Associations of elevated nonesterified fatty acids and β -hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States

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ABSTRACT

The objectives were to evaluate the effects of elevated pre- and postpartum nonesterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA) concentrations during the transition period on reproductive performance and milk production in dairy cattle. In a prospective cohort study of 91 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. All samples were stratified based on pre- or postpartum status at the time of sample collection, and 2,259 and 2,290 animals were used to evaluate reproductive and milk production performance, respectively. Reproductive performance was assessed by time to conception within 70 d post-voluntary waiting period (VWP) and milk production was assessed using mature-equivalent 305-d (ME305) milk yield estimated at 120 d in milk. While controlling for body condition score (BCS), calving season, median ME305 milk production, and parity, NEFA and BHBA concentrations were evaluated with time to event analysis to investigate reproductive performance. These same predictor variables were used to determine the effects of elevated NEFA and BHBA concentrations on ME305 milk yield with herd as a random effect. Heifers and cows were grouped in the final analyses if the results between groups were similar. In all animals sampled prepartum, the risk of pregnancy within 70 d post-VWP was reduced by 19% when NEFA concentrations were ≥ 0.27 mEq/L. In all animals sampled postpartum, those with NEFA concentrations ≥ 0.72 mEq/L had a 16% decrease in risk of pregnancy and those with BHBA concentrations ≥ 10 mg/dL had a 13% decrease in risk. In cows and heifers, ME305 milk yield was decreased by 683 kg when prepartum NEFA concentrations were ≥ 0.33 mEq/L. In heifers sampled postpartum, ME305 milk yield was increased by 488

kg when NEFA concentrations were ≥ 0.57 mEq/L and increased by 403 kg when BHBA concentrations were ≥ 9 mg/dL. In cows sampled postpartum, ME305 milk yield was decreased by 647 kg when NEFA concentrations were ≥ 0.72 mEq/L and decreased by 393 kg when BHBA concentrations were ≥ 10 mg/dL. With the exception of milk production in heifers, this study indicates that increased concentrations of serum NEFA and BHBA had a detrimental effect on reproductive performance and milk production.

Keywords: nonesterified fatty acids, β -hydroxybutyrate, milk production, pregnancy

INTRODUCTION

The ability of dairy cattle to partition available energy for milk production at the expense of reproduction early in lactation has made the role of energy balance a key factor in the study of reproductive performance and milk production. The concept of prioritization of energy and other nutrients, also known as homeorhesis, was described in 1980 (Bauman and Currie, 1980) and has set the foundation for the study of negative energy balance (NEB) in transition dairy cattle. The circulating metabolites NEFA and BHBA are commonly used indices of NEB or ketosis in transition animals. Although some elevation of these metabolites is normal as these animals balance energy intake and energy demands in early lactation, excessive elevation of NEFA or BHBA can indicate poor adaptation to NEB (Herd, 2000).

Previous reports suggest that there is an association between milk yield and decreased fertility; however, the results were controversial. Some studies reported that higher producing cows had lower fertility (Butler, 2003), whereas others did not (Hansen et al., 1983b; Butler and Smith, 1989). This unexplained contradiction between milk yield and fertility coupled with the concept of homeorhesis focused the investigation toward energy balance deficits during the transition period and their effect on fertility. Subsequent studies revealed that there was an association between excessive NEB

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and decreased reproductive performance (Walsh et al., 2007); however, that study only examined BHBA concentrations, did not evaluate NEFA, and did not define a concentration threshold above which detrimental downstream outcomes were most likely. Diminished reproductive effects may also be related to uterine disease (Reist et al., 2003; Hammon et al., 2006), and delayed luteal activity (Wathes et al., 2007), both of which have been shown to be associated with elevated BHBA concentrations.

Excessive NEB has also been linked to milk yield (Duffield et al., 2009). However, defining the level at which elevated NEFA or BHBA are associated with milk production has been difficult because some elevation in these metabolites is characteristic of the transition period and because many other factors affect milk yield. Duffield et al. (2009) described an objective BHBA concentration threshold for the prediction of milk yield early in lactation but the effects of pre- or postpartum NEFA concentrations were not evaluated.

