Predicting glutamate concentration in milk using mid-infrared spectrometry for routine detection of energy-deficient cows

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Abstract

Identifying energy-deficient cows is difficult on farm. To calculate their energy balance, it is necessary to know the feed intake of each cow and their energy requirements. An alternative way would use biomarkers of energy deficiency, that are more accessible on farm. Candidate biomarkers have previously been identified as metabolites, proteins, and microRNAs. Among them, glutamate proved to be an interesting biomarker. The present project aimed to predict the concentration of glutamate in milk based on the mid-infrared (MIR) spectra. 577 MIR spectra were available with known glutamate concentration. Of these, 514 data were from a 6-day feed restriction trial conducted on 18 mid-lactating cows. The feed allowance was restricted to 50% of the energy requirements estimated during the previous *ad libitum* week. The 63 remaining data were from 26 cows that calved next autumn. For these cows, the feed restriction was based on a dilution of the diet with straw during mid-lactation the following spring. Two datasets were then created: a calibration and a validation dataset. The calibration dataset included 70% of the data (mean glutamate concentration = 338.7 µM/L; standard deviation (SD) = 177.3 µM/L) and was used to develop the equation using spare partial least squares regression.

The validation dataset included 30% of the data (mean glutamate concentration = 346.0 µM/L; SD = 138.9 µM/L) and was used to apply the equation to calculate its accuracy [coefficient of determination (R^2) and residual standard deviation (Sy,x)]. Milk glutamate concentrations were predicted with a calibration R^2 = 0.78 (Sy,x = 82.7 µM/L) and a validation R^2 = 0.65 (Sy,x = 82.5 µM/L). This equation is original. Its inclusion in panel of biomarkers paves the way for its use in the detection of energy-deficient dairy cows.

Keywords: dairy cattle, feed restriction, milk metabolite, mid-infrared spectrometry. Presented at the ICAR Anual Conference 2024 in Bled at the Session 2: New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

Dairy cows are very sensitive to negative energy balance. A negative energy balance is the state reached by an animal when the energy provided by its diet is lower than its energy requirements. It can be physiological, such as in early lactation, when requirements are higher than the energy intake, or environmental, when a significant decrease in intake occurs, for example during a feed shortage. Energy deficiency has a negative impact on the profitability of the dairy farm, particularly when it is severe or long-lasting, leading to a drop in milk production, health disorders or reproductive problems.

To prevent or correct energy deficit in dairy cows, we first need to detect it. Breeders detect it through body condition score decrease, but it is usually not sensitive enough for early detection and consequently for early intervention. Blood tests are accurate to diagnose consequences of energy deficit such as ketosis but are invasive. Milk analyses have the advantage of being easy to access but are not specific enough.

In this context, the Biomarq'lait project aimed to identify new biomarkers of energy deficit. A review of the literature (Leduc *et al*., 2021a) has shown that energy deficit has an impact on hormonal regulation, mobilisation of body reserves, mammary gland activity and milk production and composition. Panels of biomarkers, including macro-components, proteins (Leduc *et al*., 2022), microRNAs (Leduc *et al*., 2023), and metabolites (Billa *et al*., 2020; Leduc *et al*., 2021b) have been proposed.

Among milk metabolites, glutamate appears to be a good indicator for several reasons. A rapid decrease in its concentration during dietary restriction, followed by a return to baseline levels during *ad libitum* re-feeding, has been observed (Billa *et al*., 2020, Leduc *et al*., 2021b, Pires *et al*., 2022). In addition, the positive correlation between glutamate concentration and energy balance is very strong (coefficient of correlation = 0.61 according to Billa *et al*., 2020). The response range seems to depend on the intensity of the feed restriction and on the lactation stage. Finally, no difference of glutamate concentration was observed at the beginning of lactation compared to the mid-lactation with ad libitum feeding, suggesting that glutamate could be an indicator of dietary restriction following an ad libitum period (Leduc *et al*., 2021b). Therefore, the objective was to determine the potential of measuring milk glutamate concentrations to detect energy-deficient cows using mid infrared (MIR) spectra already obtained routinely.

Material and methods

To carry out this study, two feed restriction trials were set up. The glutamate concentration was measured in milk, and MIR spectra of milk were collected. A mathematical model was then developed to predict the glutamate concentration in milk from the MIR spectra.

