Can ruminal microbial information help improve selection for low-methane emitting dairy cows?

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Abstract

Greenhouse gas emissions from livestock have been at the centre of a worldwide discussion. To achieve tangible results in mitigation strategies, all tools in the available toolset need to be used. One tool is the rumen microbiome, which are partly under the host genetics' control, and could help explain variation in methane emissions from ruminants. Data on methane emissions and rumen fluid samples were collected from 179 dairy cows from Dutch commercial herds. Genotypes from 70 cows were used to estimate heritability for methane intensity. Our goal was to assess the power of our pipeline, for subsequent application to a bigger dataset, by evaluating the value of adding microbial information to the analysis. Microbial estimates were highly confounded with the effect of farm. Our preliminary results show that our workflow is appropriate and that we will be able to estimate microbiability and heritability when our full dataset becomes available.

Introduction

The Dutch government has agreed on a set of guidelines, named the National Climate Agreement, with the goal to decrease by 2030 greenhouse gases emissions (GHG) by 49% from the 1990 levels. Five different sectors have been targeted, among them agriculture, where a reduction of 3.5 Mton CO_2eq is expected to be achieved. One source of GHG from agriculture is methane emissions from ruminants, which release methane into the atmosphere as a by-product of their digestion. A community of microorganisms produce, amongst other things, CO_2 and H_2 in the rumen while digesting the feed, which in turn are converted to methane and eructed by cattle (Janssen and Kirs, 2008).

Genetic selection is an important tool for breeders, as it brings cumulative and permanent progress for a given trait of interest (Wall *et al*., 2010). Methane emissions from dairy cattle are known to be partly under genetic control (Difford *et al*., 2020; Zetouni *et al*., 2018), which means selecting for cows that emit less methane is possible, and could therefore be used as a tool to help GHG mitigation in livestock. Another feature that seems to be partly under host control is the vast population of microbes, i.e. the microbiome, found in the rumen (Malmuthuge and Guan, 2017; Roehe *et al*., 2016). Variation in the relative composition of the microbiome, termed microbiability, has been shown to explain 13% of the variation in methane emissions from dairy cattle (Difford *et al*., 2018). Therefore, microbial information could be used to help shed some light in understanding the genetic factors behind methane variation in cattle. Our goal was to evaluate the power of our sampling strategy and subsequent pipeline in this preliminary dataset, by evaluating to which extent microbial information could explain the variation in methane intensity from dairy cows from Dutch commercial herds. This paper focuses on preliminary data collection as an indicator on how to optimize sampling of a larger dataset for the estimation of methane microbiability and heritability, and in evaluating and developing our chosen methodology. Our study is part of an ongoing project with the aim to collect methane phenotypes, genotypes and rumen fluid samples from 1,000 Dutch dairy cows.

Material and Methods

Data Collection. Methane phenotypes and rumen samples were collected from 179 Holstein Friesian dairy cows, from 17 commercial herds spread across the Netherlands. Methane emissions were measured non-invasively using the GreenFeed system (C-Lock Inc., Rapid City, SD) depending on farm. Methane production per cow was expressed in g/day, which was used to estimate methane intensity (MI), defined here as the ratio between methane production and fat and protein corrected milk (FPCM). Genomic information from 70 cows was incorporated into the model to estimate heritability of MI. Cows were genotyped with the Eurogenomics 10K chip and imputation was routinely performed by CRV to 76,438 SNPs. The rumen fluid samples were collected on the last day of the seven-day methane measurement period using the oral stomach tube according to standard operating procedures developed at WUR (Muizelaar et al. 2020). The samples were immediately frozen on dry ice and subsequently stored in the freezer. Thereafter, microbial DNA was extracted, followed by library construction of the hypervariable region V4 (from the 16S rRNA gene). Sequencing was performed on an Illumina HiSeq platform at Genotypic Technology Pvt. Ltd. in Bangalore, India. Reads were pre-processed using QIIME2 suite v2020.8 and FAST QC v 0.11.9. The resulting Amplicon Sequence Variants (ASV) were identified using the SILVA v.138 classifier. Cows with less than 20 methane measurements during the measurement period were discarded due to unreliable methane results. The final dataset contained 48,478 ASVs for 179 samples and an average number of reads per sample of 431,476. The ASVs were rarefied to a uniform sampling depth of 81,771 reads, resulting in 42,799 ASVs. Results from the sequencing of individual rumen fluid samples were used as the microbial phenotype of each cow, in order to estimate microbiability.

