Evaluation of nonesterified fatty acids and β -hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases

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ABSTRACT

The objectives of this study were to 1) establish cow-level critical thresholds for serum concentrations of nonesterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA) to predict periparturient diseases [displaced abomasum (DA), clinical ketosis (CK), metritis and retained placenta, or any of these three], and 2) investigate the magnitude of the metabolites' association with these diseases within 30 d in milk. In a prospective cohort study of 100 freestall, total mixed ration-fed herds in the northeastern United States, blood samples were collected from approximately 15 prepartum and 15 different postpartum transition animals in each herd, for a total of 2,758 samples. Serum NEFA concentrations were measured in the prepartum group, and both NEFA and BHBA were measured in the postpartum group. The critical thresholds for NEFA or BHBA were evaluated with receiver operator characteristic analysis for all diseases in both cohorts. The risk ratios (RR) of a disease outcome given NEFA or BHBA concentrations and other covariates were modeled with multivariable regression techniques, accounting for clustering of cows within herds. The NEFA critical threshold that predicted any of the 3 diseases in the prepartum cohort was 0.29 mEq/L and in the postpartum cohort was 0.57 mEq/L. The critical threshold for serum BHBA in the postpartum cohort was 10 mg/dL, which predicted any of the 3 diseases. All RR with NEFA as a predictor of disease were >1.8 ; however, RR were greatest in animals sampled postpartum (e.g., RR for DA = 9.7; 95% CI = 4.2 to 22.4). All RR with BHBA as the predictor of disease were >2.3 (e.g., RR for DA = 6.9; 95% CI = 3.7 to 12.9). Although prepartum NEFA and postpartum BHBA were both significantly associated with development of clinical disease, postpartum serum NEFA concentration was most associated with the risk

of developing DA, CK, metritis, or retained placenta during the first 30 d in milk.

Key words: dairy cow, nonesterified fatty acids, β -hydroxybutyrate, disease

INTRODUCTION

Most transition dairy cows enter a state of negative energy balance (**NEB**) for 3 primary reasons: increased energy demands at parturition, decreased DMI shortly before parturition, and lagging DMI compared with energy demands due to milk production (Gerloff, 2000; Hayirli et al., 2002). The energy need of a transition cow increases from approximately 1 kg/d of glucose during late gestation to 2.5 kg/d during the first 3 wk post-calving (Reynolds et al., 2003). Because of increased energy demand and decreased DMI, the transition cow must meet this challenge by mobilizing adipose tissue.

Some degree of NEB, which can be identified by an increase in circulating concentrations of NEFA and BHBA, is expected in the transition period as the cow adjusts to new energy demands and energy intake catches up with production. Stored energy from fat is mobilized as NEFA, some of which is taken up by the liver. In the liver some NEFA are oxidized or reesterified into triglycerides that are either exported as very low density lipoproteins or stored in the liver. During the periparturient period, high rates of NEFA enter the liver and sometimes exceed the liver's capacity to secrete triglycerides as very low density lipoproteins, resulting in an accumulation of triglycerides (Drackley et al., 2001). Increased amounts of NEFA removed by the liver along with carnitine palmitoyltransferase-1 activity regulate ketogenesis and thus, BHBA production (Hegardt, 1999).

Excessive NEB (**ENEB**) had detrimental effects on the health and production of dairy cows because of the suspected relationship between energy deficiency and immunosuppression (Kehrli et al., 1989; Hammon et al., 2006; Scalia et al., 2006). Although a study in Ontario reviewed the association between ENEB and metabolic and reproductive disease in transition cows,

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that study focused on smaller, usually component-fed herds (Duffield et al., 1999).

The ability to predict at the cow level which animals are more likely to develop disease based on NEFA and BHBA concentrations might help producers prevent diseases proactively by focusing on management and nutritional strategies to prevent subclinical and clinical disease. The objectives were to 1) establish cow-level critical thresholds for NEFA and BHBA concentrations to predict key periparturient disease conditions, and 2) investigate the magnitude of the association of these metabolites with disease conditions within 30 DIM in free-stall, TMR-fed herds in the northeastern United States. The periparturient conditions investigated were 1) displaced abomasum (**DA**), 2) clinical ketosis (**CK**), 3) either retained placenta (**RP**) or metritis (**MET**) or both, or 4) any of these 3 disease conditions.

