Potential use of milk mid-infrared spectra to predict individual methane emission of dairy cows

F. Dehareng[1,a,b], C. Delfosse[1,a], E. Froidmont[2], H. Soyeurt[3], C. Martin[4], N. Gengler[3] , A. Vanlierde[1] and P. Dardenne[1]

[1] *Valorisation of Agricultural Products Department, Walloon Agricultural Research Centre, B-5030 Gembloux, Belgium*

[2] *Department of Production and Sectors, Walloon Agricultural Research Centre, B-5030 Gembloux, Belgium*

[3] *Animal Science Unit, Gembloux Agro Bio-Tech, University of Liège, B-5030 Gembloux, Belgium; National Fund for Scientific Research, B-1000 Brussels, Belgium.*

[4] *UR1213 Herbivores, INRAClermont-Theix Research Centre, France*

[a] These authors contributed equally to the study

[b] Corresponding author: Frédéric Dehareng. E-mail: dehareng@cra.wallonie.be

ABSTRACT

This study investigates the feasibility to predict individual methane (CH_4) emissions from dairy cows using milk mid-infrared (MIR) spectra. To have a large variability of milk composition, two experiments were conducted on 11 lactating Holstein cows (2 primiparous and 9 multiparous). The first experiment aimed to induce a large variation in CH_4 emission by giving two different diets: the first one was mainly composed of fresh grass and sugar beet pulp and the second one of maize silage and hay. The second experiment consisted of grass and corn silage with cracked corn, soybean meal and dried pulp. Twice a day, for each milking period, the milk yields were recorded and a milk sample of 50 mL was collected from each cow and analyzed by MIR spectrometry. Individual CH_4 emissions were measured daily using the SF_6 method during a 7-day period. CH_4 daily emissions ranged from 10.2 to 47.1 g CH_4/kg of milk. The spectral data were transformed to represent an average daily milk spectrum (AMS), which was related to the recorded daily CH_4 data. By assuming a delay before the production of fermentation products in the rumen and their use to produce milk components, five different calculations were used: AMS at days 0, 0.5, 1, 1.5, and 2 compared to the CH_4 measurement. The equations were built using Partial Least Squares regression. From the calculated R^2cv, it appears that the accuracy of CH_4 prediction by MIR changed in function of the milking days. In our experimental conditions, the AMS at day 1.5 compared to the measure of CH_4 emissions gave the best results. The R^2 and SE of the cross validation were equal to 0.79 and 5.14 g of CH_4/kg of milk. The multiple correlation analysis done in this study showed the existence of a close relationship between milk fatty acid (FA) profile and CH_4 emission at day 1.5. The lower square root of the R^2

(R = 0.87) obtained between FA profile and CH_4 emission compared to the one corresponding to the obtained calibration (Rc = 0.93) shows the interest to apply directly the developed CH_4 equation instead of the use of correlations between FA and CH_4. In conclusion, our preliminary results suggest the feasibility of direct CH_4 prediction from milk MIR-spectra. Additional research has the potential to improve the calibrations even further. This alternative method could be useful to predict the individual CH_4 emissions at farm level or at regional scale and it also could be used to identify low-CH_4-emitting cows.

Keywords: methane, mid-infrared, milk, spectra, cows

IMPLICATIONS

Potential use of milk mid-infrared spectra to predict the individual methane emission of dairy cows *By Dehareng et al.* The disadvantages of existing methods for CH_4 measurement are the financial and human costs and/or the practical difficulties of these methods when they are used directly on field. The mid-infrared spectrometry used for milk analysis corrects this inconvenient. Therefore, the aim of this study was to develop an accurate and robust tool to predict the individual CH_4 emission of dairy cows by using mid-infrared spectrometry.

INTRODUCTION

Livestock farming is right at the forefront of climate change issues. Agriculture is regarded as the biggest producer of anthropogenic methane (**CH_4**), mainly through ruminant gas emissions (FAO, 2010). Enteric CH_4 is produced by ruminants during the microbial digestion of feed in the rumen. Methane contributes widely to global warming, accounting for about 52 percent of the green house gases (**GHG**) emissions in both developing and developed countries (FAO, 2010). Methane absorbs 25 times as much infrared radiation than CO_2 enlarging global warming problems (Fuglestvedt, 2009). In addition to those environmental concerns, the eructed CH_4 induces a significant loss of gross energy intake for the animal. This gas loss corresponds between 3 and 10% of gross energy intake (Johnson and Johnson, 1995, Jouany, 2008, Beauchemin *et al.*, 2009). These losses are mainly explained by the animal (age and species), the diet (intake level, composition) and the level of milk production (Vermorel, 1995). However, individual variations between animals of the same breed were reported in the range of 30-60% in production of CH_4 /unit of feed ingested when these animals received the same diet at equivalent ingestion and production levels (Lassey *et al.*, 1997). They are related to different rumen microbial populations, coming from different ruminal kinetics salivation; rumination (Demeyer and Fievez, 2000) and different feeding behaviors indicating interaction between animals and their microbiota. Recent results suggest therefore a genetic component in variation of individual CH_4 production (Martin *et al.*, 2010). However, most studies showed that ruminants that are genetically different for feed efficiency (e.g., Hegarty *et al.*, 2007) and productivity showed reduced daily CH_4 production (e.g., Bell *et al.*, 2010). Given the complexity of this variation, large scale studies should be

