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Comparison of methane production, intensity, and yield throughout lactation in Holstein cows

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ABSTRACT

Genetic selection to reduce methane (CH₄) emissions from dairy cows is an attractive means of reducing the impact of agricultural production on climate change. In this study, we investigated the feasibility of such an approach by characterizing the interactions between CH₄ and several traits of interest in dairy cows. We measured CH₄, dry matter intake (DMI), fat- and protein-corrected milk (FPCM), body weight (BW), and body condition score (BCS) from 107 first- and second-parity Holstein cows from December 2019 to November 2021. Methane emissions were measured using a GreenFeed device and expressed in terms of production (MeP, in g/d), yield (MeY, in g/kg DMI), and intensity (MeI, in g/kg FPCM). Because of the limited number of cows, only animal parameters were estimated. Both MeP and MeI were moderately repeatable (>0.45), whereas MeY presented low repeatability, especially in early lactation. Mid lactation was the most stable and representative period of CH₄ emissions throughout lactation, with animal correlations above 0.9. The average animal correlations of MeP with DMI, FPCM, and BW were 0.62, 0.48, and 0.36, respectively. The MeI was negatively correlated with FPCM (<-0.5) and DMI (>-0.25), and positively correlated with BW and BCS. The MeY presented stable and weakly positive correlations with the 4 other traits throughout lactation, with the exception of slightly negative animal correlations with FPCM and DMI after the 35th week. The MeP, MeI, and MeY were positively correlated at all lactation stages and, assuming animal and genetic correlations do not strongly differ, selection on one trait should lead to improvements in all. Overall, selection for MeI is probably not optimal as its change would result more from CH₄ dilution in increased milk yield than from

real decrease in methane emission. Instead, MeY is related to rumen function and is only weakly associated with DMI, FPCM, BW, and BCS; it thus appears to be the most promising CH₄ trait for selection, provided that this would not deteriorate feed efficiency and that a system of large-scale phenotyping is developed. The MeP is easier to measure and thus may represent an acceptable alternative, although care would need to be taken to avoid undesirable changes in FPCM and BW. **Key words:** methane, dairy cow, GreenFeed, animal correlations

INTRODUCTION

Methane (CH₄) is one of the greenhouse gases with the largest impact on climate change (United Nations Environment Programme and Climate and Clean Air Coalition, 2021). The main source of anthropogenic CH₄ is agriculture (Jia et al., 2019), of which around 70% originates from enteric fermentation (Nabuurs et al., 2022), a natural process of ruminant digestion. Among ruminants, cattle are the main source of CH₄ emissions (Gerber et al., 2013), and reducing their enteric CH₄ production is a key climate mitigation strategy.

Various approaches have been discussed, of which the most permanent option, with cumulative benefits, is genetic selection. This could be carried out in a variety of ways; for example, selection to increase lifespan productivity (especially when baseline productivity is low) can result in a reduction in the total number of animals or the number of heifers needed for renewal. This can be achieved by increasing productive lifespan and decreasing age at first calving, through selection for health, fertility, and precocity. However, studies have shown that it is also possible to directly select against CH₄ emissions, as it presents a heritability in the range of 0.12 to 0.45 (Pszczola et al., 2017; Breider et al., 2019; López-Paredes et al., 2020; Manzanilla-Pech et al., 2021) and a genetic coefficient of variation (CV) of around 20% (Manzanilla-Pech et al., 2021).

Methane emissions can be expressed in the following units (de Haas et al., 2017): CH₄ production measures

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CH₄ per animal per day (g/d); CH₄ intensity measures CH₄ per kilogram of output product (milk or meat, g/kg); and CH₄ yield measures CH₄ per kilogram of DMI, expressed in grams/kilogram. They are different traits and reflect different objectives. The CH₄ production per animal primarily reflects individual feed intake and, consequently, size and milk production. Methane intensity is strongly dependent on milk production and on the energy requirements of this process. Finally, for a given feed, CH₄ yield reflects the methanogenic potential of the digestive process. The relative importance of the 3 traits in a breeding objective will vary among production systems and economic conditions.

To design a selection program targeting CH₄ emissions, it is first essential to understand the variability in these 3 traits and their correlations with other traits of interest, especially with feed intake, which is also strongly related to milk production (Li et al., 2018). Considering the biological mechanisms underlying ruminal CH₄ production, one might expect that CH₄ would be mostly driven by the nature and quantity of feed intake (Fonty and Morvan, 1996). This type of analysis is especially critical for CH₄ intensity and yield, as these traits have been examined in only a few studies (Kandel et al., 2017; Manzanilla-Pech et al., 2021; Richardson et al., 2021).

The aim of this study was 2-fold: first, to provide estimates of the variance for the 3 CH₄ traits and characterize their correlations with feed intake, milk production, and body traits throughout lactation, and second, to determine which CH₄ trait has the most potential for inclusion in a selection program.

MATERIALS AND METHODS

Animals and Feeding Management

Data were obtained from first- and second-parity Holstein dairy cows reared at the French Experimental Unit Le-Pin-au-Haras (INRAE, <https://doi.org/10.15454/1.5483257052131956E12>), from December 2019 to November 2021. All animals were handled with care following INRAE's ethics policy and in compliance with the guidelines on animal research issued by the French Ministry of Agriculture (<https://www.legifrance.gouv.fr/eli/decret/2013/2/1/2013-118/jo/texte>). Cows were housed in a freestall barn and fed individually using an electronic gate feeding system and ear-tag identification. Feed was distributed automatically 4 to 5 times a day, between 0900 and 1700 h. Cows received a TMR of 60.6% maize silage, 27.5% grass silage, 10.9% rapeseed meal, 0.7% minerals, 0.2% clay, and 0.1% salt.

