ABSTRACT

Rotational crossbreeding has not been widely studied in relation to the enteric methane emissions of dairy cows, nor has the variation in emissions during lactation been modeled. Milk infrared spectra could be used to predict proxies of methane emissions in dairy cows. Therefore, the objective of this work was to study the effects of crossbreeding on the predicted infrared proxies of methane emissions and the variation in the latter during lactation. Milk samples were taken once from 1059 cows reared in 2 herds, and infrared spectra of the milk were used to predict milk fat (3.79 ± 0.81%) and protein (3.68 ± 0.36%) concentrations, yield (21.4 ± 1.5 g/kg DMI), methane intensity (14.2 ± 2.0 g/kg corrected milk), and daily methane production (358 ± 108 g/d). Of these cows, 620 were obtained from a 3-breed (Holstein, Montbéliarde, and Viking Red) rotational mating system, and the rest were purebred Holsteins. Milk production data and methane traits were analyzed using a nonlinear model that included the fixed effects of herd, genetic group, and parity, and the 4 parameters (a, b, c, and k) of a lactation curve modeled using the Wilmink function.

Milk infrared spectra were found to be useful for direct prediction of qualitative proxies, such as methane yield and intensity, but not quantitative traits, such as daily methane production, which appears to be better estimated (450 ± 125 g/d) by multiplying a measured daily milk yield by infrared-predicted methane intensity. Lactation modeling of methane traits showed daily methane production to have a zenith curve, similar to that of milk yield but with a delayed peak (53 vs 37 DIM), while methane intensity is characterized by an upward curve that increases rapidly during the first third of lactation and then slowly till the end of lactation (10.5 g/kg at 1 DIM to 15.2 g/kg at 300 DIM). However, lactation modeling was not useful in explaining methane yield, which is almost constant during lactation. Lastly, the methane yield and intensity of cows from 3-breed rotational crossbreeding are not greater, and their methane production is lower than that of purebred Holsteins (452 vs 477 g/d). Given the greater longevity of crossbred cows, and their lower replacement rate, rotational crossbreeding could be a way of mitigating the environmental impact of milk production.

Keywords: infrared spectrometry, FTIR, methane production, Montbéliarde, Viking Red

INTRODUCTION

In recent years, the study of traits associated with global climate change has become increasingly important (Moumen et al., 2016). Enteric methane emissions (EME) from ruminants are credited with being the most impactful source of greenhouse gases from food production at the world level (Roos et al., 2017; FAO, 2020), even though they have a short life in the atmosphere and their impact is much lower than previously thought (Place et al., 2022). Strategies have been developed for predicting traits related to EME of dairy cows by adjusting and calibrating measurements obtained from collected milk samples using Fourier transform infrared (FTIR) spectroscopy. This approach is becoming increasingly common and FTIR is currently one of the most routinely used technologies worldwide (de Haas et al., 2017).

The main metabolic link between the fermentation activity of the rumen microbiota and the infrared spectrum of milk is represented by the relationship between the proportion of volatile fatty acids (Williams et al., 2019) produced in the rumen (acetate production is directly related to hydrogen and methane production) and the proportion of some fatty acids among the triglycerides of milk (van Gastelen and Dijkstra, 2016;...)
Bougouin et al., 2019; Pitta et al., 2022) and other dairy products (Bittante and Bergamaschi, 2020). It is common knowledge that several milk fatty acids can be predicted by FTIR spectrometry (Engelke et al., 2018), and that their proportions in milk vary during lactation in relation to the energy balance of cows and the mobilization or storage of their body reserves. The phenotypic and genetic relationships between some milk fatty acids and EME traits also vary during lactation (Vanrobyays et al., 2016; Bittante and Cecchinato, 2020).

Predictation of EME proxies through FTIR is a promising technique, as it is much simpler and less expensive than methods based on the sampling and analysis of gases in respiration chambers, automatic feeders, automatic milking, etc., and is not surrounded by ethical controversy over animal welfare (Garnsworthy et al., 2019). The predictions based on FTIR allow the EME proxies of a large number of cows to be estimated, and the effects of different factors, like herd, breed, parity, and lactation stage, to be tested.

The EME are represented by traits with different meanings and properties (de Haas et al., 2017). The first trait to consider is methane production, that is the total daily production of methane per cow (dCH₄, g/d). This is a quantitative trait affected by cow’s feed intake, milk yield, and body size; therefore, dCH₄ is not a direct measure of the ecological efficiency but rather of milk production and feed intake (de Haas et al., 2017). Other traits that appear to be more closely related to the concept of efficiency (van Lingen et al., 2014) are methane yield, expressed as the methane emitted per kg of dry matter ingested by the cow (CH₄/DMI, g/kg), and methane intensity, expressed per kg of fat-and-protein-corrected milk (CH₄/CM, g/kg) or per kg of cheese obtained from the milk (Bittante et al., 2018). Finally, there are other traits measured by sniffer devices, which are based on the methane to carbon dioxide ratio (CH₄/CO₂) or the concentration of methane in the air (CH₄/air) (Sorg et al., 2018; Lee et al., 2022; Wang and Bovenhuis, 2019). All these traits are expected to be differently affected by the major sources of variation in milk yield, including genetics and lactation stage (Brito et al., 2018).