conducted, but no *in vivo* method to measure CH_4 emissions in ruminants is sufficiently accurate to be applied on a large number of animals (Clark, 2010). Indeed the available methods such as calorimeter or methane chambers, the tunnel system and the sulfur hexafluoride (**SF_6**) tracer technique, are not easy to use on farm and are laborious to estimate individual methane production of many cows in a short period. Therefore, a simpler and appropriate method is required to predict individual CH_4 emissions. For this, the method investigated here was based on the fact that CH_4 synthesis depends on the kind of rumen fermentation which also influences many other parameters such as milk composition (Ørskov *et al.*, 1969).

Carbohydrates are the most important source of energy and the primary precursors of fat and lactose in cow's milk. The end products of carbohydrates degradation are the volatile fatty acids (**VFA**), CO_2, CH_4 and H_2. Demeyer and Fievez (2000) have developed an equation that shows clearly the relationship between VFA and CH_4. During the fermentation process acetate and butyrate promote CH_4 production while propionate maintains a competitive role for the use of hydrogen. According to Miettinen and Huhtanen (1996), an increased ratio butyrate/propionate decreases the lactose content and increases the fat content in milk and the rumen CH_4 synthesis. Thus, a relationship among CH_4, lactose, and fat contents could be assumed as suggested by Vlaeminck and Fievez (2005). In addition, the rumen VFA composition also influences milk fat composition. In ruminants, the milk fatty acids (**FA**) come from two sources, uptake from circulation and *de novo* synthesis within the mammary gland. About one-half of the milk FA (molar percent) is derived from *de novo* synthesis, based on acetate and butyrate (Bauman and Griinari, 2003). Short and medium-chains FA (4 to 14 carbons) arise totally from *de novo* synthesis. Long-chain

FA (>16 carbons) are collected in the circulating lipids, and FA of 16 carbons depend on these two sources (Bauman and Griinari, 2003). Thus, the proportions of different FA reflect the ruminal fermentation via VFA and then CH_4 production (Weill *et al.*, 2008, 2009). Moreover, several authors have already reported, for different diets, relationships between milk FA (measured by gas chromatography) and CH_4 emission of dairy cows (Chilliard *et al.*, 2009; Dijkstra *et al.*, 2010; Delfosse *et al.*, 2010). These results suggest that in general, CH_4 emissions are linked to milk composition. As the Mid-Infrared (**MIR**) spectrum reflects the milk composition (Soyeurt *et al.*, 2006, 2011), it is logical to postulate that the MIR spectrum could predict directly the individual emission of CH_4. Therefore, the aim of this study was to have a first evaluation of the potential use of milk spectra obtained by Fourier Transform InfraRed analysis (**FTIR**) apparatus directly to predict the quantities of CH_4 eructated by individual dairy cows. The development of a MIR CH_4 equation will permit to predict and therefore to study at large scale the methane emission of dairy cows because the MIR technology is already implemented in milk labs to quantify the major milk components used for the milk payment and the routine milk recording.

MATERIALS AND METHODS

Animals and Diets

Two experiments were carried out with different experimental conditions in order to maximize the variability of individual CH_4 emission needed to establish a robust calibration model.

In the first experiment, 2 primiparous and 6 multiparous (3 of 2nd lactation and 3 of 3rd lactation) lactating Holstein cows were divided into two groups of four cows

each. At the beginning of the trial, the groups were similar in terms of lactation stage (180 days in milk) and of average milk production (17.4 ± 3.9 kg/d). Two isoenergetic experimental diets (17 kVEM) were offered according to a 2x2 cross-over design. Experimental diets were fed twice daily at 0900 and 1600 as total mixed rations (**TMR**). The diets were fed at an intake level of 18.5 kg/d and aimed at producing 20 kg/d of milk. Diet 1 consisted of: fresh-cut pasture grass (third cutting, *Holcus lanatus*), 550 g/kg DM; dried beet pulp, 200 g/kg DM; soybean meal, 150 g/kg DM; and soybean hulls, 100 g/kg DM. Diet 2 consisted of: corn silage, 400 g/kg DM; meadow hay, 200 g/kg DM; cracked corn, 130 g/kg DM; rapeseed meal, 150 g/kg DM; palm meal, 55 g/kg DM; soybean meal, 55 g/kg DM; a 50:50 mix of coconut and flaxseed oil, 10 g/kg DM. Both diets contained a mixture of vitamins and minerals.