Variance Components Estimation and Statistical Models. In order to test the power of our sampling strategy and chosen methodology when assessing microbial variance in our preliminary dataset, we first estimated microbiability using only the microbial data on all 179 cows – not including genotypes. A microbial relationship matrix was built with the relative abundance of ASVs as described in Ross *et al*. (2013). The relative abundance (RA_{ij}) of ASVs is considered for the elements $X_{ij} = \log(\mathrm{RA}_{ij} - \overline{\mathrm{RA}_j})$, where *i* is the rumen sample and *j* is the taxonomic unit. From that a metagenomic profile (**X**) is derived, with dimensions $n \times m$ (n is the number of rumen samples; m is the number of ASVs), which are then used to create the microbial relationship matrix, computed as $\mathbf{M} = \mathbf{XX}'/m$.

Microbiability was estimated by the following mixed model equation,

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zm} + \mathbf{Uc} + \mathbf{e} \quad (1)$$

where **y** is a vector of the trait observations; **X**, **Z**, and **U** are incidence matrices associated with the vector of fixed effects **b** of parity (1 to 4+), grazing type (barn pasture or summer barn feeding), season the microbiome sample was taken (fall, spring, summer or winter), soil type (clay, peat or sand), and days in milk, the vector of ASV effects $\mathbf{m} \sim \mathrm{N}(\mathbf{0}, \mathbf{M}\sigma_m^2)$, the vector of random farm effects caused by a common environment $\mathbf{c} \sim \mathrm{N}(\mathbf{0}, \mathbf{I}\sigma_c^2)$; and $\mathbf{e} \sim (\mathbf{0}, \mathbf{I}\sigma_e^2)$ is a vector of residuals. The terms σ_m^2, σ_c^2 σ_e^2, is for the microbial, farm, and residual variances respectively, and **I** is an identity matrix. Following Difford *et al*. (2018), microbiability was estimated as the proportion of phenotypic variance associated with differences in the microbiome, calculated as $\hat{m}^2 = \hat{\sigma}_m^2/(\hat{\sigma}_m^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2)$. The proportion of variance caused by the common environment effect of farm was estimated as $\hat{c}^2 = \hat{\sigma}_c^2/(\hat{\sigma}_m^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2)$.

For estimation of additive genetic variance for MI, a genomic relationship matrix was computed following the second version of the method proposed by VanRaden (2008) and based on the 70 genotypes available. The following mixed model was used,

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wg} + \mathbf{Uc} + \mathbf{e} \quad (2)$$

where terms are the same as in (1), with the exception of **W,** which is an incidence matrix associated with the vector of genotype effects $\mathbf{g} \sim \mathrm{N}(\mathbf{0}, \mathbf{G}\sigma_g^2)$.

In order to determine if microbial information could help refine the heritability for MI, a third model was evaluated, where both the genomic and microbial relationship matrices were fitted and farm was kept as common environmental effect, as follows,

$$\mathbf{y = Xb + Zm + Wg + Uc + e} \quad (3)$$

where the terms are as described for (1) and (2). All variance components and model effects were estimated with ASReml 4.1 (Gilmour *et al.*, 2015). Our main objective behind testing three different models was to investigate how well they would perform on our preliminary dataset. With that, we wanted to assess our the sampling strategy, what could be improved in our workflow, and what lessons we could learn for the 1000 samples we aim to collect.

Due to the limited size of our dataset and the nature of our analysis, our expectation was that our estimates would present large standard errors.

Results

Estimates for MI heritability (h^2), microbiability (m^2) and common environmental effect of farm (c^2) for the models tested are shown in Table 1. Microbiability based on microbial information from all 179 (analysis 1_a) was of 0.10 ± 0.13 (m^2 ± SE) and it was not significant. When we moved on to compute m^2 using data from cows with genotypes (analysis 1_b), the estimated value was zero. In analysis 1_a, c^2 was fairly small, 0.06 ± 0.07 (c^2 ± SE); however, once we limited the model to use only data from cows with genotypes, c^2 increased to 0.20 ± 0.19, while m^2 was zero. Heritability for MI, estimated based on 70 genotypes (analysis 2), was 0.06 ± 0.37 (h^2 ± SE) and it was not significant, while c^2 was 0.19 ± 0.19. When fitting both genomic and microbial relationship matrices (analysis 3), estimates for h^2, m^2 and c^2 were the same as the ones obtained for analysis 1_b and 2.

