Within day variation in milk and blood metrics for hyperketonemic and non-hypoketonemic dairy cows

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Abstract

Dairy cows often enter a state of energy deficit in early lactation, leading to an increase in plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). Currently, diagnosis of excessive energy deficit is done on farms using handheld blood BHB meters. However, this process is laborious and can become costly when used as a whole-herd screening method. Several studies have investigated the use of Fourier transform mid-infrared (FTIR) estimates to predict excessive energy deficit, but these studies relied on a single, test-day DHIA milk sample with no knowledge of actual blood NEFA or BHB concentrations. We determined the diurnal variation in plasma NEFA and BHB as well as FTIR estimates of milk BHB, milk predicted blood NEFA, and milk fatty acids with particular focus on differences between groups of cows that were hyperketonemic or non-hyperketonemic. We collected blood samples every 2 h for 5 consecutive days from 28 multiparous Holstein cows that were between 3 and 9 days in milk. Cows were housed in a tie-stall facility and offered free choice access to water and a TMR that was delivered once a day at 0900 h. Blood samples were analyzed for BHB and NEFA concentrations, and cows were classified into hyperketonemia groups based on their average daily BHB concentration. If a cow's average daily BHB was ≥1.2 mmol/L for ≥3 study days, she was assigned to the hyperketonemia group (n=13). Alternatively, if her average daily BHB was ≥1.2 mmol/L for ≤2 study days, she was assigned to the non-hyperketonemia group (n=15). We found clear and consistent diurnal patterns in plasma BHB and NEFA as well as FTIR estimates of milk BHB, milk predicted blood NEFA, and milk fatty acids. Interestingly, these diurnal differences were much more predictable when analyzing milk, with a greater ability to separate hyperketonemic from non-hyperketonemic cows. Our results support the use of FTIR estimates of milk BHB and milk predicted blood NEFA as a tool in diagnosing HYK, however time relative to feeding should be considered when analyzing results. Milk fatty acid metrics on a relative basis may also be useful to separate hyperketonemic from non-hyperketonemic cows. In particular, these results support the use of high frequency milk monitoring and measurement to detect alterations in early lactation health of dairy cows.

Introduction

Dairy cows often enter a state of energy deficit in early lactation, leading to an increase in plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). Currently, diagnosis of excessive energy deficit is done on farms using handheld blood BHB meters. However, this process is laborious and can become costly when used as a whole-herd screening method. Several studies have investigated the use

of Fourier transform mid-infrared (FTIR) estimates to predict excessive energy deficit through milk (Denis-Robichaud *et al*., 2014; Santschi *et al*., 2016; Bach *et al*., 2019), but many of these studies relied on a single, test-day DHIA milk sample with no knowledge of actual blood NEFA or BHB concentrations. Here we present our investigation of the diurnal variation in plasma NEFA and BHB as well as FTIR estimates of milk BHB and predicted blood NEFA, with particular interest in differences between groups of cows that were hyperketonemic or non-hyperketonemic. This information will improve knowledge and usability of on-farm testing results and promote discussion of the benefits of routine milk testing and analysis.

Study design and results

We collected blood samples every 2 h for 5 consecutive days from 28 multiparous Holstein cows that were between 3 and 9 days in milk. Cows were housed in a tie-stall facility and offered free choice access to water and a TMR that was delivered once a day at 0900 h. Blood samples were analyzed for BHB and NEFA concentrations, and cows were classified into hyperketonemia groups based on their average daily BHB concentration. If a cow's average daily BHB was ≥1.2 mmol/L for ≥3 study days, she was assigned to the hyperketonemia group (n=13). Alternatively, if her average daily BHB was ≥1.2 mmol/L for ≤2 study days, she was assigned to the non-hyperketonemia group (n=15).

Blood results

We saw the lowest concentrations of BHB just prior to feeding, at 0700 h, with a steady rise following feed delivery (Figure 1A). Not surprisingly, BHB was higher in the hyperketonemic cows than the non-hyperketonemic cows (Figure 1B). Unlike BHB however, we saw a peak in NEFA just prior to feeding at 0700 h, with concentrations falling quickly after feed delivery (Figure 1C). The hyperketonemic cows had greater concentrations of NEFA than the non-hyperketonemic cows (Figure 1D).