MATERIALS AND METHODS

Study Population and Study Design

A prospective cohort study was conducted from a convenience sample of 104 dairy herds in New York, Pennsylvania, and Vermont between January 2006 and July 2007. Inclusion criteria for herds were 1) >250 milking cows, 2) free-stall housing, 3) fed a TMR, and 4) participated in DHIA or used Dairy Comp 305 (version 2009; Valley Ag Software, Tulare, CA) or both.

A convenience sample of apparently healthy heifers and cows in the transition period were selected. At the time of sample collection, healthy heifers or cows were defined as not being in the sick pen, not currently receiving any medical treatment, and not displaying sick cow behavior based on the subjective interpretation of the research staff. At sample collection, 2 cohorts of animals were identified: those 14 to 2 d prepartum and those 3 to 14 d postpartum. In each herd, cross-sectional sampling of approximately 15 animals from each group was done to achieve approximately 90% confidence of within-herd prevalence. To reflect common herd demographics, approximately one-third of the animals sampled were primiparous (both before and after first parturition).

Animals were followed through 30 DIM for incidence of the selected periparturient diseases. In the cohort sampled prepartum, the diseases of interest were DA, CK, and MET or RP, a combination of RP and MET, and any combination of the 3. In the cohort sampled postpartum, diseases of interest were DA, CK, MET, and any combination of these 3. Metritis and RP were evaluated as one disease in animals sampled prepartum because of possible misclassification of metritis. Retained placenta was not evaluated in the cohort

sampled postpartum because cows sampled at 3 to 14 DIM were no longer at risk for RP.

Farm Survey and Case Definitions

Efforts were made to limit differences between farms on all levels of data collection. All farmers received a standardized consent form, survey, and case definitions for diseases of interest. All farmers consented to participate, and the study was approved by the Cornell University Institutional Animal Care and Use Committee. The survey included demographic information, and feeding times in relation to blood collection (Eicher et al., 1999) to be used as potential risk factors. Farm personnel were instructed to document any incident cases of diseases both on the farm survey and in Dairy Comp 305. Specifically, they were to document cases of DA, CK, and MET or RP.

For consistency of documentation, the diseases were defined and case definitions were provided to farm personnel: 1) DA = movement of the fourth compartment of the stomach to a location on the right (**RDA**) or left side (**LDA**) of the cow and detected by auscultating a “ping” sound with finger percussion. Often, a cow had an abrupt decrease in milk production and was off feed; 2) CK = cow that was off feed, had sudden weight loss, and decreased milk production, but had no other detectable signs of disease and was treated with dextrose, propylene glycol, steroids, or a combination (Duffield et al., 1999); 3) MET = sick cow (dull, decreased milk yield) that had a fever greater than 39.5°C with a fetid (purulent or red to brown color or both) discharge from the vulva and was <21 DIM (Sheldon et al., 2006); and 4) RP = failure to expel fetal membranes within 24 h after calving.

Blood Collection and Analysis

Blood samples were collected from each cow and BCS were assigned (Ferguson et al., 1994) concurrently with blood sample collection. Guidelines for blood collection and sample handling were based on Stokol and Nydam (2005). Briefly, a plain evacuated red-top tube was used to collect 10 mL of blood from the coccygeal vein or artery. The blood was stored in a cooler (4°C), separated from cells within 24 h, and analyzed at the Cornell Animal Health Diagnostic Center (Ithaca, NY) within 48 h of collection. All samples were analyzed using an automated wet chemistry analyzer (Hitachi 917, Roche Diagnostics, Indianapolis, IN). The sera from the prepartum cohort were analyzed for NEFA (NEFA-C, Wako Chemicals USA Inc., Richmond, VA) and hemolysis (Stokol and Nydam, 2006). The sera from animals sampled postpartum were analyzed for

NEFA, BHBA (β -HB, Catachem Inc., Bridgeport, CT), and hemolysis.

Individual animal observations were excluded for the following reasons: the sample was severely hemolysed (Stokol and Nydam, 2006) or day of sample collection was out of sampling range for inclusion; for example, animals that were >14 d prepartum when sampled or >14 DIM at the time of sample collection.

