DEVELOPMENT OF A METHOD TO PREDICT INDIVIDUAL ENTERIC METHANE EMISSIONS FROM COWS BASED ON MILK MID-INFRARED SPECTRA

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INTRODUCTION

Agriculture is directly confronted with the problem of climate change, especially concerning methane (CH_4) emissions. Indeed, livestock is considered the largest CH_4 producer from anthropogenic sources, mainly by ruminant methanogenesis. Methane contributes widely to global warming and absorbs 25 times as much infrared radiation as CO_2. In addition to those environmental concerns, the eructed CH_4 induces a significant loss (of 3-10%) of gross energy intake for the animal. Methane emissions mainly vary with the animal (genetics, age and species), the diet (intake level, composition) and the level of milk production.

To be able to decrease CH_4 emissions from dairy cows, it is important to acquire an effective individual method to measure them that is also cheap, fast, accurate, and easily applied to a large number of cows. Based on the physiological mechanisms of ruminal digestion and lactation, it has been established that there is an indirect relationship between milk composition (including fatty acids) and the production of CH_4. Therefore, examining milk mid-infrared (MIR) spectra, which reflect milk composition, may be a way to predict enteric CH_4 emissions from individual dairy cows.

MATERIALS AND METHODS

Animals and diets

Four experiments were performed on Holstein cows selected according to number of lactations. They received different diets to ensure variation in CH_4 emissions, necessary to establish a robust calibration model.

In the first experiment, 8 lactating Holstein cows were divided into two groups of four cows each. The groups had similar mean milk production (17.4 ± 3.9 kg/d). Two isoenergetic experimental diets (17 kVEM) were offered according to a 2 x 2 cross-over design. Per kg DM, diet 1 (**fresh pasture**) consisted of 550 g fresh-cut pasture grass (third cutting), 200 g dried beet pulp, 150 g soybean meal, and 100 g soybean hulls. Per kg DM, diet 2 (**maize silage**) consisted of 400 g maize silage, 200 g meadow hay, 130 g cracked maize, 150 g rapeseed meal, 55 g palm meal, 55 g soybean meal, 5 g coconut oil, and 5g flaxseed oil. Both diets contained a mixture of vitamins and minerals.

In the second experiment, 3 lactating Holstein cows with a similar mean milk production (26.2 ± 1.9 kg/d) were fed the same basal diet. Per kg DM, this diet (**grass silage**) consisted of 520 g grass silage, 130 g maize silage, 130 g cracked maize, 110 g soybean meal, and 110 g dried beet pulp.

The third experiment was conducted on 12 lactating Holstein cows with a similar mean milk production (25.5 ± 3.7 kg/d). Per kg DM, their total mixed ration (**TMR 1**) contained 140 g maize silage, 560 g grass silage, 100 g dried beet pulp, 100 g Nutex CLA, and 100 g of a concentrate mix.

Finally, in the fourth experiment 6 lactating Holstein cows with mean milk production of 26.0 ± 2.1 kg/d were fed a total mixed ration (**TMR 2**) consisting of 70 g straw, 200 g haylage, 350 g maize silage, and 380 g of a concentrate mix.

For all of them, the adaptation period was 21 days, and milk and CH_4 samples were then collected during 5 or 10 days. Fresh water was available at all times.

Sampling and analyses

The reference method used to measure the quantity of CH_4 eructed within 24 hours was the tracer gas sulfur hexafluoride (SF_6). A representative breath-gas sample, containing respired and eructated gas, was collected in a canister through a capillary tube kept in place between the nostril and the mouth of each animal with a halter. In the third and fourth experiments, two samples were collected each time from each animal to have a replicate. The canister was changed every 24 hours after the morning feeding. CH_4 and SF_6 concentrations were then analyzed by a gas chromatographer (Varian-Chrompack, CP-9003, Les Ulis, France) fitted with a flame ionisation detector (CH_4) and an electron-capture detector (SF_6).

In parallel, individual milk samples (50 ml) containing sodium azide were collected during each milking and analyzed with a FTIR Lactoscope spectrometer (Delta Instruments, Drachten, the Netherlands). This instrument gave the MIR spectral data as well as the direct measurement of milk components such as lactose, protein, fat, and non-protein nitrogen.

For each type of analysis (gases and milk), reference analyses were made in duplicate.

Spectral data treatment

For each test day and for each cow, one individual CH_4 measurement and two milk MIR spectra (one for each milking) were available. Therefore, the recorded spectral data were transformed to represent one daily spectrum related to one daily CH_4 record. The methodology used to create the average milk spectra (**AMS**) was the weighted average. It corresponds to the average of the two milk spectra of the day in proportion to the amount of milk produced by the cow in each respective milking (AM and PM). This should be the best representation of the biological background of the process.