To understand the effect of hyperketonemic on the daily fluctuations of BHB and NEFA, we calculated the difference between the daily maximum and minimum concentrations for each metabolite by hyperketonemia group. The hyperketonemic cows experienced a nearly two-fold greater difference between daily maximum and minimum BHB concentration as compared to the non-hyperketonemic cows. Interestingly, the difference between daily maximum and minimum concentrations of NEFA were relatively similar for both the hyperketonemic and non-hyperketonemic cows.

Milk results

We saw similar diurnal findings with mid-FTIR milk predicted metabolites, however with a general lag in peak or nadir concentrations than blood. The lowest milk BHB and milk predicted blood NEFA concentrations were at the morning milking just prior to feeding (Figure 2A, 2C). As for blood, predicted milk BHB and milk predicted blood NEFA were higher in hyperketonemic than non-hyperketonemic cows (Figure 2B, 2D). However, unlike blood, difference in milk BHB between hyperketonemic groups was more consistent, and the pattern of diurnal variation in milk predicted blood NEFA never overlapped between the two groups.

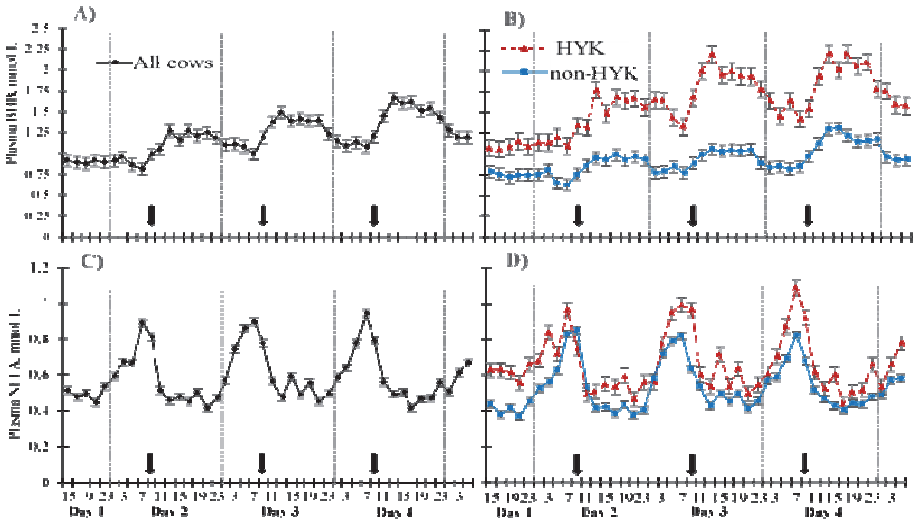

Figure 1. Concentrations of BHB and NEFA from multiparous Holstein cows classified as hyperketonemic (HYK; average daily mean BHB ≥1.2 mmol/L for ≥3 study days, red dashed line) or non-hyperketonemic (non-HYK; average daily mean BHB ≥1.2 mmol/L for ≤2 study days, solid blue line). Black arrows indicate time of feed delivery (Adapted from Seely et al., Journal Dairy Science, In Press).

