The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance

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ABSTRACT

The objective was to examine the associations of peripartum concentrations of nonesterified fatty acids (NEFA), β -hydroxybutyrate (BHBA), and calcium with milk production in early lactation and pregnancy at the first artificial insemination (AI) across different management systems. Fifty-five Holstein freestall dairy herds located across the United States and Canada were visited weekly for blood sample collection from 2,365 cows. For each week of sampling (from wk –1 through wk 3 relative to calving) and for each metabolite, serum concentrations were dichotomized at various thresholds to identify the thresholds with the best negative associations with milk production and pregnancy at first AI. These thresholds were used to categorize the serum concentrations into higher and lower risk categories. Repeated-measures ANOVA and multivariable logistic regression were conducted for milk production and pregnancy at the first AI data, respectively, considering cow as the experimental unit and herd as a random effect. In the week before calving, serum NEFA ≥ 0.5 mEq/L, BHBA ≥ 600 μ mol/L, and calcium ≤ 2.1 mmol/L were associated with 1.6 to 3.2 kg/d milk loss across the first 4 Dairy Herd Improvement Association (DHIA) milk tests. High levels of NEFA and BHBA in wk 1 and 2 after calving (≥ 0.7 and ≥ 1.0 mEq/L for NEFA, and $\geq 1,400$ and $\geq 1,200$ μ mol/L for BHBA), and low levels of calcium (≤ 2.1 mmol/L) in wk 1, 2 and 3 after calving were associated with milk loss at the first DHIA milk test. Serum concentrations of NEFA and BHBA were not associated with pregnancy at first AI in any sampling week, whereas calcium < 2.2 to 2.4 mmol/L from wk 1 through wk 3 postpartum were associated with reduced pregnancy at first AI. In conclu-

sion, high serum concentrations of NEFA, BHBA, and low concentrations of calcium around parturition were associated with early lactation milk loss, and low calcium concentration around parturition was associated with impaired early lactation reproduction.

Key words: nonesterified fatty acid, β -hydroxybutyrate, hypocalcemia, negative energy balance

INTRODUCTION

During the transition period, defined as the period from 3 wk before to 3 wk after calving (Grummer, 1995), an increase in energy demand occurs that is caused by fetal growth and the onset of the lactation, at a time when DMI often decreases. Consequently, virtually all high-producing dairy cows go through some degree of negative energy balance (NEB) immediately following calving (Herdt, 2000). The mean duration of NEB postcalving is 45 d, with a standard deviation of 21 d (Grummer and Rastani, 2004).

The initiation of lactation alters metabolism to supply the mammary gland with the nutrients necessary for milk synthesis (Bauman and Currie, 1980). If cows are unable to alter their energy metabolism rapidly enough for milk synthesis, they either produce milk below their capability or are susceptible to metabolic disorders. Body fat stores are mobilized into the bloodstream in the form of NEFA and contribute to overall energy requirements during early lactation. Feed DMI and serum NEFA concentrations usually have an inverse relationship (Overton and Waldron, 2004). Glucose, insulin, epinephrine, norepinephrine, and growth hormone are the major controls of lipid metabolism (Bauman and Currie, 1980; Herdt, 2000). When serum insulin concentrations are reduced, lipolysis increases, releasing more NEFA into the blood. An increase in stress can stimulate increases in lipolysis through epinephrine secreted from the adrenal medulla. The liver removes a large portion of the NEFA circulating in the

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bloodstream by metabolizing NEFA into ketone bodies, such as BHBA, or re-esterifying them into triglycerides (Herdt, 2000).

Previous studies have reported associations between metabolite concentrations and milk production. Increased concentrations of ketone bodies in milk in early lactation have been associated with a reduction in milk yield (Dohoo and Martin, 1984). Duffield et al. (2009) reported early and overall lactation milk losses associated with elevations in serum BHBA in the first week after calving. Elevated serum BHBA in the second week after calving was associated with milk losses in early lactation but higher overall milk production. Recently, Ospina et al. (2010b) sampled a cohort of animals within 2 wk before calving for serum NEFA concentration and a cohort of animals within 2 wk after calving for serum NEFA and BHBA. Elevated precalving NEFA in cows and heifers, and postcalving NEFA and BHBA in cows were associated with overall milk loss. Interestingly, elevated postcalving NEFA and BHBA in primiparous animals were associated with an overall milk increase.

