

Milk fatty acid profiles in early lactation as potential indicators for reproduction success

D. Warner¹, L. Fadul-Pacheco¹, R. Gervais² and D.E. Santschi¹

¹Lactanet, 555, boul. des Anciens-Combattants, Sainte-Anne-de-Bellevue, H9X 3R4, Québec, Canada

²Département des sciences animales, Université Laval, 2425, rue de l'Agriculture, Québec, G1V 0A6, Québec, Canada
Corresponding Author: dwarner@lactanet.ca

The reproductive performance of a dairy cow can be influenced by her metabolic status during early lactation. As this period is also characterized by important changes in the milk yield and milk fatty acid (FA) profile, we aimed to assess the potential of first test date milk FA profiles analysed by Fourier-transform infrared (FTIR) spectroscopy to predict the subsequent reproduction performance. First-test date records from 246,345 Holstein cows in early lactation (5–35 DIM) across Quebec, Canada, were included in the analysis. Records pertained to 2,835 herds and spanned over 2 years (2020 and 2022). Cows were clustered in similar cohorts based on their first test date milk composition, FA (expressed as g/100 g of total FA) and yield using principal components. Clustering was based on the CLARA concept with a k-medoid approach on subsamples to overcome the limitation of computational resources in clustering a large dataset. Three clusters were identified after iteration. Reproduction success was assessed based on the interval between first service to conception (FSTC) and culled by 60 DIM (CULL) using a linear regression and binomial logistic regression mixed effect model, respectively. Cluster 3 was composed of cows with high preformed FA (56.2% on total FA basis), 18:1-to-14:0 ratio (4.74), BHB (0.17 mM) and fat-to-protein ratio (1.51), but low de novo FA (17.1%). In contrast, Cluster 2 was composed of cows with low preformed FA (39.9%), 18:1-to-14:0 ratio (2.08), BHB (0.08 mM) and fat-to-protein ratio (1.17), but high de novo FA (25.8% total FA). Cluster 1 was in between Cluster 3 and 2 and had the highest milk yield. The FSTC was greatest ($P < 0.001$) for Cluster 3 (62.2 days) followed by Cluster 1 (60.7 days) and Cluster 2 (57.9 days). Likelihood for CULL was greatest ($P < 0.001$) for Cluster 3 (odds ratio of 2.6). This preliminary analysis suggests that FTIR milk FA profiles at first test date could be used as early indicators for the following reproduction success of dairy cows and help improve the transition management through continuous monitoring.

Abstract

Keywords: Decision support, DHI, fertility, early lactation.

The increased mobilization of body reserves after parturition is reflected by changes in milk composition and milk fatty acid (FA) profiles, in particular through increased uptake of FA by the mammary gland and decreased de novo synthesis of FA. As early lactation is also a critical phase for later production and reproduction performance with metabolically challenged cows more likely to suffer from early lactation disorders, first test date milk composition might be used as early indicator for reproductive success. The objective of this study was to assess whether first test milk components and FA

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profiles are associated with the subsequent reproductive performance in commercial dairy herds using a large DHI dataset.

Material and methods

Test day records with milk components, milk and reproduction performance were obtained by Lactanet database (Lactanet, Sainte-Anne-de-Bellevue, Canada). For this retrospective study, we restricted the dataset to first test date records from Holstein cows in early lactation (5-35 DIM) with test results over two complete years (2020 and 2021). The dataset included 246,345 cows (30% in parity 1; 26% in parity 2; and 44% in parity 3 or more). In total, 2,835 dairy herds from Quebec, Canada, equipped with a pipeline (N = 2,226), parlour (N = 251) or automatic (N = 358) milking system were included in the study. As a confirmed gestation was not recorded, the subsequent lactation start date was used to define the actual conception date. Therefore, for reproduction performance indicators requiring a confirmed gestation, the dataset was limited to 28,254 test dates from 100,711 cows with tests dates until July 2021.