Variations in EME traits throughout lactation have not been investigated in depth, as trials using measured gas emissions are often relatively short. Methane emissions during lactation depend on feed intake and diet composition which change during lactation in relationship to the variation of daily milk yield, and of the nutrients requirements of lactating cows (Beauchemin et al., 2022; de Ondarza and Tricario, 2017). Further knowledge could be obtained by comparing the pattern of variations during lactation of EME traits with those of milk yield. An interesting approach would be to test the use of parametric models developed to study milk yield throughout lactation to analyze EME traits (Rekaya et al., 2000; Bonallegue and M’Hamdi, 2019). The Wilmink model (Wilmink, 1987) has been widely used for modeling lactation curves, not because of its better statistical performance compared with other models, but because of its flexibility, simplicity (it is based on only 4 parameters), and the ease with which function parameters can be interpreted attributing them a physiological meaning (Macciotta et al., 2011). This last aspect could be very interesting if the physiological meanings used for explaining the development of milk production during lactation could be applied also to EME patterns.

Crossbreeding is increasingly used in the dairy sector (Guinan et al., 2019) due to its favorable effects on cow fertility (Malchiodi et al., 2014a; Hazel et al., 2020), longevity (Buckley et al., 2014; Clasen et al., 2017), and milk quality (Malchiodi et al., 2014b), which results in increased profitability despite reduced milk yield (Dezetter et al., 2017; Clasen et al., 2020; Hazel et al., 2021). The prevalent mating strategy in pasture-based dairy systems is a 2-breed alternate scheme using Holstein and Jersey bulls (Vance et al., 2012; Ferris et al., 2018). More common in indoor intensive dairy systems is the 3-breed rotational mating scheme, especially that using Holstein (HO), Montbéliarde (MB), and Viking Red (VR) breeds. Following early research carried out by the University of Minnesota (Heins and Hansen, 2012; Hazel et al., 2017), this mating scheme is now also used in European countries (Malchiodi et al., 2014b; Dezetter et al., 2017; Saha et al., 2017), although its effects on the ecological footprint, and specifically on enteric methane emissions, is not well known.

Therefore, the objectives of this study are: a) to describe and compare the lactation patterns of EME proxies predicted by FTIR spectrometry (CH₄/DMI, g/kg; CH₄/CM, g/kg; and dCH₄, g/d) using the models and physiological interpretation of the parameters tested for milk production traits; and b) to assess the influence of either a 3-breed rotational crossbreeding system or Holstein purebred group on EME traits.

**MATERIALS AND METHODS**

**Experimental design**

The present study is part of the project “Improving the ecological footprint of dairy farms through a three-way crossbreeding program (ProCROSS/Genesi project).” The ProCROSS rotational crossbreeding scheme (https://www.procross.info/) is managed by the leading suppliers of Montbéliarde genetics (Coopex...
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The experimental design involved the selection of 2 large commercial herds belonging to the 2 main dairy farming systems of Northern Italy. The first farm (GP, 331 cows involved) is in the Lombardy region and follows the production regulations for Grana Padano cheese, the most important European Protected Designation of Origin (PDO) cheese in terms of quantity produced and revenue. The milk aimed at Grana Padano cheese-making comes mainly from specialized intensive dairy herds, like GP herd, located in the lowlands of the northern part of the Po Valley, and from dairy cows generally fed total mixed rations (TMR) based on corn silage, concentrates, soybean meal and roughages. The second farm (PR, 785 cows involved) follows the production regulations for Parmigiano Reggiano PDO cheese, the second most important Italian PDO cheese. The milk aimed at Parmigiano Reggiano production comes mainly from the Emilia-Romagna region, and is produced in specialized farms located on the plains and in the hills of the southern part of the Po Valley. Production regulations forbid the use of silages in the diet, so the feed consists of dry forages (especially alfalfa and meadow hay) and concentrates administered separately, or as TMR given dry or moistened with water.

Both herds have both been using the ProCROSS rotational crossbreeding mating scheme for at least 12 years. The 2 farms have kept both genetics (HO purebreds and CR crossbreds) throughout this period and continue to mate several purebred HO cows with MB or VR bulls every year, so they both have 5 crossbreeding generations, including first and second generation cows also among the younger animals. The GP farm followed the rotational sequence VR-MO-HO-VR-…, breed of sires, beginning initially from purebred HO cows; the PR farm followed the same sequence, but also the reverse sequence MO-VR-HO-MO-… . Mating is not seasonal, but takes place all year round, and different parities were represented in both genetic types and the different crossbreeding generations.

On both farms, the HO and CR cows were kept together, managed as one herd, and fed the same TMR. As a consequence, the effects of the farm, genetic type, parity, and lactation stage could be considered substantially independent.

Animals, milk samples, and acquisition of FTIR spectra

Data for this study come from previous research aimed to investigate production traits and the cheese-making properties of milk of 1116 purebred HO and crossbred cows (Saha et al., 2020), where details about crossbreeding generation and rotational sequence can be found. In total 475 cows were HO and 641 CR (185 belonging to the first generation of crossbreeding, 164 to the second, 222 to the third, and 70 to the fourth). The distribution of parity was: 423 cows in first lactation, 343 in second lactation, 350 in third and later lactations. The DIM was 155 ± 87 d.

