Accuracy of methane emissions predicted from milk mid-infrared spectra and measured by laser methane detectors in Brown Swiss dairy cows

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ABSTRACT

Since heritability of CH₄ emissions in ruminants was demonstrated, various attempts to generate large individual animal CH₄ data sets have been initiated. Predicting individual CH₄ emissions based on equations using milk mid-infrared (MIR) spectra is currently considered promising as a low-cost proxy. However, the CH₄ emission predicted by MIR in individuals still has to be confirmed by measurements. In addition, it remains unclear how low CH₄ emitting cows differ in intake, digestion, and efficiency from high CH₄ emitters. In the current study, putatively low and putatively high CH₄ emitting Brown Swiss cows were selected from the entire Swiss herdbook population (176,611 cows), using an MIR-based prediction equation. Eventually, 15 low and 15 high CH₄ emitters from 29 different farms were chosen for a respiration chamber (RC) experiment in which all cows were fed the same forage-based diet. Several traits related to intake, digestion, and efficiency were quantified over 8 d, and CH₄ emission was measured in 4 open circuit RC. Daily CH₄ emissions were also estimated using data from 2 laser CH₄ detectors (LMD). The MIR-predicted CH₄ production (g/d) was quite constant in low and high emission categories, in individuals across sites (home farm, experimental station), and within equations (first available and refined versions). The variation of the MIR-predicted values was substantially lower using the refined equation. However, the predicted low and high emitting cows (n = 28) did not differ on average in daily CH₄ emissions

measured either with RC or estimated using LMD, and no correlation was found between CH₄ predictions (MIR) and CH₄ emissions measured in RC. When individuals were recategorized based on CH₄ yield measured in RC, differences between categories of 10 low and 10 high CH₄ emitters were about 20%. Low CH₄ emitting cows had a higher feed intake, milk yield, and residual feed intake, but they differed only weakly in eating pattern and digesta mean retention times. Low CH₄ emitters were characterized by lower acetate and higher propionate proportions of total ruminal volatile fatty acids. We concluded that the current MIR-based CH₄ predictions are not accurate enough to be implemented in breeding programs for cows fed forage-based diets. In addition, low CH₄ emitting cows have to be characterized in more detail using mechanistic studies to clarify in more detail the properties that explain the functional differences found in comparison with other cows.

Key words: digestion, feed efficiency, methane prediction, proxy

INTRODUCTION

Methane is a greenhouse gas with a global warming potential that is more than 20 times greater than that of carbon dioxide. The global livestock sector accounts for 18% of anthropogenic greenhouse gas emissions, and CH₄ from ruminants is the main source (Steinfeld et al., 2006). The research effort toward CH₄ mitigation is ongoing. Apart from the available set of efficient dietary interventions (Hristov et al., 2013), targeted animal breeding has emerged as a promising and, if successful, sustainable mitigation strategy (de Haas et al., 2017). Breeding progress is possible if a trait is sufficiently

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heritable and if phenotypic data are available from populations relevant for genetic selection purposes. The first is given because CH₄ emissions were found to be a heritable trait (e.g., Lassen and Løvendahl, 2016; Jonker et al., 2017) and the phenotype seems to be persistent throughout lactation (Garnsworthy et al., 2012b). However, it should be noted that Münger and Kreuzer (2008) did not find such a persistence. With respect to the need for individual animal data sets, the well-established techniques for measuring CH₄ from cows, respiration chambers (RC) and sulfur hexafluoride (SF₆), are not fast and cheap enough. The laser CH₄ detector (LMD) has been used to measure CH₄ concentrations in cow's breath over a short time period to estimate daily CH₄ emissions, and it might allow a quick ranking of animals by CH₄ emission on farm (Chagunda et al., 2013; Sorg et al., 2018). Similar to RC, the LMD technique can be applied for all ruminant species and production purposes. One of the most promising proxies for CH₄ emission is based on the mid-infrared (MIR) spectra of the milk (Vanlierde et al., 2016, 2018). This proxy is currently limited to lactating cattle for which the milk recording scheme is in place and calibrated. The underlying equation has been, and is, continuously refined by extending calibration and reference CH₄ measurement data sets. Mid-infrared spectra are available from national milk recording schemes. Therefore, this proxy only requires electronic storage efforts.

The next logical step in validating the MIR approach relies on a backward approach, namely screening entire cow populations for low CH₄ emitters and measuring the accuracy of the corresponding CH₄ predictions. This process was recently done with a single herd (Denninger et al., 2019), but it has not yet been applied to cow population data. If this validation is successful, the proxy could be used in breeding programs. In addition, the data could be useful for national inventory purposes, as well as potential payment or taxing regimens based on greenhouse gas emissions from dairy cows. As cows are exposed to a variety of farm-specific influences on CH₄ emissions, including diet type, intake, feeding frequency, and physiological state of the animal (Garnsworthy et al., 2012b; Hristov et al., 2013; Goopy et al., 2014), this step also has to clarify whether the differences in CH₄ prediction between cows are of sufficient magnitude to be detected when cows from different locations are kept in the same housing and feeding environment. In the development of the equation, data from various breeds, sites, and feeding regimens were integrated, but they nonetheless originated from experimental herds. Finally, it is still unclear how low CH₄ emitters differ from high CH₄ emitters in terms of specific traits and the extent to which they vary. Low

CH₄ emitters might exhibit a greater feed efficiency (ECM/DMI), as shown by a lower residual feed intake (RFI; Hegarty et al., 2007; Alemu et al., 2017). Previous research indicated that low CH₄ emitting sheep could have a proportionately smaller rumen (Goopy et al., 2014), and low CH₄ emitting cattle have been reported to have a low cell wall digestibility (Cabezas-Garcia et al., 2017). The latter differences, however, would be expected to result in a lower feed efficiency.

The objective of the current study was to test the feasibility of using CH₄ predictions from milk MIR spectra to identify truly low CH₄ emitting dairy cows on the basis of milk recording data. The hypotheses tested were the following: (1) MIR-based predictions of CH₄ production of individual cows on farm are recoverable on a uniform diet at the experimental station; (2) the MIR predictions closely correlate with individual CH₄ emission measurements made with RC and LMD; and (3) compared with high CH₄ emitters, low CH₄ emitting cows are superior in feed and digestive efficiency.

MATERIALS AND METHODS

Screening of the Cow Population and Selection of Low and High CH₄ Emitters

A milk MIR prediction equation (Vanlierde et al., 2016; modified as described below) was used to predict the daily enteric production of CH₄ (P_m ; g/d) of individual cows from MIR spectra stored from each test day, between January 2016 and July 2017, for 175,980 Brown Swiss and Braunvieh dairy cows in Switzerland. Only cow data that met the following criteria were included when identifying low and high CH₄ emitting cows: milk yield 5 to 60 kg/d, 4 to 306 DIM, 150 to 950 g/d $P_{m\text{ MIR}}$, and availability of data from at least 5 milk recordings per cow. Records from summer alpine grazing periods and from farms located in the highest mountain regions were excluded. A linear mixed model considering log-transformed milk yield, log-transformed DIM, parity, and season within year as fixed effects, as well as cow and farm as random effects, was applied to model P_m by using the nlme R package (Pinheiro et al., 2017). The conditional modes (difference between the average predicted response at population level for a given set of fixed-effect values and the response predicted for a particular individual) for the cow effect were used to select the extreme values (15% of cows in both directions) for which the predicted P_m was either greater or lower than expected from the linear mixed model. All cows were used for the first selection step, but later selection was restricted to cows in second parity to exclude a further potential factor of influence. This screening procedure resulted in 318 candidate cows (159 low $P_{m\text{ MIR}}$ cows,

159 high $P_{\text{m MIR}}$ cows). From among these cows, 30 (15 low, 15 high $P_{\text{m MIR}}$ cows), preferably in late lactation, were randomly selected for the experiment, followed by getting the approval of the cow owner. The 30 cows originated from 29 different farms.

Although this categorization of the cows did not substantially change when MIR spectra obtained during the experiment were analyzed and different prediction equations were used, cows were later recategorized because cow allocation to the categories was largely different when the RC data were used (measurements described below). Therefore, new groups were formed based on the cows' CH_4 yield (Y_{m} ; here, g/kg of DMI) as measured with RC. This adjusted trait was chosen to exclude the advantage small cows with low feed intake would have when DMI is not considered. To be able to clearly distinguish between categories and in response to the missing preselection of cows for $Y_{\text{m DMI}}$ in RC, only the 2×10 cows with either the lowest or the highest $Y_{\text{m DMI}}$, respectively, were used for the detailed comparison of the characteristics of low and high CH_4 emitters.

