHBA concentrations and development of mastitis in early lactation but contradict the results of Nyman et al. (2008) with primiparous cows, where they observed that the cows with elevated BHBA concentrations prepartum had lesser Box-Cox transformed SCC at first test-day milking in early lactation. The authors suggested that the increased BHBA concentrations prepartum may be associated with microbial butyrate production from ruminal carbohydrate fermentation, which is converted to BHBA in the rumen epithelium. The researchers also noted that BHBA is related to positive energy balance during late lactation and the early dry period. Although the fixed effect of parity was removed before our statistical analysis (Ingvartsen and Friggens, 2005), the conflicting results observed in primiparous cows (Nyman et al., 2008) and the combination of primiparous and multiparous cows (Jánosi et al., 2003) for the relationship between circulating BHBA, as well as other metabolites related to NEB, and risk of mastitis in early lactation warrants further investigation. To our knowledge, no researchers have evaluated differences in BHBA concentrations and other metabolites between primiparous and multiparous cows relative to the susceptibility to mastitis during early lactation.

Glucose concentrations were not significantly different between cows that did or did not develop mastitis during early lactation in our study (i.e., group EL). However, for cows that developed clinical mastitis during the first 7 DIM (i.e., group WK0), higher glucose concentrations were observed −1 wk relative to calving when compared with cows that did not develop mastitis during early lactation. Higher circulating NEFA and BHBA are usually associated with lower circulating glucose concentrations (Drackley, 1999). In healthy individuals, increases in glucose correlate with increases in insulin for glucose uptake into tissues (Ingvartsen and Andersen, 2000). For this study, CM cows had higher glucose levels than SM and H cows within group WK0, but between-cow variations in plasma insulin were not statistically significant ($P = 0.19; \text{Tables 2 and 3}$) among UH categories. Lack of difference in insulin concentrations may suggest a lower efficiency of glucose clearance from circulation for CM cows compared with SM and H cows. Jánosi et al. (2003) observed no significant differences in serum insulin and glucose during the first 1 to 3 DIM between cows that did and did not develop mastitis within the first 28 DIM. Contrary to our results, Nyman et al. (2008) observed that primiparous cows with higher circulating glucose prepartum had lesser Box-Cox transformed SCC at first test-day milking. Because our data set was adjusted for differences in metabolites caused by parity, we could conclude that prepartum glucose metabolism has an effect on risk of mastitis during early lactation, regardless of parity. Age at first calving had a significant effect on circulating metabolites where primiparous cows calving >27 mo of age had higher NEFA and BHBA concentrations and lower glucose, insulin, and BUN levels than cows <24 mo of age at first calving (Nyman et al. 2008). The conflicting results observed between our study and those of Nyman et al. (2008) indicate the need for additional investigation of the effect of parity on relationships between metabolites and risk of mastitis during early lactation.

Other researchers have observed elevated plasma insulin and glucose concentrations after *Escherichia coli* LPS infusion (Waldron et al., 2006). They concluded that the increased circulating glucose was attributable to enhanced glycolysis and gluconeogenesis and led to increased insulin concentrations, which might explain the elevated between-cow variations in glucose before calving in our study. Although the energy required to elicit an immune response in cattle remains unknown, glucose has been shown to be the preferred metabolic fuel rather than AA, ketones, or fatty acids for immune cell functions (Pithon-Curi et al., 2004). Further studies are needed, especially on the effect of parity, to establish clear relationships between circulating glucose and the risk for mastitis during early lactation.

Aspartate aminotransferase has been used to detect the extent of liver cell damage during diseased states. In dairy cows, recent studies have observed an association between increased circulating ASAT and increased incidence of fatty liver (Kalaitzikis et al., 2007) and hypocalcemia (Gelfert et al., 2007). Our results showed a significant ($P = 0.05$) positive relationship between circulating ASAT and the development of clinical mastitis in CM cows during early lactation with no differences observed between H and SM cows. This may indicate that the severity of mastitis may affect the relationship between ASAT and mastitis, although further research
is needed. Few researchers have examined the relationship between the incidence of mastitis and ASAT concentrations in dairy cows. Jánosi et al. (2003) observed no significant association between serum ASAT at 1 to 3 DIM and mastitis outbreak within the first 28 DIM. Babaei et al. (2007) observed no significant differences in milk ASAT between SM and H cows. In support of this, our results indicated no association between prepartum ASAT and the development of clinical mastitis and subclinical mastitis during the first week of lactation.

The growing information on the relationship between metabolism and risk of mastitis indicates that NEFA concentrations have the most consistent and least conflicting positive associations with development of mastitis (Jánosi et al., 2003; Nyman et al., 2008); therefore, NEFA concentrations may be the most useful as a potential marker for risk of mastitis during early lactation. Evidence also suggests a more positive relationship between BHBA concentrations and mastitis, although more studies are needed to resolve conflicting results, especially between parities. The use of glucose as a risk factor for mastitis is inconclusive because studies are contradictory and hormones, energy intake, calculated EBAL, and minerals have proven to be poor indicators for risk of mastitis in early lactation. Our results suggest that ASAT may be a better indicator for risk of clinical mastitis during early lactation rather than for subclinical mastitis. However, the low specificity of ASAT in circulation to a particular disease may make it a less useful marker for evaluating risk of mastitis during early lactation. The work of Nyman et al. (2008) and Jánosi et al. (2003) provides substantial insight into the relationship between metabolites and risk for mastitis. However, these studies relate blood metabolites to first test-day milk SCC (Nyman et al., 2008) or weekly milk SCC through the first month of lactation (Jánosi et al., 2003). Our data set was unique and powerful because the daily milk SCC records were available throughout an entire lactation and subsequent lactations. This allowed for a more precise evaluation of each cow’s udder health status during early lactation. Evaluating the relationships between circulating metabolites and hormones with risk of mastitis during the transition period has proven difficult because of effects (e.g., herd, breed, parity, DIM, and season) that are known to cause variations in these parameters. Our data originated from one herd, so environmental variations (e.g., nutrition, management, housing) were kept relatively constant and variations caused by such fixed effects as breed, parity, DIM, and season were previously removed (Ingvarsen and Friggens, 2005). Therefore, we are confident that the variation in metabolites, especially NEFA, was a result of UH status and not to other confounding effects that contribute to changes in metabolites and hormones during late pregnancy and early lactation. The use of metabolites as markers for mastitis during early lactation is promising but warrants further investigation. Our results indicated that NEFA concentrations may be a potential marker for the risk of mastitis during early lactation, but the mechanisms involved in the relationship between increased NEFA concentrations and risk of mastitis remain unknown. Future research also should investigate the use of several of these markers, such as NEFA, glucose, BHBA, and ASAT, in an index to predict risk of mastitis in order to provide farmers with new strategies to identify cows at risk for the development of mastitis.

REFERENCES