In the second experiment, 3 multiparous (2 of 2^{nd} lactation and 1 of 4^{th} lactation) lactating Holstein cows with a similar milk production (26.2 ± 1.9 kg/d) were fed with a same basal diet, with an intake level of 20.3 kg/d. This diet (TMR) consisted of grass silage, 520 g/kg DM; corn silage; 130 g/kg DM; cracked corn, 130 g/kg DM; soybean meal, 110 g/kg DM; and dried beet pulp 110 g/kg DM.

For both experiments, the adaptation period was 21 days and milk and CH_4 samples were then collected from day 22 to day 28. Fresh water was available at all times.

All the procedures used involving the animals were approved by the regional ethics committee in animal experimentation (protocol CRAW09/01).

153 *Sampling and Analyses*

154 Milk yield was recorded daily at each sampling period at 0730 and at 1630. A
155 50 mL aliquot of milk, containing sodium azide (0.32 g/L), was stored at 4°C until
156 infrared analyses. The sample stored at 4°C was analyzed by a FTIR Lactoscope
157 spectrometer (Delta Instruments, Drachten, the Netherlands). The Lactoscope works
158 within 925 to 3000 cm^{-1} and uses an interferometer. This instrument gave the
159 spectral data as well as the direct measurement of milk components such as lactose,
160 protein, fat, non protein nitrogen (**NPN**; considered to be essentially urea). Fatty acid
161 composition predictions were also obtained based on equations specific to this
162 machine. **Table 1** presents the statistical parameters of these equations. For each FA
163 equations, the standard error of cross-validation (SECV) given by the FTIR
164 Lactoscope equations are included between those given by Soyeurt *et al.* (2006) and
165 those of Soyeurt *et al.* (2011). A total of 154 samples were collected and analyzed
166 (spectra were in triplicate).

167 *Quantification of CH_4*

168 During the 7-day period, the SF_6 gas tracer technique was used according to
169 the method described by Martin *et al.* (2008) to measure the individual production of
170 enteric CH_4 by the studied cows. Briefly, a calibrated permeable tube containing ultra
171 pure SF_6 was placed in the rumen of each cow before the experimental period. The
172 average release rate of SF_6 from the tubes was 1189.5 ± 168.6 ng/min.

173 A representative of breath gas sample, containing respired and eructated gas,
174 was collected through a capillary tube situated between the nostril and the mouth of

each animal thanks to an halter. This gas sample was stored in a canister located near the animal (more or less one meter) in a way that the animal could not touch it. The canister was changed daily after morning feeding and the CH_4 and SF_6 concentrations were analyzed using gas chromatography (**GC**) (Martin *et al.*, 2008).

A GC (Varian-Chrompack, CP-9003, Les Ulis, France) fitted with an electron capture detector (**ECD**) (Perkin Elmer instruments; Autosystem XL, Courtaboeuf, France) and with a flame ionisation detector (**FID**) was used to determine the concentrations of SF_6 and CH_4, respectively. The samples were run on GCs equipped either with a Molecular Sieve 0.5 nm column (3 m × 3.2 mm i.d) maintained at 50°C for the SF_6, or with a Porapak N 80-100 mesh column (3 m × 3.2 mm i.d.) maintained at 40°C for the CH_4. The flow rate of the carrier gas was 30 mL/min of N_2 for the SF_6 and 40 mL/min of He for CH_4. Chromatographic analyses were performed after calibration with standard gases (Air Liquide, Mitry-Mory, France) for SF_6 (55 and 195 ppt) and CH_4 (100 ppm).

To determine the released amount of CH_4 ($\mathbf{Q_{CH4}}$), the concentration of SF_6 and CH_4 ($\mathbf{C_{CH4}}$ and $\mathbf{C_{SF6}}$) in the canister and the pre-determined released rate of SF_6 ($\mathbf{Q_{SF6}}$) were used. In parallel to each daily measurement, the concentration of the atmospheric air ($\mathbf{C^b_{CH4}}$ and $\mathbf{C^b_{SF6}}$) determined with another canister was subtracted (Johnson *et al.*, 1994):

$$Q_{CH4} = \frac{C_{CH4} - C^b_{CH4}}{C_{SF6} - C^b_{SF6}} \, Q_{SF6} \, \frac{MW_{CH4}}{MW_{SF6}}$$

where MW_{CH4} and MW_{SF6} are the molecular weight of CH_4 and SF_6, respectively.

The CH_4 emission was therefore expressed in g of CH_4/day and re-expressed later in g of CH_4/kg of milk thanks to the recorded milk yield because the global daily CH_4 production is linked to productivity.

Spectral Data Treatment

Each cow was milked twice daily providing two individual samples, which were analyzed by MIR, thus 2 spectra, whereas individual CH_4 emission was measured once daily. Therefore, to build the calibration model, 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 tested by comparing MIR predictions obtained from AMS and the real spectral data obtained from the infrared analysis of a representative daily milk sample (i.e., 50% of morning and 50% of evening milks). This ratio was chosen because it corresponds to the averaging used during the majority of milk recording process. Alternatively, the weighted average was also tested. It corresponded 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). The reason for this was that a weighted average represented better the biological background of the process.