Experimental Protocol

The experiment was conducted at the research station AgroVet-Strickhof (Eschikon, Lindau, Switzerland) from November 2017 to April 2018. The experimental protocol was approved (ZH050/17) by the Committee on Animal Experimentation of the Cantonal Veterinary Office Zurich. Owing to 4 RC being available, cows were transported in groups of 4 (2 predicted low P_{m} and 2 high P_{m} ; last time, 2 cows only) from the farms to the station and back after the experiment. This process

resulted in 8 blocks of cows. During a 10-d period of adaptation to diet and management, the cows were kept in a tie-stall barn and milked in a swing-over milking system. During this time, the cows had access to an outside area for 2 h every second day. In the following 8-d sampling period, cows were tethered all the time, which allowed complete collection of feces and urine. On sampling d 9, the collection devices were removed and rumen fluid was sampled. In the last 24 h cows were housed in a RC. All cows received the same diet (Table 1), regardless of the diet they had received at their home farm. The mixed ration was composed of 55% corn silage, 38% grass silage, 2% hay, and 5% dairy concentrate (UFA-250, UFA, Sursee, Switzerland) and was offered ad libitum. In separate troughs, cows daily received, per kilogram of milk, 250 g of an energy-rich concentrate (UFA-243, UFA) and 125 g of dried grass pellets. During morning feeding, 50 g/d NaCl and 100 g/d of a vitamin-mineral supplement were provided. The latter contained (per kg) 160 g of Ca, 80 g of P, 100 g of Mg, 32 g of Na, 10 g of S, 8.0 mg of Zn, 4.0 mg of Mn, 1.0 mg of Cu, 30 mg of Se, 100 mg of I, 30 mg of Co, 1,200,000 IU of vitamin A, 200,000 IU of vitamin D₃, 3,000 mg of vitamin E, and 150 g of biotin. The animals were milked at 0550 and 1645 h and fed at the same time. Leftovers of the mixed ration were removed before each feeding time and weighed. Energy concentrate and grass pellets were always eaten completely.

Prediction of CH_4 Emissions from Milk Sampling Combined with MIR Spectra Analysis

Analysis of MIR spectra was performed either on a stored data set (population) or on milk sampled from

Table 1. Composition (% of DM; DM: % of wet weight) of the individual experimental feeds and amounts offered and refused (arithmetic means $\pm$ SD)

Item	Diet component					
	Mixed ration				Energy concentrate ²	Grass pellets ²
	Hay ¹	Grass silage ¹	Corn silage ¹	Protein concentrate ²		
Composition						
DM	88.6 $\pm$ 1.5	35.2 $\pm$ 4.0	37.0 $\pm$ 2.8	93.1 $\pm$ 0.0	88.8 $\pm$ 0.0	90.3 $\pm$ 0.4
OM	91.0 $\pm$ 1.1	88.0 $\pm$ 1.9	96.8 $\pm$ 0.4	91.0 $\pm$ 0.0	93.7 $\pm$ 0.0	87.4 $\pm$ 0.4
CP	9.8 $\pm$ 2.3	13.9 $\pm$ 1.6	6.7 $\pm$ 0.9	31.7 $\pm$ 0.3	22.8 $\pm$ 0.3	17.3 $\pm$ 0.6
NDF	49.3 $\pm$ 4.6	39.9 $\pm$ 3.6	45.2 $\pm$ 4.0	14.5 $\pm$ 1.5	27.1 $\pm$ 1.2	40.2 $\pm$ 2.5
ADF	35.8 $\pm$ 3.8	31.9 $\pm$ 3.1	27.8 $\pm$ 3.4	11.0 $\pm$ 0.1	10.8 $\pm$ 0.1	26.3 $\pm$ 3.0
ADL	4.80 $\pm$ 0.91	4.55 $\pm$ 1.52	3.49 $\pm$ 0.95	5.78 $\pm$ 0.04	3.65 $\pm$ 0.03	4.50 $\pm$ 0.35
EE ³	1.68 $\pm$ 0.28	2.88 $\pm$ 0.42	3.41 $\pm$ 0.25	2.03 $\pm$ 0.01	7.41 $\pm$ 0.16	3.15 $\pm$ 0.46
DM amounts (kg/d)						
Offered	0.64 $\pm$ 0.19	4.94 $\pm$ 0.47	7.34 $\pm$ 0.52	1.75 $\pm$ 0.25	1.27 $\pm$ 0.43	0.63 $\pm$ 0.22
Refused	0.13 $\pm$ 0.05	0.86 $\pm$ 0.28	0.49 $\pm$ 0.15	0.11 $\pm$ 0.35	0	0

¹n = 19.

²n = 3.

³EE = ether extract.

Table 2. Overview and definitions of mid-infrared (MIR) assessments, categorization of cows into low and high CH₄ emitters, and MIR-based equations used

Item	Acronym ¹	Duration	No. of spectra per cow ²	Site	No. of cows
MIR assessments	Screening	18 mo	18	Home farm	175,980
	MIR ₁	1 d ³	1	Home farm	30
	MIR ₂ (old/new)	1 d ⁴	2 (averaged)	Station barn	30
	MIR ₃ (old/new)	8 d	16 (averaged)	Station barn	28 ⁵
	MIR ₄ (old/new)	1 d	2 (averaged)	Respiration chamber	28 ⁵
Categorization	Categorization _{MIR1}	1 d	1	Home farm	30
	Categorization _{MIR3_old}	1 d	16 (averaged)	Station barn	28 ⁵
	Categorization _{MIR3_new}	1 d	16 (averaged)	Station barn	28 ⁵

¹Old equation = equation available at the time of the experiment; new equation = further developed equation now available.

²If morning and evening milks were collected or collection was performed across several days, the spectra were averaged proportionately to milk yield to 1 spectrum following Vanlierde et al. (2015).

³From last routine performance recording before the experiment (July 2017).

⁴At arrival at station.

⁵Two high P_{m MIR} cows had severe diarrhea and were excluded.

the 30 cows either on their home farm or at the research station. The spectra were obtained using Fourier transform infrared spectrophotometry (MilkoScan FT6000 Foss Electric, Hillerød, Denmark). They were standardized according to the Grelet et al. (2017) procedure to avoid instrumental interference and ensure comparability of the spectra regardless the spectrometer used. From these spectra, P_{m MIR} was first predicted using the lactation-stage-dependent prediction equation developed by partial least-square regression from Vanlierde et al. (2016). The published prediction equation was slightly modified for this purpose by including milk spectra and corresponding CH₄ measurements from 77 Swiss cows in the calibration data set for deriving the prediction equation. This process yielded 225 RC-based CH₄ measurements in addition to the 532 SF₆-based CH₄ measurements in the original calibration data set of Vanlierde et al. (2016). The standard error of calibration (**SEC**) of this equation, later called the “old equation,” was 70 g/d, the calibration coefficient determination (**R²c**) was 0.66, the standard error of cross-validation (**SECV**) was 73 g/d, and the cross-validation coefficient of determination (**R²cv**) was 0.62. After the experiment had been completed, the prediction equation was further refined. The “new equation” was developed and calibrated in 2019 using 1,089 RC- and SF₆-based CH₄ measurements (7% from Brown Swiss cows) originating from 299 cows (13% Brown Swiss cows). This new equation had an SEC of 58 g/d, an R²c of 0.68, an SECV of 61 g/d, and an R²cv of 0.64. Following Vanlierde et al. (2016) and considering the spectral data set used to build the equation as the reference, we removed Swiss spectra obtained during this study with a standardized Mahalanobis distance (global H distance; **GH**) of >3 from the data

set. Based on the GH procedure, only 0.72% of the current data set needed to be removed. With both equations, predicted P_{m MIR} values of <150 g/d or >950 g/d were excluded from further analyses and considered as outliers as described by Vanlierde et al. (2016, 2018). The new prediction equation was applied to evaluate whether cow allocation was robust when the P_m prediction equation changes and to determine whether correlations with measured CH₄ data were improved. In detail, P_{m MIR} was predicted from MIR spectra 5 times with both the old and new equations (only the former was available at the time of the screening). Description and denominations are given in Table 2.

