J. Dairy Sci. 99:1–7
http://dx.doi.org/10.3168/jds.2016-10920
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Short communication: Association of milk fatty acids with early lactation hyperketonemia and elevated concentration of nonesterified fatty acids

S. Mann,*[1] D. V. Nydam,* A. L. Lock,† T. R. Overton,‡ and J. A. A. McArt*
*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853
†Department of Animal Science, Michigan State University, East Lansing 48824
‡Department of Animal Science, Cornell University, Ithaca, NY 14853

ABSTRACT

The objective of our study was to extend the limited research available on the association between concentrations of milk fatty acids and elevated nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) concentrations in early lactation dairy cattle. Measurement of milk fatty acids for detection of cows in excessive negative energy balance has the potential to be incorporated in routine in-line monitoring systems. Blood samples were taken from 84 cows in second or greater lactation 3 times per week between 3 to 14 d in milk. Cows were characterized as hyperketonemic (HYK) if blood BHB concentration was ≥1.2 mmol/L at least once and characterized as having elevated concentrations of NEFA ($NEFA_H$) if serum NEFA concentration was ≥1 mmol/L at least once. Composition of colostrum and milk fatty acids at wk 2 postpartum was used to investigate the potential diagnostic value of individual fatty acids and fatty acid ratios for the correct classification of cows with NEFA and BHB concentrations above these thresholds, respectively. Receiver operating characteristic (ROC) curves were used to identify thresholds of fatty acid concentration and fatty acid ratios when ROC area under the curve was ≥0.70. Correct classification rate (CCR, %) was calculated as {[(number of true positives + number of true negatives)/total number tested] × 100}. None of the colostrum fatty acids yielded a sufficiently high area under the curve in ROC analysis for the association with HYK and $NEFA_H$. The following fatty acids and fatty acid ratios were identified for an association with $NEFA_H$ (threshold, CCR): C15:0 (≤0.65 g/100 g, 68.3%); *cis*-9 C16:1 (≥1.85 g/100 g, 70.7%); *cis*-9 C18:1 (≥26 g/100 g, 69.5%), *cis*-9 C18:1 to C15:0 ratio (≥45, 69.5%); *cis*-9 C16:1 to C15:0 (≥2.50, 73.2%). Several fatty acids were associated with HYK (threshold, CCR): C6:0 (≤1.68 g/100 g, 80.5%), C8:0 (≤0.80 g/100 g, 80.5%), C10:0 (≤1.6 g/100 g, 79.3%); C12:0 (≤1.42 g/100 g, 82.9%); C14:0 (≤6.10 g/100 g, 84.1%); C15:0 (≤0.50 g/100 g, 82.9%), *cis*-9 C18:1 (≥30 g/100 g, 81.7%). The use of fatty acid ratios did not improve CCR over using individual fatty acids for the classification of HYK. Colostrum fatty acid composition was not useful in predicting $NEFA_H$ or HYK between 3 to 14 d in milk. Accuracy of milk fatty acids and fatty acid ratios to correctly classify cows with elevated concentrations of NEFA and BHB between 3 to 14 d in milk was moderate and overall higher for HYK. Determining changes in the fatty acid composition of milk fat from milk samples at wk 2 postpartum for the detection of cows with elevated concentrations of BHB and NEFA can currently not be recommended to replace direct measurement. Future applications should target repeated milk sampling between 3 to 14 d in milk to identify the best sampling for determination of milk fatty acid composition within the first 2 wk postpartum.
Key words: milk fatty acid, metabolism, diagnosis, transition cow

Short Communication

The onset of lactation and concurrent drop in DMI during the transition period in dairy cows is associated with a period of negative energy balance (**NEB**), which is most severe in the first weeks postpartum. Epidemiological data have established the association between increased blood concentrations of markers of NEB, such as BHB and nonesterified fatty acids (**NEFA**), and negative health and production outcomes at both the individual cow and herd level (Duffield et al., 2009; Ospina et al., 2010, 2013).

Whereas measurement of blood BHB concentrations can be carried out cow-side with several different hand-held meters (Iwersen et al., 2009, 2013; Mahrt et al., 2014), NEFA is currently measured in a laboratory setting only. Although NEFA concentrations are a direct measure of net adipose tissue mobilization, de-