Table 1. Chemical composition of the TMR and the concentrate fed to the automatic milking system

Item	TMR	Concentrate
DM (%)	42.8	90.0
NDF (g/kg DM)	335.3	NA ¹
CP (g/kg DM)	146.4	268.5
Starch (g/kg DM)	188.0	95.1
Energy (MJ/kg DM)	6.7	7.9

¹NDF composition of the concentrate was not available.

The nutritional values of the TMR were all within the range of those typically recommended in a high-yielding dairy herd (INRA, 2018). The TMR was allocated in amounts that allowed for approximately 10%orts to ensure ad libitum intake. In addition, cows were supplemented daily with a maximum of 4 kg of concentrate in the milking parlor of the automated milking system (AMS; Lely Astronaut A4, Lely Holding). Chemical composition of the TMR and the concentrate is presented in Table 1.

Phenotyping and Trait Definition

Milk Production Traits. Cows had free access to the AMS with a maximum of 5 milkings per day. Twice a week, milk samples were collected over a 24-h period and fat content (FC) and protein content (PC) were determined using mid-infrared spectrometry at Lilano laboratory (St-Lo, France). Daily milk yield (MY) was calculated as the sum of yields at each milking and missing daily records were interpolated from adjacent days. To obtain daily values, FC and PC were modeled following a model by Wilmink (1987):

$$y_{il} = \mu_l + b_l \times t + c_l \times e^{(-0.05 \times t)} + \alpha_{il} + e_{il}$$

where y_{il} is the FC or PC phenotype, μ_l is the overall mean of parity class l , b_l and c_l are the regression coefficients in Wilmink's equation specific for parity class l , t is the days in milk (DIM), α_{il} is the random animal effect, and e_{il} is the random residual term, assumed to be normally distributed.

Fat- and protein-corrected milk (FPCM) was computed according to the FAO formula (FAO, 2010):

$$\text{FPCM} = \text{MY} \times (0.337 + 0.116 \times \text{FC} + 0.06 \times \text{PC}).$$

Feed Intake and Body Traits. Daily individual feed intake was measured as the sum of all the feed eaten by a given cow during each visit from the first feed distribution (0900 h) to 0859 h the next day, plus the

concentrate intake at the milking parlor. Dry matter intake was calculated daily, based on the DM content of the TMR (measured weekly) and the concentrate.

The cows were weighed at the end of each visit to the AMS using an automatic weighing system to record BW. Body condition score was scored monthly on a scale from 0 to 5, with steps of 0.25, by 2 trained technicians.

Methane. The CH₄ was measured using 2 Green-Feed devices (**GF**; C-Lock Inc.) in free access. The GF devices were calibrated daily following the manufacturer's instructions. Low-energy pellets were used to bait the cows and ensure the proper head position in the bin. The GF were programmed to distribute up to 5 drops of 30 g every 45 s, with a minimum interval of 6 h between 2 visits from the same cow.

Daily CH₄ averages were obtained following 3 steps. First, CH₄ measurements at each visit were adjusted for parity, lactation stage, and diurnal variations related to feeding hours using the following model:

$$y_{iht} = \mu + a \times t + b_h \times H + c_p \times P + \alpha_i + e_i,$$

where y_{iht} is the CH₄ measurement, μ is the overall mean of CH₄ emissions, a is the regression coefficient on DIM, t is the DIM, H is the fixed effect of the hour of the day, P is the fixed effect of parity, α_i is the random animal effect, and e_i is the random residual term, assumed to be normally distributed.

Second, following Vanlierde et al. (2015), adjusted CH₄ values lower than 150 g/d or higher than 950 g/d were considered outliers and discarded (0.46% of data). Third, adjusted CH₄ values were averaged daily for each cow. In this study, CH₄ was expressed in terms of production (**MeP**, in g/d), yield (**MeY**, in g/kg DMI), and intensity (**MeI**, in g/kg FPCM). The MeY and MeI were obtained by dividing MeP by the daily DMI and daily FPCM, respectively.

Final Edits. The following filters were applied to eliminate irrelevant data. Data were recorded between 5 and 305 DIM and then averaged to obtain weekly records (except for BCS). Weekly records were retained only when based on at least 5 d of recording. Data from the last 2 wk of a cow's lactation (before drying off) were removed due to the potential bias induced by the drying protocol (involving restricted access to feeding and milking). Of the cows in the experiment, only those with at least 10 weekly records for all traits except BCS were kept. The final data set included 107 cows, 64 in first lactation and 43 in second. These filters resulted in the loss of 19% of data for CH₄, DMI, FPCM, and BW, and 13% of BCS records.

Statistical Analyses

Because of the limited number of cows, the genetic and permanent environmental effects could not be separated. Therefore, only their sum—the so-called animal effect—was investigated.

