

## Validation of a previously developed enteric methane emission prediction model using individual cow milk mid-infrared spectra in Ireland

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### Abstract

The agricultural sector in Ireland contributes 38.4% of total greenhouse gas emissions and 71.2% of the agriculture greenhouse gases is generated from methane associated with livestock production. Mid-infrared (MIR) spectral data, which are routinely collected in a fast, cheap, and non-disruptive way, have been used to predict individual cow methane emissions in Canada, Belgium, France, Netherlands, and Ireland. The objective of this study was to validate, using data from the 2023 calendar year, predictions of enteric methane from milk MIR developed in Ireland based on data from the years 2020 to 2022. The Irish prediction model was developed using 93,888 individual spot measures of methane (i.e., individual samples of animal's breath when using a GreenFeed technology) from 277 cows. T

he enteric methane phenotype was based on the average of at least 20 individual spot measures taken over a 6-day period flanking each side of the milk sample with an associated milk spectral data. Predictions were based on a neural network algorithm populated with information on the MIR spectra, milk yield, and days in milk; the correlation between the actual and the predicted values in that 2020 to 2022 data varied from 0.68 to 0.75 in cross-validation, and from 0.55 to 0.71 in leave-one-experiment treatment-out validation. The validation dataset used in this study for the 2023 calendar year consisted of 45,196 individual cow spot methane measures from 157 cows which were collapsed into 1,715 methane records with associated milk MIR; none of the cows in the validation population were in the dataset used to develop the predictive model. The correlation between the real and the predicted values, the root mean square error (RMSE), and the ratio of performance to deviation were 0.38, 79.76 g/d, and 0.69, respectively.

The validation dataset was then stratified by estimated daily methane as the highest 10% emitting cows and the lowest 10% emitting cows. The mean (standard deviations) actual methane emitted by the cows predicted from the MIR to be the highest 10% emitter cows was 417.39 g/d (31.91 g/d), while that of those predicted to be the lowest 10% emitters was 220.56 g/d (26.69 g/d); the respective predicted mean methane of those two groups of animals was 402.59 g/d (27.73 g/d) and 358.26 g/d (27.00 g/d), respectively. Results from the present study indicated a relatively poor prediction accuracy in estimating individual cow methane emissions in a subsequent year. Nonetheless, differences in the actual mean methane between groups of cows predicted to be divergent in methane materialised. Hence, while individual animal predictions was poor, actual differences in enteric methane emissions differed between groups of animals stratified on predicted methane emissions.

*Keywords: methane, mid-infrared spectroscopy, pasture based, validation.*  
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## Introduction

Irish agriculture is responsible for 38.4% of national greenhouse gas emissions (GHG) and methane from enteric fermentation accounts for the 71.2% of these emissions (EPA, 2023). Nonetheless, the total amount of GHG emissions needs to be reduced by 25% by the year 2030. Strategies which will achieve this deliverable without impacting global are required. Environmental concerns from consumers necessitate future food production systems to demonstrate they are capable of accurately quantifying and mitigating their environmental footprint. Mitigating methane emissions from dairy cows presents a complex challenge due to the biological processes involved in rumen digestion. Different strategies have been proposed to reduce cow methane emissions such as improving feed management (e.g. by adjusting the composition and timing of feed; Hristov *et al.*, 2013), inclusion of feed additives into the diet (Patra, 2012), genetic selection (Pinares-Patiño *et al.*, 2013), and rumen manipulation (e.g. inoculating the rumen with specific microbes or introducing methanogen inhibitors; Eugène *et al.*, 2015). Nonetheless, methods for estimating methane emissions are needed, ideally at an individual cow level, as they would allow for an assessment of methane output while also permitting to investigate methane-reducing strategies.

Mid-infrared (MIR) spectroscopy is a technology routinely applied to all bulk tank and individual animal milk samples to quantify the concentration of many milk components (e.g., fat, protein and lactose) in the milk sample. Moreover, it was successfully used to predict, with reasonable accuracy, the enteric methane emissions of individual animals (Vanlierde *et al.*, 2015; Shadpour *et al.*, 2022; Dehareng *et al.*, 2012). Nonetheless, these previous studies were generally small in size (from 11 cows – Dehareng *et al.*, 2012; to 202 cows - Shadpour *et al.*, 2022), often limited to cows (likely) fed indoors (Shadpour *et al.*, 2022; Wang and Bovenhuis, 2019; Dehareng *et al.*, 2012; van Gastelen *et al.*, 2018; Coppa *et al.*, 2022) or measured over a relatively short period of time (Vanlierde *et al.*, 2018; van Gastelen *et al.*, 2018). In Ireland, a methane emissions prediction equation was developed in 2023 (McParland *et al.*, 2024) using data collected between the years 2020 to 2022 from 277 grazing dairy cows. The aim of this study was to validate this Irish methane prediction equation using data collected during the 2023 calendar year.