Statistical Analysis

Multivariable Analysis. Concentrations of NEFA and BHBA were the main risk factors of interest in the evaluation of the development of any combination of the diseases. At this level of analysis, the metabolites NEFA and BHBA were treated as continuous variables. The other covariates considered were parity, season, BCS, time of blood collection in relation to feeding, and all biologically plausible 2-way interactions. They were modeled with PROC GENMOD using a Poisson distribution, a log link function, p-scale option for over-dispersion, and an exchangeable correlation matrix (Spiegelman and Hertzmark, 2005). There was no adjustment for varying time spans (offset term) because the length of the time interval was the same for every individual in the sample (Allison, 1999). This statistical method allowed for clustering of cows within herds (i.e., including herd as a random effect) while adjusting for continuous or categorical covariates.

Three full models (1 for animals sampled prepartum and 2 for animals sampled postpartum) were evaluated to predict the development of any combination of the diseases. The models were 1) prepartum NEFA, covariates, and biologically plausible 2-way interactions between main effects and covariates, 2) postpartum NEFA, BHBA, covariates, and 2-way interactions, and 3) BHBA, covariates, and 2-way interactions in animals sampled postpartum. β -Hydroxybutyrate is easily measured on dairy farms using point-of-care analyzers; therefore, its effect was evaluated in a model without NEFA. Covariates and interactions that were not significant at $P > 0.10$ were removed by manual backward stepwise elimination.

Receiver Operator Characteristic Analysis for Critical Thresholds. The continuous variables that remained in the final multivariable model were evaluated with receiver operator characteristic (ROC) analysis to determine a critical threshold for predicting disease. The ROC curves analyze sensitivity versus 100 – specificity. Sensitivity was the proportion of animals diagnosed with disease that were above a given metabolite threshold, and specificity was the proportion of animals that did not have the diseases that was below a given threshold (Greiner et al., 2000). The point on the

ROC curve that had the highest combined sensitivity and specificity was considered the critical threshold. Interpretation of this critical threshold was based on the area under the curve (AUC) such that if the AUC = 0.5 it was noninformative; if $0.5 < \text{AUC} \leq 0.7$, it was accurate; if $0.7 < \text{AUC} \leq 0.9$, it was very accurate; if $0.9 < \text{AUC} < 1$, it was highly accurate; and if AUC = 1, then it was considered perfect (Swets, 1988). The significant predictor variables from the multivariable analysis were analyzed using ROC curves to determine the critical thresholds for both individual diseases and any combination of those diseases.

Likelihood ratios (LR) were evaluated. The LR positive (LR+) was the probability that a test result at or above the threshold would be more likely to come from an animal later diagnosed with disease.

Measures of Association. Risk ratios (RR) were modeled with PROC GENMOD, with a Poisson distribution, a log link function, p-scale option for over-dispersion adjustment, and an exchangeable correlation matrix (Spiegelman and Hertzmark, 2005). At this level of analysis the significant covariates, NEFA and BHBA, were treated as categorical variables based on the thresholds from ROC analysis.

Statistical analyses of data were conducted using SAS version 9.1 (SAS Institute, 2004), and ROC curves were obtained using MedCalc version 9.5.2.0 (Schoonjans, Mariakerke, Belgium). All data were stratified based on prepartum or postpartum status at time of sample collection.

RESULTS

Descriptive Data

Of the 104 herds, 4 were excluded from the study because of missing data. There were 2,758 cows from the remaining 100 herds included and of these cows, 1,440 were sampled prepartum (35% heifers and 65% cows) and 1,318 were sampled postpartum (37% heifers and 63% cows). The number of milking cows per herd ranged from 265 to 2,770, with a median of 767 and mean of 840.

In animals sampled prepartum, the lactational incidence of each disease condition was as follows: DA, 3.3%; CK, 7.0%; MET or RP, 12.1%; and any combination of these 3 conditions, 19.6%. In animals sampled postpartum, the lactational incidences were as follows: DA, 3.1%; CK, 4.6%; MET, 2.9%; and any combination, 9.0%. The median DIM at diagnosis for diseases in animals sampled prepartum were DA 10, CK 7, MET 5, and RP 2. The median DIM at diagnosis for diseases in animals sampled postpartum were DA 15, CK 11, and MET 9.

The median BCS for heifers sampled prepartum was 4 (range: 2 to 4.75) and for cows 3.5 (2 to 4.5). The median BCS for heifers sampled postpartum was 3.5 (2 to 4.5) and for cows 3.25 (1.75 to 4.5).

