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## Short communication: Correlation of methane production, intensity, and yield with residual feed intake throughout lactation in Holstein cows



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### ABSTRACT

The environmental impact of dairy production can be reduced in several ways, including increasing feed efficiency and reducing methane ( $\text{CH}_4$ ) emissions. There is no consensus on their relationship. This study aimed at estimating the correlations between residual feed intake (RFI) and  $\text{CH}_4$  emissions expressed in g/d methane production (MeP), g/kg of fat- and protein-corrected milk methane intensity (MeI), or g/kg of DM intake methane yield (MeY) throughout lactation. We collected  $\text{CH}_4$  data using GreenFeed devices from 107 Holstein cows, as well as production and intake phenotypes. RFI was predicted from DM intake, fat- and protein-corrected milk, BW, and body condition score. Five-trait random regression models were used to estimate the individual variance components of the  $\text{CH}_4$  and production traits, which were used to calculate the correlations between RFI and  $\text{CH}_4$  traits throughout lactation. We found positive correlations of RFI with MeP and MeI ranging from 0.05 to 0.47 throughout the lactation. Correlations between RFI and MeY are low and vary from positive to negative, ranging from  $-0.18$  to  $0.17$ . Both MeP and MeI are favorably correlated with RFI, as is MeY during the first half of lactation. These correlations are mostly favorable for genetic selection, but the confirmation of these results is needed with genetic correlations over a larger dataset.

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### Implications

In the context of climate change, reducing the environmental impact of dairy cattle production is key. One way to achieve this is to reduce methane emissions and increase feed efficiency. Several studies have shown that a genetic selection is possible for these two traits when taken separately. However, it is necessary to understand how the two are related. This study focuses on residual feed intake, as an estimate of feed efficiency, and highlights favorable to slightly unfavorable correlations with methane throughout lactation, depending on the methane trait considered. Selection for reduced methane emissions may increase feed efficiency.

### Introduction

In the context of reducing greenhouse gas emissions, several studies have demonstrated the possibility of selecting dairy cows for reduced methane ( $\text{CH}_4$ ) emissions (Beauchemin et al., 2020). Before implementing a selection program, it is necessary to further investigate its relationships with other traits of interest. One of these

is feed efficiency that can affect both feed costs and environmental impact. For dairy cows, feed efficiency refers mostly to the balance between feed intake and milk production and is frequently measured by the residual feed intake (RFI). It is calculated as the difference between the observed feed intake and the predicted feed intake, calculated from the energy requirements of the animal for maintenance and production. However, since  $\text{CH}_4$  production is also biologically related to feed intake and the digestive process, a relationship between  $\text{CH}_4$  and RFI might be expected. There is no consensus in the literature regarding this relationship (Difford et al., 2020; Manzanilla-Pech et al., 2021, 2022; Sepulveda et al., 2022). This study aimed to estimate the animal correlations between  $\text{CH}_4$  and RFI throughout lactation using random regression models, for three  $\text{CH}_4$  traits: methane production, MeP (g/d); methane intensity, MeI (g/kg of fat- and protein-corrected milk [FPCM]); and methane yield, MeY (g/kg of DM intake [DMI]).

### Material and methods

#### Experimental animals

Data were collected from December 2019 to November 2021 from 64 first- and 43 second-parity Holstein dairy cows kept at

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the French experimental unit Le-Pin-au-Haras (INRAE, <https://doi.org/10.15454/1.5483257052131956E12>). The animals used in this study were described in Fresco et al. (2023). Briefly, cows were fed a total mixed ration *ad libitum* using an electronic feeding gate system and ear-tag identification and supplemented with 4 kg of concentrates in the parlor of the automated milking system (AMS; Lely Astronaut A4, Lely Holding, Maassluis, the Netherlands).

### Phenotypes

Milk production phenotypes were recorded using the AMS. We calculated FPCM using the formula proposed by the FAO in 2010:

$$\text{FPCM} = \text{MY} * (0.337 + 0.116 * \text{FC} + 0.06 * \text{PC}) \quad (1)$$

where MY is the sum of the yields at each milking over a 24-hour period, and FC and PC are the fat and protein contents, respectively. BW was recorded at each milking using an automatic weighing system in the AMS. Body condition score (BCS) was scored monthly on a scale of 0–5, with increments of 0.25, by two trained technicians.