We used random regression models to estimate the trajectories of variances and correlations through the course of lactation for the 3 CH₄ traits and the 4 traits (DMI, FPCM, BW, and BCS). Because of the relationships among the 7 traits, a single overall analysis was not possible. Therefore, 2 sets of analyses were carried out. First, correlations between CH₄ traits were estimated with a 3-trait model, and then correlations between each CH₄ trait and the 4 other traits were estimated by 3 different 5-trait models. The general form of the model was the same in both rounds and was as follows:

$$y_{ijkl} = c_j + \sum_{n=1}^6 \beta_{kln} \gamma_n(t) + \sum_{n=1}^2 \alpha_{kin} \varphi_n(t) + e_{ijkl},$$

where y_{ijkl} is the phenotype for the k th trait; c_j is the fixed effect of the contemporary group, i.e., the j th year-month combination corresponding to the record day; β_{kln} is the n th fixed regression coefficient specific to parity class l ; γ_n is the n th known covariate coefficient of a 6-knot spline (knots at wk 1, 3, 6, 13, 30, and 43) evaluated at lactation week t ; α_{kin} is the n th random regression coefficient of the animal i ; φ_n is the n th coefficient of a second-order Legendre polynomial evaluated at lactation week t ; and e_{ijkl} is the random residual term, normally distributed. Within a trait, residual effects were assumed to have different variances among lactation periods (wk 1–13, 14–30, 31–43). With these models, the (3 × nt, 3 × nt) variances-covariances were estimated for both the animal (**G**) and residual (**R**) components at each week t . From the **G** matrix and the vectors of Legendre polynomials at time t [$\varphi(t)$], estimates were obtained for the animal variance at time $t1$ for trait i by $\varphi(t1) \mathbf{G}_{i,i} \varphi(t2)$, the animal covariance between times $t1$ and $t2$ for trait i by $\varphi'(t1) \mathbf{G}_{i,i} \varphi(t2)$, and the animal covariance between times $t1$ and $t2$ for traits i and j by $\varphi'(t1) \mathbf{G}_{i,j} \varphi(t2)$, with $\mathbf{G}_{i,j}$ being the 3 × 3 submatrix **G** corresponding to traits i and j . These were used to compute repeatability, within-trait correlations, and between-trait correlations throughout lactation.

The effect of animal k for trait i at time t was predicted by $\varphi'(t)\alpha_{ik}$. Estimated animal effects were generated for each lactation week for each trait. **G** and **R** were estimated using Wombat software (Meyer, 2007) and

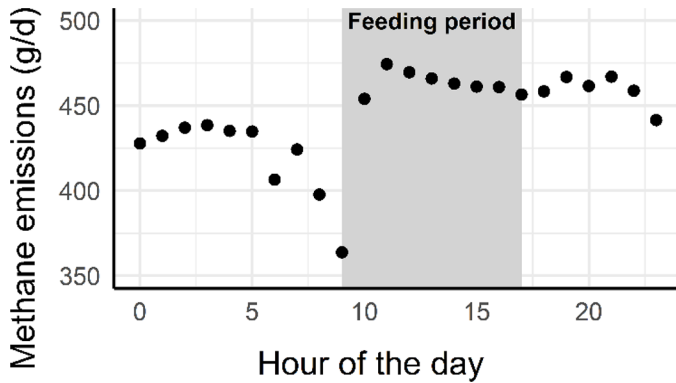


Figure 1. Diurnal variation in average methane emissions. Feed was automatically distributed 4 to 5 times between 0900 and 1700 h.

variance parameters were calculated using R software (R Core Team, 2021) using Wombat outputs.

RESULTS

Descriptive Analysis

Daily CH₄ Data. The total number of individual CH₄ measurements was 37,754, corresponding to 18,945 daily CH₄ means. On average, there were 59.9 (SD = 30.3) visits to the GF per day, corresponding to 1.3 (SD = 0.57) visits per cow per day. Individual CH₄ measurements were adjusted for the diurnal variations shown in Figure 1. The CH₄ emissions increased sharply from 0900 h (overall minimum) to 1100 h. (overall maximum), remained stable until 2100 h, then decreased for the following 12 h.

Weekly CH₄ Data and Other Traits. Statistical descriptions of the weekly means of the 7 traits (MeP,

Table 2. Number of observations, mean and SD, minimum (Min), and maximum (Max) for the weekly means of the 7 traits measured in this study

Trait ¹	n ²	Mean (SD)	Min	Max
MeP	2,939	441.2 (86.5)	156.9	847.6
MeI	2,939	11.7 (2.6)	3.5	29.3
MeY	2,899	18.3 (4.1)	6.9	54.5
DMI	3,779	24.1 (3.6)	3.9	34.0
FPCM	3,836	38.1 (7.0)	12.1	60.0
BW	3,836	678.2 (74.2)	501.3	942.6
BCS	803	3.1 (0.7)	1.5	5

¹Methane emissions in grams per day (MeP), methane emissions in grams per kilogram FPCM (MeI), methane emissions in grams per kilogram DMI (MeY), DMI (kg/d), fat- and protein-corrected milk (FPCM, kg/d), BW (kg), and BCS.

²Number of weekly means.

MeI, MeY, DMI, FPCM, BW, and BCS) are reported in Table 2. The CV for MeP, MeI, and MeY ranged from 18.4 to 19.8%, and were 13.3, 17.5, 10.3, and 21.2% for DMI, FPCM, BW, and BCS, respectively.