Multivariable Analysis

In the multivariable model for animals sampled prepartum, NEFA ($P = 0.03$) was the only predictor retained in the model, and there were no interactions with $P < 0.16$. In the postpartum multivariable model with NEFA ($P = 0.0005$) and BHBA ($P = 0.29$), NEFA was the only predictor retained. When, in a third model, BHBA ($P = 0.005$) was modeled as the main effect without NEFA, it was the only predictor retained and no interactions had a $P < 0.16$.

Critical Thresholds

Nonesterified fatty acids and BHBA were the only significant predictors identified in the multivariable models. They were then analyzed with ROC curves to determine the cow-level critical thresholds (combined highest sensitivity and specificity) to predict individual diseases as well as any combination of the diseases. Tabular results of ROC curve determination of critical NEFA (mEq/L) thresholds for the prediction of individual diseases as well as the combination of diseases are in Table 1. In summary, for the prediction of the development of any disease, the prepartum NEFA critical threshold was 0.29 mEq/L and the postpartum NEFA threshold was 0.57 mEq/L. Figure 1 is a graphical example of a ROC curve with NEFA as a predictor of DA in animals sampled postpartum. As a test for the prediction of DA, additional information on various levels of NEFA are in Table 2, showing sensitivity, specificity, and LR for various levels of prepartum and postpartum NEFA used for comparison with other studies and to provide additional information to readers.

Results for BHBA concentrations (mg/dL) are reported similarly. Table 3 identifies the critical BHBA thresholds in animals sampled postpartum when predicting individual diseases as well as any of the diseases. Briefly, the critical threshold for predicting any disease was 10 mg/dL. Table 4 provides additional information on various levels of BHBA as a test for prediction of DA.

Likelihood ratios were calculated based on critical thresholds determined by univariable ROC analysis and reported in Tables 1 to 4. In general, the LR+ reports the probability that a test result at or above a given threshold will have a greater chance of coming from an animal later diagnosed with disease. For example, the postpartum NEFA optimal threshold for predicting

Table 1. Receiver operator characteristic curve determination of critical NEFA thresholds as predictors of disease in transition dairy cows

Disease ¹	Critical threshold ²	Se ³	95% CI for sensitivity	Sp ⁴	95% CI for specificity	LR+ ⁵	AUC ⁶	P-value
Animals sampled prepartum (n = 1,440)								
DA	0.27	57	42 to 72	62	60 to 65	1.5	0.6	0.01
CK	0.26	53	43 to 64	61	58 to 64	1.4	0.6	0.001
MET, RP, or both	0.37	37	30 to 45	80	78 to 83	1.9	0.6	0.0001
Any 3	0.29	48	42 to 54	69	67 to 72	1.6	0.6	<0.0001
Animals sampled postpartum (n = 1,318)								
DA	0.72	80	65 to 91	73	70 to 75	3.0	0.8	<0.0001
CK	0.57	74	61 to 84	59	57 to 62	1.8	0.7	<0.0001
MET	0.36	97	86 to 100	30	28 to 33	1.4	0.6	0.009
Any 3	0.57	75	66 to 82	61	58 to 64	1.9	0.7	<0.0001

¹DA = displaced abomasum; CK = clinical ketosis; MET = metritis; RP = retained placenta; prepartum any 3 = DA, CK, MET or RP; postpartum any 3 = DA, CK, or MET.

²Highest combined specificity (Sp) and sensitivity (Se), mEq/L.

³Se = epidemiologic sensitivity.

⁴Sp = epidemiologic specificity.

⁵Likelihood ratio positive.

⁶AUC = area under the curve.

Table 2. Additional information on thresholds from receiver operator characteristic curves for NEFA concentrations as predictors of displaced abomasum

Threshold (mEq/L)	Sensitivity	95% CI for sensitivity	Specificity	95% CI for specificity	LR+ ¹
Animals sampled prepartum (n = 1,440)					
0.2	64	49 to 73	48	45 to 51	1.2
0.27 ²	57	42 to 72	62	60 to 65	1.5
0.4	30	17 to 45	82	80 to 84	1.7
0.5	23	12 to 38	89	87 to 91	2.2
Animals sampled postpartum (n = 1,318)					
0.4	95	83 to 99	39	37 to 42	1.6
0.72 ²	80	65 to 91	73	70 to 75	3.0
1.02	59	42 to 74	87	85 to 89	4.6

¹Likelihood ratio positive.²Highest combined sensitivity and specificity.

DA was 0.72 mEq/L; this resulted in 3.0 LR+. The interpretation is that a NEFA test value at or above this threshold (0.72 mEq/L) was 3 times as likely to come from a cow that was later diagnosed with DA.

