

The use of fatty acid profiles from milk recording samples to predict body weight change of dairy cows in early lactation in commercial dairy farms

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Most cows face energy deficits in early lactation during peak milk production, which is

Abstract

reflected in the milk fatty acid (FA) profile. These cows typically mobilize body reserves to maintain milk fat production, and synthetize less FA de novo in the mammary gland. Milk FA can be predicted routinely by Fourier-transform infrared (FT-IR) spectroscopy. This rapid milk analysis offers therefore an opportunity to develop an early indicator for body weight change (BWC) based on the milk FA profile. The objective of this study was to validate if the milk FA profile can be used to predict BWC in early lactating cows in commercial dairy farms. Data originated from 17,067 Danish Holstein cows at 7-35 days in milk across 166 herds in Denmark between March 2015 and March 2017 with body weight (BW) records from floor scales in Lely automatic milking systems at each milking. Milk FA in test-day milk samples were predicted by FT-IR on FOSS instruments providing four individual FA and seven groups of FA according to chain length and saturation. Data for BWC predictions included parity, stage of lactation, and test day data for milk production and components (fat, protein, somatic cell count, and FA concentrations). Daily BWC (median \pm standard deviation) was -0.32 \pm 2.66 g/kg of BW (first parity), -0.46 ± 2.82 g/kg of BW (second parity) and -0.60 ± 5.53 g/kg of BW (third parity). Predictions of BWC were based on a random forest model, an ensemble of multiple decision trees that can account for the nonlinear and high dimensional interactions among predictors and, to a certain extent, for a potential collinearity among single FA. The model was validated with ten-fold repeated cross-validation for which 20% of the herds were randomly withhold for validation such that data of a specific herd are used exclusively either to train or to cross-validate the model. The overall root mean square error of prediction after cross-validation was 1.66 g/kg of BW with the model explaining 89.6% of the variance. The five most important variables to develop the model were the short-chain FA group (C4:0-C10:0), oleic acid (C18:1), the medium-chain FA group (C12:0-C16:1), the saturated FA group, and palmitic acid (C16:0). The short-chain and some medium-chain FA are synthesized de novo in the mammary gland, oleic acid originates from body reserves (e.g., during energy deficits), and palmitic and palmitoleic acid (C16:1) originate either from the de novo FA pool or from body reserves and from feed. These results suggest that the FT-IR milk FA profile may be used as an early indicator of BWC in early lactation cows. Nonetheless, before this model can be used in commercial farms, the model needs to be validated for different herd management and feeding strategies, breeds

and country- or region-specific conditions. Further work is needed to assess the impact of the level of BWC on milk production, reproductive performance and health. Future models may gain from the inclusion of other milk components such as betahydroxybutyrate known to be linked to BW loss in early lactation. An early warning system may be implemented for cows with a large BW loss in early lactation based on the FT-IR milk FA profile.

Keywords: dairy herd improvement, fatty acid profile, FT-IR, body weight loss, machine learning.

Introduction

In early lactation, most dairy cows face energy deficits due to a mismatch of milk production and feed intake. Negative energy balance (NEB) varies between cows in extend and duration (Jorritsma *et al.*, 2003). These cows typically mobilize body reserves (Andrew *et al.*, 1994; Grummer and Rastani, 2003) to maintain milk fat production (Bar-Peled *et al.*, 1992). This is reflected in the milk fatty acid (FA) profile. In early lactation, *de novo* FA are synthetized less in the mammary gland and preformed FA are on a higher concentration (Palmquist *et al.*, 1993; Garnsworthy *et al.*, 2006). Furthermore the mobilization of body fat induce a reduction in BW (Grummer and Rastani, 2003). In most commercial dairy farms, BW is not routinely recorded.

In modern milk laboratories, Fourier-transform infrared (FT-IR) spectroscopy is routinely used to determine milk composition and, accordingly, to predicted the milk FA profile.

This rapid milk analysis offers therefore an opportunity to develop an early indicator for body weight change (BWC) based on the milk FA profile. Our objective was to assess whether milk FA by FT-IR analysis can be used to predict BWC in early lactating cows in commercial dairy farms.