Negative energy balance has been linked to impaired reproductive performance. Cows with elevated NEFA within 2 wk before calving and with elevated NEFA and BHBA within 2 wk after calving are less likely to become pregnant in early lactation (Walsh et al., 2007; Ospina et al., 2010b). Reproductive performance can also be impaired due to uterine disease and delayed luteal activity, which in turn are associated with elevated levels of NEFA and BHBA (Hammon et al., 2006; Wathes et al., 2007).

The objective was to examine the associations of selected indicators of energy metabolism and calcium with milk production and pregnancy at first AI. The hypothesis was that cows with high serum NEFA and BHBA and low serum calcium concentration around parturition would have reduced milk production and decreased reproductive performance. This study is novel in assembling a representative sample from most of the major dairy regions of the United States and Canada and, in contrast to a recent large field study (Ospina et al., 2010b), it follows the same cohort of animals through the peripartum period.

MATERIALS AND METHODS

Fifty-five Holstein freestall dairy herds located across the United States and Canada were selected based on the herd managers' willingness to participate in the study. Study herds were required to be subscribed to a milk recording service (DHIA) and to have a minimum of 100 milking cows (range: 100 to 6,000 cows). Selection of herds was not random and was a convenience sample

of herds within a 2-h drive of one of the authors' study sites that the investigator expected to comply with accurate record keeping. The continent was divided into 4 regions in which herds were recruited: Midwest (MN and WI; $n = 556$ cows from 17 herds), Northeast (NY and Ontario; $n = 662$ cows from 20 herds), Southeast (FL, GA, NC, SC, and VA; $n = 494$ cows from 10 herds), and West (CA; $n = 653$ from 8 herds). The median number of cows sampled per herd was 36 (range of 21 to 91). This is the same data set used in Chapinal et al. (2011) and details on sample size calculations are reported in that paper.

Data were collected from May 2006 to January 2007. Technicians visited each herd weekly, at the same time on the same day of the week. Cows and heifers were enrolled beginning 10 d before expected calving date using lists generated from herd management software programs (DairyComp 305, Valley Agricultural Software, Tulare, CA; PCDART, Dairy Records Management Systems, Raleigh, NC), with subsequent visits in wk 1, 2, and 3 after calving. During each visit, a 10-mL blood sample was collected from the coccygeal vessels into an evacuated sterile tube without anticoagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The samples were kept chilled and allowed to clot. Blood was centrifuged and the serum was separated and stored at -20°C within 8 h of collection. Body condition score was assigned at enrollment, on a scale of 1 (thin) to 5 (obese), using increments of 0.25 (Wildman et al., 1982). Herd managers recorded disease events on a standard form. Technicians educated herd managers, through the use of verbal discussion and information sheets, on case definitions of disease events to standardize the information collected. Serum was sent to the Animal Health Laboratory, University of Guelph, for analyses of NEFA, BHBA, and total calcium concentrations. All biochemical tests were conducted on an automated analyzer (Hitachi 911 Analyzer, Randox, Laval, Quebec, Canada), using reagents supplied by Randox. Serum that was sent to Guelph from distant regions was packed in coolers to maintain -20°C and shipped by overnight courier.

Dairy Herd Improvement test-day milk production data and pregnancy at the first AI were retrieved from herds using on-farm Dairy Comp 305 (Valley Agricultural Software) or PCDART (Dairy Records Management Systems) software and assembled in an Access database (Microsoft Corp., Redmond, WA). The first 4 test-day milk production records were collected for each cow to describe approximately the first 120 d of lactation.

Data Analysis

Milk Production. Univariable analyses were first performed to assess the association between milk pro-

duction across the 4 DHIA tests and different categorical variables (region, season of calving, parity, BCS, postcalving clinical disease, and occurrence of dystocia and twins) by using a mixed model (PROC MIXED; SAS Institute Inc., Cary, NC) with repeated measures within cows (nested within herd and region) and considering herd (nested within region) as a random effect. A first-order autoregressive covariance structure was chosen among others (such as unstructured or Toeplitz), based on the lowest Akaike's information criterion. Parity was categorized into primiparous and multiparous cows. Body condition score was categorized as thin (≤ 3.0), moderate (3.25–3.75), or fat (≥ 4.0). Seasons of calving were divided into warm months (May through August) and cool months (September through January). A binary disease variable was created and coded 1 if a cow was diagnosed with at least one of retained placenta, milk fever, metritis, or displaced abomasum before 30 DIM. Variables that were not associated with milk production in the univariable analysis ($P > 0.20$) were not considered further.