The objectives were to evaluate the association between elevated pre- and postpartum NEFA and BHBA concentrations on reproductive performance and milk production and to establish the metabolite concentrations above which the effects were most likely to occur.

MATERIALS AND METHODS

Study Population and Study Design

A prospective cohort study of progressive dairy herds in the northeastern United States was conducted using a convenience sample of 100 dairy herds. To be included, a herd must have 1) had more than 250 milking cows, 2) had freestall housing, 3) fed a TMR, and 4) participated in DHIA or used Dairy Comp 305 (Valley Ag Software, Tulare, CA). Herds were excluded from the study if information on herd pregnancy rate was not available and individual animals were excluded from the study if culling data or mature-equivalent 305-d (**ME305**) milk yield data at 120 DIM were not complete.

In each herd, 2 separate cohorts of animals were sampled on the same day: those 14 to 2 d prepartum and those 3 to 14 d postpartum. A representative herd sample was obtained by cross-sectional sampling of approximately 15 apparently healthy animals from each cohort. Sampling 15 animals would give 90% confidence that the sample represents the true within-herd prevalence. Healthy was defined as not being in the sick pen, not currently receiving any medical treatment, and not displaying sick-cow signs based on the investigators' interpretation. To reflect common herd demographics,

approximately one-third of the animals sampled were primiparous.

Ten-milliliter blood samples were collected into plain redtop evacuated tubes from each animal's coccygeal vessel and handled according to recommendations (Stokol and Nydam, 2005). Concentrations of NEFA and BHBA were measured in serum using standardized reagents (NEFA-C, Wako Chemicals USA Inc., Richmond, VA; β -HB, Catachem Inc., Bridgeport, CT) and an automated wet chemistry analyzer (Hitachi 917, Roche Diagnostics, Indianapolis, IN). The sera from the prepartum cohort were analyzed for NEFA and hemolysis (Stokol and Nydam, 2006). The sera from animals sampled postpartum were analyzed for NEFA, BHBA, and hemolysis (Stokol and Nydam, 2006). Hemolysed samples were discarded.

The reproductive information collected consisted of herd use of ovulation synchronization program and timed AI, voluntary waiting period (**VWP**), calving date, calving season, DIM at conception, and when applicable, culling date and pregnancy status at culling. Reproductive success was measured as pregnancy within 70 d post-individual herd VWP. This classification was used to standardize the time at risk to get pregnant and to avoid penalizing cows from herds with longer VWP. Pregnancy diagnosis was performed by the herd veterinarian by rectal palpation or ultrasound and recorded by herd personnel. Calving season was dichotomized into 2 categories: warm months (May to September) and cool months (October to April). Parity was dichotomized into 2 groups: parity 1 or ≥ 2 . Body condition score was determined at time of blood sample collection (Ferguson et al., 1994) and dichotomized into 2 groups: <3.75 or ≥ 3.75 .

Milk production information was collected as ME305 milk yield measured at approximately 120 DIM (incorporating 4 test-days) and was retrieved from DHIA or on-farm Dairy Comp 305 records.

Statistical Analysis

All data was stratified based on time of sample collection, pre- or postpartum. Initially, heifers and cows were evaluated separately, but if the association between the covariates and the outcome of interest was similar they were grouped in the final analyses. Reproduction and milk production were the 2 outcomes of interest and measured as conception within 70 d post-individual herd VWP and ME305 milk yield (kg) assessed at 120 DIM, respectively.

For both outcomes, the metabolites NEFA and BHBA were evaluated as the main categorical predictors in 3 models: prepartum NEFA; postpartum NEFA

and BHBA; and BHBA alone. β -Hydroxybutyrate is easily measured in the field; therefore, its effect as a main predictor was modeled separately from NEFA.