Data collection

The data used come from two trials set up at INRAE (Figure 1): a short and intense restriction protocol (SI) and a protocol set up in a project called DEFFILAIT.

SI protocol was led in 2016 at the Marcenat experimental farm (INRAE, Herbipôle), on 18 cows in mid-lactation. During pre- and post-restriction, the cows were fed an *ad libitum* ration consisting of maize silage (66% of diet DM), barley straw (8% of diet DM), maize grain (8% of diet DM), soybean meal (17% of diet DM) and minerals and vitamins (1% of diet DM). During the experimental period (W1 in Figure 1), feed intake was limited to 50% of cows estimated energy requirements during the previous 6 days (W-1 in Figure 1). Milk and blood sampling kinetics (one sample per day) were used to

Trial	Short & Intense (SI)	DEFFILAIT
Experimental design	W-1 W1 W+1 Day : -3 13	W1 W2 W3 (19 weeks) W-2 W-1 W+1 W+2 W+3 W+4 W+5 Calving 21 days in milk Day : -7 7 Diluting the diet with straw
	Standard feeding, Restricted feeding, Milk and blood sampling, W: week	
Headcount (n)	18 cows	30 cows
Lactation stage	165 days in milk (mean)	21 DIM & 19 weeks in milk
Location	INRAE UMRH UE Herbipôle of Marcenat)	INRAE PEGASE IEPL of Méjusseaume
Reference	Billa *et al.*, 2020	Leduc *et al.*, 2020

Figure 1. Presentation of the experimental protocols.

study responses to restriction and refeeding. The short duration of the feed restriction period meant that each cow could be considered as its own control (Billa *et al*., 2020).

DEFFILAIT protocol was carried out as part of a trial set up during the ANR DEFFILAIT programme at the INRAE experimental farm of the UMR PEGASE (IEPL), on around thirty Holstein cows chosen to be representative of the herd's variability (Fischer *et al.*, 2020). These cows calved in autumn 2017 and were fed *ad libitum* during the first few months of lactation with a constant total mixed ration based on maize silage (65% of diet DM), dehydrated alfalfa (18% of diet DM), soybean meal (18% of diet DM) and production concentrate (9% of diet DM). In mid-lactation, all cows underwent a change of diet in March 2018, moving from the control diet to a restricted diet after a week of dietary transition. For the current work, only the 2 weeks prior to feed restriction and the weeks 2 to 5 (week+1 being the transition week) of feed restriction were kept. The restricted ration was diluted in energy and protein with inclusion of straw (11.5% of diet DM) and aimed to reduce milk production by 20% while maintaining *ad libitum* DM intake. Milk and blood samples were taken at 22 ± 1 days of lactation (D21) from 34 cows that calved in autumn 2017, then at D-7 before dietary change and D+7 after the end of the transition week (Leduc *et al*., 2020).

Immediately after milk sampling, the samples were divided into two aliquots. The first was used to analyse milk glutamate concentrations using an enzymatic-fluorometric method (Larsen and *Fernández, 2017). The second was used to perform the MIR spectrum in milk analysis labs (MyLab, Chateaugiron, France for DEFFILAIT trial; Agrolab's, Aurillac, France for SI trial), which was then standardised (Grelet et al., 2015).* Finally, 577 spectra with an associated measurement of milk glutamate were used for this study (Table 1). Of these, 514 came from the SI trial and 63 from the DEFFILAIT trial.

Table 1. Presentation of the data used.

Trial	Number of data	Number of cows	Lactation number	Days in milk
Short and Intense	514	10 Montbéliarde + 8 Holstein cows	2 to 7	114 to 215 days
DEFFILAIT	63	26 Holstein cows	1 to 6	22 to 205 days

Data processing

Two sets of data were randomly generated from all the 577 spectra. We used 70% of the data from each trial (45 data from DEFFILAIT and 361 data from SI) to constitute the calibration set (n = 406). The remaining 30% (n = 171) constituted the validation set. Figure 2 shows that glutamate levels in milk and the trial of origin of the data are homogeneous between the calibration and validation sets.