Changes in CH₄ Emissions Through the Course of Lactation

The evolution of CH₄ emissions (MeP, MeI, and MeY) through the course of lactation for primiparous and multiparous cows is presented in Figure 2. The MeP was higher for multiparous cows and exhibited a more abrupt increase and an earlier plateau. Instead, MeI was lower in multiparous than in primiparous cows, although the overall pattern was very similar between the 2 groups, increasing MeI in early and late lactation, and stabilization in mid lactation. The MeY increased slightly throughout lactation for both primiparous and multiparous cows.

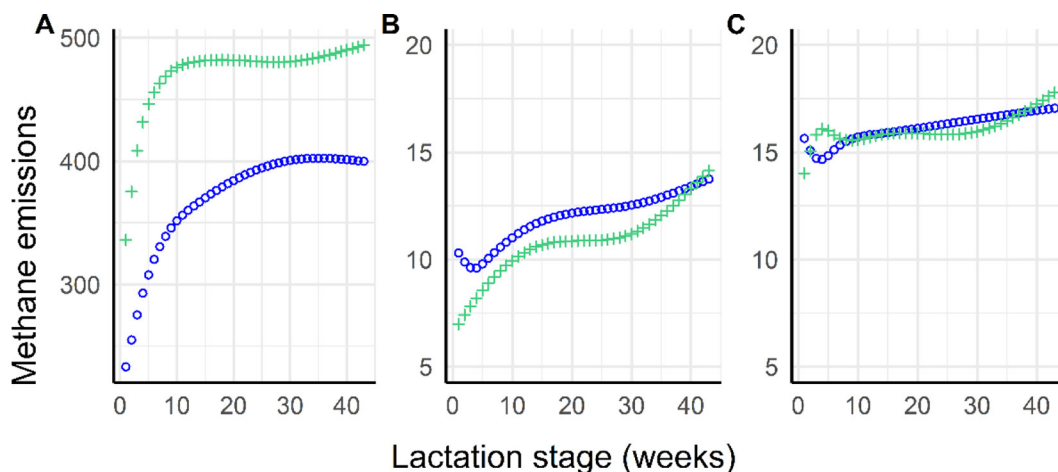


Figure 2. Methane emissions in grams per day (A), in grams per kilogram of fat- and protein-corrected milk (B), and in grams per kilogram of DMI (C) throughout lactation, in first- (○) and second-lactation (+) cows.

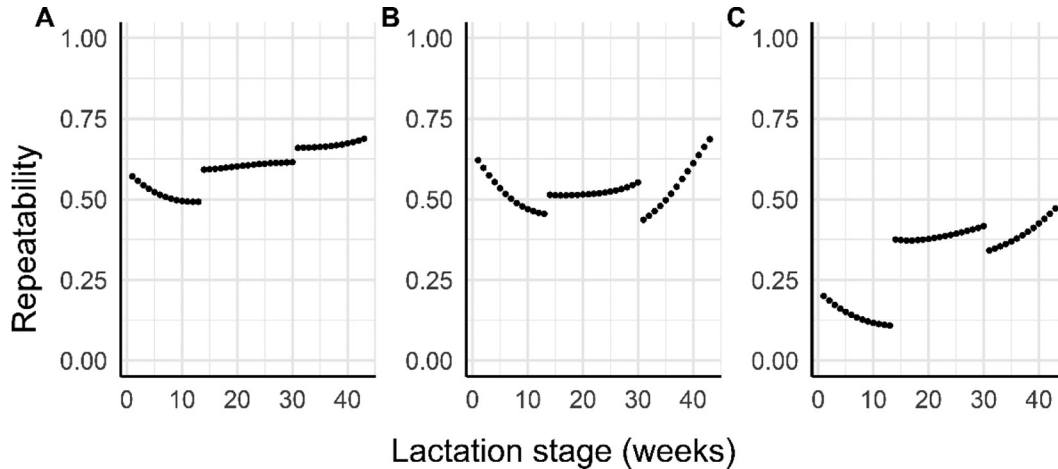


Figure 3. Repeatability of methane emissions in grams per day (A), in grams per kilogram of fat- and protein-corrected milk (B), and in grams per kilogram of DMI (C) during lactation.

Repeatability and Within-Animal Correlations Across Lactation

Repeatability was high for MeP (0.6 overall; range: 0.49–0.69) and MeI (0.52 overall; range: 0.44–0.69) but lower for MeY (0.33 overall; range: 0.11–0.47). For MeP and MeY, repeatability was lower in early lactation and then increased (Figure 3A and 3C), whereas the repeatability of MeI was lowest in mid lactation (Figure 3B). For all 3 CH₄ traits, mid lactation was characterized by stable repeatability.

Within-animal correlations were high (>0.80) between consecutive weeks (Figure 4), especially from wk 10 to 31 for MeP and, to a lesser extent, for MeI. Close to the beginning or the end of lactation, the correlations with values from the rest of the lactation were

much lower, especially for MeI and MeY, with the lowest values around 0.4. The average value of within-animal correlations was 0.88 (SD = 0.11) for MeP, 0.77 (SD = 0.18) for MeI, and 0.82 (SD = 0.16) for MeY.

