

## Methane phenotyping using different techniques and estimates of parameters for the Nordic Red cattle in Finland

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The aim of this paper is to compare the performances of different methane ( $\text{CH}_4$ ) measurement techniques, to estimate some genetic parameters and highlight  $\text{CH}_4$  phenotyping methods used in the Nordic Red cattle (RDC) in Finland. Data were from  $\text{CH}_4$  measurements of RDC cows in Jokioinen dairy research farm of the Natural Resources Institute Finland (Luke). Three  $\text{CH}_4$  measurement techniques were used and in total 32, 137 and 310 cows had daily average  $\text{CH}_4$  measurements from cattle respiration chamber (RC), GreenFeed (GF) and F10 multi gas analyser (F10 sniffer, Gasera Ltd, Turku, Finland), respectively.

### Abstract

For comparison among techniques, data from simultaneous  $\text{CH}_4$  measurements by any two techniques and Lin's concordance analyses were used for comparative assessment. Estimates of genetic correlations from repeatability animal models were used to assess the association of  $\text{CH}_4$  phenotypes with some production and functional traits included in the dairy cattle breeding goals. Methane phenotypes:  $\text{CH}_4$  production (MeP= $\text{CH}_4$  g/day),  $\text{CH}_4$  yield (MeY= g  $\text{CH}_4$ /kg DMI),  $\text{CH}_4$  intensity (MeI=g  $\text{CH}_4$ /kg ECM) and residual  $\text{CH}_4$  production (RMP) were considered. Production traits: energy corrected milk (ECM), metabolic body weight (mBW), residual feed intake (RFI) and dry matter intake (DMI) were included in the analyses.

The mean MeP, MeY and MeI from RC were  $453.0 \pm 55$ ,  $21.3 \pm 1.4$ ,  $17.1 \pm 1.6$  whilst from GF were  $467.1 \pm 61$ ,  $21.6 \pm 1.5$  and  $14.8 \pm 1.8$ , respectively. Corresponding means from the F10 technique were  $400.1 \pm 32.7$ ,  $20.6 \pm 4.3$  and  $13.9 \pm 3.5$ . The Lin's concordance correlation coefficient for MeP between the RC and GF techniques were 0.70. Whereas the 95% confidence interval of the Lin's concordance correlation coefficient for MeP between the RC and F10 techniques ranged from 0.40 to 0.85. Heritabilities using data from F10 measurement for traits: MeP, MeY, MeI and RMP were 0.04, 0.04, 0.08, and 0.16, respectively. Genetic correlations between MeP and production traits: ECM, mBW, RFI and DMI were moderate to high positive with 0.42, 0.67, 0.48 and 0.49, respectively.

Some disparities in the estimates of  $\text{CH}_4$  phenotypes from different techniques were observed. In view of the scarcity of individual animal  $\text{CH}_4$  data, to make effective use of every available  $\text{CH}_4$  measurements in livestock, methods and tools should be developed for integrating records from different techniques into standardised and harmonised set.

**Keywords:** methane, dairy cattle, measurement methods, heritability, correlations, concordance analyses.

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## Introduction

Attempts to lower the environmental footprint of milk production needs a sound understanding of the basis of  $\text{CH}_4$  emissions from the dairy production systems. Accurate and reliable  $\text{CH}_4$  measurements are therefore important not only for understanding of the basis of livestock system  $\text{CH}_4$  emissions, but also for national inventory and to identify potential mitigation strategies. However, the accurate measurement of  $\text{CH}_4$  emissions particularly from individual animals is difficult and expensive (Pickering *et al.*, 2015, Negussie *et al.*, 2017). As a result, so far, routine measurements  $\text{CH}_4$  and large-scale recordings are rare. One of the main reasons for this has been the lack of accurate, low-cost, portable, and non-invasive methods that are also suitable for application on commercial farms. Lately, with the advances in digital and sensor technologies there has been a gradual rise in new and advanced applications for  $\text{CH}_4$  measurement. There is, therefore, a need to understand the comparative performances of the different techniques and the associated challenges and opportunities to develop suitable  $\text{CH}_4$  phenotyping strategies for management, targeted nutritional studies or genetic selection. The aim of this paper is to compare the performances of few of the widely used  $\text{CH}_4$  measurement techniques and to estimate some genetic parameters for  $\text{CH}_4$  output traits in the Nordic Red cattle (RDC) in Finland.

