Can milk analysis by infrared spectroscopy reveal the welfare status of cows?

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Abstract

Assessing welfare status of cows currently requires farm visits. However, detecting welfare status of cows using unbiased analyses of milk samples is a practical alternative as millions of milk samples are routinely collected for milk recording. Thus, we set out to isolate milk infrared spectral fingerprints representing effects of housing-configuration modification on milk composition that may be associated with changes in welfare status of cows. We applied a new analysis method to milk spectra by combining principal component analysis and mixed modelling. This new method was used in trials examining the impact of housing configurations, including 4 tie rail positions and 2 chain lengths, on milk composition. Principal components extracted from the averages of milk spectra collected during weeks 8-10 of the trials revealed a significant effect of the housing configuration treatment on those spectra. This analysis method was capable of capturing changes in milk composition that were attributed to negative and positive changes in welfare status of cows resulting from housing modifications to tie rail positions and chain length, respectively. Spectral analysis revealed higher levels of biomarkers related to body fat mobilization (i.e., beta-hydroxybutyric acid, acetone, and citrate), in milk from cows subjected to the tie rail position that had most restrictive access to feed which may have resulted in a possible reduced feed intake (not measured in the trial) that led to an elevated body fat mobilization. No changes in energy reserves estimable using visual body condition scoring were observed for those cows. These results were corroborated by increased injuries at two locations on cows' necks because of pressure on their neck through repeated contact with the tie-rail, which may be indicative of laboured access to reach feed. In addition, milk from cows tied with longer chains had lower levels of biomarkers linked to episodes of ruminal acidosis (i.e., milk non-protein nitrogen and trans fatty acids). Behavioural observations showed that these cows spent more time with their heads in the manger area, assuming they might have chewed more; hence, they might have produced more saliva to balance the ruminal pH. To conclude, this novel spectral analysis methodology offers a new tool for assessing cow welfare status by detecting trends of changes in milk composition in their early stages, which will provide a mean for remote and unbiased detection of cows or herds with welfare problems before appearance of clinical signs.

Keywords: FTIR spectroscopy, cow welfare, housing configuration.

Introduction

Assessing welfare status of cows currently requires farm visits. However, detecting welfare status of cows using unbiased analyses of milk samples is a practical alternative as millions of milk samples are routinely collected for milk recording. Several studies have demonstrated that milk composition reflects the concentrations of key blood plasma metabolites, such as non-esterified fatty acids (NEFA) (Jorjong *et al*., 2014) and beta-hydroxybutyric acid (BHB) (Pralle *et al*., 2018), the nutritional state of the cow (Weber *et al*., 2013, McParland *et al*., 2014) and health conditions (Arnould *et al*., 2013) that might affect the cow's productivity. Other studies have demonstrated that health issues can be associated with behavioural changes that precede the clinical diagnosis. For example, cows that were diagnosed with a left displaced abomasum showed increased step activity in comparison to healthy animals during the week prior to the clinical diagnosis (Petersson-Wolfe *et al*., 2017). Cows at risk of subclinical ketosis were observed to have fewer feeding visits and 18% less dry matter intake in comparison with healthy controls in the week leading up to calving (Goldhawk *et al*., 2009). It was also observed that lying time was greater and average daily steps were significantly lower five days before cows were diagnosed with mastitis (Yeiser, 2011), and 2 days before onset, lying time decreased compared with that of healthy cows (Petersson-Wolfe *et al*., 2017). In this study, we hypothesized that cow welfare status might lead to physiological changes, which might be reflected in milk chemical composition. Hence, we set out to isolate milk Fourier transform infrared (FTIR) spectral fingerprints representing effects of housing-configuration modification on milk composition that may be associated with changes in welfare status of cows. We applied a new analysis method to milk spectra, which was described in a previous publication (Bahadi *et al*., 2021).

Materials and methods

The animal trials were conducted at the Macdonald Campus Dairy Complex of McGill University (Sainte-Anne-de-Bellevue, QC, Canada).

Trial 1: tie-rail configuration

Forty-eight lactating Holstein cows were assigned to 4 tie-rail (TR) configurations varying in height and position (Table 1). Details about these treatments can be found elsewhere (St John *et al*., 2021). Cows were assigned to 6 blocks to account for parity (primiparous: n = 12, multiparous: n = 36), days in milk (DIM; early: 0–100 d, mid: 101–200 d or late: 201–305 d), and location in the barn prior to the start of the experiment. Cows were housed in two separate rows of tie-stalls facing the barn wall and they were in trial for 10 weeks with 24 cows starting in summer 2016 (period 1: from July 25th to October 3rd) and the remaining 24 cows starting in fall 2016 (period 2: from October 10th to December 19th).

Trial 2: chain length configuration

Twenty-four lactating Holstein cows were assigned to 2 tie chain length (TCL) treatments (Table 1). Details about these treatments can be found elsewhere (Boyer *et al*., 2021). Cows were assigned to 12 different blocks of two cows to account for age of the cow (i.e., parity or number of lactations) and days in milk within current lactation (average DIM 129) and were placed evenly in two rows facing a wall within the barn. The trial lasted for 10 weeks from February 20th, 2017, to May 1st, 2017.