A dynamic relationship between milk composition (and, therefore, spectral data) and CH_4 was considered by assuming that there is a delay between the production of fermentation products and their use to produce milk components. Consequently, different ways exist to define the AMS. Indeed, the spectral data used

to create the average milk spectra can be generated by the milk analysis of samples collected at different times compared to the time of CH_4 measurement. The average milk spectra were calculated from five methods averaging always two samples which are illustrated in Figure 1:

- the same day of CH_4 measurement (day 0),
- evening of the same day and morning next day (day 0.5),
- next day (day 1),
- evening next day and morning 2 days later (day 1.5),
- and 2 days later (day 2).

Always two samples were averaged to reflect a whole test-day in the milk as whole day was also represented in the CH_4 measurements.

Given the stability of diets and the high degree of auto-correlation that can be therefore assumed across values for the five methods, the objective of this was not infer a definitive response what the delay between the production of fermentation products and their use to produce milk components, but to find retrospectively which definition of the AMS gives the best predictions under the condition of this study.

Calibration Model

As mentioned previously, a calibration set with a large spectral variability is required to develop robust calibration models. Therefore, a principal component analysis (PCA) was carried out on available spectra at day 0 in order to illustrate the spectral variation observed from the collected milk samples.

The calibration models were developed by Foss WINISI 4 software from the recorded spectral and CH_4 data using Partial Least Squares regressions (**PLS**). The spectral regions used for that were: 972-1,589 cm^{-1}, 1,720-1,782 cm^{-1}, and 2,746-2,970 cm^{-1}. No additional pre-treatment was applied on spectral data because with a derivative, results were not significantly better probably due to the fact that only one spectrometer was used in this experiment. The number of factors used was determined by a full cross-validation (with N observations, create N models by removing N time one sample which is predicted by the others N-1), which was also used to estimate its robustness. The accuracy of the resulting calibration models was evaluated by calculating the calibration coefficient of determination (**R^2c**), cross-validation coefficient of determination (**R^2cv**), standard error of calibration (**SEC**), and the standard error of cross-validation (**SECV**). The predictability of the calibration was evaluated through the ratio of performance to deviation (**RPD**; SD/SECV where **SD** was the standard deviation of the CH_4 measures). The RPD should be as high as possible; values between 5 and 10 are adequate for quality control, and values > 2.5 are satisfactory for screening breeding programs (Williams and Sobering, 1993).

Correlations with Milk Components

To show the interest of using a new direct MIR equation to quantify the CH_4 emissions, the best correlation between milk components already predicted by MIR and CH_4 was calculated. In this way, it was possible to verify whether the predicted CH_4 production data obtained by the developed calibration equation were due to new recombination of global spectral information or just to specific milk components already predicted by MIR and whose the equations are available. If the cross-

validation correlation is higher than the correlation between CH_4 and specific MIR milk component, it can be assumed that the developed equation provides additional information than the simple correlation between CH_4 and the already predicted milk components.

RESULTS AND DISCUSSION

Spectral Variation

The first two principal components described 56.8% of the total variation observed in the studied data set (N = 77). These principal components (Figure 2) were used to illustrate the spectral differences of studied milk samples. Spectra from cows feeding with different diets (i.e., grass silage, corn silage and fresh pasture) are separated (Figure 2). Based upon the literature (Collomb *et al.*, 2002), this suggests that the use of different diets involved a change in milk composition and therefore in the milk spectral data. Moreover, Table 2, with descriptive statistics, also shows high variations of milk composition primarily fat, protein, lactose, and saturated fatty acids (**SAT**). This variability of spectral data is needed to develop a robust calibration equation.

Table 2 also shows the variability of CH_4 expressed in g/day and g/kg of milk. The coefficients of variation were 29.84% and 33.33%, respectively. This large variation could be explained partly by the use of three different diets. A slightly larger variation was observed for the CH_4 emission expressed in g of CH_4/kg of milk because the definition of this trait took into account simultaneously the variability of CH_4 and milk yield.

Spectral Data Treatment

As mentioned previously, for each cow, two spectra measurements and one CH_4 measurement were available. In order to have one spectrum for one measure of CH_4, the collected spectral data from morning and evening milk samples were averaged. To validate the methodology, the contents of major milk components (i.e., fat, protein, lactose, true protein, and NPE) predicted from AMS derived from 50% morning and 50% evening spectral data and from the spectral data obtained from the infrared analysis of a representative 24h milk sample were compared. Table 3 shows no significant difference ($P > 0.005$) in milk components predicted by AMS and by the 24h spectral data. Therefore, AMS was considered similar to the spectrum of the representative 24h milk sample showing the pertinence of the method used to create the AMS.