Daily feed intake was recorded as the sum of the intakes weighed at each visit of the cow – from the first feed distribution (9 AM) to 8:59 AM the next day – plus the concentrate ingested in the AMS parlor. It was then converted to DMI using the weekly DM content of the total mixed ration and the concentrate.

Methane emissions were measured with two GreenFeed devices (C-Lock Inc., Rapid City, SD, USA) in free access. The GreenFeed 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. A linear model was used to adjust individual CH<sub>4</sub> measurements for diurnal variation related to feeding time (Fresco et al., 2023). Adjusted CH<sub>4</sub> measurements less than 150 g/d or greater than 950 g/d were discarded. Methane emissions, FPCM, DMI, and BW were averaged weekly. In this study, CH<sub>4</sub> was expressed in terms of production (MeP, g/d), yield (MeY, in g/kg DMI), or intensity (MeI, in g/kg FPCM).

### Statistical analyses

Due to the limited number of cows, it was not possible to distinguish the genetic and the permanent environmental effect. Therefore, we investigated the so-called animal effect, which is the sum of these two effects. No pedigree information was used.

We performed three different five-trait random regression models including one of the three CH<sub>4</sub> traits (MeP, MeI or MeY) and the four other traits (FPCM, DMI, BW, and BCS). Models are detailed in Fresco et al. (2023). They included year × month as fixed effect, a fixed regression for days in milk within parity modeled with a six-knot spline, and a random animal effect modeled with a second-order Legendre polynomial evaluated at each lactation week (1–43). Within a trait, residual effects were assumed to have a multinormal distribution with different variances across lactation periods (weeks 1–13; 14–30; 31–43). With these models, the (3×5, 3×5) variances-covariances were estimated for both the animal (**G**) and residual (**R**) components at each week. From the **G** matrix and the vectors of Legendre polynomials at week *t* ( $\varphi(t)$ ), estimates were obtained for the animal variance at time *t* for trait *i* by  $\varphi'(t1) \mathbf{G}_{i,i} \varphi(t1)$ , the animal covariance between times *t*1 and *t*2 for trait *i* by  $\varphi'(t1) \mathbf{G}_{i,i} \varphi(t2)$ , and the animal covariance between times *t*1 and *t*2 for traits *i* and *j* by  $\varphi'(t1) \mathbf{G}_{i,j} \varphi(t2)$ , where  $\mathbf{G}_{i,j}$  is the 3×3 submatrix of **G** corresponding to traits *i* and *j*. Estimated animal effects were generated for each lactation week for each trait. **G** and **R** were estimated using Wombat software (<https://didgeridoo.une.edu.au/km/wombat.php>), and variance parameters were calculated using R software (version 3.6.2, R Core Team, Vienna, Austria) from Wombat outputs.

We estimated the correlations between CH<sub>4</sub> and RFI at each lactation week using formula (2):

$$r_{\text{CH}_4, \text{RFI}}(t) = \frac{\text{cov}(\text{CH}_4, \text{RFI})(t)}{\sqrt{\text{var}(\text{CH}_4)(t) \times \text{var}(\text{RFI})(t)}} \quad (2)$$

The variances and covariances were calculated following the methodology developed by Martin et al. (2021) and formula (3):

$$\text{cov}_{\text{CH}_4, \text{RFI}}(t) = \varphi(t) \times \mathbf{G}_1 \times \varphi(t)' - \varphi(t) \times \mathbf{G}_2 \times \mathbf{G}_3^{-1} \times \mathbf{G}_4' \times \varphi(t)' \quad (3)$$

where  $\text{cov}_{\text{CH}_4, \text{RFI}}(t)$  is the animal covariance between CH<sub>4</sub> and RFI at week *t*, **G**<sub>1</sub> is the covariance matrix between CH<sub>4</sub> and DMI, **G**<sub>2</sub> is the covariance matrix between DMI and FPCM, BW, and BCS, **G**<sub>3</sub> is the variance matrix of FPCM, BW, and BCS, and **G**<sub>4</sub> is the covariance matrix between CH<sub>4</sub> and FPCM, BW, and BCS. The same method was used to estimate the correlations between RFI and FPCM, and RFI and DMI.