Reproduction

Time from individual herd VWP until pregnancy was modeled using a semiparametric proportional hazards model (Cox, 1972) accounting for clustering of cows within herds with Proc Phreg (SAS Institute, 2004). In addition to the main effects of NEFA and BHBA, the covariates BCS, parity, and ME305 milk were evaluated. The ME305 milk data were dichotomized based on the median production of the 2 sampled cohorts. The effect of an elevated metabolite concentration was evaluated by dichotomizing the metabolite concentration within the range of values identified as critical thresholds for prediction of diseases in Ospina et al. (2010). The ranges were as follows: prepartum NEFA, 0.27 to 0.37 mEq/L; postpartum NEFA, 0.36 to 0.72 mEq/L; and BHBA, 7 to 12 mg/dL. The dichotomized NEFA and BHBA concentrations that resulted in the smallest chance of committing a type I error and had the largest estimate were kept in the final model.

The Cox proportional hazards models allowed the use of information from animals that did and did not become pregnant; however, animals that were culled before the end of the VWP were excluded from the analysis. Animals not pregnant by the end of the follow-up period were right censored. The proportional hazards assumption was checked statistically by evaluating time-dependent covariates (Allison, 1995). Noninformative censoring was evaluated with sensitivity analysis. Kaplan-Meier estimator graphs (Proc Lifetest) of time to pregnancy within 70 d post-VWP for animals with elevated metabolite levels were created (SAS Institute, 2004).

Milk Production

Mature-equivalent 305-d milk yield was modeled using a mixed effects model (Proc Mixed) with herd as a random effect in SAS (SAS Institute, 2004). In addition to the main predictors (NEFA and BHBA), the covariates BCS, calving season, and when applicable, parity and the interaction between parity and the metabolite concentration were evaluated. The effect of elevated NEFA and BHBA concentrations was evaluated by dichotomizing the concentrations within the range of critical values previously identified. The dichotomized metabolite concentration that resulted in the smallest chance of committing a type I error and largest estimate was kept in the final model.

RESULTS

Descriptive Data

Ninety-one herds were included in the study, and the herd size ranged from 265 to 2,770 with a mean of 827 lactating animals. In total, 2,259 cows were used to evaluate reproductive performance, of which 1,164 were sampled prepartum (37% heifers and 63% cows) and 1,095 were sampled postpartum (41% heifers and 59% cows). In total, 2,290 animals were used to evaluate milk production, of which 1,183 were sampled prepartum (36% heifers and 64% cows) and 1,107 were sampled postpartum (41% heifers and 59% cows).

The median ME305 milk production measured at 120 DIM for heifers sampled prepartum was 12,572 kg and for those sampled postpartum was 12,550 kg. The median milk production for cows sampled prepartum and postpartum was 12,254 and 12,197 kg, respectively. The VWP ranged from 40 to 75 d. More than 95% of herds used ovulation synchronization programs. The mean pregnancy rate was 19.6 (median 19.5) and ranged from 9 to 32.

Reproduction in the Prepartum Cohort

Heifers and cows were grouped in the final analyses because the associations between prepartum NEFA concentrations and reproductive performance were similar. In animals sampled prepartum, and controlling for BCS, calving season, milk production, and parity, the effect of having a NEFA concentration ≥ 0.27 mEq/L resulted in 19% decreased risk (hazard ratio = 0.81) of conception within 70 d post-VWP ($P = 0.01$; Table 1). In this model, parity was the only other significant covariate, where animals with parity ≥ 2 resulted in a 27% decreased risk of conception within 70 d post-VWP ($P = 0.001$; Table 1). Figure 1 is a graph of the Kaplan-Meier estimation of animals sampled prepartum; animals with NEFA values ≥ 0.27 mEq/L took longer to get pregnant than animals with NEFA values < 0.27 mEq/L ($P = 0.03$).