Measures of Association

Risk ratios were calculated using multivariable modeling after critical thresholds were determined in ROC

analysis (Tables 5 and 6). All RR were significant and >1.8. In the cohort sampled prepartum, all risk ratios were >1.8 (range: 1.8 to 2.2; Table 5), meaning, for example, that the risk of getting any of the diseases later was at least 1.8 times greater in cows with a NEFA concentration higher than the threshold value (0.29 mEq/L). In the cohort sampled postpartum, all NEFA risk ratios were >4.4 (range: 4.4 to 17). An example of one of the larger risk ratios is the one associated with developing a DA, where cows with postpartum NEFA ≥ 0.72 mEq/L were almost 10 times more likely to develop a DA within 30 DIM. Risk ratios based on BHBA concentrations were significant and >2.3 (2.3 to 6.9, Table 6) meaning that there was a higher risk of developing disease if animals had a BHBA level higher than the threshold.

DISCUSSION

The analytical approach used to examine the association between both pre- and postpartum NEFA and postpartum BHBA and the development of several diseases in transition cows allowed reporting the RR directly rather than approximating it with odds ratios. The ROC curves were used to determine the critical thresholds of the metabolites as predictors of diseases in TMR-fed, free-stall herds milking an average of 840 cows. The results generally support other reports of transition cow disease: elevated NEFA and BHBA concentrations were associated with the development of the diseases of interest (DA, CK, MET or RP). This association was reported by Dohoo and Martin (1984) who examined the association between subclinical ketosis and production and disease. Cameron et al. (1998) and LeBlanc et al. (2005) explored this association with DA as the outcome and determined that elevated metabolite levels were predictive of disease. Duffield et al. (2002) reported that improved energy metabolism reduced the incidence of DA and RP, confirming the

Figure 1. Receiver operator characteristic (ROC) curve (solid line) that determines the critical threshold (upper left corner, $\square$) for NEFA concentrations (≥ 0.72 mEq/L) predicting displaced abomasum in animals sampled postpartum. The dotted curves represent the 95% confidence interval for the ROC curve. The diagonal line represents the sensitivity and specificity level at which the test is noninformative.

Table 3. Receiver operator characteristic curve determination of critical BHBA thresholds as predictors of disease in transition dairy cows in animals sampled postpartum (n = 1,318)

Disease ¹	Critical threshold ²	Se ³	95% CI for Se	Sp ⁴	95% CI for Sp	LR+ ⁵	AUC ⁶	P-value
DA	10	71	55 to 84	80	77 to 82	3.5	0.8	<0.0001
CK	10	57	44 to 70	80	78 to 82	2.8	0.7	<0.0001
MET	7	63	46 to 78	59	56 to 61	1.5	0.6	0.03
Any 3	10	57	47 to 66	82	79 to 84	3.1	0.7	<0.0001

¹DA = displaced abomasum; CK = clinical ketosis; MET = metritis; any 3 = DA, CK, or MET.

²Highest combined specificity and sensitivity (mg/dL).

³Se = epidemiologic sensitivity.

⁴Sp = epidemiologic specificity.

⁵Likelihood ratio positive.

⁶AUC = area under the curve.

association between the 2 factors. Kaneene et al. (1997) and Oetzel (2004) reported similar relationships.

Concentrations of NEFA and BHBA were the main risk factors for predictors of disease after controlling for the random effect of herd, parity, season, BCS, sample collection time before feeding, and all biologically plausible 2-way interactions. There were no significant interactions between the main effects and other covariates. Neither BCS nor its interaction with metabolites was significant. This is not surprising because it was the loss of body condition that was most closely related to problems with ENEB (Busato et al., 2002; Kim and Suh, 2003). β -Hydroxybutyrate concentrations fluctuate throughout the day and are generally highest 4 to 5 h after feeding, whereas NEFA concentrations are generally highest before feeding (Oetzel, 2004). Previous studies show that NEFA is less sensitive to time of sample collection, whereas BHBA is more sensitive (Eicher et al., 1999). In this study, the interaction between the metabolites NEFA and BHBA and the timing of blood collection relative to feeding were not significant. Days in milk or number of days before parturition were not included as covariates because the objective was to identify the critical threshold for the specified time frame, not describe the difference in metabolite levels based on DIM or days until parturition.