An equation was developed by sparse partial least square regression on the data from the calibration set to predict the glutamate concentration in milk from MIR spectra. This equation was then applied to the validation set to calculate its performance, described by the coefficient of determination (R^2), the residual standard deviation ($S_{y,x}$), and the ratio between the standard deviation of the calibration set and the residual standard deviation (RPD).

Results and discussion

The predicted glutamate using the developed equation was compared with the measured values of glutamate obtained using the enzymatic-fluorometric method (Figure 3).

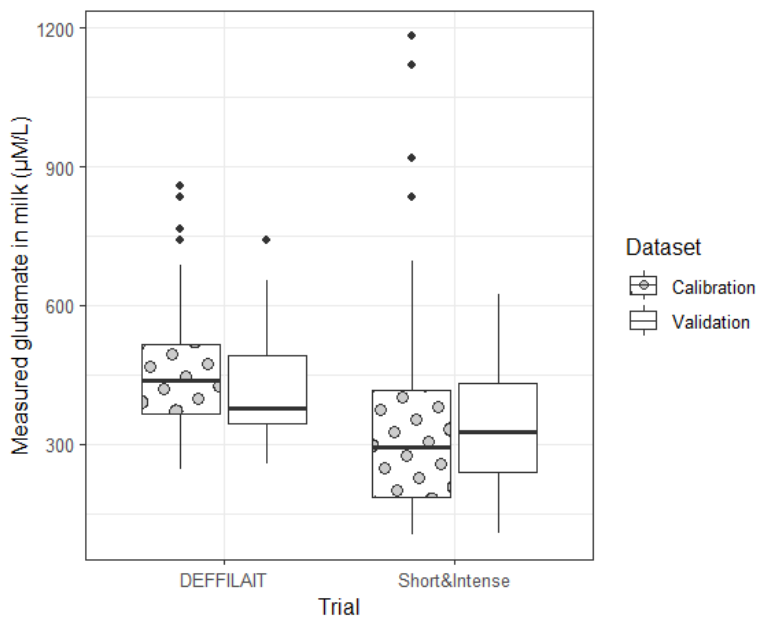

Figure 2. Glutamate levels in milk according to trials in the calibration and validation sets.

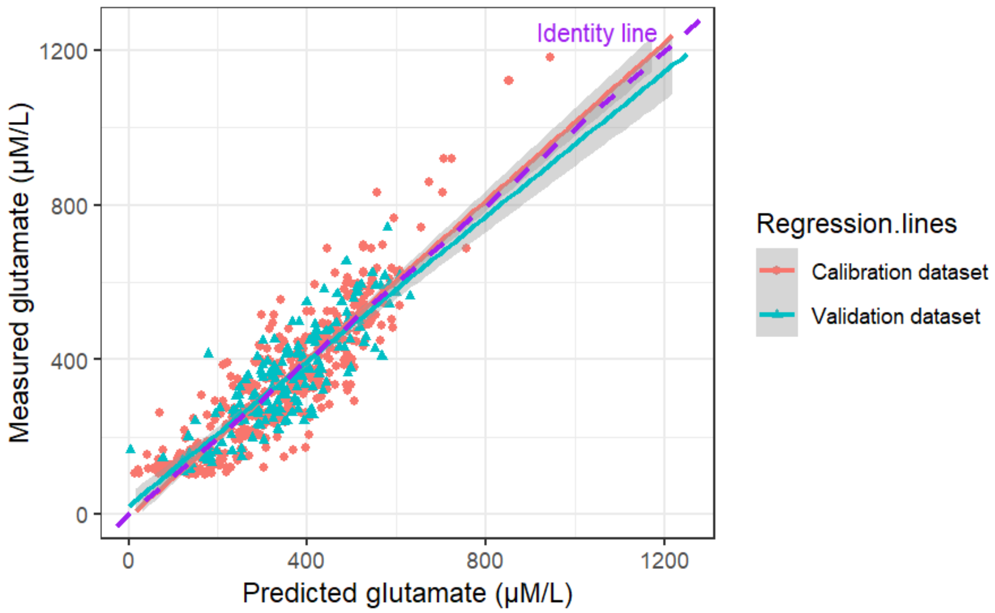

Figure 3. Scatter plot of measured (y-axis) versus predicted (x-axis) values for the glutamate concentration in milk using a partial least squares model built on all 577 milk samples. 1 point = 1 individual milk sample. Circle = data from calibration set; triangle = data from validation set; solid lines = regression lines; dashed line = first bisector.