Calibration Model

Different equations were built to predict the quantity of CH_4 produced per day in function of the five different possibilities used to create AMS as shown in Figure 1. **Erreur ! Source du renvoi introuvable.** presents the statistical parameters obtained for the 20 developed calibration equations. The prediction of CH_4 by MIR, based on R^2cv, was better by considering g of CH_4/kg of milk instead of g of CH_4/day ($R^2cv=0.79$ *vs.* $R^2cv=0.73$). This could be explained by the fact that the expression unit g of CH_4/kg of milk compared to g of CH_4/day takes into account the milk production, which is directly related to the release of CH_4 by cow (Vermorel, 1995)

306 and, therefore, reflected in the spectral data thanks to the dilution effect known for
307 the majority of milk components. For instance, the literature shows that the contents
308 of major milk components such as fat, protein, and fatty acids (Soyeurt *et al.*, 2009)
309 are negatively correlated with the milk yield. **Table 4** shows also that the use of a
310 ratio taking into account the milk produced during the evening and morning milkings
311 for the calculation of AMS compared to the ratio 50% of morning and 50% of evening
312 spectral data gave better results. This could be explained by the relationship between
313 the milk production and the CH_4 emission by dairy cows (Vermorel, 1995). Therefore,
314 the equations using the CH_4 content expressed in g/kg of milk and the morning and
315 evening milk AMS gave the best results.

316 The interval between the measurement of CH_4 by the SF_6 tracer method and
317 the spectral data used (Figure 1) gave different results (**Table 4**). By observing the
318 last 5 lines of **Table 4**, the day 1.5 gave the best result for the internal validation (i.e.,
319 $R^2cv=0.79$). The best equation seems to be the one built from the AMS created from
320 the weighted average taking into account the milk produced, the CH_4 content
321 expressed in g/kg of milk, and an interval between the measurement of CH_4 and the
322 spectral data equal to 1.5 day. This equation also seemed to be the most robust
323 because the difference between R^2c and R^2cv was the lowest (0.08). These results
324 are only first indications of the delayed response of milk composition to rumen
325 fermentation. More research is needed in other experimental conditions; for instance
326 by inducing the inhibition of the rumen methanogenesis and observing the milk
327 spectra modifications in the next few days..

328 Even if this study must be validated on a larger data set, the obtained results
329 suggest a clear indirect link between the CH_4 emission and the milk composition
330 through the direct use of the MIR spectral data. Chilliard *et al.* (2009) also showed
331 this relation through the link between the contents of milk FA analyzed by GC and the
332 production of CH_4 by dairy cows fed with diets supplemented or not with linseeds.
333 The present study also shows the feasibility of the MIR prediction of CH_4 produced by
334 dairy cows and a relative robustness of the developed equation. Indeed, despite of
335 the large variation observed for g of CH_4/kg of milk produced per cow and per diet
336 over the study period and the observed variability of milk composition (**Table 2**), the
337 MIR CH_4 predictions were good and R^2c and R^2cv were never below to 0.77 and
338 0.68, respectively. Moreover, CH_4 prediction did not seem to be affected (Figure 3)
339 by the different kind of diets used during the experiments (one consisting of corn
340 silage, another of pasture grass and other of grass silage). However, it seems that
341 the pasture grass diet contributes mainly to the large variation in CH_4 emissions. This
342 variation between animals for this diet could be due to the season and therefore a
343 higher lignification of grass at the end of the experimentation. Also individual, e.g.
344 genetic, differences among the animals could be the reason for these results. In
345 order to be able to make a generalisation of the prediction, it is important to continue
346 to collect data on different diets. Finally, The RPD, which relates the standard error of
347 prediction to the standard deviation of the original reference data, was equal to 2.19
348 suggesting a good robustness of the prediction. Indeed, Williams and Sobering
349 (1993) show that a value of 2.5 and over are satisfactory for screening. Therefore,
350 using this equation, it is currently feasible to classify the dairy cows in two groups:
351 low and high CH_4 producers. More variability in the available data can improve the

ability of the equation to predict CH_4 emission. Potential use of data obtained from genetically diverse animals, fed on different diets and producing in diverse management, should allow improving the equations.

Correlations with Milk Components

Due to the strong indirect link existing between CH_4 emissions and the overall milk composition, it was expected that CH_4 emissions would correlate strongly with milk production and milk components already predicted by MIR and whose equations are available (**Table 5**). Despite what was expected (Miettinen and Huhtanen, 1996) a low correlation was observed between lactose and CH_4 emissions. This is probably because the lactose concentration is less and milk yield is more responding to variation in propionate supply. Nevertheless Rcv for the best equation is always superior to the correlation obtained from milk production and MIR milk components suggesting that the developed equation provides additional information.

A multiple correlation (**Table 6**) was built starting from the parameters appearing in **Table 5** to predict CH_4 production/kg of milk for the day +1.5. The square root of the R^2 ($R = 0.87$) was lower than the square root of the R^2c value ($Rc = 0.93$) obtain by the equation for this parameter in **Table 4**. In addition, the results presented in **Table 6** suggest the existence of a close relationship between FA profiles and CH_4 emission, as previously reported by Chilliard *et al.* (2009). However, our first results showed that the use of the full spectral data gave more information. In the future, the use of more precise equations for the prediction of the FA, could help us to understand more in detail the role of each FA and infirm or confirm these observation. In fact, the precision (SECV) of our FA equations could be upgraded

375 and met at least the precision obtain by Soyeurt *et al.* (2011). This underlines the
376 practical interest to use MIR spectra to predict individual CH_4 emission of dairy cows.

