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Use of milk fatty acids to estimate plasma nonesterified fatty acid concentrations as an indicator of animal energy balance

J. R. R. Dórea, E. A. French, and L. E. Armentano[1]
Department of Dairy Science, University of Wisconsin, Madison 53706

ABSTRACT

Negative energy balance is an important part of the lactation cycle, and measuring the current energy balance of a cow is useful in both applied and research settings. The objectives of this study were (1) to determine if milk fatty acid (FA) proportions were consistently related to plasma nonesterified fatty acids (NEFA); (2) to determine if an individual cow with a measured milk FA profile is above or below a NEFA concentration, (3) to test the universality of the models developed within the University of Wisconsin and US Dairy Forage Research Center cows. Blood samples were collected on the same day as milk sampling from 105 Holstein cows from 3 studies. Plasma NEFA was quantified and a threshold of 600 μEq/L was applied to classify animals above this concentration as having high NEFA ($NEFA_{high}$). Thirty milk FA proportions and 4 milk FA ratios were screened to evaluate their capacity to classify cows with $NEFA_{high}$ according to determined milk FA threshold. In addition, 6 linear regression models were created using individual milk FA proportions and ratios. To evaluate the universality of the linear relationship between milk FA and plasma NEFA found in the internal data set, 90 treatment means from 21 papers published in the literature were compiled to test the model predictions. From the 30 screened milk FA, the odd short-chain fatty acids (C7:0, C9:0, C11:0, and C13:0) had sensitivity slightly greater than the other short-chain fatty acids (83.3, 94.8, 80.0, and 85.9%, respectively). The sensitivities for milk FA C6:0, C8:0, C10:0, and C12:0 were 78.8, 85.3, 80.1, and 83.9%, respectively. The threshold values to detect $NEFA_{high}$ cows for the last group of milk FA were ≤2.0, ≤0.94, ≤1.4, and ≤1.8 g/100 g of FA, respectively. The milk FA C14:0 and C15:0 had sensitivities of 88.7 and 85.0% and a threshold of ≤6.8 and ≤0.53 g/100 g of FA, respectively. The linear regressions using the milk FA ratios C18:1 to C15:0 and C17:0 to C15:0 presented lower root mean square error (RMSE = 191 and 179 μEq/L, respectively) in comparison with individual milk FA proportions (RMSE = 194 μEq/L), C18:1 to even short-medium-chain fatty acid (C4:0–C12:0) ratio (RMSE = 220 μEq/L), and C18:1 to C14:0 (RMSE = 199 μEq/L). Models using milk FA ratios C18:1 to C15:0 and C17:0 to C15:0 had a better fit with the external data set in comparison with the other models. Plasma NEFA can be predicted by linear regression models using milk FA ratios.

Key words: energy balance, linear regression, milk fatty acid, threshold

INTRODUCTION

Negative energy balance (**NEB**) can lead to a variety of early lactation metabolic disorders, reduced fertility, and ultimately decreased milk production (Collard et al., 2000). One indicator of NEB related to adipose mobilization is elevated plasma nonesterified fatty acid (**NEFA**) concentrations (Grummer, 1993). Increased plasma NEFA concentration is correlated with greater prevalence of fatty liver, mastitis, dystocia, retained placenta, and displaced abomasum (van Knegsel et al., 2005). In addition to use for monitoring successful adaptation during early lactation, a sudden elevation in plasma NEFA could be an early indicator of reduced feed intake, which may be otherwise unnoticed for individual cows in a group management setting.

Milk fatty acids (**FA**) are related to stage of lactation in a manner suggesting they relate to the energy balance of dairy cows. Craninx et al. (2008) reported that as animals increased in DIM, the ratio of milk *cis*-9 C18:1 to C14:0 and C17:0 to C15:0 decreased. This relationship suggests a negative correlation between these milk FA ratios and improved energy balance that occurs as lactation proceeds. However, neither plasma NEFA concentrations nor actual energy balance were reported.

Recent studies have proposed the use of milk *cis*-9 C18:1 proportion of total milk FA as a biomarker to diagnose plasma NEFA above a specific threshold (Jor-

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[1]Corresponding author: learment@wisc.edu

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jong et al., 2014, 2015; Mann et al., 2016) and the use of C18:1 to C15:0 ratio as a potential tool to diagnose hyperketonemia incidence. However, in those studies the thresholds and the relationship between plasma NEFA and milk FA were defined based on single studies. The milk FA were analyzed in samples collected from a single time point: 10 DIM (Mann et al., 2016), or after the second week postpartum (Jorjong et al., 2014). The use of single studies with the cohorts on the same diet and management practices likely limits the variability of milk fat composition (Mann et al., 2016) as well as the capacity to generalize the information across herds. Thus, our objectives were (1) to determine if milk FA proportions were consistently related to plasma NEFA across multiple studies and milk sample time points, (2) to determine a milk FA threshold to identify cows with elevated plasma NEFA, and (3) to test the universality of linear models developed within the University of Wisconsin and US Dairy Forage Research Center (**USDFRC**) in an external data set from other published experiments.