Milk samples (100mL) were collected once in all cows during the even milking in 2 sampling dates (one per herd) according to the sampling protocol described by Saha et al. (2020). The FTIR spectra were acquired from milk samples and analyzed with a MilkoScan™ FT+ 6000 (Foss A/S). The instrument and its operations are validated and certified according to ISO 9622:2013/IDF 141:2013 (ISO, 2013), recently updated to ISO 21543:2020/IDF 201:2020 (ISO, 2013, 2020; Niermöller and Holroyd, 2019). The complete spectrum of every milk sample analyzed was stored in the experimental database. A total of 1,060 absorbance values were recorded for each milk sample covering the infrared wavenumbers ranging from 5,000/cm (corresponding to a wavelength of 2.0 μm) in the near-infrared subdivision of the infrared area, through the mid-infrared to wave number 930/cm (corresponding to a wavelength of 10.8 μm) in the far infrared subdivision (Bittante and Cecchinato, 2013).

For this study, data on milk yield and composition of all lactating cows with 5 to 365 DIM have been matched with FTIR spectra, resulting in an overall data set of 1,059 cows: 439 HO (160, 132, and 147 for first, second, third, and more parity) and 620 CR (239, 200, 181 for first, second, and third and more parity).

Data editing

A principal component analysis was performed on the FTIR spectra with Mahalanobis distances; samples with a probability level of < 0.01 were considered outliers and removed from the analysis. A detailed description of this methodology is reported in Toledo-Alvarado et al. (2018). After editing, the data set contained the complete spectra of 1,059 milk samples collected during routine test-day milk recording paired with the measured daily milk yield obtained from milk-recording data (dMY, kg/d), and the fat (%) and protein (%) concentrations predicted from the same spectra according to the official ICAR procedure (Orlandini, 2020). From these traits, a daily fat-protein-corrected milk yield (dCMY, kg/d) was calculated (CVB, 2008; van Lingen et al., 2014). Only records within ± 3 SD for each trait were subsequently used. The parity and...
DIM of each cow on the sampling date were also reported.

**Prediction of enteric methane emissions**

“Direct” predictions of EME traits from the milk FTIR spectra were made using the calibration equations described in a previous study (Bittante and Cipolat-Gotet, 2018). The methane EME prediction equations were obtained in the previous study by using, as reference, the values estimated from milk fatty acids according to a meta-analysis of trials carried out in respiration chambers (van Lingen et al., 2014). The predicted EME traits were:

- **Methane yield** per kg of dry matter ingested by cows (CH4/DMI, g/kg);
- **Methane intensity** per kg of milk corrected for fat and protein concentration (CH4/CM, g/kg);
- **Methane production** per cow and per day (dCH4-IR, g/d).

The predicting equations used were characterized by a calibration accuracy of 80%, 84%, and 69%, a cross-validation accuracy of 70%, 75%, and 60%, and a residual error of cross-validation of 1.18 g/kg, 1.17 g/kg, and 86 g/d, respectively for CH4/DMI, CH4/CM, and dCH4-IR (Bittante and Cipolat-Gotet, 2018). Direct FTIR prediction of daily milk yield (dMY-IR) was also obtained for comparison with the measured dMY-MR and with the direct FTIR-predicted dCH4-IR. Lastly, an “indirect” prediction of dCH4 (dCH4-CMY, g/d) was made by multiplying the dCMY (kg/d) of each cow by its corresponding CH4/CM (g/kg).

**Lactation curve modeling**

The Wilmink’s function initially used for modeling dMY_MR (a “zenith” curve with negative values for the “b” and “c” parameters) was expanded to include positive and negative values (Amalfitano et al., 2021):

\[
y_t = a + b \times \exp (-k \times t) + c \times t,
\]

where:
- \(y_t\) is the response of the trait (dMY_MR, fat, protein, dCMY, CH4/DMI, CH4/CM, dCH4_IR, dCH4_CM, and dMY_IR) at time \(t\) (corresponding to days in milk, DIM);
- \(a\) is a parameter representing the potential value of the trait at the beginning of lactation, in the absence of adaptation;
- \(b\) is the parameter that quantifies the short-term adaptation fraction soon after parturition;
- \(c\) is the parameter that describes the pattern of the curve after the peak, interpreted as the long-term variation (persistency of lactation);
- \(k\) is a parameter associated with the time of the peak of lactation, and interpreted as the speed of adaptation after parturition.

We used the PROC GLM of SAS (release 9.4, SAS Institute Inc., Cary, NC) to analyze each trait using DIM to define the initial parameters \((a, b, \text{ and } c)\), and with the value of the parameter \(k\) set to 0.05, as suggested by Wilmink (1987). These values were used as references for the preliminary statistical analysis to obtain suitable parameters to apply to the population.