377 **CONCLUSION**

378 The results of our study tended to show that the prediction of CH_4 emissions
379 prediction from milk spectra is feasible. This is partially explained, by the fact, that the
380 milk spectrum reflects the milk composition which is considered linked to CH_4, due
381 the relationship of both phenomena to ruminal fermentation. Results from this study
382 showed that CH_4 is better predicted, based on R^2cv, with the weighted AMS built
383 from spectral data collected at the day 1.5 compared to the moment of CH_4
384 measurement. Additional studies providing data from genetically diverse animals and
385 different breeds, fed on different diets and producing in diverse managements are
386 needed to improve the obtained RPD and therefore clarify and potentially confirm the
387 initial relationship which is reported in this study.

388 The calibration results indicated that the best equation could be used for
389 screening purposes, differentiating high and low methane producers, even if addition
390 research is still required to make it more reliable and potentially implementable on
391 other spectrometers. Once this being achieved, as the existing methods for CH_4
392 measurement are difficult to apply on a large scale, this alternative method has the
393 potential to be very useful to predict CH_4 emission for dairy herds at individual cow
394 level from milk MIR spectra. These required data are already taken and used for the
395 milk recording and the milk payment. Through the large scale generation of predicted
396 CH_4 emission data, the method could help improve the knowledge about the sources
397 of CH_4 variation (genetic or not) and about its link to other traits of interest.

398

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500 **Table 1** Estimated statistical parameters for each calibration equation that
501 estimated the concentrations of fatty acid in milk (g / dl milk) used in this study

Fatty acids (g/dL of milk)	N	Mean	SD	SEC	R^2_C	SECV	R^2_{CV}	RPD
C4:0	137	0.11	0.03	0.01	0.82	0.01	0.73	1.93
C6:0	138	0.08	0.02	0.01	0.86	0.01	0.82	2.32
C8:0	131	0.05	0.01	0.00	0.86	0.01	0.81	2.33
C10:0	137	0.11	0.04	0.01	0.9	0.02	0.84	2.52
C12:0	138	0.13	0.05	0.02	0.88	0.02	0.84	2.48
C14:0	136	0.45	0.12	0.03	0.93	0.04	0.88	2.9
C14:1	138	0.04	0.02	0.01	0.66	0.01	0.58	1.52
C16:0	139	1.19	0.35	0.09	0.93	0.11	0.89	3.06
C16:1	143	0.08	0.04	0.02	0.82	0.02	0.77	2.06
C17:0	138	0.03	0.01	0.00	0.84	0.01	0.72	1.89
C18:0	138	0.39	0.15	0.07	0.79	0.07	0.76	2.05
C18:1 *trans*	138	0.10	0.04	0.02	0.67	0.03	0.55	1.49
C18:1 *cis-9*	138	0.80	0.42	0.08	0.97	0.09	0.95	4.5
Total C18:1 *cis*	138	0.87	0.43	0.07	0.98	0.08	0.97	5.38
Total C18:2	143	0.09	0.03	0.01	0.7	0.02	0.6	1.58
C18:2 *cis-9,cis-12*	142	0.06	0.02	0.01	0.77	0.01	0.7	1.83
C18:3 *cis-9,cis-12,cis-15*	138	0.02	0.01	0.01	0.52	0.01	0.4	1.29
C18:2 *cis-9,trans-11*	139	0.03	0.02	0.01	0.5	0.01	0.36	1.24
Saturated FA	132	2.72	0.72	0.06	0.99	0.07	0.99	10.57
Monounsaturated FA	135	1.12	0.46	0.06	0.98	0.07	0.98	6.45

502 SD : Standard deviation; SEC: Standard error of calibration; R^2_C : Calibration coefficient of determination;
503 SECV: Standard error of cross-validation; R^2_{CV} : Cross-validation coefficient of determination;RPD : Ratio of
504 standard error of cross validation to standard deviation

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507 **Table 2** *Means and range of CH_4 emissions, production and composition of*
508 *the milk, saturated fatty acid composition of the milk fat for the two experiments (day*
509 *0)*

Components	N	Mean	Minimum	Maximum	Std dev
CH_4, g/d	77	429	218	653	128
g/kg milk	77	21.9	10.2	47.1	7.3
Milk, kg/d	77	19.7	12.5	30	5.1
Milk Composition, g/kg					
Fat	77	4.11	2.86	5.63	0.61
Protein	77	3.40	2.39	4.82	0.42
Lactose	77	4.67	3.69	5.21	0.27
Milk Fat composition, g/kg					
SAT	77	66.1	55.4	76.9	5.2

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512 **Table 3** *Comparison between components prediction for average milk spectra*
513 *(AMS) and spectra of average milk in a ratio 50-50 for 37 milk sample during the*
514 *second experiment*