### Results and discussion

Statistical description of the weekly means of the seven traits (MeP, MeI, MeY, DMI, FPCM, BW, and BCS) are reported in Fresco et al. (2023). Variances for RFI, MeP, MeI, and MeY were stable in mid-lactation and increased in early and late lactation (Fig. 1). Animal correlations between CH<sub>4</sub> and RFI throughout lactation are shown in Fig. 2. Both MeP and MeI were positively – thus favorably – correlated with RFI throughout the lactation. Animal correlations between MeP and RFI varied from 0.05 to 0.47, consistent with the moderate to high genetic correlations reported by Manzanilla-Pech et al. (2021, 2022). Using CH<sub>4</sub> concentrations and RFI calculated in three different ways, Difford et al. (2020) found mostly high positive genetic correlations, although some high negative correlations were also reported. Animal correlations between MeI and RFI varied from 0.09 to 0.30, while genetic correlations were reported to be either moderately positive or moderately negative (Manzanilla-Pech et al., 2021, 2022). Animal correlations between

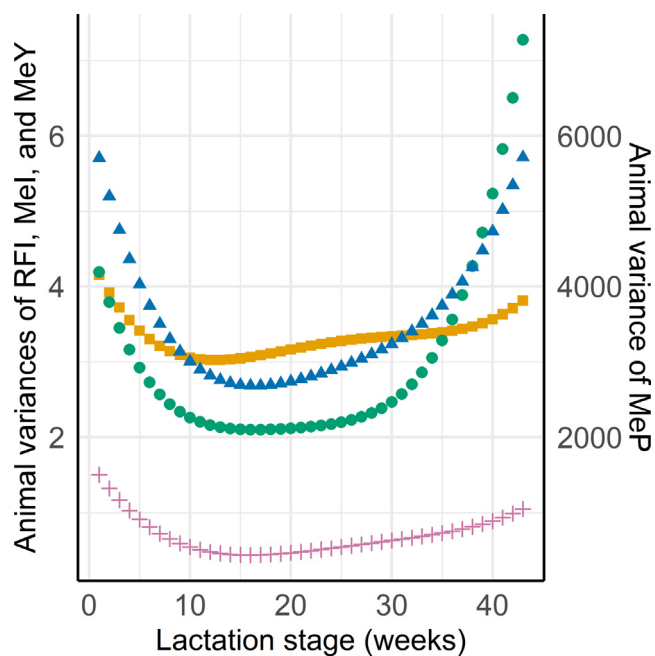


Fig. 1. Variances throughout lactation between residual feed intake (+, RFI), and methane emissions expressed in g/d (■, MeP), in g/kg of fat- and protein-corrected milk (▲, MeI), and in g/kg of DM intake (●, MeY) for Holstein dairy cows.

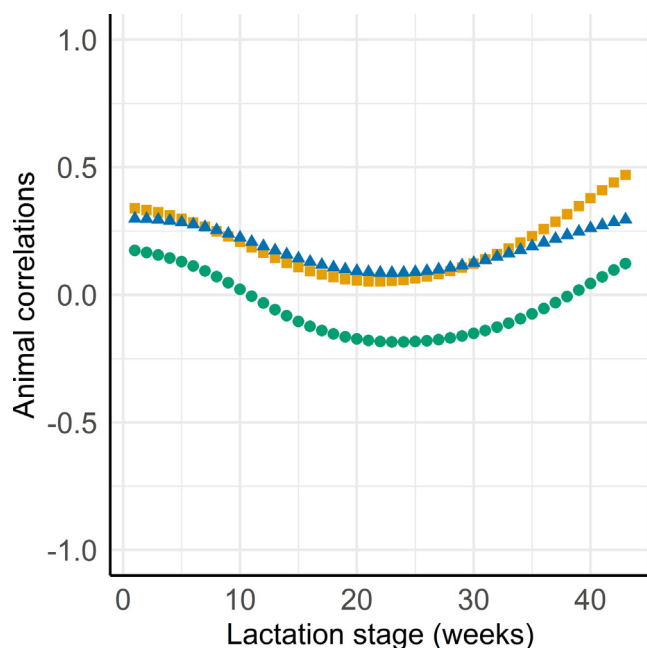


Fig. 2. Animal correlations throughout lactation between residual feed intake and methane emissions expressed in g/d (■, MeP), in g/kg of fat- and protein-corrected milk (▲, MeI), and in g/kg of DM intake (●, MeY) for Holstein dairy cows.