In general, LR+ increased as metabolite levels increased. Biologically this is logical because circulating NEFA and BHBA serum concentrations can be used as markers of energy status. Therefore, levels above a given threshold should have a positive relationship with the probability of developing disease (i.e., higher metabolite levels are more predictive of disease). The LR related to DA and prepartum NEFA (LR = 1.5), postpartum NEFA (LR = 3), and BHBA (LR = 3.5) supported results from other studies. For example, LeBlanc et al. (2005) reported that a prepartum NEFA test result ≥ 0.5 mEq/L was twice (LR = 1.9) as likely to come from a cow later diagnosed with LDA as from one without an LDA; similarly they reported that a postpartum NEFA test result ≥ 1.0 mEq/L was 3.5 times as likely to come from a cow later diagnosed with an LDA. In Duffield et al. (2009), test results of BHBA concentration ≥ 12 mg/dL measured 1 wk postpartum were 1.87 times as likely to come from a cow later diagnosed with LDA. Yet, in comparison to LR where there was a high prior probability of having the condition in question, these LR were low.

Risk ratios were reported as measures of association between the metabolites (NEFA or BHBA) and disease outcomes. An RR >1 indicated that animals with metabolite levels above the critical threshold (exposed

Table 4. Additional information on thresholds from receiver operator characteristic curves for BHBA concentrations as predictors of displaced abomasum in animals sampled postpartum (n = 1,318)

Threshold (mg/dL)	Sensitivity	95% CI for sensitivity	Specificity	95% CI for specificity	LR+ ¹
8	76	60 to 88	69	67 to 72	2.5
10 ²	71	55 to 84	80	77 to 82	3.5
12	63	47 to 78	86	84 to 88	4.6
14	51	35 to 67	90	88 to 91	4.9

¹Likelihood ratio positive.

²Highest combined sensitivity and specificity.

Table 5. Risk ratios (RR) of disease based on NEFA critical thresholds derived from receiver operator characteristic curve analysis

Disease ¹	Critical threshold (mEq/L)	Estimate	SE ²	P-value ³	RR	95% CI ⁴
Animals sampled prepartum (n = 1,440)						
DA	0.27	0.68	0.32	0.03	2.0	1.1 to 3.7
CK	0.26	0.56	0.18	0.001	1.8	1.2 to 2.5
MET, RP, or both	0.37	0.78	0.17	<0.0001	2.2	1.6 to 3.0
Any 3	0.29	0.56	0.12	<0.0001	1.8	1.4 to 2.2
Animals sampled postpartum (n = 1,318)						
DA	0.72	2.3	0.43	<0.0001	9.7	4.2 to 22
CK	0.57	1.6	0.41	<0.0001	5.0	2.3 to 11
MET	0.36	2.8	1.1	0.008	17	2 to 134
Any 3	0.57	1.5	0.26	<0.0001	4.4	2.6 to 7.3

¹DA = displaced abomasum; CK = clinical ketosis; MET = metritis; RP = retained placenta; prepartum any 3 = DA, CK, or MET or RP; postpartum any 3 = DA, CK, or MET.

²SE = standard error for estimate.

³P-value reported for estimate.

⁴CI for RR.

group) were at higher risk for development of the disease than the animals below the critical threshold (unexposed group). When NEFA was evaluated as predictor of DA in animals sampled postpartum, the risk ratio was 9.7; that is, animals sampled between 3 to 14 DIM with a NEFA level ≥ 0.72 mEq/L were approximately 10 times more likely to develop a DA than animals below this threshold. In general, postpartum NEFA concentrations resulted in the largest RR compared with those reported for prepartum NEFA and BHBA in this and other studies (Kaneene et al., 1997; Cameron et al., 1998; LeBlanc et al., 2005). When BHBA was evaluated as the main predictor of disease, all RR were significant.