Components	N	AMS	Average milk	P<
$Fat\ milk_{predicted}$, g/kg	37	4.33	4.37	NS[2]
$Protein_{predicted}$, g/kg	37	3.28	3.28	NS
$Lactose_{predicted}$, g/kg	37	0.037	0.034	NS
$True\ protein_{predicted}$, g/kg	37	3.31	3.33	NS
$NPN^{1}_{predicted}$, mg/100g $_{milk}$	37	21.75	20.69	NS

515 1 Non Protein Nitrogen

516 2 NS: P>0.05

517 **Table 4** *Evolution of accuracy of the resulting calibration with the different kind*
518 *of average milk spectra (AMS) with a ratio 50-50 and a weighted average for the two*
519 *experiments. The number of available data depends on the chosen day*

Ratio		Day	N	R^2c	R^2cv	SEC	SECV	RPD
Ratio 50-50	g of CH_4	Day 0	77	0.84	0.71	73.00	98.30	1.84
		Day 0.5	71	0.81	0.68	79.39	105.19	1.77
		Day 1	65	0.84	0.72	71.73	98.29	1.88
		Day 1.5	60	0.77	0.70	87.77	100.65	1.83
		Day 2	59	0.77	0.72	86.19	95.23	1.89
	g of CH_4/kg of milk	Day 0	77	0.80	0.72	4.81	5.69	1.91
		Day 0.5	71	0.83	0.77	4.39	5.69	2.06
		Day 1	65	0.86	0.69	4.16	6.13	1.81
		Day 1.5	60	0.85	0.75	4.25	5.61	2.01
		Day 2	59	0.93	0.72	3.06	6.05	1.89
Ratio defined from morning and evening milk yields produced	g of CH_4	Day 0	77	0.85	0.72	68.97	95.93	1.90
		Day 0.5	71	0.83	0.73	74.82	95.45	1.92
		Day 1	65	0.85	0.69	71.55	102.21	1.80
		Day 1.5	60	0.78	0.71	86.89	99.20	1.85
		Day 2	59	0.77	0.72	85.81	83.81	1.89
	g of CH_4/kg of milk	Day 0	77	0.89	0.75	3.52	5.42	2.00
		Day 0.5	71	0.86	0.78	3.96	5.00	2.12
		Day 1	65	0.84	0.69	4.35	6.22	1.78
		Day 1.5	60	0.87	0.79	4.06	5.14	2.19
		Day 2	59	0.90	0.73	3.59	5.94	1.93

520 SEC: Standard error of calibration; SECV: Standard error of cross-validation; RPD: ratio of performance to
521 deviation
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523

524 **Table 5** *Individual correlations between different milk parameters and the*
525 *CH_4/kg of milk produced for the day1.5. Rcv = square root of the R^2cv value*

Milk production and Milk components[1]	Correlation CH_4/Milk
Milk production	-0.45
Protein	0.40
Lactose	0.19
Fat	0.49
C4:0	0.26
C6:0	0.35
C8:0	0.40
C10:0	0.31
C12:0	0.34
C14:0	0.48
C14:1*cis*-9	0.62
C16:0	0.32
C16:1*cis*-9	0.48
C17:0	0.53
C18:0	0.25
Tot C18:1 *trans*	0.11
C18:1 *cis*-9	0.44
C18:2*cis*-9,*cis*-12	0.23
C18:3*cis*-9;*cis*-12,*cis*-15	0.60
c18:2*cis*-9,*trans*-11	0.32
Rcv	**0.89**

1 Milk components were predicted by FTIR spectrometer

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527

528 **Table 6** *Multiple correlations from the parameters appearing in table 5 to*
529 *predict the CH_4/kg of milk produced for the day1.5. R = square root of the R^2 value*

Correlation CH_4/Milk	Name	F test
R : 0.87	1. C18:3 n-3	121.83
	2. C4:0	61.87
	3. C12:0	49.48
	4. C6:0	30.57
	5. C8:0	12.42

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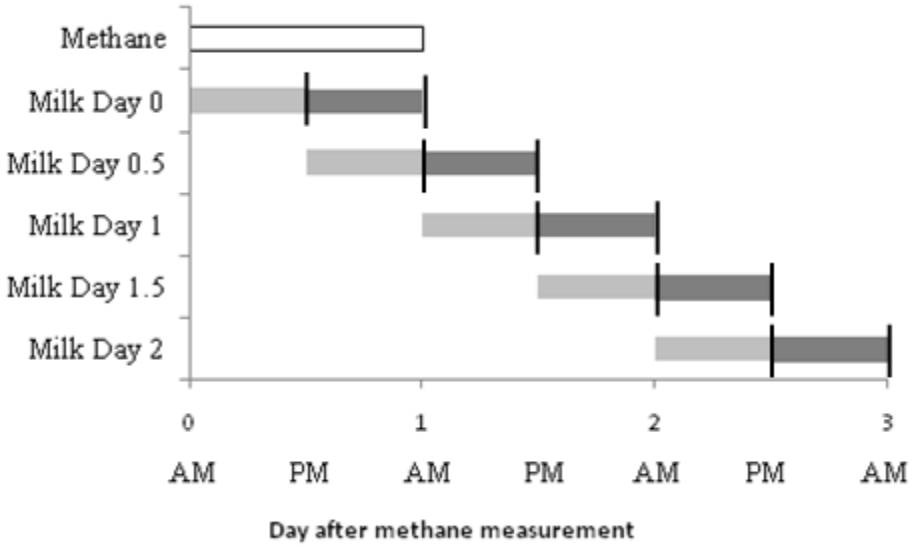