Animal Correlations Among Methane Traits

The trajectories of the animal correlations among the 3 methane traits are shown in Figure 5. Correlations were high and positive for the 3 combinations of traits all throughout lactation, with the highest values found between MeP and MeY (average = 0.8) and lowest between MeP and MeI (average = 0.4). Through the course of lactation, correlations were relatively stable for MeI and MeY, and for MeP and MeY, with a steep decrease for the last 10 weeks, but

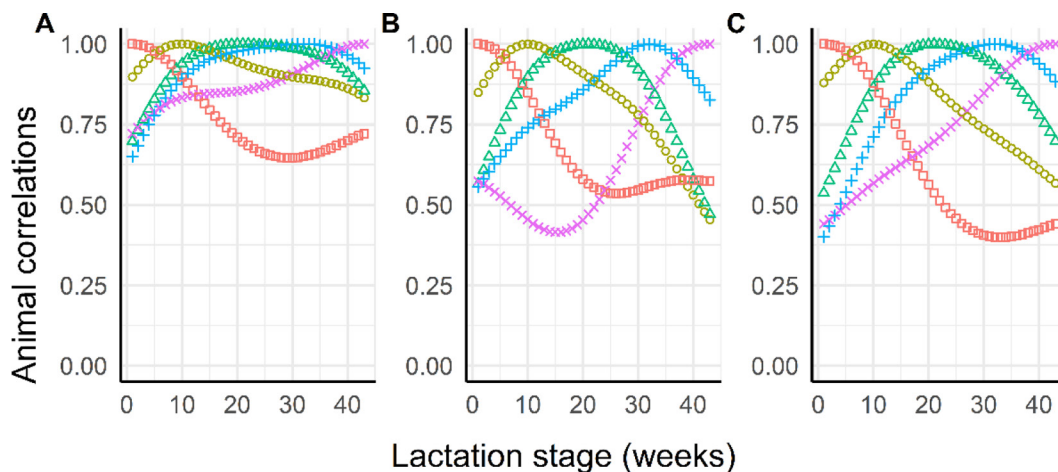


Figure 4. Animal correlations between methane emissions in the week shown on the x-axis and those in wk 1 (□), 10 (○), 21 (Δ), 31 (+), and 43 (×) for methane emissions in grams per day (A), in grams per kilogram of fat- and protein-corrected milk (B), and in grams per kilogram of DMI (C).

decreased from the beginning to the end of lactation for MeP and MeI.

Animal Correlations Between CH₄ Traits and Other Traits

The animal correlations between CH₄ traits and DMI, FPCM, BW, or BCS are shown in Figure 6. Both DMI and FPCM exhibited stable, moderate, positive correlations with MeP throughout lactation, with averages of 0.62 (SD = 0.03) and 0.48 (SD = 0.06), respectively. Correlations between BW and MeP decreased starting from mid lactation, with an average of 0.36 (SD 0.10). The correlations between MeP and BCS were low. The MeI was negatively correlated with FPCM [average of -0.55 (SD 0.06)] and, to a lower extent, with DMI, but was moderately positively correlated with BCS [average of 0.35 (SD 0.10)]. Finally, MeY presented stable and weakly positive correlations with the 4 other traits throughout lactation, with the exception of the correlations with FPCM and DMI, which became negative after the 35th week.

DISCUSSION

The first objective of this study was to provide a description of the pattern, repeatability, and stability of the 3 CH₄ traits (MeP, MeI, and MeY) through the course of lactation. We examined the animal correlations of these traits with DMI, FPCM, BW, and BCS with the aim of investigating the possibility of incorporating a CH₄ trait into a breeding goal. The second objective of this study was to determine the CH₄ trait that would be most appropriate for inclusion in a selection program, based on the objective and the characteristics of each trait.

Changes in Methane Emissions Throughout the Day

A cow's feeding pattern is known to have a strong influence on daily methane emissions, which increase after feed intake and then steadily decrease (Crompton et al., 2010; Garnsworthy et al., 2012; Judy et al., 2018). These diurnal variations were confirmed in our study: CH₄ was highest between 1000 and 2200 h, during the feeding period and the following hours. On average, each cow visited the GF devices twice per day, which was lower than expected, considering the capacity of the GF (estimated at 4 visits per cow per day on our farm). This small number of visits heightened the risk for a potentially uneven distribution of measurements during the day (e.g., cows always visiting the GF during high- or low-emission periods). To avoid over- or underestimating CH₄ emissions for cows with