**Pattern and derived variables of the curves**

The patterns of the curves for each trait were classified according to a combination of the signs of parameters \(b\) and \(c\) (\(a\) and \(k\) are always positive). They were as follows (Amalitano et al., 2021):

- **Zenith curve**, when \(b < 0\) and \(c < 0\), i.e., a curve increasing from calving to the maximum (peak) of lactation, then decreasing until the end;
- **Nadir curve**, when \(b > 0\) and \(c > 0\), i.e., a curve decreasing from calving to the minimum (negative peak) of lactation, then increasing until the end;
- **Upward curve**, when \(b < 0\) and \(c > 0\), i.e., a curve continually increasing from the beginning to the end of lactation (no peak); and
- **Downward curve**, when \(b > 0\) and \(c < 0\), i.e., a curve continually decreasing from the beginning to the end of lactation (no peak).

The detailed behavior of all the patterns can be consulted in Macciotta et al. (2005).

Other variables were also calculated from the 4 parameters of the lactation model to describe the lactation curves (Amalitano et al., 2021), such as:

- **initial value**, which is the sum of the maximum production potential (parameter \(a\), long-term compartment) and the adaptation production (parameter \(b\), short-term compartment) when DIM is equal to 0, \([a+b]\);
- **peak value**, which is the maximum (zenith curve) or minimum (nadir curve) value of the trait during lactation, \([a-(c/k) \times \ln(c/(k \times b))+(c/k)]\);
- **peak DIM**, which is the number of days from parturition to reach the peak of lactation, \([-1/(k \times \ln(c/(k \times b)))]\);
- **final value**, which is the production value when lactation ends, calculated as the sum of the maximum potential (parameter \(a\)) and the persistency...
(parameter c) multiplied by the defined final DIM (DIMf; for this study the limit was 300 DIM), \[a-c \times DIMf];

- **adaptation fraction**, which is the proportion of the adaptation in relation to the maximum potential expressed as a percentage, \[b/a \times 100\]; and
- **persistency fraction**, which is the percentage of the production variation up to the end of lactation in relation to the maximum production potential, \[(c \times DIMf)/a \times 100\].

### Statistical Analysis

All production and EME traits were analyzed with a nonlinear regression model using PROC TRANSREG and PROC NLIN of SAS (release 9.4; SAS Institute Inc., Cary, NC) with the Gauss-Newton algorithm. For each trait, the designed model was the following:

\[y_{hij} = a + b \times \exp (-k \times t) + c \times t + \text{herd}_h + \text{breed}_i + \text{parity}_j + e_{hij}\]

where \(y_{hij}\) is the response of the trait (dMY-MR, fat, protein, dCMY, CH₄/DMI, CH₄/CM, dCH₄_IR, dCH₄-CMY and dMY-IR); \(a, b, c,\) and \(k\) are the parameters of the lactation model; \(t\) is the days in milk (DIM) from 1 to 300; \(\text{herd}_h\) is the fixed effect of the herd (2 levels: GP and PR herds); \(\text{breed}_i\) is the fixed effect of the cows’ genetic type (2 levels; HO, purebred Holsteins, and CR, 3-breed rotational crossbreds); \(\text{parity}_j\) is the fixed effect of the cows’ parity (3 levels; first, second and ≥ third parity); and \(e_{hij}\) is the effect of the residual.

### RESULTS AND DISCUSSION

#### Milk production and quality traits

Table 1 summarizes the descriptive statistics of the measured and FTIR-predicted traits analyzed in this study. The production traits from milk recording (dMY-IR, and the fat and protein concentrations of the milk samples) were the subject, together with milk coagulation and cheese-making properties, of a previous study on the effects of rotational crossbreeding (Saha et al., 2020), but in that previous study lactation modeling was not tested. The results of the analysis of milk production and quality traits obtained are important to characterize the production environment of this study and to test the lactation modeling adopted here on traits that have a well-known lactation pattern (control traits) before moving on to the main objective of the study, i.e., EME proxies (experimental traits). The distribution of dCMY was, as expected, very similar to that of the uncorrected daily milk yield (dMY-IR).

#### Effects of farm, parity, and rotational cross-breeding on milk traits

The 2 selected farms belong to 2 different farming systems: the Grana Padano system (GP), with production mainly on intensive indoor farms in the lowlands using TMR based on corn silage, concentrates, and some roughages; and the Parmigiano-Reggiano system (PR), a less intensive system with production more frequently in the hills, and using dry rations without silages. As expected (Mucchetti et al., 2017), the cows kept in the Grana Padano herd showed higher milk yield (Table 2), and the difference in MY between the 2 herds was close to 12%. On the other hand, the milk of cows kept in Parmigiano Reggiano herd had a 5.3% higher protein concentration than the milk of