Milk samples were analysed as regular DHI milk samples for fat, protein, lactose, MUN, SCC, and FA by Fourier-transform infrared (FTIR) using MilkoScan FT+ and MilkoScan 7 RM instruments (Foss, Hillerød, Denmark). Fatty acids included individual and groups of FA (C14:0, C16:0, C18:0, and sum of C18:1, saturated, mono-unsaturated, poly-unsaturated, short-chain, medium-chain, long-chain, de novo, mixed, and preformed FA) as described in FOSS Application Note 64 and Schwarz *et al.* (2018).

Data were cleaned to remove implausible values and outliers by removing data below the 1st and above the 99th percentile. The milk FA profile was expressed as % of total FA (TFA; i.e., the sum of de novo, mixed, and preformed FA).

Cows were grouped in similar cohorts based on their first test date milk composition and yield using k-medoid clustering via principal components. The principal components were used to de-noise the data and to balance the influence of similar milk components. To deal with the large number of observations a sampling approach was used for clustering (CLARA; Kaufman and Rousseeuw, 1990). The procedure consists of randomly splitting the dataset into multiple subsamples and applying the PAM algorithm (Partitioning Around Medoids; Kaufman and Rousseeuw, 1990) to generate the optimal set of cluster centres for each subsample, here computed based on a dissimilarity function based on the Euclidean distance. The optimal number of clusters was evaluated using the average Silhouette approach.

Differences in milk composition and reproduction performance among the identified clusters were evaluated through a mixed effect linear regression with fixed effects assigned to cluster, milking system, lactation, DIM at test date, season and year, and random effects assigned to herd. For a binary outcome, a mixed effect logistic regression was used. All analyses were conducted using R (version 4.1.3; R Foundation for Statistical Computing, Vienna, Austria) and add-on packages computing the principal components (FactoMiner; Lê *et al.*, 2018), clusters (cluster; Mächler *et al.*, 2022), and mixed effect linear regression (lmerTest; Kuznetsova *et al.*, 2017), and mixed effect logistic regression (lme4; Bates *et al.*; 2015).

Results and discussion

Clustering animals based on their first test milk composition, yield, and fatty acid profiles resulted in three distinct clusters. Most cows were assigned to Cluster 1 but herds, herd size and housing system were similarly distributed among clusters (Table 1).

The milk composition differed among clusters ($P < 0.001$; Figure 1) which resulted in Cluster 3 having the least ideal profile with a high fat-to-protein ratio (FPR; mean of 1.51), high BHB (0.17 mmol/L), high preformed FA (56.2% of TFA), a high C18:1-to-C14:0 ratio (4.74), low de novo FA (17.1% of TFA), and low mixed FA (26.8% of TFA). Although the FPR is typically high for early lactation cows due to the higher milk fat synthesis during the postpartum negative energy balance, cows with a FPR value greater than 1.5 (Heuer *et al.*, 1999) or 2.0 (Toni *et al.*, 2011) may show an increase in postpartum diseases. Likewise, cow with milk BHB above 0.20 mmol/L were more likely to suffer from hyperketonemia (Denis-Robichaud *et al.*, 2014), and cows with de novo FA below 20% of TFA were more likely to have early-lactation diseases and be removed from the herd (Bach *et al.*, 1999). Milk composition for Cluster 2 was particular high in de novo (25.8% of TFA) and mixed (34.5% of TFA) FA, low in preformed FA (39.7% of TFA) and C18:1 to C14:0 (2.08). Cluster 1 was intermediate among the three clusters but had a higher milk yield (+9.1 kg milk to Cluster 2).

Table 1. Cluster description.