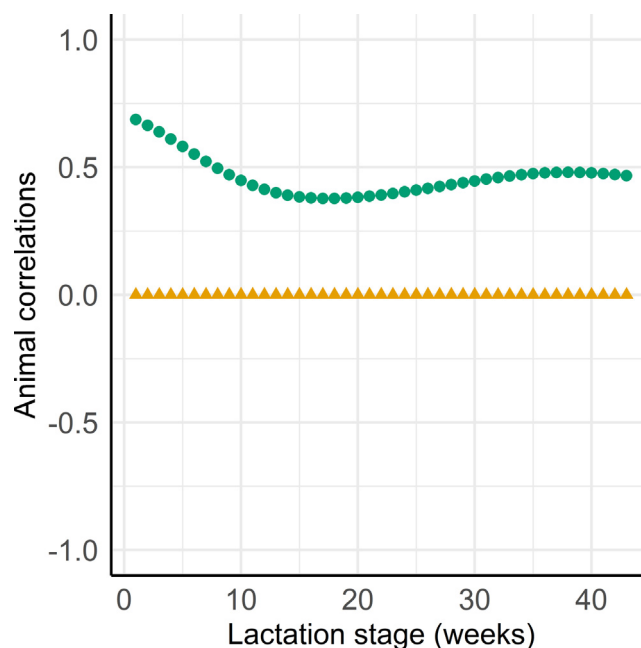


Fig. 3. Animal correlations throughout lactation of residual feed intake with fat- and protein-corrected milk (▲) and DM intake (●) for Holstein dairy cows.

MeY and RFI ranged from 0.17 to  $-0.18$  during the first 24 weeks of lactation and increased to 0.12 thereafter, i.e., they went from favorable to unfavorable throughout lactation (Fig. 2). This result is consistent with that of [Manzanilla-Pech et al. \(2021\)](#), who found genetic correlations between MeY and RFI close to zero, either positive or negative. However, other studies found these correlations to be moderately positive or strongly negative ([Manzanilla-Pech et al., 2022](#); [Sepulveda et al., 2022](#)).

Comparing our results with the literature illustrates the lack of consensus regarding the correlations between RFI and  $\text{CH}_4$  emissions, except for MeP and RFI, usually found to be positive, i.e., favorable. One hypothesis is that efficient cows lose less energy through  $\text{CH}_4$  production. It appears that efficient cows do not produce much less  $\text{CH}_4$  per unit of DMI, as the correlations between RFI and MeY are close to zero (Fig. 2). However, they have a lower intake, as the correlations between RFI and DMI and between MeP and DMI are positive (Fig. 2; [Fresco et al., 2023](#)). [Lassen and Løvendahl \(2016\)](#) also reported studies showing no difference in MeY related to RFI and that efficient cows produce less  $\text{CH}_4$ . Consequently, efficient cows produce less  $\text{CH}_4$  per unit of FPCM. Because the RFI we used in this study is independent from FPCM (Fig. 3), the correlation between RFI and MeI appears to be directly driven by the correlations between RFI and MeP.

Several factors could explain the lack of consensus on the relationship between  $\text{CH}_4$  and RFI. The first is diet composition, which influences both  $\text{CH}_4$  emissions ([Beauchemin et al., 2020](#)) and the RFI without being included in the calculation ([Difford et al., 2020](#)). For example, the forage-to-concentrate ratio affects ruminal metabolic pathways, with diets rich in concentrate stimulating the production of propionate, which is an  $\text{H}_2$  sink and thus regulates  $\text{CH}_4$  production ([Beauchemin et al., 2020](#)). The use of different diets and their unequal consideration of  $\text{CH}_4$  and RFI creates heterogeneity between studies, particularly by favoring high-producing cows that tend to be fed higher-quality diets ([Difford et al., 2020](#)).