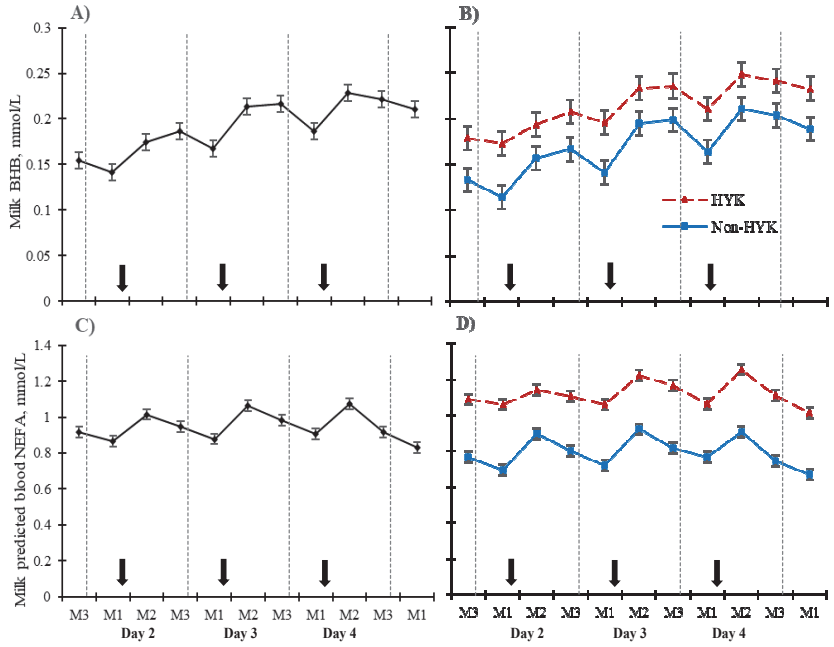

Figure 2. Concentrations of mid-FTIR milk predicted metabolites (milk BHB and milk predicted blood NEFA) from multiparous Holstein cows classified as hyperketonemic (HYK; average daily mean BHB ≥1.2 mmol/L for ≥3 study days, red dashed line) or non-hyperketonemic (non-HYK; average daily mean BHB ≥1.2 mmol/L for ≤2 study days, solid blue line). Black arrows indicate time of feed delivery; M1 = morning milking, M2 = afternoon milking, M3 = evening milking (Unpublished data from Seely, McArt, and Barbano).

Cows were milked 3 times per day. Therefore, each milk sample theoretically represents the average of what happened in the blood for the 8 h period prior to milking. We averaged the 2 h blood testing data over each 8 h period, prior to each milking, to achieve better correspondence of the time period for milk and blood results. Milk predicted blood NEFA, milk BHB, and milk fatty acids were measured at each milking using a mid-FTIR milk analysis (Delta FTA, Perkin-Elmer Corp., Drachten, The Netherlands). The data for milk predicted blood NEFA and blood NEFA over consecutive milkings for the hyper and non-hyperketonemic groups of cows are shown in Figures 3 and 4. Both the blood NEFA and milk estimated blood NEFA cycled during each 24 h period with a slight lag in timing of the cycling. Both the blood NEFA and the milk estimated blood NEFA clearly separated the two groups of cows.

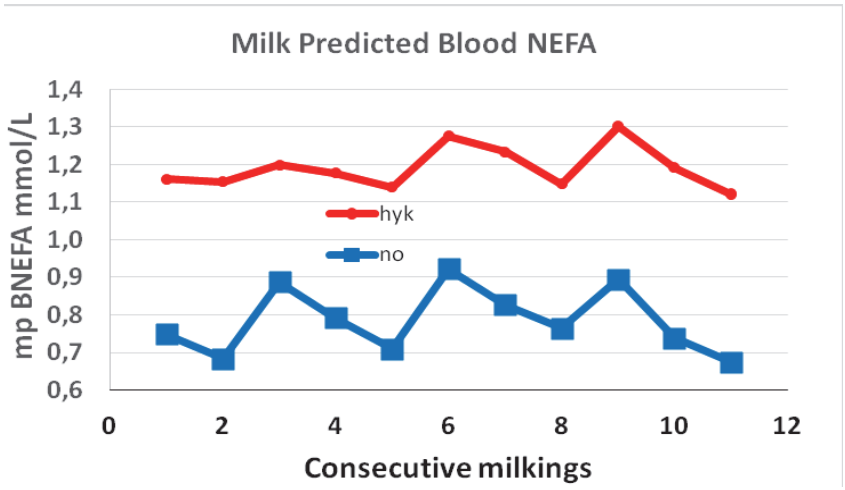

Figure 3. Milk estimated blood NEFA over consecutive milkings.