Reproduction in the Postpartum Cohort

Heifers and cows were grouped in the final analyses because the associations between postpartum NEFA and BHBA concentrations and reproductive performance were similar. In animals sampled postpartum, and controlling for BCS, calving season, milk production, parity, and BHBA dichotomized at 10 mg/dL, the effect of having a NEFA value ≥ 0.72 mEq/L resulted in a 16% decreased risk (hazard ratio = 0.84) of concep-

Table 1. Cox proportional hazards model for the effect of NEFA, covariates, and cows clustered within herds on days to conception within 70 d post-voluntary waiting period for animals sampled prepartum (n = 1,164)

Variable	Parameter estimate	SE	Hazard ratio	P-value
NEFA ¹	-0.21	0.08	0.81	0.01
BCS ²	0.02	0.10	1.03	0.8
Season ³	-0.02	0.09	0.98	0.8
Parity ⁴	-0.31	0.09	0.73	0.001
ME305 ⁵	-0.03	0.09	0.97	0.6

¹NEFA dichotomized at < or ≥0.27 mEq/L.²BCS dichotomized at < or ≥3.75.³Calving season dichotomized into warm (May to September) versus cool months (October to April).⁴Parity dichotomized into < or ≥2.⁵Mature-equivalent 305-d milk yield dichotomized into < or ≥ median for animals sampled prepartum.

tion within 70 d post-VWP ($P = 0.05$; Table 2). In this model, parity was the only other significant covariate where parity ≥2 resulted in a 19% decreased risk of conception within 70 d post-VWP ($P = 0.01$). When BHBA was evaluated as the only main predictor (i.e., without NEFA in the model) and after controlling for BCS, calving season, and parity, animals with BHBA ≥10 mg/dL had a 13% decreased risk of conception ($P = 0.1$). In this model, parity was the only significant covariate where parity ≥2 resulted in a 20% decreased risk of conception within 70 d post-VWP ($P = 0.01$; Table 3).

Milk Production in the Prepartum Cohort

Data from heifers and cows sampled prepartum were combined in the final analysis because the association between prepartum NEFA concentrations and milk

Table 3. Cox proportional hazards model for the effect of BHBA, covariates, and cows clustered within herds on days to conception within 70 d post-voluntary waiting period for animals sampled postpartum (n = 1,095)

Variable	Parameter estimate	SE	Hazard ratio	P-value
BHBA ¹	-0.14	0.10	0.87	0.1
BCS ²	0.09	0.08	1.11	0.3
Season ³	0.12	0.09	1.13	0.2
Parity ⁴	-0.23	0.09	0.80	0.01
ME305 ⁵	0.06	0.08	1.07	0.4

¹BHBA dichotomized at < or ≥10 mg/dL.²BCS dichotomized at < or ≥3.75.³Calving season dichotomized into 2 seasons: warm (May to September) versus cool (October to April).⁴Parity dichotomized at < or ≥2.⁵Mature-equivalent 305-d milk yield dichotomized into < or ≥ median for animals sampled postpartum.

production were similar for both parity groups. After controlling for parity, BCS, calving season, and the interaction between NEFA and parity, a NEFA concentration ≥0.33 mEq/L in animals sampled prepartum resulted in a decrease of 683 kg in ME305 milk ($P = 0.001$). Parity was the only other significant covariate ($P = 0.01$; Table 4).

Milk Production in the Postpartum Cohort

The final analyses were stratified by parity because the association between postpartum NEFA and BHBA concentrations and ME305 milk yield were different between heifers and cows. In heifers, after controlling for BCS, calving season, and BHBA dichotomized at 10 mg/dL, heifers with NEFA concentrations ≥0.57 mEq/L had increased ME305 milk yield of 488 kg ($P =$

Table 2. Cox proportional hazards model for the effect of NEFA, BHBA, covariates, and cows clustered within herds on days to conception within 70 d post-voluntary waiting period for animals sampled postpartum (n = 1,095)

Variable	Parameter estimate	SE	Hazard ratio	P-value
NEFA ¹	-0.17	0.09	0.84	0.05
BHBA ²	-0.07	0.10	0.93	0.4
BCS ³	0.11	0.08	1.12	0.2
Season ⁴	0.12	0.09	1.13	0.2
Parity ⁵	-0.21	0.09	0.81	0.01
ME305 ⁶	0.06	0.08	1.06	0.5

¹NEFA dichotomized at < or ≥0.72 mEq/L.²BHBA dichotomized at < or ≥10 mg/dL.³BCS dichotomized at < or ≥3.75.⁴Calving season dichotomized into 2 seasons: warm (May to September) versus cool (October to April).⁵Parity dichotomized at < or ≥2.⁶Mature-equivalent 305-d milk yield dichotomized into < or ≥ median for animals sampled postpartum. **Figure 1.** Graph of Kaplan-Meier estimator of time to pregnancy for animals with prepartum NEFA ≥0.27 mEq/L or <0.27 mEq/L ($P = 0.03$).