Calibration model

The daily CH_4 measured was related to its corresponding AMS, and several equations were built using partial least squared regressions (Foss WINISI 4 software) to predict individual CH_4 emissions from the MIR spectra. A first-derivative spectral treatment was used to correct the baseline drift. The number of factors included in the equations was determined by full cross-validation (with N observations, create N models by removing N times one sample that is predicted by the other N-1), which was also used to estimate the robustness of the developed equations. Statistical parameters were also calculated to assess the accuracy of the calibration models; the calibration coefficient of determination (**R^2c**), the cross-validation coefficient of determination (**R^2cv**), the standard error of calibration (**SEC**), and the standard error of cross-validation (**SECV**). The predictability of the equations was evaluated through the ratio of performance to deviation (**RPD**; SD/SECV where **SD** was the standard deviation of the SF_6 measures). This factor should be as high as possible but values greater than 2.5 are considered satisfactory for practical and precise applications.

RESULTS AND DISCUSSION

Equations were built to predict the quantity of CH_4 produced per day. The best CH_4 emission prediction (L CH_4/kg milk/day) was based on 165 measurements and showed a R^2cv of 0.74 and an RPD of 1.96 (**Table 1**), which was promising. Indeed, this equation allows a first screening of the population : distinguish high and low CH_4 emitters. **Fig. 1** shows the linear relationship between the SF_6-measured CH_4 and the MIR-predicted CH_4 (L CH_4/kg milk). The different diets tested showed CH_4 measurements not distributed at any specific place on the line. Thus, the animal effect was greater than the feeding effect in this study. Results suggested the need to perform more measurements (especially of relatively low and high CH_4 emissions) to confirm the results obtained in this study. Moreover, external validation should be conducted by using independent SF_6 measurements of CH_4.

Table 1 : Statistical parameters for the methane prediction equation

n	R^2c	R^2cv	SEc	SEcv	RPD
165	0.84	0.74	3.1	3.94	1.96

SEc*:* standard error of calibration ; **SEcv**: standard error of cross validation ; **RPD**: Ratio of performance to deviation

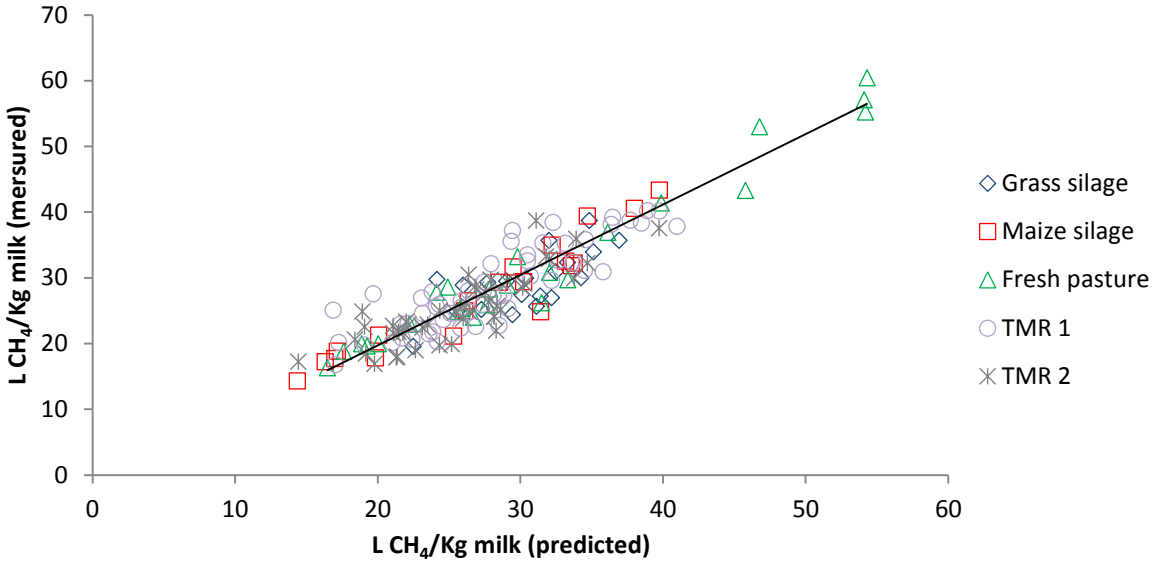

Fig. 1: Relation between measured and predicted CH_4 emissions according to the type of feeding

CONCLUSION

Results suggest a clear indirect link between CH_4 emission and milk composition assessed with MIR spectra. Therefore, prediction of enteric CH_4 emissions of individual cows seems to be feasible. Calibration results indicated that the equation could be used for screening purposes, differentiating high and low CH_4 producers. However, this equation will be refined by increasing the number of measurements to cover the range of existing CH_4 variability: genetically diverse animals from different breeds, fed on different diets and subject to diverse herd-management strategies. By applying this equation to spectral databases (e.g., related to regular milk recording), it will be possible to predict the emission of enteric CH_4 by dairy cows at small (e.g., intra-farm) and large (e.g., inter-farm, country) scales to develop management and selection tools. Through large-scale prediction of CH_4 emissions, the method could help improve knowledge about the sources of CH_4 emission variation (whether genetic or not) and about its link to other traits of interest. In this way, cows with low CH_4 emission and high milk production could be selected, and the best practices for the main production systems could be identified.