### Table 1. Descriptive statistics of milk production traits and enteric methane emission traits

<table>
<thead>
<tr>
<th></th>
<th>Measured</th>
<th>FTIR-predicted milk traits:</th>
<th>Mixed¹</th>
<th>FTIR-predicted EME traits:</th>
<th>Mixed²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dMY_MR</td>
<td>dMY_IR</td>
<td>Fat</td>
<td>Protein</td>
<td>dCMY</td>
</tr>
<tr>
<td>kg/d</td>
<td>1,059</td>
<td>1,053</td>
<td>1,042</td>
<td>1,042</td>
<td>1,041</td>
</tr>
<tr>
<td>Mean</td>
<td>32.7</td>
<td>24.6</td>
<td>3.79</td>
<td>3.68</td>
<td>32.1</td>
</tr>
<tr>
<td>SD</td>
<td>10.2</td>
<td>6.1</td>
<td>0.81</td>
<td>0.36</td>
<td>9.1</td>
</tr>
<tr>
<td>CV, %</td>
<td>31.2</td>
<td>24.8</td>
<td>21.4</td>
<td>9.8</td>
<td>28.3</td>
</tr>
<tr>
<td>Min</td>
<td>5.5</td>
<td>5.5</td>
<td>1.43</td>
<td>2.60</td>
<td>8.7</td>
</tr>
<tr>
<td>Max</td>
<td>63.0</td>
<td>42.7</td>
<td>7.47</td>
<td>5.03</td>
<td>58.4</td>
</tr>
<tr>
<td>P1</td>
<td>11.3</td>
<td>10.2</td>
<td>1.94</td>
<td>2.95</td>
<td>11.5</td>
</tr>
<tr>
<td>P99</td>
<td>57.8</td>
<td>38.6</td>
<td>6.16</td>
<td>4.55</td>
<td>54.7</td>
</tr>
</tbody>
</table>

dMY_MR = measured daily milk yield (kg/d); dCMY = daily corrected milk yield (kg/d); CH₄/DMI = methane yield per unit of dry matter intake (g/kg); CH₄/CM = methane intensity per kg of corrected milk (g/kg); dCH₄_IR = methane production per cow per day predicted directly from FTIR (g/d); dCH₄-CMY = daily methane production obtained indirectly by multiplying dCMY by FTIR-predicted CH₄/CM (g/d); CV = 100 × SD/mean; P1 = 1st percentile; P99 = 99th percentile.

¹: trait obtained from the measured dMY_MR corrected for FTIR-predicted fat and protein concentrations.

²: trait obtained by multiplying the mixed dCMY by FTIR-predicted CH₄/CM.
The effects of the parity of the cows in this study also confirm the expected increase in milk yield with advancing number of lactations (Table 2), and the small variation in quality traits. Lastly, the results obtained here confirm that the crossbred cows reared in intensive indoor farming systems tend to have a lower fluid milk yield (see dMY-MR and dCMY in Table 2) and a higher fat and protein concentrations than the purebred HO (Hazel et al., 2020).

**Effects of lactation modeling on measured milk traits.** The lactation model is statistically significant for all production traits, with at least 3 of the 4 parameters significantly different from the null value (Table 2). Consequently, Table 2 also reports the variables that describe the lactation curves derived from the model parameters.

The resulting lactation curves are depicted in Figure 1 (left side graphs). The lactation modeling adopted here fully reflects the expectations regarding these traits. It is worth noting that the daily yield traits (dMY_MR and dCMY) have a “zenith” curve with a positive peak in the first part of the lactation curve (negative short-term adaptation compartment b, and negative persistency coefficient c). On the contrary, the 2 qualitative traits (fat and protein concentration) of milk are characterized by a ‘nadir’ curve with a negative peak (positive parameters b and c). The “zenith” curve shape of recorded daily production (dMY_MR and dCMY), was well established by Wilmink's (1987) original research and subsequently many other authors (Macciotta et al., 2005).

As is well known, the model is based on the estimation of 2 compartments, one long-term and the other short-term. The first represents lactation as an initial potential value (parameter a, theoretical intercept at DIM 0) and a linear slope (parameter c, persistency), which is negative for milk yield and depicts the decrease in milk yield toward the end of lactation. The...
second is a transitory compartment that represents the cow-udder effect of the adaptation after calving. Adaptation is represented as the difference between the theoretical and actual initial production ($b$ parameter). This parameter is negative for milk yield because after parturition the cow needs to improve its feed intake capacity and the volume and productivity of the udder before reaching its potential milk production. The speed of this adaptation process is represented by the fourth parameter, $k$, in the form of an instant rate constant causing the disappearance of the second compartment, and then the increase in milk yield, with advancing lactation. After parturition, the daily milk yield increases because the favorable effect of adaptation is greater than the unfavorable effect of the decrease in persistency. The 2 opposite effects are equal at the peak of lactation, whereas the decrease in persistency prevails because the favorable effect of adaptation is greater. However, the result is that we estimated the peak at 32 DIM (Amalfitano et al., 2021). Brown Swiss cows using the same modeling, we estimated the peak would be reached at 37 DIM, which is in the range of Wilmink’s (1987) estimates. In a previous study on Brown Swiss cows we obtained an average CH4/DMI of 21.3 g/kg (Bittante et al., 2018), while van Lingen et al. (2014) reported an average of 24.6 g/kg/d for dMY$_{IR}$ vs 32.7 for dMY$_{MR}$; Table 1). It is worth noting that 24.6 kg/d is very similar to the average dMY of the data set used to calibrate and validate the prediction equations (Bittante and Cipolat-Gotet, 2018). Moreover, with regard to the effects of the 3 fixed factors considered, dMY$_{IR}$ yielded more variable results than dMY$_{MR}$. With respect to the measured production (dMY$_{MR}$), Table 2 shows that the prediction of milk yield based on milk FTIR spectra (dMY$_{IR}$) confirms the higher level of the GP herd over the PR herd, but the higher level of the HO cows over the CR cows was not, and the effect of parity reversed, with an unrealistic prediction of decreasing production from the first lactation to maturity. The lactation modeling for dMY$_{IR}$ differed little from that for dMY$_{MR}$, but the values were much lower and the pattern flatter (Figure 2).