Measurement of Daily CH₄ Emissions Using RC

Four new RC (No Pollution, Industrial Systems Ltd., Edinburgh, UK) were used to measure CH₄ emissions from the individual cows at AgroVet-Strickhof. The chambers were 4.75 m wide, 3.25 m deep, and 2.5 m tall (38.6 m³). Each chamber was fitted with 1 large back door for animal entrance, 1 smaller front door, safety opening devices, and rubber seals around the whole perimeter of each opening. The animals were tied in metabolism stalls (255 × 150 cm) equipped with water troughs and feed bins mounted on an electronic balance. The doors were opened for a very short time twice daily at the same time for milking and feeding. These openings were accounted for by interpolating about 2 × 20 min/d with values from adjacent times in which gas concentration had returned to the equilibrium. Fresh air was supplied via a common duct through 2 shutters (SPI-F-160, Systemair AB, Buchs ZH, Switzerland and LM 230, Belimo Automation AG, Hinwil, Switzerland) to prevent backflow. The air was exchanged about 12

times/h. Temperature was maintained at 16°C, with relative humidity at 60%. Spent air was removed by an extraction fan (K06-MS Blower, FPZ Blower Technology, Concorezzo, Italy), coupled with a frequency controller (3.3-kW VLT HWAC Drive, Danfoss GmbH, Offenbach, Germany) maintaining an airflow between 19.0 and 23.0 L/s. The chambers were kept under a slight negative pressure. The CH₄ concentration was determined with an MGA 3500 (ADC Gas Analysis Ltd., Hertfordshire, UK) using nondispersive infrared absorption. This measurement was done every 10 min in the outgoing chamber air and in the fresh air collected on the roof of the building where the air pipe for the RC was installed. Calibration was performed directly before and after each experimental run. To start, a pure N₂ gas (99.999%) was applied. Then, the first standard gas mixture containing 0.1% H₂ and 99.9% N₂ was delivered for 3 min until H₂ level stabilized, which was followed by pure N₂ gas for 3 min. Next, a second standard gas mixture (0.08% CH₄, 20.9% O₂, 0.4% CO₂, and 78.62% N₂) was delivered to let the instrument return to the expected concentrations. A recovery test (total calibration) for CH₄ was performed 3 times per chamber during the experiment. While the regular data collection was performed, CH₄ (99.9%) was injected at 0.35 L/min via a tube through the outside wall for 4 h. The measured concentration reached a plateau after 1 to 2 h. The flow rate was controlled by a Sierra mass flow controller (MC-5SLPM-RD, Alicat Scientific, Tucson, AZ). The calibration of the chambers and the gas analyzers provided a calibration factor for CH₄. The average recoveries in the 4 chambers were 88, 88, 90, and 89%, respectively.

Estimation of Daily CH₄ Emissions Using LMD

Two LMD units (Mini-Green Lmm-g; Tokyo Gas Engineering Solutions, Tokyo, Japan) were used. Measurement principle (tunable diode laser absorption spectroscopy) and the operation of the devices were described in detail by Chagunda et al. (2013) and Sorg et al. (2018). The duration of single measurements was set to 6 min/cow, and the distance between the LMD device and the cow's nostril was set to 1 m. The measurements were conducted on each cow during the last 3 d of the 8-d sampling period, before and after each feeding event while the animals were standing. From each LMD measurement, the CH₄ concentration (ppm × m; arithmetic mean of all peaks in a 6-min measurement) was calculated. Estimates of daily CH₄ emissions by the LMD technique ($P_{m \text{ LMD}}$; g/d) were obtained as described by Sorg et al. (2018). The 3-d $P_{m \text{ LMD}}$ values

were averaged across before feeding, after feeding, and overall.

Recordings, Sampling, and Analysis of Feed, Feces, Urine, and Rumen Fluid

Body weight was measured on a truck load scale (Waagen Döhrn GmbH & Co. KG, Wesel, Germany) upon arrival and directly before and after the sampling period. Milk yield was recorded automatically. During the sampling period, samples from each milking (50 mL) were separately conserved with bronopol. Eating and ruminating behavior were recorded on 3 consecutive days per cow during the sampling period, using RumiWatch (Itin + Hoch GmbH, Liestal, Switzerland) halters equipped with pressure sensors detecting jaw movements, acceleration sensors detecting head position, and data loggers. Data were differentiated by the software into eating, ruminating, and other activities (Rombach et al., 2018). During the sampling period, feed intake was measured daily from supply and leftovers on flat troughs with separations between cows, using electronic balances developed by Mettler-Toledo (Dübendorf, Switzerland). The leftovers were pooled per animal. Forage samples were taken once per week in the adaptation period and twice in the sampling period. Grass pellets and concentrate were sampled 3 times during the 23-d experiment. Samples were dried at 60°C to constant weight and ground to a particle size of 1 mm with a cutting mill. For concentrate samples, a centrifugal mill was used.

During the 8-d sampling period, all feces were collected on steel trays located below a grid at the end of the tie stall. Urine was collected separately from feces using urinals attached around the vulva and glued (Cyanolit 202, Panacol Elosol GmbH, Steinbach, Germany) onto hair and skin. Urine pH was maintained at <3 by the addition of 5 M H₂SO₄ to prevent ammonia volatilization. Feces and urine were weighed daily, and representative samples proportional to the amounts excreted were taken and frozen at -20°C. For the quantification of digesta retention time, 100-g samples of feces were collected 4, 8, 12, 18, 22, 26, 30, 36, 42, 46, 52, 58, 66, 74, 82, 90, 98, 106, 114, 126, 138, and 150 h after application of a marker bolus. Baseline was determined by 3 samples taken on the day before bolus application. The markers used were Co-EDTA as solute marker and mordanted grass hay following Udén et al. (1980). For that process, the hay was cut in a cutting mill (MM180S, Fuchs-Mühlen, Vienna, Austria) to pass an 8-mm screen. The cut hay was sequentially dry screened by shaking on sieves with mesh sizes of 3.55,

2, 1, and 0.5 mm to obtain 3 particle fractions of 8, 5, and 2 mm, which were mordanted with Ce, La, and Cr, respectively. For more details see Grandl et al. (2018). The samples of feces containing marker were dried at 60°C to constant weight and ground through a 1-mm screen with a centrifugal mill.

Rumen fluid was collected on d 9 of the sampling period at 4 h after morning feeding via a stomach tube (SELEKT Pump and Collector, Nimrod Veterinary Products Ltd., Gloucestershire, UK). Two duplicate samples of 10 mL were obtained. Trichloroacetic acid was added to one for ammonia analysis and sulfuric acid to the other for VFA analysis. Samples were stored at -20°C.

Feeds and feces were analyzed according to standard procedures (AOAC International, 1995). Contents of DM and total ash were determined with a thermogravimetric device (TGA-701, Leco Corp., St. Joseph, MI; AOAC method 942.05). The OM was calculated as DM minus total ash. Nitrogen was assessed in feeds, nondried feces, and acidified urine on a C/N analyzer (Type Tru-Mac CN, Leco Cooperation; AOAC method 968.06). The CP was calculated as $6.25 \times \text{N}$. Ether extract was determined with a Soxhlet extraction system (model B-811, Büchi, Flawil, Switzerland). Ash-corrected contents of NDF (AOAC method 2002.04; with heat-stable α -amylase [Sigma-Aldrich, St. Louis, MO]) and ADF (AOAC method 973.18) in feeds and feces were determined using the Gerhardt Fibertherm FT 12 (Gerhardt GmbH and Co. KG, Königswinter, Germany). Determination of ADL in feed items was performed sequentially after ADF analysis by incubation in sulfuric acid (72%) for 3 h. Gross energy (GE) contents were measured in feeds and feces with a bomb calorimeter (C7000, IKA-Werke GmbH & Co. KG, Staufen, Germany). The bronopol-conserved milk was analyzed for contents of fat, protein, and lactose using a Fourier transform infrared spectrophotometer (MilkoScan FT6000 Foss Electric) at SuisseLab AG (Zollikofen, Switzerland). The spectra obtained during this process were also used to determine $P_{\text{m MIR}}$. Milk protein was divided by 6.38 to calculate N content. The element concentrations in the Co-EDTA, the mordanted hay, and the feces were analyzed after wet ashing using inductively coupled plasma optical emission spectrometry (Optima 8000, Pekin Elmer, Rodgau, Germany). The markers contained (per kg DM) 32.8 g of Cr, 49.5 g of La, 41.5 g of Ce, and 151 g of Co. Rumen fluid ammonium was measured with a potentiometer equipped with a corresponding glass electrode (6.0506.100, Metrohm AG, Herisau, Switzerland) calibrated by using NH_4Cl at 0.1, 1, and 10 mM. The VFA were analyzed by HPLC (LaChrom, L-7000 series, Hitachi Ltd., Tokyo, Japan) with a UV detector.