Table 4. Mixed model for the effect of NEFA, covariates, and herd as a random effect on milk production measured by 120 DIM mature-equivalent 305-d milk yield (ME305, kg) for animals sampled prepartum (n = 1,183)

Variable	Difference in ME305 (kg)	SE	P-value
NEFA ¹	-683	180	0.0001
BCS ²	-9	130	0.9
Season ³	170	220	0.4
Parity ⁴	-556	225	0.01
Parity × NEFA ⁵	246	270	0.4

¹NEFA dichotomized at < or ≥0.33 mEq/L.²BCS dichotomized at < or ≥3.75.³Calving season dichotomized into 2 seasons: warm (May to September) versus cool (October to April).⁴Parity dichotomized at < or ≥2.⁵Interaction between parity and NEFA.

0.02). There were no other significant covariates in this model (Table 5). In heifers sampled postpartum, when BHBA was evaluated as the main predictor, without NEFA in the model, and after controlling for calving season and BCS, heifers with BHBA ≥9 mg/dL had an increase of 403 kg more ME305 milk ($P = 0.04$; Table 6).

In cows sampled postpartum, after controlling for BCS and calving season, those with NEFA ≥0.72 mEq/L had a 647-kg decrease in ME305 milk ($P = 0.001$; Table 7). No other covariates were significant in this model. When BHBA was evaluated as the main predictor, without NEFA in the model, and after controlling for BCS and calving season, those with BHBA ≥10 mg/dL had a 393-kg decrease in ME305 milk yield ($P = 0.04$; Table 8). No other covariates were significant in this model.

DISCUSSION

Reproduction

Cows with excessive NEB (i.e., concentrations of pre- and postpartum NEFA and BHBA above the

Table 6. Mixed model for the effect of BHBA, covariates, and herd as a random effect on milk production measured by 120 DIM mature-equivalent 305-d milk yield (ME305, kg) for heifers sampled postpartum (n = 449)

Variable	Difference in ME305 (kg)	SE	P-value
BHBA ¹	403	195	0.04
BCS ²	38	193	0.8
Season ³	270	273	0.3

¹BHBA dichotomized at < or ≥9 mg/dL.²BCS dichotomized at < or ≥3.75.³Calving season dichotomized into 2 seasons: warm (May to September) versus cool (October to April).

thresholds determined in this study) had a decreased risk of pregnancy within 70 d post-VWP (Tables 1 to 3). This observation is consistent with previous reports that NEB is detrimental to reproductive performance (Villa-Godoy et al., 1988; Wathes et al., 2007). Although elevated concentrations of both NEFA and BHBA resulted in decreased risk of conception, NEFA concentrations generally had a stronger association with reproductive performance than did BHBA. When both postpartum NEFA and BHBA were in the same model, BHBA was not as strong a predictor as NEFA. The strong association between NEFA concentrations and reproductive performance is likely because of the more direct physiological relationship between NEFA concentrations and NEB (Herdt, 2000). The concentration of NEFA increases because of lipolysis, which is positively stimulated by glucagon. Glucagon release is stimulated by hypoglycemia, a direct effect of NEB. Although BHBA concentrations are also related to NEB, they are regulated by several other factors including NEFA supply to the liver, activity of carnitine palmitoyltransferase (CPT-1), and intramitochondrial activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (Hegardt, 1999; Drackley et al., 2001).