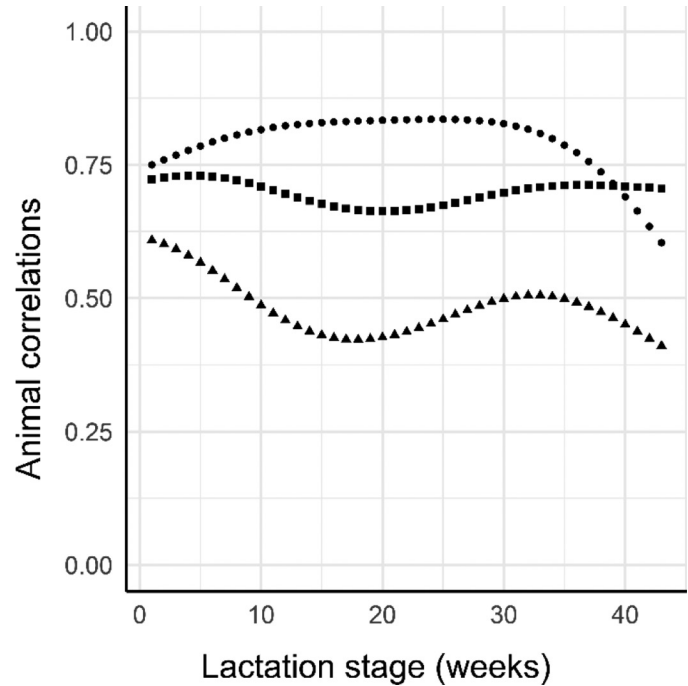


Figure 5. Animal correlations between methane emissions (▲) in grams per day (MeP) and in grams per kilogram of fat- and protein-corrected milk (MeI), (●) MeP and methane emissions in grams per kilogram of DMI (MeY), and (■) MeI and MeY through the course of lactation.

few visits to the GF, we corrected for diurnal variations before computing the daily CH₄ average. The average value measured during this study for MeP was higher than those reported by Coppa et al. (2021) and Li et al. (2020), in studies of lactating dairy cows fed high-energy TMR without grazing, whereas that for MeY was very similar. Concerning average MeI value of 11.7, it was consistent with the values ranging from 9.6 to 11.7 reported by Coppa et al. (2021), but lower than the value of 36.7 reported by Liu et al. (2022) due to lower FPCM values.

Changes in Methane Emissions Throughout Lactation

As was observed with DMI, MeP increased in early lactation before reaching a plateau, with primiparous cows producing less CH₄ than multiparous ones (Figure 2). The MeP pattern observed in this study was similar to those reported by Pszczola et al. (2017) using Sniffer measurements, and by de Haas et al. (2011) and Kandel et al. (2017) using MeP predicted from feed intake and mid-infrared spectra, respectively.

The MeI increased during the course of lactation, with primiparous cows having a higher MeI than multiparous cows (Figure 2). The same observations were

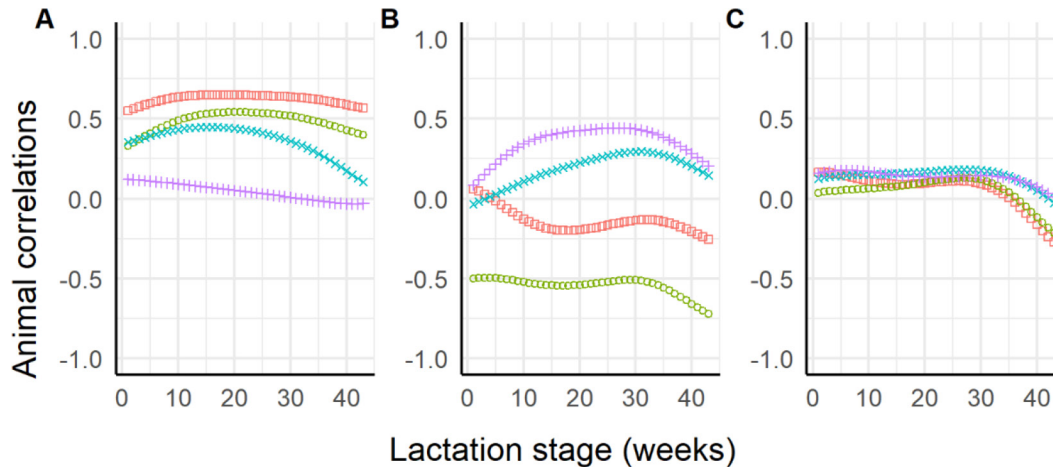


Figure 6. Animal correlations between DMI (\square), fat- and protein-corrected milk yield (FPCM; \circ), BW (\times), BCS ($+$), and methane emissions throughout lactation. Methane emissions expressed in grams per day (A), in grams per kilogram of FPCM (B), and in grams per kilogram of DMI (C).

made by de Haas et al. (2011) and Kandel et al. (2017) based on MeP predicted from feed intake and milk mid-infrared spectra, respectively. The relative evolution of MeP and FPCM throughout lactation can explain both the higher MeI for primiparous cows and the evolution of MeI during lactation. Before the peak in FPCM, MY was increasing more sharply than MeP, which we would expect to result in a decrease in MeI. This was observed for primiparous cows but not for multiparous ones, for which after the peak in FPCM, MeP increased while FPCM decreased, leading to an increase in MeI. In mid lactation, both FPCM and MeP tended to stabilize, yielding relatively constant values of MeI. In late lactation, MeI increased again due to a decrease in FPCM, whereas MeP remained stable and high.

The MeY increased very slightly during lactation (Figure 2), consistently with the results of Lyons et al. (2018). We hypothesized this increase in MeY being the result of adaptation by both the rumen and the microbiota with lactation stages. This is supported by the results from Lyons et al. (2018), that showed differences in bacterial and archaeal community structure comparing rumen samples from early, mid, and late lactation along with an increased MeY. In addition, high DMI is associated with high passage rate of particles from the rumen to the rest of the digestive tract, which in turn is associated with low ruminal degradation, leading to low MeY (Janssen, 2010). One may think that rumen adapts to the changes in DMI, slowing down the passage rate from early to late lactation, allowing a more-complete digestion and resulting in the increase in MeY along the lactation. These ideas are speculations that require further investigation.