Received January 20, 2016.
Accepted March 28, 2016.
[1]Corresponding author: sm682@cornell.edu

termination of serum NEFA concentrations is therefore more costly and associated with a substantial lag time compared with BHB measurements, which has hampered knowledge regarding the benefits of individual identification and treatment of cows with high concentrations of NEFA. The large amount of epidemiological data concerning the association of negative health and production outcomes with BHB and NEFA as markers of NEB on the herd level have increased interest in on-farm measurement. Particularly, the search for testing methods not requiring blood sampling that could be incorporated into the daily milking routine with simple, fast, and inexpensive methods has increased (Hamann and Krömker, 1997; Barbano et al., 2015). Researchers have focused on milk composition (fat and protein) for several years using the ratio of fat to protein as a herd-level screening tool for lipomobilization, NEB, and hyperketonemia (**HYK**; Duffield et al., 1997; Krogh et al., 2011; Toni et al., 2011). However, this method does not take the variability in different milk fatty acids into account. Because the elevation of certain milk fatty acids originating from adipose tissue mobilization is associated with the degree of NEB and HYK (Van Haelst et al., 2008; Gross et al., 2011; Barbano et al., 2015), studying the association of markers of NEB with milk fatty acid profile is of particular importance.

Recently, Jorjong et al. (2014, 2015) proposed cut-off values of milk fatty acids and milk fatty acid ratios for the early diagnosis of elevated blood plasma concentrations of NEFA and as biomarkers for HYK. However, cows were only sampled once per week in this study (Jorjong et al., 2014, 2015), and classification of cows was based on concentrations of NEFA and BHB in wk 2 to 8 postpartum, possibly leading to an underestimation of the incidence of an increase of these markers above established thresholds, as discussed by the authors. Because incidence of HYK peaks around 4 to 5 d postpartum and the median length of an episode of HYK is 5 d (McArt et al., 2012; McCarthy et al., 2015), case detection is improved through frequent sampling of cows, particularly in wk 1 and 2 postpartum. In addition, NEFA concentrations are highest during the first 2 wk of lactation (McCarthy et al., 2015). Our objective was therefore to extend the research already available and examine the association between concentrations of milk fatty acids with elevated NEFA and BHB concentrations during the highest at-risk period between 3 and 14 DIM, and to compare threshold values established by receiver operating characteristic (**ROC**) curves to those proposed in the studies by Jorjong et al. (2014, 2015).

Data and samples originated from the experiment described in detail in Mann et al. (2015) studying the effect of different nutritional planes of energy during the dry period. All cows with colostrum milk fatty acid data were included in the current study. A detailed description of diets, DMI, milk production, and fatty acid analysis has previously been described (Mann et al., 2015), and the detailed analysis of colostrum samples can be found elsewhere (Mann et al., 2016). In brief, blood samples were taken from the coccygeal vessels 3 times per week before the morning feeding and analyzed cow-side for BHB concentration using a handheld device (Precision Xtra meter, Abbott Diabetes Care Inc., Alameda, CA), whereas serum was analyzed for NEFA concentration using a colorimetric measurement of an enzymatic reaction [HR Series NEFA-HR (2), Wako Life Sciences, Mountain View, CA]. Cows were considered positive for HYK if one or more samples yielded a BHB concentration of ≥1.2 mmol/L between 3 to 14 DIM. Cows were considered as having elevated concentrations of serum NEFA (**$NEFA_H$**) when concentrations were ≥1.0 mmol/L for one or more samples between 3 to 14 DIM and otherwise classified as having low concentrations (**$NEFA_L$**). Colostrum samples as well as a composite sample of morning and afternoon milk samples from the second week after calving were analyzed for fatty acid composition as previously described (Lock et al., 2013).

Univariable logistic regression analysis was carried out with the binary dependent variables HYK/non-HYK and $NEFA_H$/$NEFA_L$, respectively, where HYK and $NEFA_H$ were considered as test positive outcomes. Each fatty acid or fatty acid ratio was screened using the statistical software package JMP (v. 11.0; SAS Institute Inc., Cary, NC) for an area under the curve (**AUC**) of the ROC curve ≥0.70, which was considered a useful cut-off according to Swets (1988). The closer the ROC AUC gets to 1, the more accurate the fatty acid threshold is in correctly classifying cows in each group. Ratios of fatty acids included those between *cis*-9 C18:1 and C15:0 as proposed by Jorjong et al. (2014, 2015) as well as between those fatty acids with ROC AUC ≥0.70 that were increased in HYK or $NEFA_H$ with C15:0 (Jorjong et al., 2014, 2015). Threshold values were determined based on the concentrations and ratios that yielded maximum sensitivity and specificity as previously described (Ospina et al., 2010). Sensitivity, specificity, and positive and negative predictive values were calculated after classification of cows according to the established threshold values identified by our analysis. In addition, those thresholds proposed by Jorjong et al. (2014, 2015) were also evaluated. The accuracy of the test was calculated as the correct classification rate (CCR, %) = {[(number of true positives + number of true negatives)/total number tested] × 100}.

In addition to using fatty acid ratios to improve accuracy, and because of the interest in having the high-

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