For each week of sampling (from wk -1 to wk 3 relative to calving) and for each metabolite, a series of thresholds of concentrations was created, ranging from 0.2 to 1.4 mEq/L with 0.1 mEq/L increases for NEFA, from 400 to 2,000 $\mu\text{mol/L}$ with 200 $\mu\text{mol/L}$ increases for BHBA, and 1.8 to 2.6 mmol/L with 0.1 mmol/L increases for calcium. Hierarchical dummy variables were created, designating a value of 1 for the group considered at higher risk (samples at or above the threshold for NEFA and BHBA, and at or below the threshold for calcium) and a value of 0 otherwise. To maintain temporal associations between metabolites and milk production, only serum samples collected before the first DHIA milk test were considered.

Differences in milk production across the 4 DHIA tests between the high and low risk metabolite concentration groups were tested by using mixed models (PROC MIXED; SAS Institute Inc.) with repeated measures within cows (nested within herd and region) and considering herd (nested within region) as a random effect. A Toeplitz covariance structure was chosen based on the lowest Akaike's information criterion. Separate multivariable mixed models were first created for each metabolite and week of sampling, including the dichotomized metabolite (1 dummy variable tested at a time), the linear and quadratic effect of DIM at DHIA test, the categorical covariates (region, season of calving, parity, BCS, disease that occurred before the blood sample being tested was obtained, and occurrence of dystocia and twins) that were significant in the univariable analyses, and the biologically plausible 2-way interactions. Covariates and interactions were

removed from the final model if $P > 0.05$ by manual backward selection. Because disease events occurred after the precalving samples were obtained, the precalving models were built with disease included or excluded to investigate the associations of precalving metabolites with milk production, with and without knowledge of future disease events. Similar models, but without repeated measures, were used to assess differences in milk production at the first DHIA test only between the high and low risk groups, testing one dummy variable at a time. From each model, the metabolite threshold for the dummy variable having a negative β coefficient and the lowest P -value was chosen as the optimal threshold for defining the group at risk based on milk production loss. For each week of blood sampling, the selected thresholds for the 3 metabolites were combined in mixed models similar to the models described previously. These models included both the current week of interest as well as the previous weeks in postcalving models. Spearman correlation coefficients between the different selected dummy variables were calculated to avoid submitting highly correlated variables to the same model. The correlation coefficients were ≤ 0.30 in all cases. Residuals were examined after each model to verify normality and homogeneity of variances as well as to detect possible outliers and influential observations.

Pregnancy at the First AI. For each week of sampling and for each metabolite, the same series of thresholds of concentrations as described above was tested for association with pregnancy at the first AI. Initial screening of metabolite thresholds for simple associations with pregnancy at the first AI was performed with Chi-squared tests using 2×2 contingency tables. Among associations with $P < 0.05$, the highest combined sensitivity and specificity was chosen as the optimal threshold and was used to categorize the concentrations of NEFA, BHBA, and calcium into high and low risk groups. Univariable associations between categorical cow-level covariates (listed above) and pregnancy at the first AI were tested with Chi-squared tests. For each week of sampling, dichotomized metabolites concentrations, significant covariates in the univariable analysis and the biologically plausible 2-way interactions were submitted to multivariable logistic regression (PROC GLIMMIX), using a binary distribution and logit link function, and herd as a random effect. Covariates and interactions were removed from the final model if $P > 0.05$ by manual backward selection. The predicted probabilities of pregnancy were estimated from the model (LSMEANS statement). Residuals were examined after each model to detect possible outliers and influential observations.