This means that FTIR predictions of a quantitative trait, like dMY, are unreliable, even though the spectra capture some of the variations in production, especially when they are (negatively) correlated with quality traits (such as in early and late lactation). On the other side, there is no theoretical justification linking a qualitative picture of milk, like its FTIR spectrum, to the quantity of milk produced.

**Enteric methane emission traits**

According to the main aims of this study, an important topic of discussion is the use of EME proxies predicted from FTIR spectra acquired from milk samples (Shetty et al., 2017; Negussie et al., 2017; Bittante et al., 2020). The 4 EME traits had very different sources of variation and patterns during lactation according to their nature and the estimation method.

**Methane yield.** The average CH4/DMI of HO and CR cows in this study (21.4 g/kg, Table 1) is within the interval (20.1 ± 4.3) obtained by Hristov et al. (2018) in an extensive review covering different experimental methods and dairy systems. The same authors reported that of the various EME traits, this one had the lowest coefficient of variation across studies (21.6%). In a previous study on Brown Swiss cows we obtained an average CH4/DMI of 21.3 g/kg (Bittante et al., 2018), while van Lingen et al. (2014) reported an average of 22.5 g/kg.
21.5 g/kg in their meta-analysis of several trials with HO cows. It is worth noting from Table 1 that the coefficient of variation of CH₄/DMI (7.0%) is the lowest among all the traits studied.
FTIR spectra (dCH$_4$-IR). Methane yield (CH$_4$/DMI) of any significant effect of cow parity on CH$_4$/DMI. Modest differences in DMI could explain the absence of any significant effect of cow parity on CH$_4$/DMI. Modest differences in DMI from cows of different dairy breeds reared in intensive farming systems. The same factors (the same diet and modest differences in milk yield are not expected to differ between HO and CR cows for this trait, as on each farm they received the same diet. Given the same diet as in the present study, the fermentation pattern in the rumen (microbiota composition, proportions of volatile fatty acids, etc.) is not expected to differ between HO and CR cows. Therefore, potential differences in the rumen fermentation patterns of the 2 genetic types could arise from differences in DMI, while similar body weights and modest differences in milk yield are not expected to have much effect on this trait. It is worth noting that no scientific article the authors are aware of reports the opportunity of different prediction equations for milk from cows of different dairy breeds reared in intensive farming systems. The same factors (the same diet and modest differences in DMI) could explain the absence of any significant effect of cow parity on CH$_4$/DMI.

The pattern of this trait during lactation as depicted in the lactation modeling is almost flat (Figure 1, right side graphs). In fact, none of the lactation model parameters of this trait was significant (Table 3) and so the derived traits were not calculated. Some small variations could be a consequence of variations in the amount of DM ingested daily by the cows, which is expected to increase until about mid-lactation and to decrease thereafter (Mäntysaari et al., 2012). In a previous study in Brown Swiss cows we found that DIM classes affected the CH$_4$/DMI trait, but the maximum difference among the LSM values was only 0.7 g of methane produced per kg of dry matter ingested by cows (Bittante and Cipolat-Gotet, 2018; Bittante et al., 2018).

**Methane intensity.** The average CH$_4$/CM found in this study (14.2 g/kg, Table 1) is within the interval (15.6 ± 8.5) obtained in the articles reviewed by Hristov et al. (2018), who also found that this trait had the highest coefficient of variation across the different studies (54.4%). This average value is very similar to those previously found by us for Brown Swiss cows (Bittante et al., 2018) and by van Lingen et al. (2014) for HO cows. It is probable that this trait has an inverse relationship with milk production (denominator of the ratio), so the lower values found in the GP herd and in mature cows (Table 3) are expected. Similarly, a marginally lower value for HO cows is expected compared with CR cows (no significant difference).

Regarding the lactation curves, in a previous study on milk protein fractions (Amalfitano et al., 2021), where we adapted the model used for dMY to several other milk traits, we obtained 2 other curve patterns without peaks: an upward curve with positive b and positive c parameters and a downward curve with positive b and negative c parameters. Here (Table 3), CH$_4$/CM exhibited an upward pattern, increasing rapidly at the beginning of lactation and more slowly toward the end (Figure 1). This curve is very similar to the polynomial trend found in our previous work on Brown Swiss cows (Bittante et al., 2018). The curve intercept in that work (10.9 g/kg) is almost identical to that in the present study in HO and CR cows (10.5 g/kg), and the values calculated after 300 DIM are also very similar (15.5 and 15.2 g/kg, respectively). Obviously, the linear, quadratic, and cubic slopes of the polynomial curve cannot be interpreted from a physiological point of view. Moreover, after 300 DIM the value obtained in this study (14.2 g/kg, Table 1) is within the interval (15.6 ± 8.5) obtained in the articles reviewed by Hristov et al. (2018), who also found that this trait had the highest coefficient of variation across the different studies (54.4%). This average value is very similar to those previously found by us for Brown Swiss cows (Bittante et al., 2018) and by van Lingen et al. (2014) for HO cows. It is probable that this trait has an inverse relationship with milk production (denominator of the ratio), so the lower values found in the GP herd and in mature cows (Table 3) are expected. Similarly, a marginally lower value for HO cows is expected compared with CR cows (no significant difference).