Materials and methods

Data source, fatty acid analysis, and body weight Cow information, test day production data, test day milk FA concentrations analyzed by Fourier-transform infrared were obtained by the Danish Cattle database (SEGES, Skejby, Denmark). Milk samples were analyzed as regular DHI milk samples for fat, protein, lactose, MUN, SCC, BHB, and acetone in addition to FA concentrations. Analyses were performed using a Foss MilkoScanFT+/FT 6000 (Foss Electric A/S, Hillerød, Denmark) for infrared evaluation of milk component, equipped with special software (Foss Application Note 0064 / Rev.5; Foss, Hillerød, Denmark) for predicting 11 FA. These FA are 4 individual FA, namely C14:0, C16:0, C18:0 and C18:1, and 7 FA fractions according to their degree of saturation, namely saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), and *trans*-unsaturated (TFA) FA, and their chain length, namely short-chain (SCFA), medium-chain (MCFA), and long-chain (LCFA) FA.

Cow information and BW data recorded by floor scales in Lely automatic milking systems at each milking were obtained by the Danish Cattle database (SEGES, Skejby, Denmark).



The data set of the Danish Cattle database contained:

- The actual weight measured in robot (body weight start [BWs]).
- The BWs corrected for differences between robots in one herd if differences were present (body weight corr [BWc]).
- A smoothed body weight based on locally weighted smoothing (body weight end [BWe]) of BWc.

The initial data set of FA included 232,575 test day records between days in milk (DIM) 5 to 305 of 39,209 Danish Holstein cows from 170 herds in Denmark, collected from March 2015 to March 2017, with records from parity 1 throughout 6. The initial data set of BW included 28,581,762 BW records between DIM 5 to 305 of 35,787 Danish Holstein cows from 168 herds in Denmark, collected from January 2015 to September 2017, with records from parity 1 throughout 3. The number of robots per herd spanned from 1 to 11, whereby 36.3% of the farms held two robots, 23.2% three robots, and 14.3% four robots.

The data sets were edited with numerous consecutive procedures to ensure a high quality of observations and to eliminate abnormal records. According to Hein *et al.* (2018) observations in the FA data set were removed if any of the following conditions was met:

- one or more of the 11 FA fractions was missing from an observation;
- the PUFA concentration was greater or equal to the MUFA concentration;
- the ratio of the sum of the SFA, MUFA, and PUFA contents to the total fat content was less than 0.825 or greater than 1.075 (values chosen such that 5% of remaining observations were removed);
- the ratio of the sum of the SCFA, MCFA, and LCFA contents to the sum of the SFA, MUFA, and PUFA contents was less than 0.84 or greater than 1.04 (values chosen such that 1% of remaining observations were removed).

Based on present parities of 1 to 3 in the data set of BW, we removed parities equal to or greater than 4 in the data set of FA. Therefore, the edited data set of FA included 197,058 test day records of 34,917 Danish Holstein cows from 168 herds.

Observations of BWs in the data set of BW were removed if:

- The weight were less than 300 kg or greater than 1100 kg, and if
- BWs were more than 3 times the standard error away from the smoothed weight of BWs of each cow and each robot.

For further calculations BWe was used since these values were corrected for differences in robots in each herd and were already smoothed for daily differences in body weight. To estimated BWC, we calculated a relative daily BWC for each single DIM as follows:

$$\frac{BW_{DIMx} - BW_{DIMx^{-1}}}{BW_{DIMx^{-1}}} * 100 = daily BWC_{DIMx} \%$$
⁽¹⁾

Data and data editing

To combine daily BWC to a test day, we calculated an average of the last three daily BWC before test day whereby the test day was included (Figure 1). DIM 5 and 6 were removed as a calculation of an average of the last three daily BCW was not achievable. Furthermore early lactation period were defined from DIM 7 to 35. So the final data set included 19,371 test day records with the BWC of 17,067 Danish Holstein cows from 166 herds with an average milk yield of 35.87 kg (SD=10.90, range from 2.10 kg to 76.80 kg). The relative BWC is shown in g/kg of BW. All editing procedures of the initial data sets are listed in Figure 2.

The descriptive statistics of characteristics of milk components and FA of early lactation cows are presented in Table 1.