Table 1. Descriptive data on DHIA test-day milk production (kg/d) from 1,919 Holstein cows in 45 herds in 4 regions of Canada and the United States in 2006–2007

Test-day	Samples (no.)	DIM		Milk production	
		Mean	SD	Mean	SD
1	1,842	20	11	34.8	11.4
2	1,836	52	15	40.1	10.3
3	1,699	84	19	40.1	9.7
4	1,748	117	24	38.7	9.2

RESULTS

From the 55 herds selected, 2,365 cows were sampled across the 4 study regions. Only 45 herds recorded monthly DHIA milk production data. In total, 102 cows within the 45 herds were discarded because they were sold or culled before the first DHIA milk test. As a result, a total of 1,919 cows and 7,125 DHIA test-day records were considered in the milk production analysis. A total of 60, 420, and 781 blood samples collected in wk 1, 2, and 3, respectively, from the 1,919 cows were not included in the analyses because data were missing or the samples were collected after the first DHIA milk test. Data on pregnancy at first AI were available for 48 herds only. In total, 110 cows within the 48 herds were sold or culled after calving and before pregnancy data at first AI were available. As a result, 2,069 cows were considered in the analysis. A total of 20, 23, and 79 blood samples collected in wk 1, 2 and 3, respectively, from the 2,069 cows were not included in the analyses because the data were missing.

Milk Production

Data on test-day production in the 45 herds are shown in Table 1. In the univariable analyses, no significant differences in milk production were observed across the 4 DHIA tests among regions or between the 2 calving seasons, whereas DIM at DHIA test, BCS, parity, postcalving clinical disease, and dystocia were associated with differences in milk production. Cows with BCS ≥ 4.0 in the week before calving produced 1.4 ± 0.5 kg/d more milk compared with cows with BCS ≤ 3.0 ($P = 0.006$). Multiparous cows (59% of the cows sampled) produced 8.6 ± 0.3 kg/d more milk compared with primiparous cows ($P < 0.001$). Cows with at least one disease event after calving (25.5% of the cows) produced 1.5 ± 0.4 kg/d less milk ($P < 0.001$), whereas cows with difficulties at calving (10.5% of the cows) produced 2.4 ± 0.6 kg/d less milk ($P < 0.001$). Giving birth to twins (3.2% of the cows) had no effect on milk production ($P = 0.2$).

Higher concentrations of NEFA and BHBA and lower concentrations of calcium in the week before calving

were associated with milk loss across the first 4 DHIA milk tests, when each metabolite was tested in a separate model (Table 2). The thresholds with the highest association with milk loss were NEFA ≥ 0.5 mEq/L, BHBA ≥ 600 $\mu\text{mol/L}$, and calcium ≤ 2.1 mmol/L in the week before calving. A significant parity by NEFA ≥ 0.5 mEq/L interaction ($P = 0.02$) was observed such that the association between NEFA ≥ 0.5 mEq/L and milk loss was only significant in multiparous cows. Results were very similar when only the first DHIA test was considered. The models controlling for clinical disease that occurred after calving yielded very similar coefficient estimates, and interactions between disease and precalving metabolites concentrations were not significant in any case ($P > 0.3$).

When the 3 metabolites (dichotomized at the selected optimal thresholds) were combined in the same model for wk -1 relative to calving, only BHBA ≥ 600 $\mu\text{mol/L}$ (estimate = -1.5 ± 0.4 kg/d; $P < 0.001$) and calcium ≤ 2.1 mmol/L (estimate = -2.9 ± 0.4 kg/d; $P < 0.001$) remained in the model after controlling for the effect of parity, DIM (linear and quadratic), and BCS. When only NEFA ≥ 0.5 mEq/L and BHBA ≥ 600 $\mu\text{mol/L}$ were considered in the same model, a significant NEFA by parity interaction ($P = 0.02$) was observed. When the model was stratified by parity, only BHBA (estimate = -1.7 ± 0.6 kg/d; $P = 0.03$) remained in the model in the case of primiparous cows, and both NEFA (estimate = -1.2 ± 0.5 kg/d; $P = 0.02$) and BHBA (estimate = -1.4 ± 0.5 kg/d; $P = 0.006$) remained in the model in the case of multiparous cows.