The critical thresholds at which the metabolites were predictive of disease were lower than in previous reports. Some previous studies sampled over different time frames and focused on different populations, often smaller, component-fed herds. LeBlanc et al. (2005) sampled cows 10 to 4 d prepartum, in small, often component-fed herds and reported that the critical threshold for predicting an LDA with NEFA was at ≥ 0.5 mEq/L, with an odds ratio of 3.6. They found that

animals sampled up to 1 wk postpartum with NEFA ≥ 1 mEq/L yielded an odds ratio of 4.8; and a BHBA concentration of ≥ 12 mg/dL resulted in an odds ratio of 8. Kaneene et al. (1997) did not report a critical value for NEFA or BHBA, but animals sampled postpartum (3 to 35 DIM) presented a probable association between metabolic events associated with energy insufficiency and the risk of MET and RP. In high-producing Michigan dairies, Cameron et al. (1998) sampled animals 3 to 35 d prepartum and found that animals with NEFA > 0.3 mEq/L were twice as likely to develop an LDA. Sampling from the first week postpartum, Geishauser et al. (2000) reported that the odds of developing a LDA were 4 times higher in animals with BHBA levels ≥ 14 mg/dL. They reported that if BHBA was at this level in the second week postpartum, the odds were 8:1 that animals would develop an LDA.

There were some limitations to this study including possible disease misclassification and loss to follow-up. Although several steps were taken to prevent disease misclassification (e.g., case definitions and careful monitoring), MET may not have been properly diagnosed in all groups. Metritis can be difficult to diag-

Table 6. Risk ratios (RR) of disease based on postpartum BHBA critical thresholds derived from receiver operator characteristic curve analysis in animals sampled postpartum (n = 1,318)

Disease ¹	Critical threshold (mg/dL)	Estimate	SE ²	P-value ³	RR	95% CI ⁴
DA	10	1.9	0.32	<0.0001	6.9	3.7 to 12.9
CK	10	1.6	0.21	<0.0001	4.9	3.2 to 7.3
MET	7	0.85	0.41	0.04	2.3	1.1 to 5.2
Any 3	10	1.5	0.18	<0.0001	4.4	3.1 to 6.3

¹DA = displaced abomasum; CK = clinical ketosis; MET = metritis; any 3 = DA, CK, or MET.

²SE = standard error for estimate.

³P-value reported for estimate.

⁴CI for RR.

nose, especially if it coincides with RP. Cases of CK may have been misclassified because ketone levels were not directly measured. Loss to follow-up is a limitation inherent to prospective cohort studies. A small degree of loss to follow-up was experienced because cows from 4 farms were excluded due to missing cow disease information. At the cow level in the postpartum cohort, cows that were sick at time of sample collection were not eligible to be part of the study. This may have influenced the median DIM at disease diagnosis. In addition, as many cows as intended were not sampled in all herds for several reasons: smaller herds did not have enough eligible cows at time of sampling and some samples were discarded because of hemolysis.

Postpartum NEFA had a higher association with the development of disease than did prepartum NEFA or postpartum BHBA as reflected by larger RR. This association suggested that the energy status as measured by NEFA from animals sampled postpartum (3 to 14 DIM) may have a more direct association with the development of disease than their energy status measured by BHBA or prepartum NEFA. When compared with prepartum NEFA, time of sample collection may play a role: samples collected postpartum were temporally much closer to the disease event and were perhaps better able to predict this event. Postpartum NEFA concentration as a predictor of disease has not been investigated as thoroughly as BHBA concentration or prepartum NEFA concentration. The AUC values from ROC analysis coupled with larger risk ratios suggested that postpartum NEFA could be used similarly to BHBA and prepartum NEFA.

CONCLUSIONS

The effects of elevated concentrations of NEFA and BHBA in the transition period predicted clinical disease (e.g., DA, CK, MET, or RP) in cattle from TMR-fed northeastern US free-stall dairies with an average of 840 cows. The following cow-level critical thresholds should be considered general guidelines for monitoring cattle: NEFA concentrations ≥ 0.3 mEq/L for cattle 14 to 2 d prepartum; and NEFA concentrations ≥ 0.6 mEq/L and BHBA ≥ 10 mg/dL for cattle 3 to 14 d postpartum. Both pre- and postpartum NEFA concentrations and BHBA concentrations above these critical thresholds were associated with increased risk for subsequent disease.