Mid Lactation as the Most Representative Period of Methane Emissions During Lactation

The 3 CH_4 measures—MeP, MeI, and MeY—were found to have a stable animal component all throughout the lactation (Figure 4). Indeed, the animal correlations between the values of a given CH_4 unit at different weeks of lactation were high, with an average value greater than 0.77. The strongest correlations were obtained between mid lactation and other points during lactation, a pattern that is not specific to CH_4 but is true for many traits such as MY and composition or feed intake (Martin et al., 2021). Values obtained during the first 5 wk of lactation were less correlated with those from other periods, probably due to the disruption caused by the mobilization of body reserves for initial milk production, as well as the significant increase in DMI at this time, which can modify the composition and function of the ruminal microbiota. Similar results were obtained by de Haas et al. (2011) and Kandel et al. (2017), working on MeP and MeI predicted from feed intake and MeP measured by Sniffer, respectively. Due to the difficulties associated with directly measuring methane emissions (e.g., high costs, heavy workload, low accuracy, or alterations in animal behavior; (Garnsworthy et al., 2019), methane is generally measured only over short time periods, typically a few weeks. For these measurements to be as representative as possible of the whole lactation, we recommend that they be taken during mid lactation, as this period is the most stable and presents the highest animal correlations between CH_4 values emissions at different weeks of lactation.

Variability and Repeatability of CH₄ Emissions

To select for changes in a trait, 3 elements are essential: it must exhibit variability in the population, it should have a genetic basis (i.e., be heritable), and it must be routinely measurable on a sufficient number of animals.

In our study, the CV of the CH₄ traits varied between 18.4 and 19.8%, in line with the values found by Coppa et al. (2021) using a GF system (between 17.1 and 21.1% for weekly averages). This degree of variation is similar to that of other traits currently under selection, such as FPCM, meaning that there may be enough variation in the population to consider selection on one of the 3 CH₄ traits.

Regarding the second criterion, heritability, the small number of animals in this study did not permit the estimation of genetic parameters, as we were unable to distinguish between genetic and permanent environmental variances. However, the sum of these 2 variances (i.e., the animal variance) was estimated, as well as the ratio between the animal variance and the phenotypic variance (i.e., the repeatability), which represents an upper limit to heritability. The average repeatabilities obtained in this study for MeI (0.52) and MeY (0.33) were in line with the repeatabilities of 0.46 and 0.39 reported by Coppa et al. (2021) for the same traits. The repeatability of MeP was higher (0.60), but consistent with previously reported values ranging from 0.50 to 0.69 for weekly averages (Breider et al., 2019; Coppa et al., 2021); it was, however, notably higher than the value of 0.25 reported by Pszczola et al. (2017) for daily measures, more affected by random variations. Because of this similarity with previous work, it is reasonable to expect the heritability in our population to be close to that obtained in these other studies (e.g., heritability varying from 0.12 to 0.45 through the course of lactation), estimated by Breider et al. (2019) using a random regression model on CH₄ measured with a Sniffer, but it should be further investigated.

Animal Correlations Between Methane Emissions and Other Traits

Before a new trait can be incorporated into a breeding goal, it is critical to first consider the correlations with traits currently under selection to evaluate the possibility that the new trait might undermine progress on others. Although animal correlations are only a proxy for genetic correlations, we still consider it useful to compare our values to genetic correlations from the literature, keeping in mind that the permanent environmental effect might be a source of discrepancy between the results. The large diversity of direct and

indirect methods used in different studies to measure CH₄ emissions, as well as the different statistical analyses, are additional factors explaining potential discrepancies. In our study, MeP appeared to be moderately and positively correlated with DMI, FPCM, and BW, with a weak correlation with BCS throughout lactation (Figure 6). The animal correlation between MeP and DMI was the highest (~0.6), but lower than we originally anticipated. This result clearly shows that, contrary to our initial expectation, CH₄ is far from being almost directly proportional to feed intake, and that significant variation exists in ruminal activity among animals. Recent studies (Manzanilla-Pech et al., 2021; Richardson et al., 2021) have reported similar results, with a genetic correlation of 0.42. As FPCM was highly positively correlated with DMI (result not shown), we expected the positive and moderate correlation we obtained between MeP and FPCM. However, results in the literature are inconsistent on this point: our results were similar to those of certain studies [0.43 for Lassen and Løvendahl (2016); 0.45 for Manzanilla-Pech et al. (2021)], whereas others found a weak negative genetic correlation [−0.08, Richardson et al. (2021)] or variable genetic correlations ranging from −0.66 to 0.70 at different points in lactation (de Haas et al., 2011). Similarly, results are inconsistent in the literature concerning the correlation between MeP and BW. We found an average value of 0.36, whereas some studies reported values of 0.01, −0.16, and 0.65 (Breider et al., 2019; Lassen and Løvendahl, 2016; Manzanilla-Pech et al., 2021). We found a very weak correlation between MeP and BCS (positive in early and mid lactation and negative in late lactation) that was similar to the value of 0.11 reported by Manzanilla-Pech et al. (2021), whereas Zetouni et al. (2018) found a value of −0.28. This set of correlations is shaped by the fact that DMI is the best predictor of MeP, and DMI is strongly associated with FPCM and BW.