Calculations and Statistical Analysis

Feed conversion efficiency (ECM/DMI), milk production efficiency (ECM/BW), and RFI (difference between observed and predicted DMI) were calculated as measures of efficiency. For RFI, the predicted DMI was calculated using Equation 1 of Gruber et al. (2004), which was developed based on measured DMI data recorded in Switzerland, Austria, and Germany, thus reflecting similar farming systems. This equation considered breed, lactation number, DIM, BW, milk yield, concentrate amount, and forage composition. The ECM (kg/d) was calculated as $\text{milk (kg/d)} \times [0.38 \times \text{fat (\%)} + 0.24 \times \text{protein (\%)} + 0.17 \times \text{lactose (\%)}] / 3.14$ (Agroscope, 2019).

Fecal baseline marker concentrations were used to correct for individual animal background levels. Mean retention time (MRT) in the gastrointestinal tract (GIT) was computed for each marker according to Thielemans et al. (1978), as $\text{MRT GIT} = (\sum C_i \times t_{i-1,i} \times dt_i) / (\sum C_i \times dt_i)$, where $t_{i-1,i}$ = mean time (h) after application of markers of 2 subsequent samplings $i - 1$ and i calculated as $t_{i-1} + (t_i - t_{i-1}) / 2$, C_i = marker content in the fecal sample voided in the interval represented by time t_i and t_{i-1} , and dt_i = sampling interval (h) of the respective sample calculated as $[(t_{i-1} - t_i) + (t_i - t_{i-1})] / 2$. The MRT of Co-EDTA in the reticulorumen (RR) was calculated following Grovum and Williams (1973) and that of the particles according to Huhtanen and Kukkonen (1995), as $\text{MRT RR particles} = \text{MRT GIT particles} - (\text{MRT GIT solute} - \text{MRT RR solute})$. Dry matter gut fill was calculated following Munn et al. (2015) and considering DMI, DM digestibility, and the MRT GIT of the 5-mm particle marker (La).

All statistical analyses were performed with R version 3.3.1 (R Core Team, 2018). As a measure for accuracy, Lin's concordance correlation coefficients (CCC) were computed between the CH_4 emission data measured and predicted, as well as linear regressions. Pearson correlation coefficients were calculated between P_{m} and non- CH_4 variables. Data from the 10 low and 10 high $Y_{\text{m RC}}$ cows were subjected to ANOVA performed with a linear mixed model using the nlme R-package (Pinheiro et al., 2017). Emission category (low, high), experimental block (run 1–7 with 4 cows, run 8 with 2 cows) and their interaction were fixed effects, and cow was the random effect. The same model was used for the MIR categorized P_{m} data (MIR_1 , $\text{MIR}_{3 \text{ old and new}}$, $\text{MIR}_{4 \text{ old and new}}$, RC, LMD). Homogeneity of variances was checked with the Bartlett test and normality of the residuals with the Shapiro-Wilk test. To evaluate the accuracy of the prediction of $P_{\text{m MIR}}$, the root mean square error of prediction (RMSEP) for predicted CH_4

(MIR_{3_old} and MIR_{3_new}) was also calculated according to Vanlierde et al. (2015).

RESULTS

Categorization of Cows by MIR CH_4 Predictions and Its Recovery by Measured CH_4

Based on the population screening, the groups of 159 low and 159 high $P_{m\ MIR}$ cows differed in each month during the entire 1.5-yr period of assessment (Figure 1C), and this outcome was also observed during the course of the lactation (Figure 1D). The 2 groups of 15 cows selected for the on-station experiment also

differed in almost every month and during lactation (Figures 1A and 1B). The characteristics of the cows in the experiment are given in Table 3. Accordingly, the average difference (high in relation to low CH_4 emitting cows) in $P_{m\ MIR}$ was 16% (old equation) when determined directly before the start of the experiment on the home farm (MIR_1), and was 18% (old equation) and 10% (new equation) in spectra obtained on the day of arrival at the research station (MIR_2). The absolute $P_{m\ MIR}$ levels differed between the 3 assessments, especially in the high $P_{m\ MIR}$ cows. The 2 categories were similar in average DIM and milk yield.

Values for CCC ($P < 0.01$) in $P_{m\ MIR}$ were close across all time points ($MIR_{1,3,4}$) when using the old

Figure 1. Methane production (g/d) before the experiment predicted from milk mid-infrared (MIR) spectra obtained in the 18 mo from January 2016 to July 2017 (means $\pm$ SE; A and C: changes with calendar time; B and D: changes with progressing DIM) of either all lactating Brown Swiss cows identified as low and high methane emitters ($n = 2 \times 159$; upper and lower quartile of preselected cows; C and D) or the cows selected for the present experiment ($n = 2 \times 15$; A and B).

Table 3. Description of the experimental animals selected for presumed low and high CH₄ emissions (P_m) based on milk mid-infrared (MIR) spectra predictions before the experiment (arithmetic means $\pm$ SD or ranges)

Item	MIR-CH ₄ emission category		Overall characterization of the cows (n = 30)
	Low (n = 15)	High (n = 15)	
Methane ¹ (P _m ; g/d)			
MIR ₁ ²	409 $\pm$ 23	507 $\pm$ 36	455 $\pm$ 57
MIR _{2_old} ³	450 $\pm$ 42	529 $\pm$ 28	487 $\pm$ 61
MIR _{2_new} ³	411 $\pm$ 26	443 $\pm$ 34	426 $\pm$ 54
BW ³	656 $\pm$ 39	637 $\pm$ 39	651 $\pm$ 42
DIM ²	244 $\pm$ 29	238 $\pm$ 31	241 $\pm$ 30
Milk yield ⁴ (kg/d)	20.2 $\pm$ 2.62	20.7 $\pm$ 2.8	20.3 $\pm$ 5.3
BW range ⁴ (kg)	570–740	550–680	570–740

¹MIR = methane emission predicted from mid-infrared spectra of milk samples analyzed. For further explanations see Table 2.

²Measured during last monthly milk performance recording before the start of the experiment (July 2017).

³Measured on the first day at the experimental station.

⁴Measured during the experimental period.

equation, and between the 2 time points assessed with the new equation ($P < 0.001$) (Table 4). This result is illustrated through the regression analysis for the MIR assessments on the home farm and during the 8-d collection period (Figure 2A). The changes caused by using the new equation were moderate (Figure 2B). By contrast, no significant correlations were found between individual cow data from either RC or LMD values with the MIR predictions or between RC and LMD (Table 4, Figure 2C–F). Accordingly, relating P_m RC with P_m MIR₃ (“old” and “new”) by means of a linear regression did not result in significant relationships ($R^2_{old} = 0.014$, $P = 0.23$; $R^2_{new} = 0.026$, $P = 0.19$). The RMSEP was smaller using RC data and MIR_{3_new} (30.6 g/d) compared with using RC data and MIR_{3_old}

(48.1 g/d). No significant CCC was found between the 3 LMD-predicted P_m variables (Table 4).