Reproductive performance was evaluated with time to event analysis. Individual herd VWP was incorporated

Table 5. Mixed model for the effect of NEFA, BHBA, covariates, and herd as a random effect on milk production measured by 120 DIM mature-equivalent 305-d milk yield (ME305, kg) for heifers sampled postpartum (n = 449)

Variable	Difference in ME305 (kg)	SE	P-value
NEFA ¹	488	203	0.02
BHBA ²	-143	238	0.5
BCS ³	-0.3	193	0.9
Season ⁴	340	273	0.2

¹NEFA dichotomized at < or ≥0.57 mEq/L.²BHBA dichotomized at < or ≥10 mg/dL.³BCS dichotomized at < or ≥3.75.⁴Calving season dichotomized into 2 seasons: warm (May to September) versus cool (October to April).**Table 7.** Mixed model for the effect of NEFA, BHBA, covariates, and herd as a random effect on milk production measured by 120 DIM mature-equivalent 305-d milk yield (ME305, kg) for cows sampled postpartum (n = 658)

Variable	Difference in ME305 (kg)	SE	P-value
NEFA ¹	-647	195	0.001
BHBA ²	-165	205	0.4
BCS ³	-70	203	0.7
Season ⁴	270	242	0.3

¹NEFA dichotomized at < or ≥0.72 mEq/L.²BHBA dichotomized at < or ≥10 mg/dL.³BCS dichotomized at < or ≥3.75.⁴Calving season dichotomized into 2 seasons: warm (May to September) versus cool (October to April).

Table 8. Mixed model for the effect of BHBA, covariates, and herd as a random effect on milk production measured by 120 DIM mature-equivalent 305-d milk yield (ME305, kg) for cows sampled postpartum (n = 658)

Variable	Difference in ME305 (kg)	SE	P-value
BHBA ¹	−393	195	0.04
BCS ²	−165	202	0.40
Season ³	335	247	0.20

¹BHBA dichotomized at < or ≥10 mg/dL.

²BCS dichotomized at < or ≥3.75.

³Calving season dichotomized into 2 seasons: warm (May to September) versus cool (October to April).

into the time to event analysis to create equal time at risk for pregnancy; thus, avoiding penalizing herds with longer VWP. The VWP could be incorporated as a factor in the time to event analysis because over 95% of the study population used ovulation synchronization protocols and timed artificial insemination. In this population, other commonly used measures of reproductive performance, such as time to first insemination or display of estrus, were not useful indicators because these events were either scheduled or not measured.

Seventy-day post-VWP was chosen as a measure of reproductive performance because we wanted to evaluate the effects of elevated NEFA and BHBA concentrations during the transition period on reproduction during this time frame. In addition, conception during this time interval represents an optimal calving interval, such that loss associated with days open may be minimized (Schmidt, 1989; Meadows et al., 2005). A potential limitation of this study was that reproductive performance was not followed until the end of lactation; however, given the scope of this project, extending the follow-up time may have introduced additional confounders and diluted the effect of NEFA and BHBA concentrations.

In addition to NEFA or BHBA concentrations, several other covariates were examined to control for potential confounding: parity, BCS, calving season, and median ME305 milk production. Of these covariates, parity was the only one that was significant in addition to the main effects of NEFA or BHBA. Cows had a decreased risk of pregnancy compared with heifers. This was expected because heifers usually have a higher conception risk than cows. The effect of BCS on reproductive performance was not significant in this analysis, as has been reported previously (Ruegg and Milton, 1995). However, contrary to other reports (Oseni et al., 2003; Huang et al., 2008), season was not significantly associated with reproductive performance. Interestingly, milk production did not significantly affect reproductive performance (Hansen et al., 1983b; Leroy et al., 2008). Although there has been controversy regarding the as-

sociation between milk production and reproductive performance, it is not surprising that there was no significant association in the present study. Several studies report that NEB as measured by NEFA or BHBA has a stronger association with reproductive performance than milk production (Reist et al., 2003; Walsh et al., 2007; Wathes et al., 2007). It is important to note that NEFA concentrations generally increase closer to calving; therefore, studies that sample closer to calving may have higher cut-points associated with downstream outcomes (e.g., milk production, disease events, or reproduction) than those reported here, which included cows sampled as far as 2 wk prepartum.