## Material and methods

To evaluate the performances of GF in measuring  $\text{CH}_4$  emission compared to respiration chambers, thirty-two lactating Nordic Red cows (RDC) were used. Measurements of  $\text{CH}_4$  emission were done using two GF units and cattle respiration chambers ( $n = 4$ ) in a complete block design (8 blocks). The experimental period for every block lasted for 5 weeks; in the first 2 weeks the cows were measured in GF units, on the 3<sup>rd</sup> week cows were measured in the chambers and on the 4<sup>th</sup> and 5<sup>th</sup> weeks they were measured again in the GF units.

The cows were fed a grass silage-based diets with 55:45 forage to concentrate ratio. The data collected from the study was edited in that GF records above or below  $2.5 \times \text{SD}$  of all measurements for every cow over 4 weeks period were considered as outliers and were deleted resulting in removal of 217. In a similar but separate study, F10 multigas analyser (F10, sniffer) was compared to respiration chambers ( $n = 4$ ) using twenty-one first lactation RDC cows to assess the agreement between the two techniques. Here  $\text{CH}_4$  measurements on cows were taken in a three-weeks sequence, where a one-week F10 measurements was followed by another one-week  $\text{CH}_4$  measurements in the chamber which was then followed by a one-week F10 measurements.

Data on  $\text{CH}_4$  measurements of twenty-one cows from the two techniques were made available for analysis. In both comparative assessments of  $\text{CH}_4$  measurement techniques: the GF *versus* respiration chamber and F10 sniffer *versus* respiration chambers, the agreement between the techniques was assessed using the Lin's concordance correlation analyses (Lin, 1989). Data on  $\text{CH}_4$  measured from a relatively large number of RDC cows using the F10 technique was then used to estimate genetic parameters for the different  $\text{CH}_4$  output traits and to assess their genetic and phenotypic associations with some production traits.

Data was from 309 RDC cows including 13,573 weekly average records. Production traits were energy corrected milk (ECM), metabolic body weight (mBW), residual feed intake (RFI) and dry matter intake (DMI). Methane phenotypes included were:  $\text{CH}_4$  production ( $\text{MeP} = \text{CH}_4$  g/day),  $\text{CH}_4$  yield ( $\text{MeY} = \text{g CH}_4/\text{kg DMI}$ ),  $\text{CH}_4$  intensity ( $\text{MeI} = \text{g CH}_4/\text{kg ECM}$ ) and residual  $\text{CH}_4$  production (RMP). Univariate and bivariate repeatability animal models were used for the estimation of heritabilities and genetic correlations between traits, respectively. The models included fixed effects of age, feeding kiosk number, lactation week, measurement year-month and random permanent environment and animal effect.

Respiration chambers are calibrated to be accurate and precise, and are the gold standard for benchmarking new methods (Garnsworthy *et al.*, 2019). Where an alternative method may be cheaper, less invasive, easier to implement, or have a wider scope of applications, it is of value to assess their relative accuracy and agreement with the gold standard. In actual comparison between methods simultaneous repeated measures per cow with two or more techniques are required in order to establish agreement between the techniques. In such comparisons, it is also important to have short time interval between repeated measures per subject to ensure that the underlying biology of the cow has not changed (Garnsworthy *et al.*, 2019).

The main difficulty faced in most comparisons involving  $\text{CH}_4$  measurement techniques is that not all techniques can be recorded simultaneously on the same individual and the methane emission of cows changes both throughout the day and over the lactation period. In such cases, either cross-over designs are needed, or else matched-pair repeated measures designs. In both cases, the Lin's concordance correlation analysis (Lin, 1989) is useful to validate and establish agreement between any two methods.