![Figure 2. Comparison between the lactation patterns of milk yield measured during milk recording (dMY-MR, in blue) and milk yield predicted by FTIR milk spectra (dMY-IR, in green). Dashed lines refers to the long-term compartment (y = a+c, where y is the independent variable, a is the potential value at the beginning of lactation, and c is the persistency during lactation). The difference between the solid line and the dashed one represents the effect of the short-term adaptation compartment depending on b (adaptation fraction at the beginning of lactation) and k (speed of adaptation) parameters.](image-url)
part of the milk is produced using energy and nutrients mobilized from the cow’s body reserves (Roche et al., 2009). The use of these reserves does not affect the actual production of methane in the rumen, but reflects part of the methane emitted by the cow before parturition when the body reserves were stored. The short-term compartment of the CH4/CM lactation curve can then be interpreted as the quantity of milk produced at the beginning of lactation from body reserves without producing methane. At the same time, the positive persistency of CH4/CM in the long term compartment could be interpreted as the cow’s need to eat more than required for maintenance and lactation to restore the body reserves and support pregnancy. The values of the variables “adaptation” (−25%) and “persistency” (+8%) shown in Table 3 are therefore a numerical indication of these opposing phenomena.

### Methane production directly or indirectly predicted from FTIR spectra

The average dCH4/IR found in this study (358 g/d) is identical to the average value (358 ± 105) reviewed by Hristov et al. (2018), and almost identical to the average value found in our earlier study on Brown Swiss cows (357 g/d), but lower than the value for HO cows reported by van Lingen et al. (2014). Hristov et al. (2018) also showed this trait to have an intermediate coefficient of variation across studies (29.2%).

It is worth noting, however, that by dividing this value of dCH4/IR by dCMY (Table 1) we obtain an indirect estimate of CH4/CM of only 10.2 g/kg, much lower, as previously seen, than the values obtained in the present study through FTIR prediction for HO and CR cows (14.2 g/kg), in the Hristov et al.’s (2018) review of different breeds and methods (15.6 g/kg), in
van Lingen et al. (2014) review of HO cows in respiratory chambers, and for Brown Swiss cows (14.2 g/kg) using the milk fatty acid profile of milk (Bittante et al., 2018).

As previously seen, dCH4 is expected to be closely related to DMI, which in turn is expected to reflect the cow’s energy requirement (except at the beginning of lactation). Milk energy is by far the largest and most variable component of the total energy requirement of a dairy cow. So we expect a close relationship between dMY (or rather dCMY) and dCH4 (Martínez-Marín et al., 2023). Summarizing the trials carried out in respiration chambers, Hristov et al. (2018) calculated a correlation of 68% between dCH4 and dCMY. The dCMY was 32.1 kg/d in this study, but was lower in the studies of Hristov et al. (2018), van Lingen et al. (2014), and Bittante et al. (2018): 28.4, 29.1, and 25.6 kg/d, respectively. Therefore, the average value of dCH4-IR obtained here seems too low if the productive level of the cows must be taken into account.

It is worth noting that, if the CH4/CM calculated dividing dCH4-IR by dMY-IR is unreliable (10.2 g/kg), the CH4/CM became fully congruent (14.5 g/kg) when we divide the dCH4-IR by dMY-IR (Tables 2 and 3). This means that the inability of FTIR spectrometry in capturing information on the quantity of milk produced daily, is paralleled by its inability in capturing the quantity of methane produced daily (both quantities are unreliable low, but their ratio seems correct).

In the present study, dCH4-IR was found to be higher for cows on the PR farm, cows obtained from rotational crossbreeding (not significantly) and those in their first and second lactations (Table 3), which is similar to dMY-IR, but contrary to expectations based on dMY-MR (Hristov et al., 2018).

Regarding the lactation modeling, we obtained an “upward” pattern (Figure 1) and this is also contrary to the expectation of a “zenith” curve simulating the classical lactation curve and DMI curve. In the previous study on Brown Swiss cows (Bittante et al., 2018) we obtained a cubic polynomial curve characterized by a growing phase during the first 3 mo of lactation followed by a decrease till end of lactation, not very different to the expected “zenith” curve.

It is evident that direct prediction of a quantitative trait, like dCH4-IR, using FTIR spectra alone is as unreliable as prediction of dMY-IR. A similar conclusion was also reached recently by Denninger et al. (2020). This is also confirmed by the much lower R2 of the equation predicting this trait compared with the equations predicting qualitative EME traits (Bittante and Cipolat-Gotet, 2018; Coppa et al., 2022).

Vanlierde et al. (2015) also found that dCH4 predictions using FTIR spectra alone produced a lactation pattern (“nadir” type curve), contrary to expectations. The inclusion of DIM information (linear and quadratic Legendre polynomials) in the prediction equations did not increase the R2 of cross-validation, but yielded a lactation pattern according to expectations (“zenith” type curve) and increased the robustness of the predictions when adopted at the population level. The need to combine spectra information with a variable expressing quantitative information was further demonstrated by van Lingen et al. (2014), Vannierde et al. (2015), who concluded that the explanatory models based on FTIR spectra and DIM were improved by also including dMY, parity and breed information, and also reported that when these 3 variables were included in the predictive equations the predictions of dCH4 were in closer agreement with the literature.