High serum concentrations of NEFA and BHBA in wk 1, 2, and 3 after calving were not associated with milk loss when all 4 DHIA tests were considered. In fact, low concentrations of these metabolites, particularly in wk 1 and 2 after calving, were associated with milk production loss over the 4 DHIA tests. All NEFA thresholds below 0.5 and 0.7 mEq/L in wk 1 and 2 after calving, respectively, and all BHBA thresholds below 800 $\mu\text{mol/L}$ in wk 1 and 2 after calving were associated with higher milk yield (i.e., cows with NEFA and BHBA concentrations at or above each of those thresholds produced more milk than cows with concentrations below the same thresholds). Calcium ≤ 2.1 mmol/L in

Table 2. Milk losses across the first 4 DHIA tests in lactation associated with serum metabolites sampled in wk -1 relative to calving for 1,919 Holstein cows¹

Variable	Proportion of animals in the high-risk group (%)	Estimate (kg of milk/d)	SE	P-value
NEFA ≥ 0.5 mEq/L ²	25	-1.6	0.5	0.001
BHBA ≥ 600 μ mol/L	26	-1.7	0.4	<0.0001
Calcium ≤ 2.1 mmol/L	7	-3.2	0.8	<0.0001

¹Each metabolite was tested in a separate model, controlling for the effect of BCS before calving, the linear and quadratic effect of DIM, parity, and the random effect of herd. Metabolite concentrations were dichotomized at different thresholds and only the threshold with the highest association with milk loss is shown.

²Parity by NEFA interaction ($P = 0.02$). The association of NEFA ≥ 0.5 mEq/L was only significant for multiparous cows.

wk 2 (8% of the cows) and wk 3 (4% of the cows) was associated with milk loss across the 4 DHIA tests of 2.6 ± 0.6 and 3.5 ± 0.9 kg/d, respectively ($P < 0.001$).

When only the first DHIA milk test was considered (Table 3), high levels of NEFA and BHBA in wk 1 and 2 after calving, and low levels of calcium in wk 1, 2, and 3 after calving were associated with milk loss when each metabolite was tested in a separate model. An interaction was found between NEFA concentration and parity in wk 1 after calving ($P = 0.04$), such that high NEFA concentrations were only associated with milk loss in multiparous cows.

When the 3 metabolite concentrations measured in wk -1 and wk 1 relative to calving (dichotomized at the selected optimal thresholds) were combined in the same model, considering only the first DHIA milk test, only calcium ≤ 2.1 mmol/L (estimate = -2.6 ± 0.5 kg/d; $P < 0.001$) measured in wk 1 remained in the model after controlling for the effect of parity and DIM

(linear and quadratic). When only NEFA and BHBA measured in wk -1 and wk 1 were considered, only BHBA $\geq 1,400$ μ mol/L measured in wk 1 remained in the model in the case of primiparous cows (estimate = -2.9 ± 1.0 kg/d; $P = 0.004$), and only NEFA ≥ 0.7 mEq/L measured in wk 1 remained in the model in the case of multiparous cows (estimate = -1.6 ± 0.6 kg/d; $P = 0.01$), after controlling for the effect of DIM (linear and quadratic).

When the 3 metabolite concentrations measured in wk -1, wk 1, and wk 2 relative to calving (dichotomized at the selected optimal thresholds) were combined in the same model, considering only the first DHIA milk test, only precalving BHBA (estimate = -1.5 ± 0.6 kg/d; $P < 0.001$) and calcium measured in wk 1 and wk 2 relative to calving remained in the model (estimate = -2.0 ± 0.6 and 3.9 ± 1.0 kg/d, respectively; $P < 0.001$) after controlling for the effect of parity, DIM (linear and quadratic), and disease that occurred before

Table 3. Milk losses at the first DHIA test in lactation associated with serum metabolites sampled in wk 1 (n = 1,858), wk 2 (n = 1,499), and wk 3 (n = 1,138) relative to calving

Metabolite	Proportion of animals in the high-risk group (%)	Estimate (kg of milk/d)	SE	P-value
Wk 1 after calving ²				
NEFA ≥ 0.7 mEq/L ³	41	-1.8	0.6	0.001
BHBA $\geq 1,400$ μ mol/L	12	-2.4	0.6	<0.001
Calcium ≤ 2.1 mmol/L	23	-2.6	0.5	<0.001
Wk 2 after calving ⁴				
NEFA ≥ 1.0 mEq/L	12	-1.7	0.8	0.03
BHBA $\geq 1,200$ μ mol/L	20	-1.5	0.6	0.01
Calcium ≤ 2.1 mmol/L	8	-4.8	1.0	<0.001
Wk 3 after calving ⁵				
Calcium ≤ 2.1 mmol/L	4	-7.1	1.6	<0.001

¹Each metabolite was tested in a separate model. Metabolites concentrations were dichotomized at different thresholds and only the threshold with the highest association with milk loss is shown.