## Materials and methods

### Calibration dataset

The calibration dataset included data from 93,888 individual spot measures of methane (i.e., individual samples of animal's breath when using a GreenFeed technology) from 277 dairy cows collected between the years 2020 and 2022. Different methane phenotypes were investigated and the one which produced the most accurate prediction results was based on the average of at least 20 individual spot measures taken over a 6 day period surrounding each side of the milk sample (McParland *et al.*, 2024). The quantified phenotype was then merged with the same cow's daily average milk spectrum quantified as the milk yield weighted average of the milk spectrum originating from an evening milking and the milk spectrum originating from the following morning milking. A total of 531 wavelengths were used for the analyses (i.e., after discarding the water regions). The mean and standard deviation of the calibration dataset was 324.0 g/d and 94.0 g/d, respectively. The prediction equation was developed using a neural networks algorithm that was populated with data on the MIR spectra, milk yield, and days in milk. The R package brnn (Perez Rodriguez and Gianola, 2020) was used to develop the prediction equation, and the default tuning parameters were chosen, which

included two hidden layers and a Bayesian regularization to the input layer to improve generalizability. Prediction results in the calibration dataset resulted in a correlation between the actual and the predicted values of 0.68 to 0.75 in cross-validation and from 0.55 to 0.71 in leave-one-experiment treatment-out validation.

A further 45,196 individual spot methane measures from 157 cows were collected during the 2023 calendar year. These records were collapsed into 1,715 daily methane records with associated daily weighted average milk MIR; none of the cows in the validation population were in the calibration population. The mean and the standard deviation in the validation dataset was 313.20 g/d and 55.52 g/d, respectively.

### Validation dataset

The correlation between the real and the predicted values, the root mean square error (RMSE), and the ratio of performance to deviation when validated in the 2023 data were 0.38, 79.76 g/d, and 0.69, respectively. The actual versus the predicted methane emissions values are in Figure 1.

## Results

The actual and predicted lactation profile for methane is in Figure 2. Actual daily emitted methane increased as the lactation progressed until week22 after which it declined; in contract, predicted daily methane reduced as the lactation progressed.

The correlation between the actual and the predicted methane emissions was then investigated within stage of lactation, where each stage was approximately 60 days in duration. The correlation between the actual and the predicted methane emissions was always 0.50 for the records collected between 5 to 59 DIM, between 60 and 119 DIM, and between 120 and 179 DIM, but weakened to 0.46 between 180 and 239 DIM and weakened further to 0.27 post 240 DIM.