The MeI appeared to be negatively correlated with FPCM, which was expected due to the way MeI is computed; for a given MeP, an increase in FPCM leads to a decrease in MeI. This negative correlation was stable throughout lactation, hovering around −0.50 before decreasing to −0.75 at the end of lactation. Our results are consistent with previously reported values of −0.39, −0.73, and −0.87 (de Haas et al., 2011; Manzanilla-Pech et al., 2021; Richardson et al., 2021). Likewise, the correlation between MeI and DMI was similar to the genetic correlations reported by Manzanilla-Pech et al. (2021) and Richardson et al. (2021).

The MeY appeared to be weakly positively correlated with all the measured traits throughout lactation, with the exception in late lactation of DMI and FPCM, with which it was weakly negatively correlated. Our results

are similar to those of (Manzanilla-Pech et al., 2021), who found genetic correlations of -0.01 between MeY and BW and 0.15 between MeY and FPCM, whereas Richardson et al. (2021) found moderate negative correlations between MeY and DMI or FPCM.

The animal correlations obtained in our study represent a first step in analyzing the relationships of CH₄ with DMI, FPCM, BW, and BCS; any efforts to develop a selection program will certainly require investigations of genetic correlations. However, the weak and moderate animal correlations obtained here suggest that selecting against methane emissions whereas maintaining progress on other traits should be possible by balancing the weights associated with each trait in the breeding goal. Furthermore, correlations between CH₄ and traits related to fertility and health must also be considered to avoid unintended negative repercussions of a selection against CH₄, as suggested by the first studies on this topic (Pszczola et al., 2019; Zetouni et al., 2018).

Selecting for a Specific CH₄ Trait

After analyzing each CH₄ trait, we wanted to determine which one seemed most suitable for selection based on the following criteria: biological significance and the objectives addressed by each, ease of measurement, variability, repeatability, and correlations with other traits. In this paragraph, our conclusions depend on the assumption that genetic and animal parameters do not strongly differ, as only animal parameters were estimated in this study. However, this assumption may not hold and more research is needed on the genetic correlations.

First, it is essential to consider the objective targeted by each CH₄ trait. The MeY provides the possibility of reducing CH₄ per kilogram of DMI, which could lead to a net reduction in an individual's methane emissions via modifications in rumen function, with likely limited effect on production and BW. It would be a very attractive trait, provided it is not associated with poor feed efficiency, which requires careful investigation. Selecting for MeI means selecting for a reduction in CH₄ per kilogram of milk. The MeI would be directly related to financial income in case mandatory constraints on CH₄ were to emerge in the future. But because it is a ratio, it can be reduced via a decrease in the numerator (i.e., CH₄ per animal), or in an increase in the denominator (i.e., individual MY). As MY is already under strong selection and associated with unfavorable responses in functional traits, adding another indirect milk production criterion such as MeI brings little added value and novelty in the breeding objective. However, it could be interesting for low-producing animals or systems. Finally, selection against MeP would generate an overall

decrease in CH₄ per animal, likely via multiple avenues such as decrease in BW and stature, decrease in production (both inducing a decrease in feed intake), and a change in rumen function (i.e., a decrease in MeY). Because of this complexity, selecting against MeP alone is not optimal, but it may be possible by associating it with other traits to obtain the desired response.

As mentioned above, CH₄ is difficult to measure. With respect to ease of measurement, we consider MeP and MeI roughly equivalent because data on MY and FC and PC are readily available in dairy selection programs. However, DMI recording is labor-intensive and requires expensive equipment that is not widely available, and this represents a serious obstacle to the implementation of selection based on MeY. However, it may be possible to indirectly predict MeY from, for instance, milk mid-infrared spectra, and this needs to be further investigated.

On average and at any lactation stage, all 3 CH₄ traits were positively correlated, showing that selection on one should improve the components held in common among all 3 (Figure 5). In terms of their relationships with other traits, though, the 3 CH₄ traits behave very differently. In the context of multitrait selection, MeY, with its relative independence from the other traits, appears to be the most attractive target, despite its likely lower heritability and its difficulty of measurement. Indeed, it is the one trait that truly adds new information to the breeding objective. Considerations of BW or stature or capacity could further reinforce genetic gain for CH₄, as this information is not included in MeY. If MeY is not available or feasible, an acceptable alternative would be MeP, as it is highly correlated with MeY, more repeatable, more stable throughout the lactation, and easier to measure or predict. However, selection on MeP would also reduce milk production and feed intake and would have to be counterbalanced by additional emphasis on production.

CONCLUSIONS

The 3 CH₄ traits examined here—MeP, MeI, and MeY—were all sufficiently variable among animals to serve as targets for selection, and all remained relatively stable through the course of lactation, especially around the middle of the lactation period. Each CH₄ trait differed in its animal correlations with DMI, FPCM, BW, and BCS, reflecting the fundamental biological differences among them. Of the 3, MeY is promising for incorporation into a breeding objective, provided that it can be measured or predicted by proxies on large scale. The MeP may be able to serve as a satisfactory alternative, but its undesirable effect on production must be counterbalanced. These conclusions are

necessarily provisional because many questions remain to be answered: Are the animal correlations examined here similar to true genetic parameters? Is there any adverse effect of MeY on feed efficiency? How can these phenotypes be predicted on a large scale for genomic prediction? Further investigations are needed to shed light on these issues.

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