²Controlling for the linear and quadratic effect of DIM, parity, and the random effect of herd.

³Parity by NEFA interaction ($P = 0.04$). The association of NEFA ≥ 0.7 mEq/L was only significant for multiparous cows.

⁴Controlling for the linear and quadratic effect of DIM, parity, BCS before calving, clinical diseases that occurred before blood sampling, and the random effect of herd.

⁵Controlling for the effect of parity, clinical diseases that occurred before blood sampling, and the random effect of herd.

the blood sample was collected. When only NEFA and BHBA measured in wk -1, wk 1, and wk 2 were considered, none of the metabolites remained in the model.

Pregnancy at First AI

Body condition score, postcalving clinical disease, and dystocia were associated with the odds of pregnancy at first AI in univariable analyses. Cows classified as thin or moderate BCS had lower odds of pregnancy at first AI than cows classified as fat [odds ratio (OR) = 0.54; confidence limits (CL) = 0.40, 0.73, and OR = 0.71; CL = 0.56, 0.91, for thin and moderate, respectively; $P = 0.002$]. The occurrence of postcalving clinical diseases (OR = 0.60; CL = 0.48, 0.77; $P = 0.001$) and dystocia (OR = 0.71; CL = 0.52, 0.98; $P = 0.03$) decreased the odds of pregnancy.

The initial screening showed no association between NEFA and BHBA concentrations and the odds of pregnancy at first AI. Higher calcium concentrations from wk -1 through wk 3 relative to calving were associated with increased odds of pregnancy. The optimal thresholds ranged from 2.2 to 2.4 mmol/L, with 18 to 44% of the animals at or below the threshold. Yet, the specificity associated with the optimal thresholds was low, ranging from 19 to 44%, whereas the sensitivity ranged from 63 to 87%. After controlling for parity, BCS, and disease that occurred before blood sampling, the odds of conceiving was 1.5 times higher for cows with precalving calcium >2.3 mmol/L (CL = 1.1, 2.0; $P = 0.005$), and 1.3 times higher for cows with calcium >2.2 mmol/L in wk 1, >2.3 mmol/L in wk 2, and >2.4 mmol/L in wk 3 relative to calving (CL = 1.0, 1.6; $P \leq 0.03$). The predicted probabilities of pregnancy were 26 to 29% and 35 to 36% for cows with low and high calcium serum concentrations, respectively.

DISCUSSION

This study was a large, multi-region analysis of the association of serum metabolites in the transition period with milk production and early lactation reproductive performance on commercial dairies. Although the study herds were a convenience sample, the results are likely applicable to cows in freestall dairies in general because of the variety of management conditions represented. Cows that were culled before the first DHIA test or before first AI could not be included in the respective analyses. Although such cows were likely less healthy than those remaining in the analyses, the incidence of removal from the herd in early lactation (approximately 5%) is similar to that reported for modern dairy herds (Dechow and Goodling, 2008).

Milk Production

The majority of the DHIA test-day samples were collected in the summer and fall for Northeast and Midwest herds, whereas the Southeast and Western herds were sampled from fall into early winter. The difference in season of sampling was due to available labor at the trial sites. Milk yield did not differ among regions. Other studies report differences in milk production among regions in the United States. Losinger and Heinrichs (1996) found that Western herds had a higher rolling average milk production than herds in the Midwest, the Northeast, and the Southeast. Ely et al. (2003) found that cows in the southeast produced less milk in comparison to cows in the Midwest and northeast, and they speculated these results were driven by environmental factors such as heat stress in the southeast. The majority of cows in the Southeast and Western regions being test sampled in the late fall and early winter for this study may explain why no regional difference was found.