Lin's concordance analysis computes agreement on a continuous measure obtained by two methods. It is widely used in validation studies because of its ability to combine measures of both precision and accuracy to determine how far the observed data deviate from the line of perfect concordance. In this study, the overall average daily  $\text{CH}_4$  emission using GF units was  $467 \pm 61.4$ , g/d and was  $453 \pm 55$  g/d for respiratory chambers. For  $\text{CH}_4$  production, the Lin's concordance correlation coefficients between the GF and chamber was 0.68.

On the other hand, in the F10 sniffer *versus* chamber comparison, the concordance correlation coefficient for the first week before chamber F10 measurements was 0.70 with 95% lower and upper confidence limits of 0.41 and 0.85, respectively. Whereas the concordance correlation coefficient for the third week after chamber F10  $\text{CH}_4$  measurements was 0.69 with corresponding lower and upper confidence limits of 0.37 and 0.86, respectively.

The concordance correlation coefficient for combined before and after chamber F10 measurements was 0.84 with the 95% lower and upper confidence limits of 0.65 and 0.93, respectively. Here the indications are that when the week before and week after chamber F10  $\text{CH}_4$  measurements were combined, the agreement between the methods has markedly improved. The result shows that a combined weekly mean F10 measurements taken in a week interval can provide a much closer prediction of the respiration chamber measurements.

Comparing different  $\text{CH}_4$  measurement methods, Garnsworthy *et al.*, (2019) reported that for the methods with repeated measures per cow, the mass flux-based methods had the highest repeated measures correlations which outperformed the concentration-based methods. They have reported a concordance correlation coefficient of 0.87 and 0.81 for comparison between SF6 *versus* chamber and GF *versus* chamber which is close to the results obtained in this study.

## Results and discussion

### Comparison of $\text{CH}_4$ measurement techniques

Genetic selection provides a reliable route towards permanent and cumulative reductions in quantitative traits such as enteric  $\text{CH}_4$  emissions. This requires estimation the amount of available genetic variations for the  $\text{CH}_4$  traits and their genetic associations with other

### Estimates of genetic parameters

dairy breeding goal traits. Unfortunately, such estimates particularly for dairy cattle are rare in literature. In this study, the mean MeP, MeY and MeI from the F10 technique CH<sub>4</sub> measurements were 400.1±32.7 g/d, 20.6±4.3 g/kg, 13.9±3.5 g/kg. The estimates of heritability for the CH<sub>4</sub> output traits: MeP, MeY, MeI and RMP were 0.04, 0.04, 0.08, and 0.16, respectively.

The genetic correlations between MeP and production traits: ECM, mBW, RFI and DMI were moderate to high positive with 0.52, 0.67, 0.48 and 0.49, respectively. The estimated genetic associations among the traits ranged from moderate to high and are in line with literature estimates. Analysing a combined dairy cows data from four countries Manzanilla-Pech *et al.* (2021) reported heritability (SE) for MeP of 0.21 (0.04), and heritabilities of 0.30 and 0.38, respectively for the MeY and MeI, respectively. Difford *et al.* (2020) on the other hand reported 0.26 for CH<sub>4</sub> concentration (in ppm) for Danish Holstein, whereas Breider *et al.* (2018) reported 0.33 for MeP using SF6 in Australian Holstein.

Compared to these literature estimates, our estimates of heritability for MeP is slightly lower than the reported estimates for Holstein cattle. However, our estimate is in line with an earlier estimate obtained on part of the same data. Any disparity between the present estimates and above cited literature reports could be in part due to the methods of CH<sub>4</sub> measurement, data size, the population under consideration and the model used for the evaluation.

## Conclusion

In general, results from comparing the performances of different methane measurement methods have shown some differences. The main question is if we measure CH<sub>4</sub> by sniffer or by GF or respiration chamber are they the same phenotype. The answer for this is clear and particularly in analyses involving animal evaluations, efforts should be made, and methods should be developed to standardize and harmonize CH<sub>4</sub> measurements coming from the different methods. This enables us to make effective use of the small, scattered, rare and unique CH<sub>4</sub> data sets for accurate estimation of the genetic merit of animals and planning mitigation strategies.

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