A second factor influencing the correlations between RFI and  $\text{CH}_4$  is the method used to calculate RFI. In this study, we did not calculate RFI directly, following the methodology proposed by

[Martin et al. \(2021\)](#) while other studies presented RFI calculated as a partial regression of DMI on its predictors ([Manzanilla-Pech et al., 2021, 2022](#); [Sepulveda et al., 2022](#)). Moreover, different traits are used to predict RFI. Most of the studies include a trait related to milk production – energy-corrected milk or FPCM – and a trait related to body size – BW or metabolic BW. Some studies consider additional predictors such as changes in BW or BCS. Such variability influences the correlations of RFI with MeP, MeI, and MeY, both in terms of sign and value ([Manzanilla-Pech et al., 2021](#)).

A third factor influencing the correlation between RFI and  $\text{CH}_4$  is the recording method of  $\text{CH}_4$  emissions. Several tools have been developed, that either measure  $\text{CH}_4$  quantities or concentrations. Apart from the respective interests of each recording method, it is necessary to estimate and consider the differences between them to estimate how they affect the correlations with RFI. It has been reported that methane emissions recorded with respiration chambers are phenotypically highly correlated with  $\text{CH}_4$  measured with other tools, while the correlations were frankly lower among the other methods ([Garnsworthy et al., 2019](#)).

The correlation between  $\text{CH}_4$  and RFI is also influenced by the lactation stage. In this study, we were able to estimate the correlations throughout the lactation using random regression models, and thus highlight the variations of the correlation. Days in milk influences the correlation between RFI predictor traits (DMI, energy-corrected milk, and BW), mostly due to their genetic heterogeneity between early and mid/late lactation ([Li et al., 2018](#)). Consequently, analyses considering days in milk are strongly recommended ([Li et al., 2018](#)), such as random regression models or multi-trait analysis. Some of the discrepancies in the literature could be related to the consideration or not of the genetic heterogeneity of the RFI predictor traits. Noteworthy, most of the previous studies had access to data over a limited period, which did not allow for analysis throughout lactation. Furthermore, since both  $\text{CH}_4$  and RFI traits vary over time, it could create discrepancies if they are not measured simultaneously ([Difford et al., 2020](#)).

In this study, we estimated animal correlations, because the number of cows was too low to disentangle the permanent environmental effect from the additive genetic. This is a likely source

of discrepancies when comparing our results to the genetic correlations reported in the literature, obtained by including either pedigree (Difford et al., 2020; Manzanilla-Pech et al., 2021, 2022) or genomic information (Sepulveda et al., 2022).

The favorable or slightly unfavorable correlations between RFI and CH<sub>4</sub> emissions need to be confirmed on a larger dataset before implementing a selection, but they are already a good indication. Selection to reduce the environmental impact of cattle production can be achieved either by selecting for increased feed efficiency, which is already being considered in some breeding programs, or by selecting directly for reduced CH<sub>4</sub> emissions. However, care should be taken because both traits are unfavorably correlated with DMI, and such a selection could result in animals with lower intake. In turn, reduced DMI may be detrimental to milk production (Fresco et al., 2023), or even to the animal health and reproduction.

## Conclusion

In conclusion, the results suggest that MeP and MeI are favorably associated with RFI, while MeY has a low favorable or unfavorable association with RFI throughout the lactation. These results show a potential for genetic selection of those traits, provided that these correlations are confirmed on a larger dataset.

## Ethics approval

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.legi-france.gouv.fr/eli/decret/2013/2/1/2013-118/jo/texte>).

## Data and model availability statement

None of the data were deposited in an official repository. Data can be obtained from the authors upon reasonable request.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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## CRediT authorship contribution statement

**S. Fresco:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **D. Boichard:** Writing – review & editing, Visualization, Supervision, Methodology. **R. Lefebvre:** Resources. **S. Barbey:** Resources. **M. Gaborit:**

Resources. **S. Fritz:** Project administration. **P. Martin:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization.

## Declaration of interest

None.

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