In this study, although information on disease incidence (displaced abomasum, clinical ketosis, metritis, or retained placenta) was available for the animals, it was not included in the investigation of reproductive performance or milk production. The metabolite concentrations used as main predictors in these models are correlated with the development of disease (Ospina et al., 2010) and, as such, would introduce multicollinearity if they were in the same model. Further, the study design employed a cross-sectional sampling strategy such as those typically used in field investigations or in monitoring (Oetzel, 2004). In these scenarios, only information concurrently available would be used to predict reproductive performance or milk production. For example, information on parity and calving season could be incorporated easily, but information on future development of diseases or serial measurement of NEFA or BHBA concentrations would not.

Milk Production

Milk production was estimated using ME305 milk yield measured at 120 DIM. Mature-equivalent 305-d milk yield was used in this study because it allows us to compare heifers and cows, and, with information from 4 test-days, ME305 is a precise estimate of total milk production (Quist et al., 2007). Generally, the results of this study are similar to those of other reports; elevated concentrations of BHBA were associated with decreased milk production (Dohoo and Martin, 1984; Duffield et al., 2009). In addition, we also investigated the association of NEFA with milk production and the effect was similar. However, in this study, heifers sampled postpartum showed an inverse relationship; heifers with elevated metabolite levels (NEFA ≥0.57 mEq/L, BHBA ≥9 mg/dL) produced more ME305 milk compared with heifers with concentrations below these levels. Duffield et al. (2009) also found a similar relationship, but did not distinguish between parities. In that study, cows ketotic in wk 2 postpartum had lower first test milk yield, but had higher second and third test milk yields

compared with their nonketotic counterparts. Heifers find themselves in a unique physiological circumstance: they have to balance maintenance, growth, and milk production, and as a result may mobilize energy resources such as lipid more readily than cows. This concept of homeorhesis may help explain our findings (Bauman and Currie, 1980).

Although elevated concentrations of NEFA and BHBA were significantly associated with ME305 milk production, NEFA concentrations generally had a stronger association with milk production than did BHBA. Prepartum NEFA concentrations resulted in the low-est chance of committing a type I error ($P = 0.0001$). When both postpartum NEFA and BHBA were in the same model, NEFA concentrations were so strongly associated with ME305 milk that BHBA was no longer a significant effect in the model. The strong association between NEFA concentrations and milk production is likely because of the direct physiological relationship between NEFA concentrations and lipid mobilization.

In addition to NEFA and BHBA concentrations, BCS, calving season, parity, and, when applicable, the interaction between parity and the metabolite concentration were examined. In all groups, the single measure of BCS in the transition period was not a significant predictor of milk production. Ruegg and Milton (1995) showed that the change in BCS, and not the single measurement, was predictive of milk production, which may explain our result. Mature-equivalent 305-d milk yield incorporates season into the total calculation; therefore, it was unsurprising that season was not a significant predictor of ME305 milk. Parity was the only significant covariate in animals sampled prepartum. This is likely because ME305 would be higher for heifers versus cows in progressive herds. It is important to note that the interaction between parity and NEFA concentration was not significant, which means that although there was a difference based on parity, it was not dependent on whether the NEFA concentration was above or below the threshold used in this study.

CONCLUSIONS

The current analysis allowed the opportunity to examine the effect of elevated concentrations of pre- and postpartum NEFA and postpartum BHBA on reproduction and milk production at the cow level. The NEFA and BHBA concentrations above which reproduction and production effects were most likely to occur were identified. Generally, compared with animals with metabolite concentrations below the identified thresholds, animals with prepartum NEFA ≥ 0.27 mEq/L, postpartum NEFA ≥ 0.72 mEq/L, and postpartum BHBA ≥ 10 mg/dL had a decreased risk of pregnancy within

70 d post-VWP. Milk production was decreased in animals with prepartum NEFA concentrations ≥ 0.33 mEq/L. In heifers sampled postpartum, there was an increase in milk production when postpartum NEFA was ≥ 0.57 mEq/L and BHBA ≥ 9 mg/dL; however, in cows, milk production was decreased when postpartum NEFA and BHBA were ≥ 0.72 mEq/L and 10 mg/dL, respectively.

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