On the other side, it is evident that after adding DIM, dMY, parity, and breed to the prediction equation, the contribution of the FTIR spectra to the accuracy of the results becomes less certain.

**Methane production indirectly predicted from FTIR spectra and measured dCMY.** In our previous study on the development of FTIR-based EME prediction equations (Bittante and Cipolat-Gotet, 2018), we concluded that direct FTIR predictions of CH4/DMI and CH4/CM yielded informative results, but not dCH4. We suggested that a much more informative result could be achieved by estimating methane production by simply multiplying the measured dCMY from milk recording by the FTIR-predicted CH4/CM. This is why we calculated this EME trait (dCH4-MR) here.

The first difference between dCH4-MR and dCH4-IR (Table 1) is that the average value of the former is much larger than that of the latter (450 vs 358 g/d), and is closer to that found for HO cows with a high dMY, like ours. The second difference is that the fixed effects included in the dCH4-MR statistical model (Table 3) were all in agreement with expectations (whereas those of dCH4-IR were all the inverse): GP > PR herd, HO > CR cows, and third > second > first lactation. The third difference is that, as expected, dCH4-MR showed a “zenith” curve, and not an “upward” curve as dCH4-IR (Figure 1).

**Effects of 3-breed rotational crossbreeding on enteric methane emission traits**

Some information is available on the effects on some EME traits of cow breed (van Lingen et al., 2011; Coppa et al., 2012) and of HO-Jersey crossbreds (Xue et al., 2011; Hynes et al., 2016), while the authors are not aware of any information on the effects of rotational crossbreeding on the ecological footprint of the intensive dairy production chain. The EME is only one aspect
of the ecological footprint, and EME during lactation is only a fraction of this aspect. Nonetheless, EME is known to be the most important part of the ecological footprint of ruminants, and lactation the most important segment of the cow’s lifetime (Berton et al., 2020).

It is generally accepted that the environmental impact is reduced, first of all, by increasing production efficiency and that efficiency is related to animal productivity (Kandel et al., 2017). Crossbreeding is expected to reduce the daily milk yield of cows, so it can be expected to reduce feed intake, but also production efficiency. Possible crossbreeding expectations for EME traits could be: a) no effect on CH4/DMI, if the diet is the same as that given to HO cows; b) improvement (decrease) in dCH4, due to an expected lower intake of feed for milk production (but a constant intake for maintenance and pregnancy); c) worsening (increase in) CH4/CM, because the numerator (dCH4) is expected to decrease less than the denominator (dCMY).

In the present study, the first expectation is confirmed since the LSM of the purebred HO and the 3-breed CR for CH4/DM were not significantly different. The second expectation is also confirmed because HO cows have a higher dCH4-MCM than CR cows. The third expectation was not confirmed because the HO and CR cows presented very similar LSMS for CH4/CM. This better-than-expected CH4/CM in CR cows could be due to the better feed efficiency of these cows, which can compensate for their slightly lower dCMY (Pereira et al., 2022). Shonka-Martin et al. (2019) found that Viking Red-Montbéliarde-Holstein rotational crossbred cows are both nutritionally and economically more efficient than purebred Holstein cows.

Therefore, we can conclude from this study that rotational crossbreeding does not appear to increase the production of methane per kg of milk produced during lactation, although it should be remembered that the ecological footprint of dairy production also includes the EME of dry cows and replacement heifers. Rotational crossbreeding has proven to be particularly effective in improving the fertility (Malchiodi et al., 2014a) and longevity of cows, reducing the risk of culling (Piazza et al., 2023a). This means that the (environmental) costs of heifer production are mitigated by a longer productive life-time and overall milk production in the case of CR cows (Piazza et al., 2023b). Taking an LCA approach (Beauchemin et al., 2022) at the individual cow, we found that overall methane production (including not only lactating cows, but also heifers and dry cows) per kg of milk produced in the career is lower in CR than in purebred HO cows (Piazza et al., 2022).

CONCLUSIONS

Milk FTIR spectra have proven to be useful for the direct prediction of proxies of qualitative EME traits, such as CH4/DMI and CH4/CM, but not for quantitative traits, such as dCH4. Estimation of the daily production of methane seems better done by multiplying a measured dMCY by a FTIR predicted CH4/CM. Lactation modeling of EME traits confirms that dCH4-MR has a zenith curve, similar to that of milk yield but with a delayed peak, as expected for the cows’ DMI. CH4/CM is characterized by an upward curve that increases rapidly after calving in parallel with the reduction in the negative energy balance, then slowly until the end of lactation, in parallel with the recovery of body reserves and the progression of pregnancy. In contrast, lactation modeling was not useful for explaining CH4/DMI, which is almost constant during lactation. Lastly, 3-breed rotational crossbreeding involving HO, MO and VR did not unfavorably affect CH4/DMI and CH4/CM during lactation, and reduced dCH4 compared with purebred HO. Taking into account the greater longevity of CR cows and their lower replacement rate, rotational crossbreeding could be seen as a way to mitigate the environmental impact of milk production.

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