The categories established before the experiment (MIR₁) and those based on spectra obtained in the 8-d sampling period (MIR₃) were mostly different on average ($P < 0.05$ to 0.01) in P_m MIR with any equation (old, new; Table 5). Using the new equation for MIR-based predictions largely reduced SE of the category means. Recategorization resulted in a certain regrouping of the 28 cows, with 1 cow each from low to high P_m MIR and vice versa when moving from Categorization_{MIR1} to Categorization_{MIR3_old}. When changing either from Categorization_{MIR1} to Categorization_{MIR3_new} or from Categorization_{MIR3_old} to Categorization_{MIR3_new}, 3 cows each were regrouped. Other than predicted, P_m RC and

Table 4. Lin’s concordance correlation coefficients between different predictions and measurements of methane production (g/d; n = 28)¹

	MIR ₁	MIR _{3_old}	MIR _{4_old}	MIR _{3_new}	MIR _{4_new}	RC	LMD _{avg}	LMD ₁
MIR _{3_old}	0.70** (0.37; 0.84)							
MIR _{4_old}	0.65** (0.36; 0.83)	0.89*** (0.77; 0.95)						
MIR _{3_new}	0.36 ^{NS} (0.21; 0.57)	0.32 ^{NS} (0.13; 0.48)	0.41 ^{NS} (0.17; 0.60)					
MIR _{4_new}	0.31 ^{NS} (0.05; 0.53)	0.28 ^{NS} (0.09; 0.44)	0.37 ^{NS} (0.14; 0.57)	0.90*** (0.81; 0.94)				
RC	0.05 ^{NS} (−0.15; 0.08)	0.05 ^{NS} (−0.13; 0.04)	0.03 ^{NS} (−0.12; 0.07)	0.11 ^{NS} (−0.06; 0.28)	0.16 ^{NS} (−0.05; 0.37)			
LMD _{avg}	0.02 ^{NS} (−0.01; 0.02)	0.003 ^{NS} (−0.004; 0.12)	0.01 ^{NS} (−0.03; 0.15)	−0.01 ^{NS} (−0.01; 0.01)	0.001 ^{NS} (−0.01; 0.01)	−0.004 ^{NS} (−0.01; 0.001)		
LMD ₁	0.11 ^{NS} (−0.16; 0.37)	0.13 ^{NS} (−0.09; 0.34)	0.12 ^{NS} (0.12; 0.35)	0.06 ^{NS} (−0.14; 0.24)	0.01 ^{NS} (−0.20; 0.23)	0.05 ^{NS} (−0.18; 0.08)	0.05 ^{NS} (0.02; 0.08)	
LMD ₂	−0.18 ^{NS} (−0.51; 0.19)	−0.10 ^{NS} (−0.38; 0.20)	−0.02 ^{NS} (−0.31; 0.35)	−0.09 ^{NS} (−0.39; 0.22)	0.04 ^{NS} (−0.28; 0.35)	−0.05 ^{NS} (0.20; 0.11)	0.01 ^{NS} (−0.01; 0.02)	−0.28 ^{NS} (−0.53; 0.01)

¹Values in parentheses are 95% CI. MIR = CH₄ values predicted by mid-infrared analysis (for further explanations see Table 2); RC = respiration chamber; LMD = laser methane detector (1 = used before feeding; 2 = used after feeding; avg = daily average).

** $P < 0.01$; *** $P < 0.001$.

$P_{m\text{ LMD}}$ levels measured in low and high $P_{m\text{ MIR}}$ cows did not differ ($P > 0.10$; Table 5).

Characteristics of Cows Categorized by the RC Results

The animals were recategorized into low and high CH_4 emitters based on their $Y_{m\text{ DMI}}$ as measured by RC (Table 6). Group differences (high in relation to low $Y_{m\text{ RC}}$) accounted for 18, 21, 19, and 19% when CH_4

was related to intakes of DM, digestible OM, digestible NDF, and GE, respectively ($P < 0.01$ to 0.001). The difference ($P = 0.001$) was even larger with 21% for CH_4 emission intensity (I_m ; CH_4/ECM), but not for CH_4/BW . The $P_{m\text{ RC}}$, in contrast, was not different between the categories, similar to the $P_{m\text{ MIR}}$ averages obtained at 2 different time points and with 2 different equations. When relating $P_{m\text{ MIR}}$ to measured DMI ($Y_{m\text{ MIR DMI}}$), $Y_{m\text{ RC}}$ -categorized cows differed (16%; $P < 0.05$) with MIR_1 , and trends for such differences

Figure 2. Relationships between individual cow CH_4 production data (g/d) predicted by the milk mid-infrared (MIR) spectra obtained from the last milking before the experiment (MIR_1 ; July 2017) and across the 8 d of sampling (A: MIR_{3_new} and B: MIR_{3_old} ; using the newest and the first available equation, respectively) and those measured in respiration chambers (RC; on sampling d 9; C, D) and with the laser methane detector (LMD; across the last 3 d within 8 d of sampling; E); and those measured in RC and with LMD (F).

(13 and 18%; $P < 0.10$) were found using MIR_3 (old and new equations). Group differences in LMD results before and after feeding were reversed, leading to almost the same average $P_{m\ LMD}$ in the 2 categories. In addition, $Y_{m\ DMI}$ did not differ between groups when measured with LMD.

Compared with the high $Y_{m\ RC}$ cows, the low $Y_{m\ RC}$ cows were characterized by a higher DMI (+11%; $P < 0.01$) and a higher ECM yield (+27%; $P < 0.05$) (Table 7). The diets as consumed contained $10.0 \pm 1.5\%$ and $7.4 \pm 0.6\%$ concentrate for low and high $Y_{m\ RC}$ cows, respectively. The low $Y_{m\ RC}$ cows were higher ($P < 0.05$) in ECM/BW compared with the high $Y_{m\ RC}$ cows. The RFI was higher ($P < 0.05$) for low compared with high $Y_{m\ RC}$ cows. Cow categories did not differ in daily eating and ruminating times; total-tract apparent digestibility of nutrients; and balance, losses, and utilization of N for milk protein formation (milk N, % of N intake or digested N intake). The low $Y_{m\ RC}$ cows had a different VFA pattern compared with the high $Y_{m\ RC}$ cows, with higher proportions of propionate ($P < 0.01$), lower proportions of acetate ($P < 0.05$), and a consequently lower acetate-to-propionate ratio ($P < 0.001$). Ruminal ammonia concentration and most variables describing MRT of the digesta in RR and GIT did not differ between categories. Only GIT MRT of small particles was shorter ($P < 0.05$) in low compared with

high $Y_{m\ RC}$ cows, associated with a slightly higher ($P < 0.05$) DM gut fill.

The data on $P_{m\ RC}$ correlated with DMI and N intake, digestibility of OM and NDF, urinary N losses, propionate, isobutyrate, and isovalerate proportions of total VFA, as well as acetate-to-propionate ratio ($P < 0.05$ to 0.01; Table 7). In addition, trends for Pearson correlations ($P < 0.10$) were apparent in RFI, CP digestibility ($P < 0.05$ –0.01), and milk N relative to intake of digested N. Only a few correlations were found between $P_{m\ MIR}$ and these variables ($P < 0.05$ –0.01). These included ECM per unit of DMI and milk N proportion of intake of N and digested N (MIR_{3_old} only).

DISCUSSION

The current study attempted to confirm the usefulness of predictions of daily CH_4 emissions based on MIR spectra of milk samples or estimates of daily CH_4 emissions made using LMD detectors to predict actual daily CH_4 emissions of dairy cows. Our study showed that the predictions based on currently available equations derived from MIR spectra as well as the estimates based on short-term measurements made with the LMD devices did not have a close phenotypical correlation with the measured values. This outcome does not necessarily exclude the presence of genetic

Table 5. Methane production (P_m , g/d) of cows categorized into low ($n = 15$) or high ($n = 13$) emitters based on values predicted from milk mid-infrared (MIR) spectra obtained before or during the experiment (LSM $\pm$ SE)¹

Milk spectra database	Categorization based on	MIR emission category		P-value
		Low	High	
MIR_1	MIR_1	407 $\pm$ 14.1	501 $\pm$ 14.5	0.002
	MIR_{3_old}	417 $\pm$ 11.1	492 $\pm$ 10.7	0.010
	MIR_{3_new}	422 $\pm$ 17.1	479 $\pm$ 13.7	0.14
MIR_{3_old}	MIR_1	486 $\pm$ 55.6	563 $\pm$ 55.2	0.002
	MIR_{3_old}	477 $\pm$ 48.2	561 $\pm$ 47.8	0.007
	MIR_{3_new}	526 $\pm$ 85.5	587 $\pm$ 84.6	0.023
MIR_{4_old}	MIR_1	550 $\pm$ 87.4	622 $\pm$ 86.2	0.004
	MIR_{3_old}	535 $\pm$ 83.7	611 $\pm$ 82.9	0.020
	MIR_{3_new}	556 $\pm$ 103.2	603 $\pm$ 102.1	0.057
MIR_{3_new}	MIR_1	448 $\pm$ 7.9	411 $\pm$ 7.0	0.029
	MIR_{3_old}	447 $\pm$ 7.6	411 $\pm$ 7.9	0.050
	MIR_{3_new}	405 $\pm$ 6.5	451 $\pm$ 6.3	0.002
MIR_{4_new}	MIR_1	441 $\pm$ 9.5	403 $\pm$ 8.5	0.080
	MIR_{3_old}	440 $\pm$ 9.2	404 $\pm$ 9.5	0.14
	MIR_{3_new}	391 $\pm$ 8.7	447 $\pm$ 7.2	0.005
RC	MIR_1	384 $\pm$ 5.7	382 $\pm$ 6.4	0.24
	MIR_{3_old}	386 $\pm$ 6.2	385 $\pm$ 5.9	0.56
	MIR_{3_new}	380 $\pm$ 6.5	385 $\pm$ 6.6	0.72
LMD ²	MIR_1	423 $\pm$ 16.5	426 $\pm$ 18.5	0.95
	MIR_{3_old}	416 $\pm$ 18.4	428 $\pm$ 17.7	0.60
	MIR_{3_new}	429 $\pm$ 17.5	426 $\pm$ 18.0	0.47

¹MIR = values predicted by mid-infrared analysis (for further explanations see Table 2); RC = respiration chamber; LMD = laser methane detector.