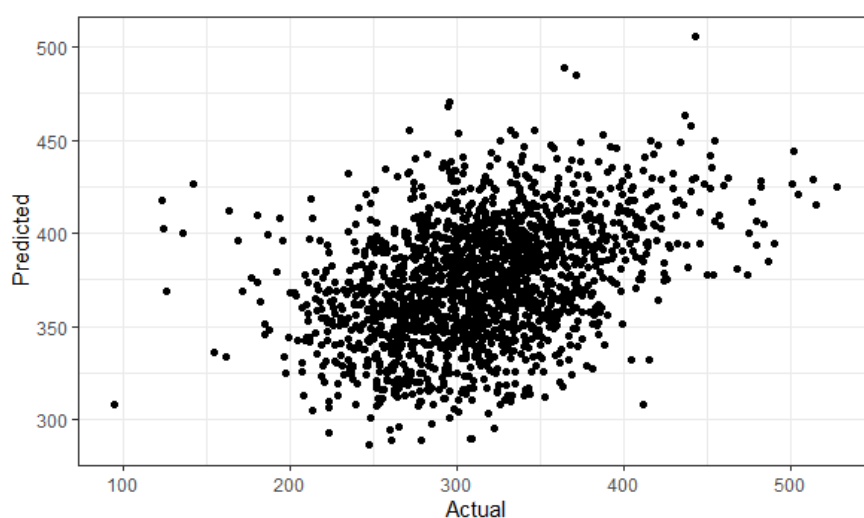
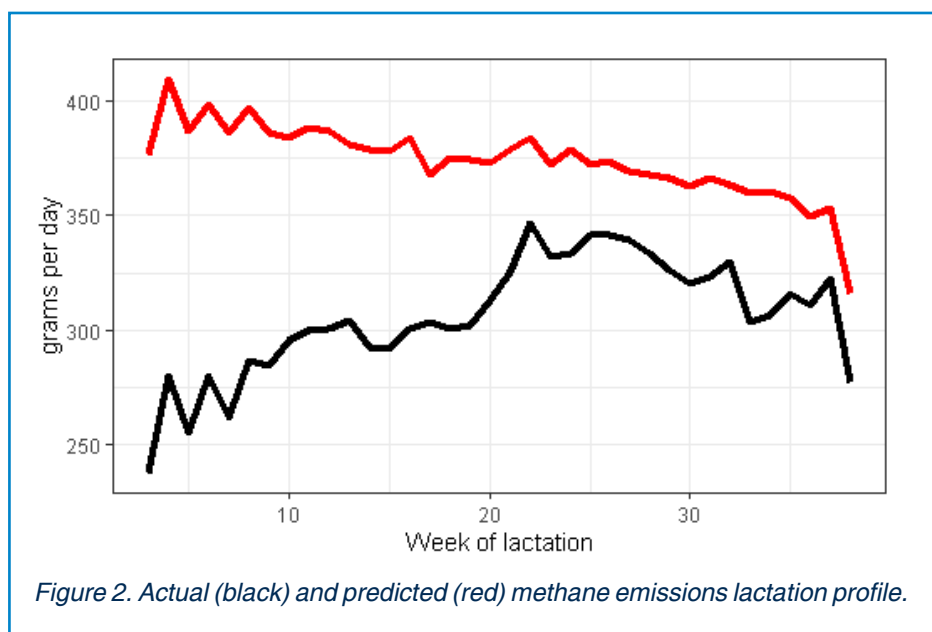


Figure 1. Actual (x-axis) versus predicted (y-axis) methane emissions (g/d).



The validation dataset was then stratified by predicted daily methane as the highest 10% emitting cows and the lowest 10% emitting cows. The mean actual methane emitted by the cows estimated to be the highest 10% emitter cows was 417.39 g/d, while that of those estimated to be the lowest 10% emitters was 220.56 g/d; the respective predicted mean methane of those two groups of animals was 402.59 g/d and 358.26 g/d, respectively.

## Discussion

Quantification of methane emissions is essential to study the effect of different diets or the inclusion of feed additives on cow methane emissions, as well as to include methane emissions as a trait in national genetic evaluations. The reference method used for the quantification of methane emission are the respiratory chambers, which may not be a good reflection of the actual enteric methane emissions in grazing cows, since the cows are removed from their natural environment. Indeed, activities like walking, the grazing process itself (i.e., diet selection when grazing pasture), and the influences of weather conditions on grass quantity and quality are not detected with the respiratory chambers.

Therefore, alternative approaches to quantify methane emissions in the grazing system need to be explored. The utilization of milk MIR spectral data coupled with previously developed prediction equations generated reasonably accurate predictions. Nonetheless, the developed equations have to be properly validated before being used. Indeed, Wang and Bovenhuis (2019) reported a coefficient of determination of 0.49 when methane emissions were quantified from MIR in dairy cows using random cross-validation, but a coefficient of determination of 0.01 when methane emissions were quantified using block cross-validation, with farm as blocks. As prediction equations are generally generated using data collected in a relatively small number of farms (often research farms), the developed equations need to be able to accurately quantify methane emissions for farms with no records in the calibration dataset. The validation dataset used in the present study included records collected from cows not included in the calibration dataset, collected in the successive year to the records in

the calibration dataset, and majority of the data in the validation dataset were collected in a farm which was not included in the calibration dataset. While the accuracy metrics used to assess the model predictive ability were acceptable, the mean of the predicted methane emissions of the high 10% emitting cows was different ( $P < 0.05$ ) to the mean of the predicted methane of the low 10% emitting cows.

The results from the present study demonstrated that even if the actual methane emissions value for the different cows was not accurately quantified, groups of cows (i.e., high and low emitting cows) can be correctly identified. Therefore, the methane emission phenotypes quantified using the milk MIR spectra and the already developed equation could potentially be used for selection of lower emitting cows.

## Conclusions

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