Table 2. Performances of the milk glutamate prediction equation in the calibration set and validation set.

Dataset	Mean of measured glutamate	SD of measured glutamate	Mean of predicted glutamate	SD of predicted glutamate	SD of Residuals	R^2	RPD
Calibration set	338.7	177.6	340.6	152.2	82.7	0.78	2.15
Validation set	346.0	138.9	348.2	123.4	82.5	0.65	1.68

The performances of the milk glutamate prediction equation developed are showed in Table 2. The equation achieved a calibration coefficient of determination (R^2) of 0.78 and a residual standard deviation ($S_{y,x}$) of 82.7 µM/L. On the validation set, R^2 reached 0.65 with a $S_{y,x}$ of 82.5 µM/L. There is no equivalent equation in the literature to compare with our results. However, this equation appears promising.

To detect cows with energy deficits, the energy status of the animals was projected in Figure 4, with a distinction being made between cows in early lactation, cows in mid-lactation with energy deficit and cows in mid-lactation with energy surplus. The low glutamate values (less than 200 µM/L) only concern cows in mid-lactation with an energy deficit, i.e., on feed restriction. Milk glutamate therefore appears to be specific to feed restriction. This finding opens interesting prospects for improving the prevention of energy deficit when advising livestock farmers.

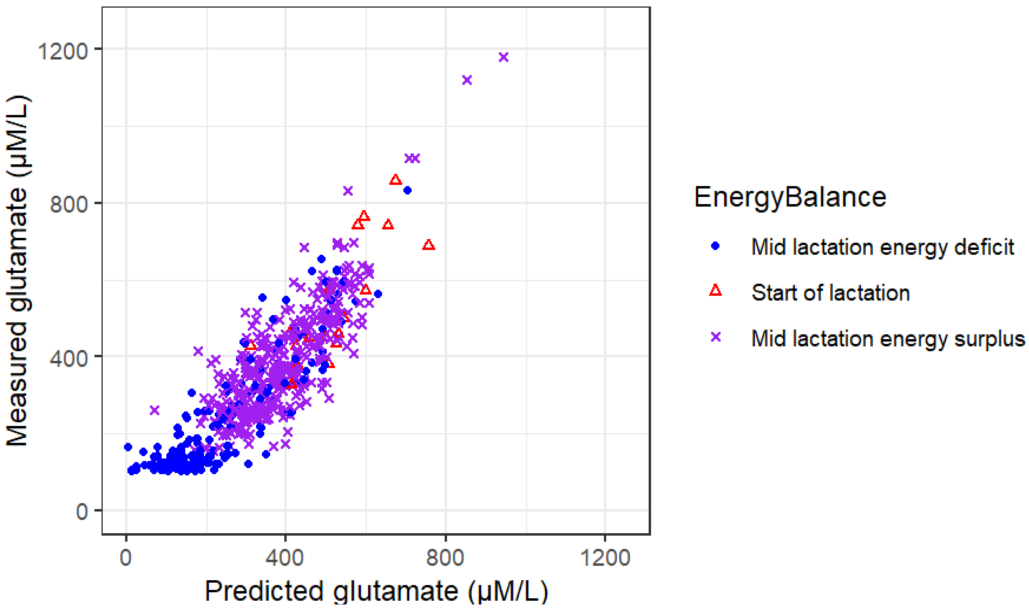

Figure 4. Scatter plot of measured (y-axis) versus predicted (x-axis) values for the glutamate concentration in milk using a partial least squares model built on all 577 milk samples. 1 point 1 individual milk sample. Circle = Mid lactating cows in energy deficit; triangle = early lactating cows; cross = Mid lactation cows without any energy deficit.

To go further, it would be interesting to explore the possibility of predicting the variation in glutamate content to detect situations penalising cows at an early stage, or even the possibility of qualitatively identifying milks with low glutamate concentrations.

Conclusion

Glutamate concentration in milk seems to be an interesting indicator of energy deficit in dairy cows, when it is caused by feed restriction, and can be predicted using MIR spectrometry is possible. However, as the accuracy of the equation is not high enough for routine use, this indicator could be used in combination with other MIR-based indicators to provide more accurate information about the physiological state of the cows.

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