²Measurements in ppm $\times$ m were converted to g/d using the regression of Sorg et al. (2018).

Table 6. Methane emission of cows categorized into low or high CH₄ emitters (n = 2 × 10) based on methane yield per unit of DMI (Y_m) measured in the respiration chambers (RC; LSM ± SE)¹

Item	Emission category		P-value
	Low Y _m RC	High Y _m RC	
Respiration chamber			
Methane production (P _m ; g/d)	379 ± 6.9	391 ± 6.5	0.22
Methane yield (Y _m)			
g/kg of DMI (Y _m DMI)	21.7 ± 0.35	25.6 ± 0.33	<0.001
g/kg of digestible OM	34.6 ± 0.71	41.7 ± 0.66	<0.001
g/kg of digestible NDF	104 ± 3.8	124 ± 3.6	0.003
% of gross energy (Y _m GE)	6.77 ± 0.129	8.04 ± 0.134	<0.001
Methane emission intensity (I _m)			
per ECM (g/kg; I _m ECM)	16.6 ± 1.15	21.1 ± 1.09	0.001
per BW (g/kg)	0.60 ± 0.022	0.59 ± 0.023	0.81
MIR predictions			
MIR ₁ (g/d; P _m)	473 ± 20.0	483 ± 18.8	0.97
MIR _{3_ol} d (g/d; P _m)	453 ± 19.0	450 ± 17.9	0.58
MIR _{3_ne} w (g/d; P _m)	412 ± 23.5	415 ± 24.0	0.66
MIR ₁ (g/kg of DMI; Y _m)	27.2 ± 1.45	31.6 ± 1.37	0.044
MIR _{3_ol} d (g/kg of DMI; Y _m DMI)	26.1 ± 1.39	29.5 ± 1.32	0.090
MIR _{3_ne} w (g/kg of DMI; Y _m DMI)	23.5 ± 1.04	27.8 ± 1.08	0.058
Laser methane detector ²			
Before feeding (g/d)	384 ± 39.2	445 ± 37.7	0.48
After feeding (g/d)	455 ± 17.1	412 ± 17.4	0.051
Average (g/d; P _m)	422 ± 22.0	414 ± 20.7	0.77
Average (g/kg of DMI; Y _m DMI)	24.3 ± 1.63	27.0 ± 1.54	0.26

¹MIR = values predicted by mid-infrared analysis (for further explanations see Table 2); Average = averages of measurements before and after feeding using the laser methane detector.

²Measurements of methane in ppm × m were converted to g/d using the regression of Sorg et al. (2018).

correlations, and further developed MIR spectra based equations might perform better. An in-depth investigation of potential factors of influence from ingestion and digestion that may cause CH₄ emissions to differ among animals was carried out. In addition, the utility of the LMD technique was assessed. For both the MIR-based prediction and the LMD spot-sampling measurements, the RC technique was used as a reference method because it captures total CH₄ emissions and is generally considered highly accurate. Indeed, the current results found with RC are highly plausible, which can be concluded from comparing levels of P_m RC, Y_m RC DMI, and Y_m RC GE with published data and from the expected significant positive relationships between P_m RC and DMI, apparent fiber digestibility, and ruminal acetate-to-propionate ratio (e.g., Niu et al., 2018).

Robustness and Accuracy of the CH₄ Prediction from Milk MIR Spectra

Among the MIR-based prediction methods intended to be implemented into breeding schemes, the Belgian approach (Vanlierde et al., 2015, 2016, 2018) is probably the most developed because a high number of CH₄ measurements and different diets are considered. Shetty et al. (2017) also attempted to predict CH₄ via P_m MIR by using reference data obtained from a nondispersive infrared analyzer installed (sniffer) in an automated

milking system. Good agreement existed between this sniffer method and RC measurements (Garnsworthy et al., 2012a). However, when using the full milk MIR spectra and including DIM, the correlations were clearly lower with the sniffer method compared with those described by the Belgian group (Shetty et al., 2017). The Belgian equation was at first based on data derived from the SF₆ method, but RC data were included later. This inclusion reduced the R²c from 0.74 to 0.66 (old equation used in the present study), but enhanced the applicability by generating additional variability (Vanlierde et al., 2016, 2018). Including more RC-based P_m data in the most recent equation (new equation) improved the R²c to 0.68.

The MIR predictions developed by Vanlierde et al. (2015), which were based on SF₆ data, had shown a good correlation to a reference data set (R²c = 0.75). Further, when predicted CH₄ emissions were compared with RC-based measurements from an external data set, the correlation was still moderate (r = 0.48). The SECV of a refined equation using SF₆ and RC was 61 g/d. As the correlation is highly dependent on the distribution of the considered data set, the error of prediction also needs to be considered when evaluating the performance of a method. Additionally, prediction equations have known errors to consider. For the equation of Vanlierde et al. (2018), the SECV was 47 g/d for a RC-based prediction and 70 g/d for a SF₆-based

prediction. As a lower SECV indicates that the equation is closer to actual values (Vanlierde et al., 2018), the new equation was an improvement compared with the SF₆-based equation. The RMSEP can be used to evaluate the predictive ability of the obtained calibration models (Shetty et al., 2017). Indeed, the RMSEP decreased from 48 to 31 g/d when MIR_{3_new} was used instead of MIR_{3_old}. This RMSEP of the new equation is even lower than the known errors established during calibration and cross-validation processes.

To be useful for breeding purpose, the genetic variability of a trait among cows has to persist over time and different feeding regimens (Pinares-Patiño et al., 2011). The prediction equation indeed turned out to be robust over different countries, feeding regimens, and measurement techniques (Vanlierde et al., 2016, 2018). Persistence was also observed for the cohort of 318 cows of the present study when MIR spectra were followed across 1.5 yr. A suitable equation also has to consider that the P_m of dairy cows changes over the course of

Table 7. Variables characterizing intake, performance, and digestion of cows categorized into low or high CH₄ emitters (n = 2 × 10) based on CH₄ yield per unit of DMI (Y_m) measured in the respiration chambers (RC; LSM ± SE) and correlations of these variables (n = 28) with methane production (g/d) measured with RC and predicted with MIR based on first available (old) and further developed equation (new)