Milk samples

One composite milk sample per week was collected from each cow participating in the trials. The sample consisted of milk collected during the evening milking and the morning milking of the next day. All collected milk samples were analysed for milk composition by FTIR spectroscopy at the Lactanet laboratory (Sainte-Anne-de-Bellevue, QC, Canada) using the same CombiFoss FT+ analyser (FOSS, Hillerød, Denmark).

Spectral analysis

Baseline and treatment application average spectra were calculated for each cow from spectra of samples collected from weeks 1 to 3 and from weeks 8 to 10, respectively. To detect the housing treatment effect, we applied a new analysis method to those spectra by combining principal component analysis (PCA) and mixed modelling. Details of this methodology are described elsewhere (Bahadi *et al*., 2021) and are summarized in Figure 1. The statistical models that were used in the analysis were:

For the TR trial

$$Y_{ijk} = \mu + trt_i + period_j + block_{kji} + e_{ijk} \quad (1)$$

where trt_i was the fixed effect of the i^{th} TR configuration treatment, $period_j$ was the fixed effect of the j^{th} period, $block_{kji}$ was the fixed effect of k^{th} parity, DIM and location in the barn from the period on the TR configuration treatment and was the random residual error.

For the TCL trial

$$Y_{ijkl} = \mu + trt_i + row_j + block_k + e_{ijk} \quad (2)$$

where trt_i was the fixed effect of the i^{th} TCL treatment, $block_k$ was the fixed effect of the k^{th} parity and lactation stage combination, row_j was the random effect of the j^{th} row in the barn and e_{ijk} was the random residual error.

Results and discussion

Principal components (PC) 7 and 6 revealed significant housing treatment effects for the TR (P = 0.011) and TCL (P = 0.032) trials, respectively (Table 2). Both PCs did not reveal significant effects for the other studied factors that were included in their respective statistical models. This observation suggests that the TR configuration and TCL might have influenced milk composition during these two trials. Differences in the least-squares means for scores of PC7 and PC6 for the TR and TCL trials, respectively, suggest that composition of milk from cows enrolled in T3 was significantly different from that of cows enrolled in T1 for the TR trial and the composition of milk from cows enrolled in T1 was significantly different from that of cows enrolled in T2 for the TCL trial (Table 3).

For the TR trial, inspection of the integral of the loading spectrum for PC7 revealed an inverse relationship between lactose and biomarkers related to body fat mobilization

Table 1. Housing configuration treatments across the 2 trials.

Trial	Housing configuration	Treatments			
		T1	T2	T3	T4
1	Tie rail[1]	122 cm x 36 cm	122 cm x 18 cm	112 cm x 18 cm	112 cm x 36 cm
2	Chain length	1.0 m	1.4 m	-	-

[1] Height from the stall base x forward position from the manger wall

Table 2. Principal components extracted from treatment application spectral datasets that revealed significant effects. The table also lists P values obtained from the SAS Mixed Procedure for tested effects in each trial.

Trials	Spectral Dataset[1]	PC	Eigenvalue	Explained Variation %	P Values		
Tie rail	VN FD	7	3.82	1.37	Trt[2] 0.011	Period 0.559	Block 0.060
Chain length	VN FD	6	4.75	1.70	Trt 0.032		Block 0.088

[1] FD = first derivative, VN = vector normalized
[2]Trt = treatment

Table 3. Differences of least squares means for the scores of the principal components that revealed significant treatment effect.

Trial	Trt[1]	Trt	Estimate	Standard Error	DF[2]	t Value	P Value	Scheffé Adj. P Value
Tie rail	T1	T3	2.1442	0.68	30	3.15	0.004	0.033
Chain length	T1	T2	-1.6819	0.6650	9	-2.53	0.032	0.032

[1]Trt = treatment
[2]DF = degrees of freedom

(i.e., BHB, citrate, acetone). This observation can be interpreted that cows subjected to the tie rail position T3 had the most restrictive access to feed which may have resulted in a possible reduced feed intake that led to an elevated body fat mobilization. No changes in energy reserves estimable using visual body condition scoring were observed for those cows (St John *et al*., 2021). The behavioural results (St John *et al*., 2021) showed that cows in T3 had increased injuries at two locations on cows' necks because of pressure on their neck through repeated contact with the tie-rail, which may be indicative of laboured access to reach feed.

For the TCL trial, inspection of the integral of the loading spectrum for PC6 revealed that milk from cows tied with longer chains had lower levels of biomarkers linked to episodes of ruminal acidosis (i.e., milk non-protein nitrogen and trans fatty acids). This observation suggests that cows with a longer chain may have a more stable ruminal pH, which is an indication of better digestive health. Behavioural observations showed that these cows spent more time with their heads in the manger area (Boyer *et al*., 2021). Increased access to the manger could mean easier access to feed, and by doing so, more time was available for rumination and chewing; hence, they might have produced more saliva to balance the ruminal pH.