MATERIALS AND METHODS

This study was divided into 3 parts. First, we screened 30 milk FA (% total milk FA; C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, *iso*-C14:0, *cis*-9 C14:1, C15:0, *iso*-C15:0, *anteiso*-C15:0, C16:0, *iso*-C16:0, *cis*-9 C16:1, C17:0, *iso*-C17:0, *anteiso*-C17:0, *cis*-9 C17:1, C18:0, total C18:1, *cis*-9 *cis*-12 C18:2, *cis*-9 *trans*-11 C18:2, *trans*-10 *cis*-12 C18:2, *cis*-9 *cis*-12 *cis*-15 C18:3, and *cis*-6 *cis*-9 *cis*-12 C18:3) and 4 milk FA ratios [C17:0 to C15:0, C18:1 to C15:0, C18:1 to C14:0, and C18:1 to even short-medium-chain fatty acids (**eSMCFA**)] from 3 short-term experiments (French et al., 2012; Lobos Sandoval, 2009; R. Grummer, emeritus professor, University of Wisconsin, Madison, unpublished data) to evaluate the capacity of those FA proportions to classify cows with elevated plasma NEFA concentration (NEFA $\geq$600 μEq/L) according to a determined milk FA threshold. The eSMCFA were considered as the sum of milk C4:0, C6:0, C8:0, C10:0, and C12:0. The milk FA thresholds were identified through receiver operating characteristic (**ROC**) curves when ROC area under the curve (**AUC**) was $\geq$0.80. Second, we developed 6 linear regression models using the milk FA selected based on AUC $\geq$ 0.80. Two models were developed based on individual milk FA proportions, and 4 models were developed using the milk FA ratios. Finally, we assessed if the linear models developed using individual animals from the University of Wisconsin and USDRFC studies fit an external data set from a wider population using treatment means from the literature.

Experiment Descriptions

The first of the 3 Wisconsin studies study was a 4 × 4 Latin square experiment (4 cows, n = 16 observations, French et al., 2012). Four mid-lactation cows averaging 119 DIM were ruminally infused with acetate, propionate, isovalerate, or anteisovalerate while being fed the same TMR. Measurements were taken over 8 d total (four 2-d periods). The infusion of both 3-methylbuyrate (isovalerate) and 2-methylbutyrate (anteisovalerate) markedly decreased DMI in comparison with acetate and propionate, but only 3-methylbuyrate increased plasma NEFA and BHB. In this study (French et al., 2012), some treatments induced high plasma NEFA concentration because the cows used in that trial were mid-lactation animals, and normally those animals would not have high NEFA.

The second study (60 cows, n = 173 observations used, Lobos Sandoval, 2009) was a transition cow study in completely randomized block design, where cows were fed the same basal ration until 3 wk postpartum. The diet contained (g/kg of DM): 300 corn silage, 200 alfalfa silage, 218 rolled high-moisture shelled corn, 25 alfalfa hay, and 235 concentrate mixture, with supplemental vitamins and minerals. Milk and blood samples were collected weekly over the first 3 wk of lactation for milk FA and plasma NEFA analysis (7, 14, and 21 d postpartum).

The third study used in the data set was another transition cow study where animals were used in a completely randomized block design (45 cows, n = 15 sampled observations; R. Grummer, emeritus professor, University of Wisconsin, Madison, unpublished data). The 2 different experimental diets fed were (g/kg of DM): 240 corn silage, 240 alfalfa silage, and 520 of a concentrate mixture containing supplemental vitamins and minerals. The difference between the 2 diets was the addition of 20 g/kg of DM calcium salts with different FA profiles. Diet A contained the majority of total diet FA as C16:0 (45.6%) and *cis*-C18:1 (36.5%). Diet B contained the majority of total diet FA as *cis*-C18:1 (18.8%) and *trans*-C18:1 (35.7%). Milk FA and plasma NEFA concentrations were determined over the first 2 wk of lactation (2, 6, and 12 d postpartum).

In all 3 studies, milk was composited by cow by day based on milk volume produced at each milking. The composited milk samples were centrifuged for collection of fat cake for FA isolation by hexane-isopropanol extraction according to Hara and Radin (1978). Isolated milk fat was methylated and prepared for GLC analysis of individual known and unknown milk FA from C4:0 to C24:1 (Chouinard et al., 1999). Milk fat samples were analyzed on a PerkinElmer Clarus 500 (Norwalk, CT) using the column and temperature program described

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