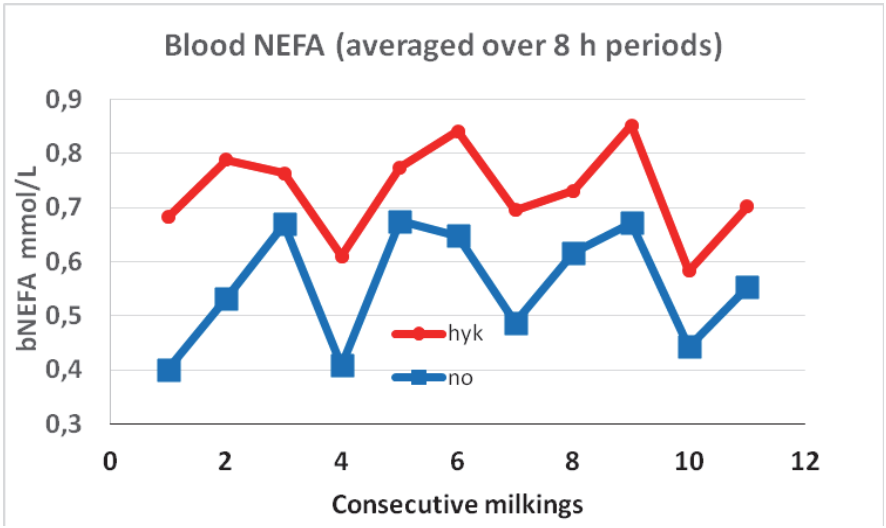

Figure 4. Blood NEFA (2 h samples averaged across the 8 h period prior to milking) plotted as a function of consecutive milkings.

The concentration of milk BHB was also measured by infrared milk analysis and concentration in milk also cycled (data not shown).

Milk fatty acids (denovo, mixed origin, and preformed) were also measured at every milking by mid-infrared milk analysis (Figures 5, 6, and 7), as described by Wojciechowski *et al.,* 2016 and Woolpert *et al.,* 2016. The comparison of relative concentration for the 3 different groups of milk fatty acids between hyper and non-hyperketonemic groups of cows was clearly separated for all three milk fatty acid metrics. The non-hyperketonemic cows had higher relative concentrations of de novo and mixed origin milk fatty acids and lower performed milk fatty acids than the

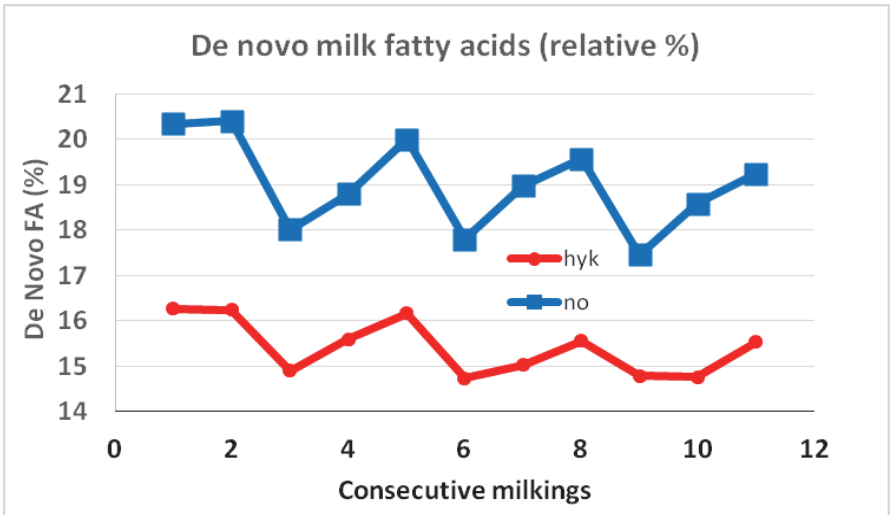

Figure 5. De novo milk fatty acids (relative %) measured by mid-infrared milk analysis. Fresh feed offered immediately after milking 3, 6, and 9.