In the current study, cows with precalving BCS ≥ 4.0 produced more milk compared with cows with BCS ≤ 3.0 . Other studies have found similar patterns of overconditioned cows producing more milk in early lactation (Domecq et al., 1997; Duffield et al., 1999). Not surprisingly, developing one or more early lactation diseases negatively affected milk production. An association of disease in early lactation with reduced milk production before diagnosis and throughout subsequent lactation has been well documented (Edwards and Tozer, 2004; Sheldon et al., 2008).

We observed a relationship of precalving NEFA with milk production at the first test and through the first 4 test-days in multiparous cows. The optimal threshold of ≥ 0.5 mEq/L for predicting a reduction in milk yield was the same as that associated with an increased risk of displaced abomasum in LeBlanc et al. (2005) and Chapinal et al. (2011). The association was very similar when disease was accounted for in the model, showing that having elevated NEFA without subsequent clinical disease was still associated with decreased milk yield. Similarly, Ospina et al. (2010b) found that precalving serum NEFA concentrations ≥ 0.33 mEq/L resulted in a decrease of 683 kg in mature-equivalent 305-d milk yield in cows and heifers sampled within 2 wk before calving in 91 freestall herds in the northeastern United States. The increase in NEFA concentration typically starts approximately 5 d before calving (LeBlanc et al., 2005), which may explain why Ospina et al. (2010b) found a lower threshold, because their sampling time-frame was larger. On the other hand, a study looking at the effect of administering propylene glycol before

calving found lower NEFA concentrations in cows that received the treatment, but no significant difference was found in milk yields (Studer et al., 1993). With only 24 cows in that study, the statistical power may not have been high enough to find a difference. We found no association in the current study between higher NEFA concentrations and milk loss in primiparous cows. This supports a similar lack of association of clinical diseases (retained placenta and metritis) with milk production in primiparous dairy cows (Dubuc et al., 2011). This result might be explained in part by the concept of homeorhesis (Bauman and Currie, 1980), such that primiparous cows that already balance demands for growth with milk production might more readily shift metabolic partitioning in response to health challenges.

To our knowledge, this is the first study to report an association between precalving BHBA and milk loss. The optimal threshold of ≥ 600 $\mu\text{mol/L}$ was slightly lower than the threshold of ≥ 800 $\mu\text{mol/L}$ associated with an increased risk of displaced abomasum in Chapinal et al. (2011). When precalving NEFA and BHBA were tested together, both stayed in the model in the case of multiparous cows, but only BHBA remained in the case of primiparous cows. This result again suggests differences in physiological response between primiparous and multiparous cows as well as a potential use of precalving BHBA as a predictor of milk loss. This finding is very interesting because, in contrast to NEFA, ketones can be easily measured in the field (Carrier et al., 2004).

Lower NEFA and BHBA concentrations in wk 1, 2, and 3 postcalving were associated with milk loss through the first 4 test-day milk samples, showing that a moderate degree of fat mobilization in early lactation may be critical to obtain high milk yields. Nevertheless, excessive magnitude or rate of mobilization of fat reserves will cause suboptimal metabolic performance and is likely an indicator of a reduced adaptive response to NEB. High concentrations of NEFA and BHBA after calving have been associated both with higher and lower overall milk production. Harrison et al. (1990) reported higher NEFA concentrations in wk 1 and 2 postcalving in high-producing cows (10,800 kg of 305-d milk yield production; 1.0 and 0.9 mEq/L NEFA for wk 1 and 2 postcalving, respectively) compared with low-producing cows (6,900 kg of 305-d milk yield production; 0.7 and 0.5 mEq/L NEFA for wk 1 and 2 postcalving). Ospina et al. (2010b) found that primiparous cows with NEFA concentrations ≥ 0.57 mEq/L and BHBA concentrations ≥ 900 $\mu\text{mol/L}$ within the first 2 wk postcalving had higher 305-d milk yield, although the relationship was reversed in the case of multiparous

cows with NEFA ≥ 0.72 mEq/L and BHBA $\geq 1,000$ $\mu\text{mol/L}$. Duffield et al. (2009) also found contradictory results: increased BHBA was associated with decreased 305-d milk production when measured in wk 1 after calving (with an optimal threshold of $\geq 1,800$ $\mu\text{mol/L}$), but with increased 305-d milk production when measured in wk 2 after calving (with an optimal threshold of $\geq 1,000$ $\mu\text{mol/L}$). Some of the controversy in the literature could be explained by differences in sampling time and by differences in the inflection point at which fat mobilization stops being beneficial depending on other metabolic and environmental conditions.