Statistical analysis

A random forest algorithm (Breiman, 2001) was used to predict BWC based FA and milk components. A random forest is an ensemble of multiple decision trees and can be understood as the sum of piecewise linear functions. The dataset is divided into smaller regions that become more manageable. As such a random forest model can deal with a multitude of linear and nonlinear relationships among predictors, and the complexity inherent to high-dimensional dataset. The model was prepared using 10-fold cross-validation with three iterations. For each iteration, a model was trained on nine splits of the data set and cross-validated on the remaining part of the data set (i.e., one split), for which 20% of the herds were randomly withhold for validation such that data of a specific herd are used exclusively either to train or to cross-validate the model. Model accuracy was estimated based on the average of the 10-fold repeated cross validation. The optimal parameter configuration for each model was evaluated for each model based on the repeated cross-validation and was set at 500 trees. The models were implemented in R (version 3.5.0; R Foundation for Statistical Computing, Vienna, Austria) using the caret modelling package workflow (Kuhn, 2008).

Results and discussion

Milk fatty acid profile

The milk FA profile is a result of complex interactions among dry matter intake, diet composition, rumen fermentation, body reserve mobilization, liver metabolism, and mammary absorption and *de novo* synthesis of FA (Garnsworthy *et al.*, 2006). The onset of lactation is a delicate period for the metabolism of the cow. Body fat mobilization (Andrew *et al.*, 1994; Grummer and Rastani, 2003) to maintain milk fat production (Bar-Peled *et al.*, 1992) lead to a change in milk FA profile and BW loss in early lactation.

In Figure 3, the distribution of the FA groups SCFA, MCFA and LCFA in g/day by lactation 1, 2, and 3 plus higher Lactations of Danish Holstein cows are shown. In early lactation (DIM 7 to 35), the concentrations of SCFA and MCFA were increased by DIM. Concentrations of LCFA are decreased rapidly by DIM. Differences in concentrations of milk FA across parity are also apparent. With increasing lactation, the concentration of FA increased due to a higher production of milk fat and a stronger mobilization of body fat due to a stronger NEB.

SCFA are synthetized *de novo* and the synthesis of these FA in the mammary gland is less in early lactation (Palmquist *et al.*, 1993; Garnsworthy *et al.*, 2006). According to Garnsworthy *et al.* (2006), the molar proportions of the FA C10:0 to C14:0 were significantly lower in early lactation than in mid lactation. Palmquist *et al.* (1993) reported in early lactation lower concentrations of FA C6:0 to C14:0 and concluded that *de novo* synthesis of FA was inhibited by LCFA from body fat.

Table 1. Characteristics of milk components and milk fatty acids (g/100g milk) in early lactation (DIM 7-35) Danish Holstein cows by parity.

	Parity 1 (n = 8,323 an imals)					Р	Parity 2 (n = 6,716 animals)					Parity 3 (n = 4,332 animals)				
Trait ¹	Mean	SD	р1	Median	p99	Mean	SD	p1	Median	p99	Mean	SD	p1	Median	p99	
Fat (%)	4.50	0.97	2.56	4.38	7.44	4.25	0.91	2.43	4.16	6.86	4.33	0.98	2.41	4.25	7.25	
Prote in (%)	3.41	0.31	2.78	3.39	4.22	3.37	0.33	2.72	3.37	4.28	3.34	0.34	2.71	3.30	4.26	
Fat:Protein	1.32	0.28	0.77	1.29	2.19	1.26	0.27	0.73	1.23	2.10	1.30	0.29	0.75	1.27	2.28	
SFA	2.68	0.58	1.48	2.62	4.39	2.57	0.57	1.33	2.53	4.03	2.60	0.60	1.40	2.55	4.32	
MUFA	1.33	0.41	0.66	1.27	2.67	1.23	0.37	0.62	1.17	2.42	1.28	0.42	0.62	1.21	2.65	
PUFA	0.17	0.05	0.08	0.17	0.31	0.16	0.04	0.01	0.16	0.28	0.16	0.04	0.07	0.16	0.30	
SCFA	0.43	0.11	0.23	0.43	0.74	0.43	0.10	0.22	0.43	0.69	0.44	0.10	0.23	0.43	0.72	
MCFA	1.59	0.37	0.84	1.55	2.68	1.52	0.38	0.75	1.49	2.51	1.52	0.39	0.76	1.48	2.64	
LCFA	1.90	0.59	0.85	1.81	3.74	1.76	0.54	0.78	1.69	3.42	1.83	0.60	0.79	1.75	3.77	
C 14:0	0.37	0.09	0.20	0.36	0.63	0.36	0.09	0.19	0.35	0.59	0.36	0.09	0.18	0.35	0.61	
C 16:0	1.11	0.25	0.62	1.09	1.87	1.05	0.25	0.56	1.03	1.74	1.05	0.26	0.56	1.03	1.81	
C 18:0	0.59	0.17	0.29	0.57	1.09	0.54	0.15	0.25	0.52	0.98	0.56	0.17	0.26	0.54	1.08	
C 18:1	1.20	0.40	0.54	1.14	2.46	1.11	0.35	0.51	1.05	2.22	1.15	0.40	0.51	1.09	2.48	