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532 **Figure 1** Possibility to average the spectra milk in function of CH_4 day collect.

533 Vertical lines corresponds to milking time; surface shaded to milk production.

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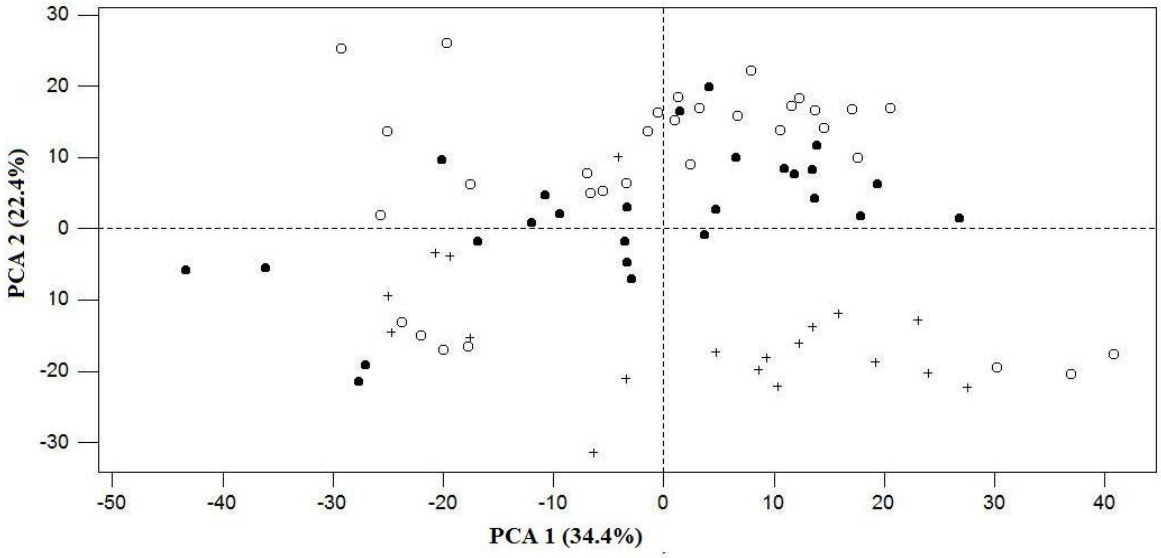

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536 **Figure 2** Plot of the principal component analysis of milk spectra for individual cows

537 fed three differing diets: corn silage (•), fresh pasture (o) and grass silage (+).

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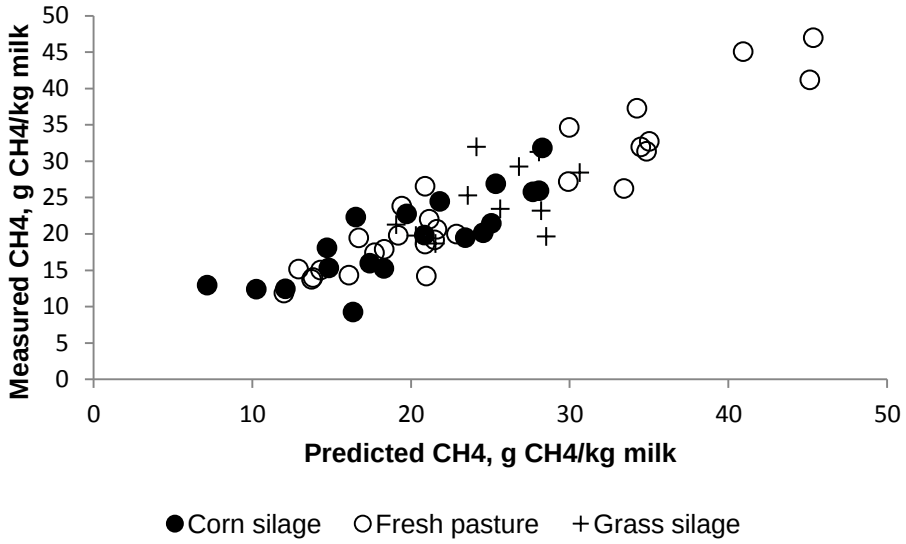

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540 **Figure 3** Infrared CH_4 prediction on basis of milk spectra of the day1.5 for the
541 different diets: corn silage (•), fresh pasture (o) and grass silage (+).