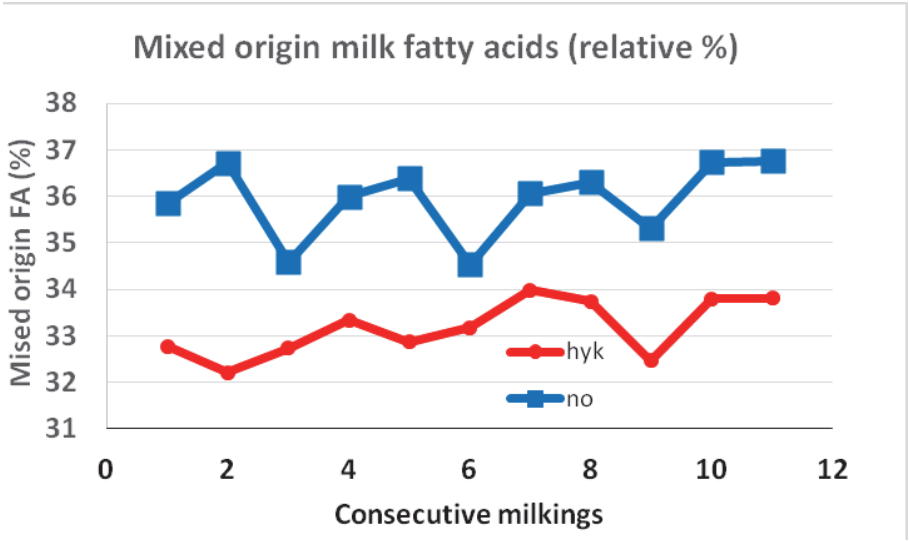

Figure 6. Mixed origin milk fatty acids (relative %) measured by mid-infrared milk analysis. Fresh feed offered immediately after milking 3, 6, and 9.

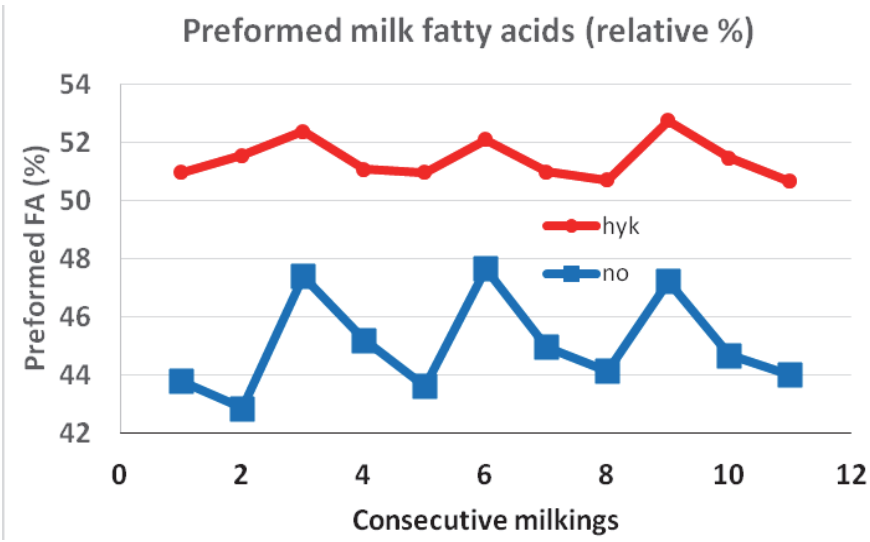

Figure 7. Preformed milk fatty acids (relative %) measured by mid-infrared milk analysis. Fresh feed offered immediately after milking 3, 6, and 9.

hyperketonemic cows. The cycle phasing of the relative concentrations of the de novo and mixed origin fatty acids had the opposite phasing when compared with the phasing of the preformed milk fatty acid cycling. Cycling of the relative concentration of milk fatty acid groups (Figures 5, 6, 7) was related to cycling of blood NEFA and the cycling of the fatty acid groups was consistent with the milk estimated blood NEFA cycling (Figure 3). When milk estimated blood NEFA was at a maximum of a cycle, preformed fatty acids were also at the maximum. Immediately before the cows were given fresh feed, milk estimated blood NEFA and relative concentration of milk preformed fatty acids were at a maximum and the de novo and mixed origin fatty acids were at a minimum.

We hypothesize that the differences between peak and nadir blood and milk metabolites are due to milk having a higher correlation with an 8-hour average of blood metabolite concentrations rather than a single blood sample. This makes biological sense and also supports the idea that milk analysis might be an improved method of representing a cow's overall energy status than a single snapshot in time as currently provided with blood sampling.

Acknowledgement

We would like to acknowledge Dr. Kathryn Bach, the post-doctoral associate who assisted with study organization. Our study was funded by the USDA National Institute of Food and Agriculture, Hatch project no. 1017096.

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