¹Trait: SFA = saturated fatty acids; MUFA = mono unsaturated fatty acids; PUFA = poly unsaturated fatty acids; SCFA = short-chain fatty acid; MCFA = medium-chain fatty acid; LCFA = long-chain fatty acid.

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Figure 2. Editing procedures of initial data sets.

MCFA originate either from the *de novo* FA pool or from body reserves and from feed. Generally, 60% of C16:0 originate from *de novo* synthesis (Moore and Christie, 1981). In the study of Garnsworthy *et al.* (2006) the yield of C16:0 was 11% higher in early lactation than mid lactation. This is contrary to our results as seen in Figure 3. According to the Foss Application Note 0064 / Rev. 5 (Foss, Hillerød, Denmark), the prediction model estimated for MCFA the total group of C12, C14 and C16 FA. This could explain the lower concentrations of MCFA in early lactation compared to later stages in lactation.

LCFA are preformed FA and originate from body fat and from feed. In early lactation these FA are on a higher concentration than in mid lactation due to increased mobilization of body fat in early lactation (Palmquist *et al.*, 1993; Garnsworthy *et al.*, 2006). In adipose tissue, C18:1c9, C16:0 and C18:0 account for nearly 90% of the FA and are contained in approximately equal molar proportions (Christie, 1981). The mobilization of body reserves, especially body fat, would be expected to increase incorporation of these FA into milk fat. Garnsworthy *et al.* (2006) reported an 80% higher yield of C18:1c9 for early lactation cows than for mid lactation cows. 50% higher concentrations for C18:1 and C18:0 in milk fat in week 1 than week 16 was reported by Palmquist *et al.* (1993).

BW characteristics in early lactation Danish Holstein cows by parity are shown in Figure 4. Distribution of initial BW at DIM 7 showed a normal distribution and differed across parity (570, 641, and 681 kg for first, second, and third parity, respectively).

Time from calving to nadir BW differed across parity (26, 37, and 39 DIM for first, second, and third parity, respectively). Other studies showed similar results. Maltz (1997) showed in a visual analysis of 40 primiparous and 64 multiparous Israeli Holsteins cows a BW trough by days 25-30. The BW weight data were collected after each milking (thrice daily at 8-h intervals) by a walk-through scale and averaged daily. Van Straten *et al.* (2008) reported a similar mean DIM from calving to nadir BW with 29, 34, and 38 for first, second, and third and above parity, for high-producing cows. In their study 250,920 daily BW measurements were included to constructed standard relative BW curves, which were corrected for the random effect of farm and the correlation between repeated measurements in the same cow (van Straten *et al.*, *al.*, *and al.*, *and*, *and*,



Figure 3. Distribution of the FA groups SCFA, MCFA, and LCFA in g/day for days in milk of Danish Holstein cows. The green range represent the early lactation from DIM 1 to 35.

Relative BWC and BWC curves



2008). The distribution of DIM from calving to nadir BW increased with parity. These results suggest that the duration of NEB increased with an increase in parity, whereby the NEB was lower for first parity as compared to older cows (van Straten *et al.*, 2008).