On the other hand, high levels of NEFA and BHBA in wk 1 and wk 2 postcalving were associated with milk loss at the first DHIA test, with optimal thresholds within the range of those associated with postpartum disease (LeBlanc et al., 2005; Ospina et al., 2010a; Chapinal et al., 2011) and milk loss (Duffield et al., 2009; Ospina et al., 2010b). Duffield et al. (2009) found that cows with serum BHBA concentrations $\geq 1,400$ $\mu\text{mol/L}$ in wk 1 and $\geq 2,000$ $\mu\text{mol/L}$ in wk 2 postcalving produced 1.9 kg and 3.3 kg less milk, respectively, at first DHIA test. These results suggest that high levels of NEFA and BHBA postcalving are associated with milk loss in early lactation but not necessarily with overall milk loss, which might be explained by the hypothesis that cows with a greater potential to produce milk are at greater risk of ketosis if their greater metabolic, nutritional, or management demands are not met.

No association was found between high serum concentrations of NEFA and BHBA in wk 3 and milk loss at the first DHIA test. These results are not surprising, because NEFA and BHBA concentrations peak in wk 1 and 2 after calving, respectively (Duffield et al., 1998; LeBlanc et al., 2005). This lack of association should be viewed with caution because the sample size in this study decreased substantially in wk 3 and thus, statistical power might have been too low to test the association between the metabolites sampled in that week and milk loss at the first DHIA test.

We found an association between low pre- and postcalving calcium concentrations and milk loss, although the optimal thresholds were within the reference range of 2.1 to 2.8 mmol/L for the laboratory in which the samples were measured, and within the range (1.64–2.61 mmol/L) for cows sampled in the first week after calving with no clinical disease in the transition period (Quiroz-Rocha et al., 2009). Furthermore, the percentage of animals below the threshold was very small in most of the weeks tested. Therefore, results should be interpreted with caution. It is likely that lower calcium concentrations are an indicator of inadequate DMI rather than metabolic disease.

Pregnancy at the First AI

Overconditioned cows at calving were more likely to become pregnant at the first AI. This result was unexpected and contributes to the body of variable reports on this question. A meta-analysis (López-Gatius et al., 2003) found no benefit of high BCS at calving for pregnancy at first AI, but a detrimental effect of BCS <2.5 at calving. The magnitude of loss of BCS may be a more important predictor of reproductive performance. A review has argued that cows should generally be managed to calve in lean to moderate body condition (Garnsworthy, 2008). In support of other studies, the occurrence of dystocia or clinical disease after calving decreased reproductive efficiency (Berry et al., 2007; Dubuc et al., 2011).

Contrary to our hypothesis, we found no relationship between NEFA and BHBA concentrations around calving and the odds of pregnancy at the first AI. Previous studies reported an association between reproductive efficiency in early lactation and elevated NEFA within 2 wk before calving and elevated NEFA and BHBA within 2 wk after calving (Walsh et al., 2007; Ospina et al., 2010b). Nevertheless, the underlying mechanism through which NEB in the transition period has an effect on reproductive performance some weeks later is unclear. Butler et al. (2006) attributed this delayed effect to increased risk of delayed commencement of ovulation, whereas Leroy et al. (2006) showed a direct toxic effect of NEFA and BHBA on in vitro maturation of oocytes.

An association between low pre- and postcalving calcium concentration and pregnancy at first AI was identified in the current study. Similar to the thresholds found for milk production, the optimal thresholds were within the reference range for the laboratory in which the samples were measured, and the specificity was very low. Therefore, these results should be interpreted with caution.

CONCLUSIONS

Elevated serum concentrations of NEFA and BHBA and low concentrations of calcium within 1 wk before calving were associated with milk loss through the first 4 DHIA test days. Elevated serum concentrations of NEFA and BHBA in wk 1 and 2 after calving and low concentrations of calcium in wk 1 through wk 3 after calving were associated with milk loss at the first DHIA test. Low calcium concentrations in wk -1 through wk 3 after calving were associated with lower probability of pregnancy at the first AI.

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