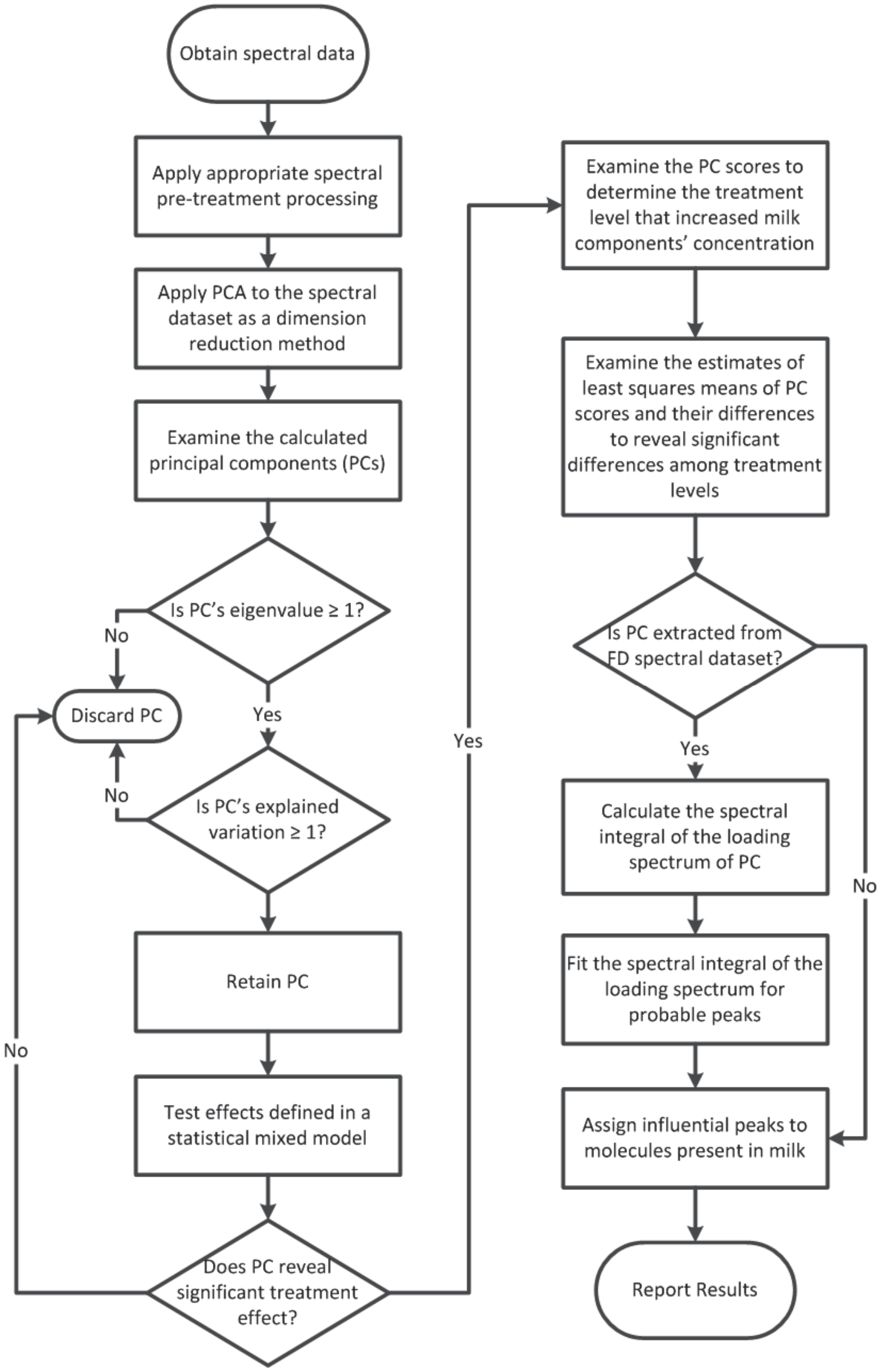

Figure 1. Workflow of hybrid data analysis approach of FTIR milk spectral data that combines multivariate analysis with mixed modelling to detect housing treatment effect (Bahadi et al., 2021).

Conclusion

Milk FTIR spectra could detect changes in milk composition that could be attributed to negative and positive welfare status in cows. Behavioural and clinical results corroborated the findings of the new spectral analysis methodology. This methodology offers a new tool for assessing cow welfare by detecting trends of changes in milk composition in their early stages, which will provide a mean for remote and unbiased detection of cows or herds with welfare problems before appearance of clinical signs.

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Jessica St John (McGill University) conducted the tie rail trial and developed the SAS code to detect the treatment effect on milk components by the mixed procedure as part of her M. Sc. Thesis. Behavioural and other animal welfare outcomes for the tie-rail trial are published in St John *et al*. (2021).

Véronique Boyer (McGill University) conducted the chain length trial and developed the SAS code to detect the treatment effect on milk components by the mixed procedure as part of her M. Sc. Thesis. Behavioural and other animal welfare outcomes for the chain length trial are published in Boyer *et al*. (2021).

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