Item	Emission category			Pearson correlation coefficient		
	Low Y _m RC	High Y _m RC	P-value	RC	MIR _{3_old}	MIR _{3_new}
DMI (kg/d)	17.5 ± 0.47	15.7 ± 0.44	0.004	0.55**	-0.21 ^{NS}	0.06 ^{NS}
Feeding behavior						
Eating time (min/d)	371 ± 34.8	351 ± 38.1	0.70	-0.22 ^{NS}	0.16 ^{NS}	-0.09 ^{NS}
Ruminating time (min/d)	412 ± 19.1	394 ± 20.9	0.54	-0.32 ^{NS}	0.06 ^{NS}	0.02 ^{NS}
BW (kg)	634 ± 17.9	665 ± 16.9	0.25	0.27 ^{NS}	-0.22 ^{NS}	-0.09 ^{NS}
ECM (kg/d)	23.8 ± 1.58	18.8 ± 1.49	0.041	0.29 ^{NS}	0.27 ^{NS}	0.33†
Efficiency						
ECM (kg/kg of DMI)	1.35 ± 0.073	1.22 ± 0.061	0.17	0.08 ^{NS}	0.49**	0.43*
ECM (kg/100 kg of BW)	3.73 ± 0.241	2.85 ± 0.232	0.020	0.21 ^{NS}	0.32 ^{NS}	0.35†
Residual feed intake (kg/d)	-1.58 ± 0.224	-2.47 ± 0.201	0.012	0.33†	-0.16 ^{NS}	0.01 ^{NS}
Apparent digestibility (%)						
OM	69.5 ± 0.82	68.2 ± 0.78	0.29	0.51**	-0.12 ^{NS}	0.16 ^{NS}
NDF	51.9 ± 1.46	51.1 ± 0.84	0.84	0.41*	-0.02 ^{NS}	0.16 ^{NS}
ADF	49.1 ± 2.10	50.3 ± 1.98	0.69	0.25 ^{NS}	-0.31 ^{NS}	0.36†
CP	60.8 ± 2.91	57.4 ± 2.80	0.83	0.34†	-0.12 ^{NS}	0.03 ^{NS}
N balance (g/d per cow)						
N intake	433 ± 25.1	446 ± 56.3	0.39	0.52**	-0.15 ^{NS}	0.09 ^{NS}
Fecal N	166 ± 10.1	175 ± 21.4	0.48	0.30 ^{NS}	-0.10 ^{NS}	0.07 ^{NS}
Urinary N	136 ± 8.0	135 ± 8.3	0.57	0.59**	-0.31 ^{NS}	-0.10 ^{NS}
Milk N	127 ± 9.0	110 ± 17.0	0.26	0.18 ^{NS}	0.27 ^{NS}	0.29 ^{NS}
N losses (% of N intake)						
Fecal N	38.8 ± 1.51	40.8 ± 1.53	0.26	-0.34†	0.12 ^{NS}	-0.03 ^{NS}
Urinary N	34.2 ± 1.56	38.9 ± 1.57	0.14	-0.11 ^{NS}	-0.22 ^{NS}	-0.23 ^{NS}
Milk N	30.9 ± 1.14	28.7 ± 1.44	0.83	-0.24 ^{NS}	0.57**	0.37†
Milk N (% of digestible N)	50.8 ± 1.92	48.5 ± 1.94	0.96	-0.34†	0.57**	0.32†
Urine N (% of total N loss)	48.7 ± 1.62	46.9 ± 1.60	0.50	0.30 ^{NS}	-0.22 ^{NS}	-0.15 ^{NS}
VFA in rumen fluid (mol % of total VFA)						
Acetate	73.1 ± 3.14	86.8 ± 2.97	0.013	0.19 ^{NS}	0.02 ^{NS}	-0.06 ^{NS}
Propionate	22.6 ± 0.59	19.3 ± 0.56	0.002	-0.47**	0.11 ^{NS}	-0.14 ^{NS}
<i>n</i> -butyrate	15.5 ± 0.88	14.4 ± 0.83	0.42	-0.19 ^{NS}	0.19 ^{NS}	0.09 ^{NS}
isobutyrate	1.46 ± 0.11	1.33 ± 0.10	0.38	-0.38*	-0.12 ^{NS}	-0.18 ^{NS}
<i>n</i> -valerate	1.85 ± 0.08	1.84 ± 0.07	0.92	-0.23 ^{NS}	-0.07 ^{NS}	-0.29 ^{NS}
isovalerate	1.64 ± 0.13	1.86 ± 0.12	0.23	-0.50**	0.03 ^{NS}	-0.25 ^{NS}
Acetate-to-propionate ratio	3.26 ± 0.19	4.51 ± 0.18	<0.001	0.42*	-0.08 ^{NS}	0.07 ^{NS}
Ruminal ammonia (mM)	10.8 ± 1.41	13.8 ± 1.32	0.16	0.09 ^{NS}	0.08 ^{NS}	-0.02 ^{NS}
Reticuloruminal retention time (h)						
Small particles (2 mm)	26.6 ± 1.58	29.0 ± 1.88	0.93	0.09 ^{NS}	0.00 ^{NS}	0.01 ^{NS}
Medium particles (5 mm)	34.1 ± 2.46	35.7 ± 2.60	0.88	0.03 ^{NS}	-0.21 ^{NS}	-0.18 ^{NS}
Large particles (8 mm)	34.9 ± 2.47	36.3 ± 2.61	0.95	0.03 ^{NS}	-0.21 ^{NS}	-0.13 ^{NS}
Solute	16.5 ± 1.61	17.0 ± 1.69	0.42	-0.04 ^{NS}	-0.05 ^{NS}	-0.12 ^{NS}
Gastrointestinal (GIT) retention time (h)						
Small particles (2 mm)	37.3 ± 1.83	40.5 ± 1.94	0.03	-0.09 ^{NS}	0.28 ^{NS}	0.25 ^{NS}
Medium particles (5 mm)	44.7 ± 2.09	47.1 ± 2.21	0.11	-0.18 ^{NS}	0.09 ^{NS}	0.08 ^{NS}
Large particles (8 mm)	45.7 ± 2.18	47.7 ± 2.30	0.16	-0.17 ^{NS}	0.09 ^{NS}	0.12 ^{NS}
Solute	29.9 ± 1.34	28.1 ± 1.42	0.84	-0.30 ^{NS}	0.35 ^{NS}	0.24 ^{NS}
GIT fill (kg of DM)	35.0 ± 1.64	29.9 ± 1.55	0.042	-0.15 ^{NS}	0.10 ^{NS}	0.24 ^{NS}

* $P < 0.05$ ** $P < 0.01$; † $P < 0.10$.

the lactation (Garnsworthy et al., 2012a). The $P_{m\text{ MIR}}$ equation developed by Vanlierde et al. (2016) therefore considers DIM. Indeed, a biologically reasonable change in predicted $P_{m\text{ MIR}}$ with DIM was found for both the 318 and the 30 cows. This change was also observed by Garnsworthy et al. (2012a) in an automated milking system fitted with an infrared sensor. To further exclude bias caused by DIM, we considered only cows with ≥ 5 milk performance records obtained at different DIM when selecting the experimental cows.

Despite the good performance of the Belgian prediction equation (Vanlierde et al., 2018), especially in its newest version (unpublished), with respect to RMSEP and the biological meaningful change in CH_4 emission over the course of the lactation (Figure 1C), the relationship (CCC) of the predicted with the RC data in the present study was weak. Different from a previous assessment on a single herd using GreenFeed instead of RC as standard method (Denninger et al., 2019), the low MIR predicting ability in our study also concerned categorization and not only that of individual cow values. The MIR predicting ability of category differences was better when the 2×10 cows were categorized retrospectively by using RC data. When $P_{m\text{ RC}}$ and $P_{m\text{ MIR}}$ were related to DMI, the differences were in a similar direction, and $P_{m\text{ MIR}}$ was consistent across different stages of refinement of the prediction equation. However, none of the anticipated correlations of $P_{m\text{ MIR}}$ with DMI, NDF digestibility, and ruminal VFA pattern were apparent. Also, the average $P_{m\text{ MIR}}$ level predicted was too high (amounting to 9.9 and 9.3% of GE in high $Y_{m\text{ MIR}}$ cows, predicted with MIR_1 and MIR_{3_old}). This result clearly exceeded the default value of the IPCC (2006) of 6.5%, which may have been due to including data from cows beyond the range of P_m of 350 to 450 g/d in the reference data set for the equation by Vanlierde et al. (2016). With further refinement of the equation (MIR_{3_new}), the predicted Y_m was lower, with 8.5% of GE. The number of animals used for the development of the equation whereby CH_4 was measured by RC was still limited, but this cohort only included cows for which strong scrutiny or preselection against unusual animals or measurements had been practiced. This could also be a reason why the difference in $P_{m\text{ MIR}}$ did not exceed 20% between low and high $P_{m\text{ MIR}}$ cows and why the MIR prediction equations did not clearly discriminate between the categories distinguished by RC. However, the main goal of the present study was to recover the variability observed by MIR spectra on farm ($P_{m\text{ MIR}}$) by means of RC measurements, which was not successful. The findings based on the present data set support the claim of van Gastelen and Dijkstra (2016) that MIR data alone might be insufficient for a reliable prediction, at least to distinguish between ani-

mals that are not at extremes in their CH_4 emission levels. In this context, MIR spectra might not sufficiently predict indicative milk fatty acids related to processes associated with CH_4 formation. van Gastelen and Dijkstra (2016) suggested that the MIR-based prediction might be improved by implementing more factors, such as milk yield, DMI, and others. However, care has to be taken against giving the correlated factors included too much weight in the equation, which would diminish the weight of the milk spectral information. Further improvements can be expected from continuing to develop the equation by adding new data of interest.