The relative daily BWC differed across parity and lactation stage (Figure 5). The relative daily BWC was (median ± standard deviation) -0.32 ± 2.66 g/kg of BW, -0.46 ± 2.82 g/kg of BW, and -0.60 ± 5.53 g/kg of BW for first, second, and third parity. In the study of Maltz (1997), 77% of the multiparous cows lost 5-15% of their post-calving weight in the period of minimal BW between days 25-40. From calving to nadir BW, the standard first-parity cow lost 6.5% of its initial BW, for standard second- and greater-parity cows, relative BW loss was 8.5 and 8.4% (van Straten, 2008). Zachut and Moallem (2017) found an average BW loss from week 1 to 5 of 5.87 and 7.27 % for first-parity cows, 4.83 and 6.49 % for second-parity cows, and 5.45 and 7.80 % for third-parity cows. In the study of Zachut and Moallem (2017), BW was measured 3 times a day from calving and they distinguished between 2 groups of different BW loss: low weight loss and high weight loss. Difference in relative mean daily BWC across parity and lactation stage are shown in Figure 5. The initial rate of BWC seemed similar in the 3 parity groups. First-parity cows reached a positive daily BWC at an earlier DIM and gained BW at a greater rate than second- and third- and greater-parity cows, respectively. Second- and third- and greater-parity cows showed similar attainment of a positive daily BWC but second-parity cows gained BW at a greater rate than third- and greater-parity cows. Similar findings were reported by van Straten et al. (2008). By 120 DIM, first-parity cows reached 98.8% of their original BW. Second- and greater-parity cows reached 93.9 and 92.8% of their original BW (van Straten, 2008).

Maltz et al. (1997) described BWC as a result of two factors:

- Changes in body reserves (mobilization in early lactation, and deposition and late lactation), and
- Changes in gastrointestinal (GI) size and content (increased in early lactation, decreased in late lactation) as well as other metabolism-supporting organs (liver, kidney etc.).



Figure 4. Daily body weight of dairy cows across 168 Danish Holstein herds with nadir body weight obtained at 26 days (1st parity), 37 days (2nd parity) and 39 days of lactation (3rd parity).

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To predict BWC of early lactation (7 to 35 DIM) Danish Holstein cows based on FA profile we used a random forest regression model. The random forest regression model included all FA groups and FA, milk yield, fat percentage, protein percentage, a ratio of C18:1 to SCFA, a ratio of fat percentage to protein percentage, DIM, parity, SCC, year and month, and predicted BWC at an accuracy after cross-validation of $R^2_{cv} = 0.896$, MAE_{cv} = 0.761, and RMSE_{cv} = 1.66 g/kg of BW (Figure 6).

The random forest regression model are shown that SCFA had the highest influence, followed by C18:1, MCFA, SFA, and C16:0 (Figure 7). The effects of fat percentage and protein percentage were low as well as year and month although a seasonality of FA concentration in milk is known.

The results have shown that the prediction of the BWC in early lactation would make it possible for the herd manager to continuously know the current metabolism status of the animals in this lactation period, in the context of the monthly milk recording. The use of FA in milk samples is an economically feasible approach without the need of using technical equipment such as floor scales or 3-dimensional vision systems. BW measurements on floor scales showed daily fluctuations (Maltz *et al.*, 1997) due to milk yield, feed intake, water intake and milking times compared to weight times. Therefore, the first step in data analysis has to overcome this obstacle in order to differentiate changes of physiological significance from normal daily fluctuations (Maltz and Metz, 1994).



Figure 5. Relative mean daily body weight change of dairy cows across 168 Danish Holstein herds.

Prediction model for BWC





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Figure 6. Random forest regression model for prediction of body weight changes based on milk fatty acid profiles.



Figure 7. Relative importance of selected variables in random forest prediction model.



The results suggest that BWC can be estimated by FT-IR milk FA profiles in DHI samples. Nonetheless, before this model can be used in commercial farms, the model needs to be validated for different herd management and feeding strategies, breeds and country- or region-specific conditions. Further work is needed to assess the impact of the level of BWC on milk production, reproductive performance and health. Future models may gain from the inclusion of other milk components such as beta-hydroxybutyrate known to be linked to BW loss in early lactation. A prediction of BWC for a real-time decision support tool may not be feasible as any suggested modification in management strategies will likely not improve reproductive performance in the current lactation anymore. However, our prediction model might be useful to identify herd-level deficiencies and improve overall herd management to improve herd performance in future lactations.

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Conclusions

Acknowledgments

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