Variable	Cluster		
	1	2	3
Number of herds	2722	2726	2717
Number of cows	107,657	72,889	57,913
Number of lactating cows (median)	71.7	71.9	67.9
Number of herds in tie stalls	2,195	2,200	2,194

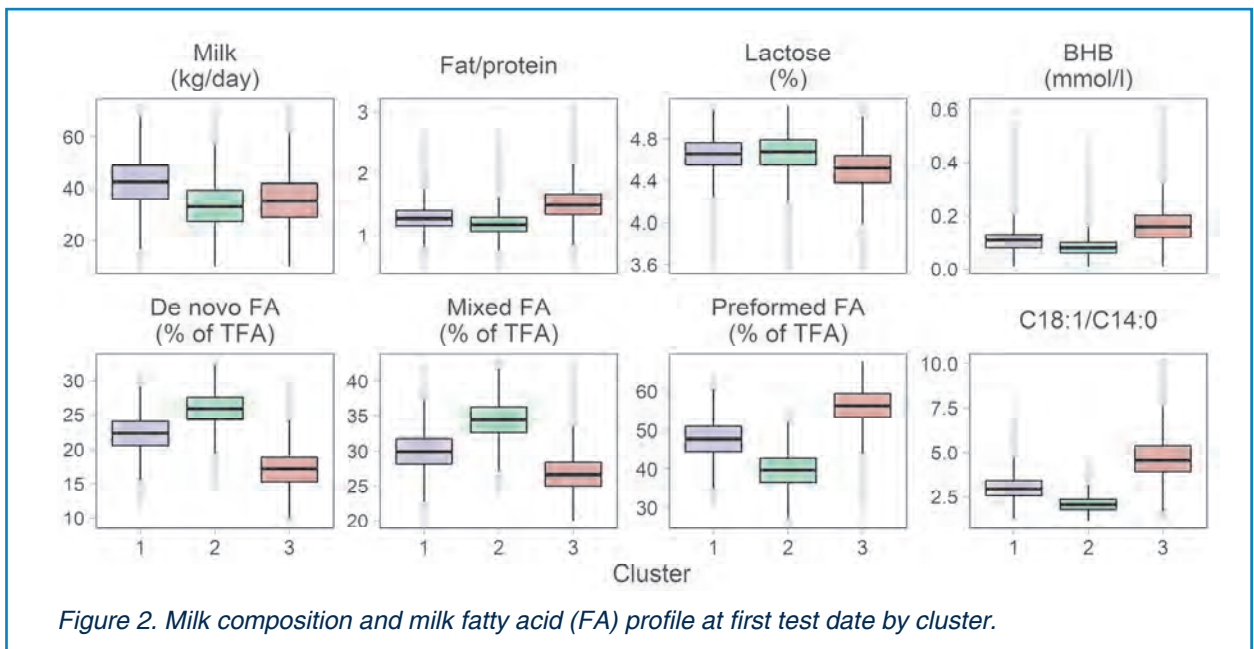


Figure 2. Milk composition and milk fatty acid (FA) profile at first test date by cluster.

Preliminary analyses suggested that the reproduction performance differed among clusters. Cluster 3 with the least ideal milk composition and FA profile was associated with the longest ($P < 0.001$) interval between first breeding to conception at 62.2 days (confidence interval of 32.2-92.3 days) compared to 57.9 (27-8-87.9) days for Cluster 2 and 60.7 (30.6-90.8) days for Cluster 1. Likewise, cows assigned to Cluster 3 were more likely ($P < 0.001$) to be culled before 60 DIM than cows in Cluster 2 (odd ratio of 2.6 and 1.3, respectively). The contrast among clusters is expected to be even more greater considering that cows left before the event occurred were not considered for first breeding to conception (e.g., cows removed before the first breeding or before the start of the next lactation). Future studies should therefore use a survival analysis approach to take into account censored observations in the analyses.

These findings suggest that the milk composition and milk FA profile at first test date can be indicative for the following reproduction success. As such, these findings can be useful for prevention measures and can help with transition management and decision-making support for future lactations but will be of limited use for current early lactation cows due to the monthly DHI test sampling scheme generally used in practice. A more frequent test sampling during the first two weeks of lactation should be considered for a timely intervention. Likewise, test day information from the previous lactation might be relevant for the current reproduction performance and add predictive power to forecast future reproduction issues.

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