One other important drawback of the MIR-based predictions is that they currently only aim at absolute CH_4 production and not at CH_4 yield or CH_4 emission intensity. However, the latter might be easily implemented because the milk recording events also provide data on milk yield. Currently, the knowledge of genetic correlations between different CH_4 proxies and reference CH_4 values is extremely limited.

Accuracy of the CH_4 Measurement with the LMD

The LMD has been shown to be potentially useful in fast phenotyping the P_m of cows under some circumstances because measurements require only a few minutes per cow (Sorg et al., 2018). The circadian pattern of P_m is mainly driven by feed intake as determined by Bell et al. (2018) in a study of cows in a freestall barn. We therefore measured $P_{m\text{ LMD}}$ before and after feeding. We found the expected lower P_m before feeding, but only in the low $Y_{m\text{ RC}}$ group. It is possible that, for a short time, low CH_4 emitters have greater postfeeding emissions due to particularly effective fermentation, whereas greater “basal” (prefeeding) emissions lead to the overall higher emissions of high CH_4 emitters. In this situation, the LMD technique may indeed be able to detect differences in P_m caused by feeding events, as shown earlier by Sorg et al. (2017), but it also means that no reliable categorization may be possible with LMD values obtained after feeding. Chagunda (2013) reported a positive relationship between LMD and RC data and concluded that the LMD would rank cows for P_m in a very similar way. When compared with the GreenFeed system and 2 different infrared sensors installed in an automatic milking system, the LMD method ranked cows similarly with respect to their P_m (Sorg et al., 2018). Nevertheless, we found no correlation of any of the LMD measurements with the RC data (nor with any MIR prediction) despite repeating measurements over 3 d. This outcome coincides with the report of low agreement by Ricci et al. (2014). The level of $P_{m\text{ LMD}}$ was high compared with $P_{m\text{ RC}}$ and in the range found with MIR_{3_new} , and categorization for $Y_{m\text{ DMI}}$ with

RC was only weakly recovered with LMD. The reversal of categories in $P_{m\text{ LMD}}$ rank before and after feeding was especially puzzling. The LMD operates indirectly and relies on an assumed relationship between breath CH_4 concentration and other parameters. As such, it is subject to greater variance and uncertainty compared with quantitative direct measurements. In addition, the accuracy is affected by the proximity of other animals, the distance to the cows' head, the angle of the laser beam, and as shown, the time point in relation to feeding. All of these factors were controlled in the present study, but they may be difficult to control on farm and thus add further uncertainty.

Characteristics of Low-Emitting Cows Identified by CH_4 Yield in RC

Recategorizing cows for low and high emission by the RC CH_4 data was limited to the 28 remaining cows with complete data sets. It can, therefore, be expected that the difference in CH_4 yield between the 2 categories in the Brown Swiss population in Switzerland is clearly larger, as that between the 2×10 cows eventually selected. Still several clear differences between these 2 groups were found. These differences included higher RFI and ECM/BW ratios in low versus high $Y_{m\text{ RC}}$ cows, whereas differences in feed efficiency were not statistically significant despite similar DMI and different ECM. A low RFI is an indicator for good feed utilization. Hegarty et al. (2007) demonstrated that beef cattle selected for low RFI have a lower P_m but not a decreased $Y_{m\text{ DMI}}$. The RFI itself is also a heritable trait ($h^2 = 0.40$), and genetic correlations in the range of 0.18 to 0.84 between RFI and predicted P_m indicate that selection for lower RFI might also reduce P_m (de Haas et al., 2011). The indirect effect of differences in feed efficiency of ruminants fed on the same diet explains at least half of the heritability of $Y_{m\text{ DMI}}$ (Pinares-Patiño et al., 2013). This explanation is independent of the side effect of breeding for ECM yield on $I_{m\text{ ECM}}$ (−15% per kg of ECM; Knapp et al., 2014). Here, $I_{m\text{ ECM}}$ is mainly declining because of the lower dilution by maintenance and more concentrate (less fiber) associated with higher ECM yield (Grandl et al., 2016). The lower dilution of maintenance also explains the higher ECM/BW ratio of the low $Y_{m\text{ RC}}$ cows. In addition, the recategorization also slightly increased ECM difference (on farm: 22 vs. 20 kg/d in low and high $P_{m\text{ MIR}}$ cows; recategorized on station: 23 vs. 19 kg/d in low and high $Y_{m\text{ RC}}$ cows) and thus minimally enhanced the need for allocation of concentrate (10 vs. 7.5% of diet).

Flay et al. (2019) showed that $Y_{m\text{ DMI}}$ was smaller in high RFI animals (consistent with our findings) and hypothesized that this result might be due to a de-

creased ruminal NDF digestibility, which is consistent with the present study regarding the RFI. Accordingly, Cabezas-Garcia et al. (2017) found that a reduced $Y_{m\text{ DMI}}$ was associated with a lower DM and cell wall digestion. The fiber degrading microbes produce less hydrogen, the main substrate for CH_4 formation, possibly as a consequence of a faster ruminal digesta passage rate. Indeed, MRT in RR or GIT was suggested to be a main contributor leading to differences in CH_4 emissions in ruminants because a shorter MRT leaves less time for CH_4 formation from the same amount of feed (Goopy et al., 2014). Goopy et al. (2014) showed that low $Y_{m\text{ DMI}}$ sheep have a lower rumen particulate content and a proportionately smaller rumen. However, only part of these findings were recovered in the current study. Indeed the proportion of ruminal acetate was declining at cost of propionate proportion in low compared with high $Y_{m\text{ RC}}$ cows, a clear sign of a shift in fermentation from fiber toward starch (Hristov et al., 2013). This possibility might have been supported by the concomitant small difference in concentrate allocation. However, total-tract fiber digestibility was not different in the present study. The same is true (with one exception) for RR and GIT MRT of all particle fractions, for which only the smallest particles had a shorter GIT MRT in the low $Y_{m\text{ RC}}$ cows. The estimated gut DM fill was even higher for the low $Y_{m\text{ RC}}$ cows. The present results therefore point toward a shift in fiber fermentation from the rumen to the hindgut in low compared with high $Y_{m\text{ RC}}$ cows. In the hindgut, CH_4 formation per unit of fiber degraded is lower due to the absence of protozoa and the higher competitiveness of the reductive acetogens (Fievez et al., 1999). In case the category differences measured in the present study were caused by genetics, the genotype indeed appears to have some control over the gut microbial community. Accordingly, transcription of methanogenesis pathway genes was found by Shi et al. (2014) to be lower in low CH_4 producing sheep even though methanogen abundance was unaffected. Goopy et al. (2014) also reported that host genetics may influence the rumen ecosystem, which itself might affect ruminal CH_4 production.

CONCLUSIONS

The present study demonstrated that the MIR spectra-based predictions for CH_4 production of individual cows on farm could be recovered on a uniform diet could be recovered on farm at the experimental station thus confirming hypothesis 1. However, the CH_4 production of individuals or categorized groups as predicted with MIR did not correspond to that measured in RC, although a slight improvement was noted with the most refined equation (a numerical increase in the

concordance correlation coefficient to 0.16). This outcome disproves hypothesis 2 and indicates that, at least with forage-based diets and with this range of variation in CH₄ values, the proxy is not yet accurate enough to be implemented for selection purposes in Brown Swiss breeding. This assessment also applies to the laser CH₄ detector technology. The current study provided detailed information about the characteristics of low CH₄ emitting cows in terms of intake, efficiency, and digestion. Compared with high CH₄ emitters, low CH₄ emitting cows are superior in some variables describing feed and digestive efficiency, which partially confirms hypothesis 3. Still, cows with low CH₄ yield will have to be further characterized by mechanistic studies to understand the relative importance of different physiological aspects contributing to the lower CH₄ emissions and to clarify the extent to which these aspects are under genetic control.

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