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REFERENCES

- Allison, P. D. 1999. Logistic Regression Using the SAS System: Theory and Application. SAS Institute Inc., Cary, NC.
- Busato, A., D. Faissler, U. Kupfer, and J. W. Blum. 2002. Body condition scores in dairy cows: Associations with metabolic and endocrine changes in healthy dairy cows. *J. Vet. Med. A* 49:455–460.
- Cameron, R. E., P. B. Dyk, T. H. Herdt, J. B. Kaneene, R. Miller, H. F. Bucholtz, J. S. Liesman, M. J. VandeHaar, and R. S. Emery. 1998. Dry cow diet, management, and energy balance as risk factors for displaced abomasum in high-producing dairy herds. *J. Dairy Sci.* 81:132–139.
- Dohoo, I. R., and S. W. Martin. 1984. Subclinical ketosis: Prevalence and associations with production and disease. *Can. J. Comp. Med.* 48:1–5.
- Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long chain fatty acid metabolism in liver of dairy cows during the periparturient period. *J. Dairy Sci.* 84(E Suppl.):E100–E112.
- Duffield, T., R. Bagg, L. DesCoteaux, E. Bouchard, M. Brodeur, D. DuTremblay, G. Keefe, S. LeBlanc, and P. Dick. 2002. Prepartum monensin for the reduction of energy associated disease in postpartum dairy cows. *J. Dairy Sci.* 85:397–405.
- Duffield, T. F., K. E. Leslie, D. Sandals, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1999. Effect of a monensin-controlled release capsule on cow health and reproductive performance. *J. Dairy Sci.* 82:2377–2384.
- Duffield, T. F., K. D. Lissemore, B. W. McBride, and K. E. Leslie. 2009. Impact of hyperketonemia in early lactation dairy cows on health and production. *J. Dairy Sci.* 92:571–580.
- Eicher, R., A. Liesegang, E. Bouchard, and A. Tremblay. 1999. Effect of cow-specific factors and feeding frequency of concentrate on diurnal variations of blood metabolites in dairy cows. *J. Vet. Res.* 60:1493–1499.
- Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703.
- Geishauser, T., K. Leslie, and T. Duffield. 2000. Metabolic aspects in the etiology of displaced abomasum. *Vet. Clin. North Am. Food Anim. Pract.* 16:255–265.
- Gerloff, B. J. 2000. Dry cow management for the prevention of ketosis and fatty liver in dairy cows. *Vet. Clin. North Am. Food Anim. Pract.* 16:283–292.
- Greiner, M., D. Pfeiffer, and R. D. Smith. 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Prev. Vet. Med.* 45:23–41.
- Hammon, D. S., I. M. Evjen, T. R. Dhiman, J. P. Goff, and J. L. Walters. 2006. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet. Immunol. Immunopathol.* 113:21–29.
- Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. *J. Dairy Sci.* 85:3430–3443.
- Hegardt, F. G. 1999. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase: A control enzyme in ketogenesis. *Biochem. J.* 338:569–582.
- Kaneene, J. B., R. Miller, T. H. Herdt, and J. C. Gardiner. 1997. The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. *Prev. Vet. Med.* 31:59–72.
- Kehrli, M. E. Jr., B. J. Nonnecke, and J. A. Roth. 1989. Alterations in bovine neutrophil function during the periparturient period. *Am. J. Vet. Res.* 50:207–214.

- Kim, I. H., and G. H. Suh. 2003. Effect of the amount of body condition loss from the dry to near calving periods on the subsequent body condition change, occurrence of postpartum diseases, metabolic parameters and reproductive performance in Holstein dairy cows. *Theriogenology* 60:1445–1456.
- LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. *J. Dairy Sci.* 88:159–170.
- Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. *Vet. Clin. North Am. Food Anim. Pract.* 20:651–674.
- Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J. Dairy Sci.* 86:1201–1217.
- SAS Institute. 2004. SAS and STAT User's Guide, Release 9.1. SAS Institute Inc., Cary, NC.
- Scalia, D., N. Lacetera, U. Bernabucci, K. Demeyere, L. Duchateau, and C. Burvenich. 2006. In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. *J. Dairy Sci.* 89:147–154.
- Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. *Theriogenology* 65:1516–1530.
- Spiegelman, D., and E. Hertzmark. 2005. Easy SAS calculations for risk or prevalence ratios and differences. *Am. J. Epidemiol.* 162:199–200.
- Stokol, T., and D. V. Nysdam. 2005. Effect of anticoagulant and storage conditions on bovine nonesterified fatty acid and beta-hydroxybutyrate concentrations in blood. *J. Dairy Sci.* 88:3139–3144.
- Stokol, T., and D. V. Nysdam. 2006. Effect of hemolysis on nonesterified fatty acid and beta-hydroxybutyrate concentrations in bovine blood. *J. Vet. Diagn. Invest.* 18:466–469.
- Swets, J. A. 1988. Measuring the accuracy of diagnostic systems. *Science* 240:1285–1293.