Table 1. Heritability, microbiability and common environmental effect estimates for the different models.

Analysis	Group size	h^2 (SE)	m^2 (SE)	c^2 (SE)
1_a	179	NE	0.10 (0.13)	0.06 (0.07)
1_b	70	NE	0 (0.00)	0.20 (0.19)
2	70	0.06 (0.37)	NE	0.20 (0.20)
3	70	0.06 (0.37)	0 (0.00)	0.20 (0.20)

h^2 = heritability for methane intensity; m^2 = microbiability for methane intensity; c^2 = common environmental effect of farm; SE = standard error; NE = not estimable

Discussion

There has been a plethora of literature over the past decade showing that there is a genetic component influencing methane production from cattle, regardless of the methane phenotype chosen (de Haas *et al*., 2017). In this study, we looked at methane intensity. Heritability was low and not significant. High SE were expected due to the limited size of our preliminary dataset. Using methane data from a combined database, where methane was measured with different equipment but where GreenFeed data was present, Manzanilla-Pech *et al.* (2021) obtained a heritability of 0.38 for methane intensity; therefore, we can speculate that our estimates will improve as our dataset grows, and the lessons we have learned by conducting this study will increase the chances of obtaining significant heritability estimates for MI.

In our study, m^2 estimates were highly confounded with c^2. We believe that due to the limited dataset, the models are not able to properly separate the variance explained by the differences in microbiome from the common environmental effect of farm. Data on the 70 cows with genotypes available came from eight different herds, and the number of cows per herd is not evenly distributed. When looking at m^2 based on 179 cows, the model more efficiently separated the microbial effect from the common environmental effect, indicating that data on more animals could help separating both effects.

With the experience of the confounding between farm and microbiome, we propose sampling on fewer farms and sampling as many cows as possible within a farm (here the number of cows per farm was smaller than the number of farms). Due to GreenFeed having a throughput limitation, we will need a method that allows for large scale phenotyping such as the sniffers, but that will mean compromising on the methane trait used. We were also limited by the ratio of cows that had both microbiome and genotype, therefore farms should be selected based on the proportion of animals in the herd already genotyped.

Conclusion

Even though more genotypes are needed in order to obtain significant h^2 estimates for MI, our results have highlighted the importance of modifying our sampling strategy for the next step of our project. The focus will be on data collection from fewer farms, increasing the number of cows per farm, in order to minimize the effect of c^2 on m^2, therefore improving our estimates of microbial effects. With that, we hope to get a deeper look into how the microbiome can be useful in explaining the variance we observe in methane emissions from dairy cows.

Acknowledgement

The research leading to these results was part of the Climate Approach project funded by the Dutch ministry for Agriculture, Nature and Food Quality (LNV) (project number BO-53-003).

References

Difford G.F., Plichta D.R., Løvendahl P., Lassen J., *et al.* (2018) PLoS Genet 14(10): e1007580. https://doi.org/ 10.1371/journal.pgen.1007580

Difford G.F., Løvendahl P., Veerkamp R.F., Bovenhuis H., *et al.* (2020) J Dairy Sci 103:2442-2459. https://doi.org/10.3168/jds.2019-16966

Gilmour A., Gogel B., Cullis B., Welham S., and Thompson R. (2015) VSN International Ltd, Hemel Hempstead, HP1 1ES, UK. www.vsni.co.uk

de Haas Y., Pszczola M., Soyeurt H., Wall E., and Lassen J. (2017) J Dairy Sci 100:855-870. https://doi.org/10.3168/jds.2016-11246

Janssen P.H. and Kirs M. (2008) Appl Environ Microbiol 74(12):3619–3625. https://doi.org/10.1128/AEM.02812-07

Malmuthuge N. and Guan L.L. (2017) Microbiome 8:8. https://doi.org/10.1186/s40104-016-0135-3

Manzanilla-Pech C.I.V., Løvendahl P., Gordo D.M., Difford G.F., *et al.* (2021) J Dairy Sci 104:8983–9001. https://doi.org/10.3168/jds.2020-19889

Muizelaar W., Bani P., Kuhla B., Larsen M., *et al.* (2020) Rumen fluid sampling via oral stomach tubing method. In: Mesgaran S.D., Baumont R., Munksgaard L., Humphries D., Kennedy E., Dijkstra J., Dewhurst R., Ferguson H., Terré M., Kuhla B. (eds) Methods in cattle physiology and behaviour – Recommendations from the SmartCow consortium. PUBLISSO, Cologne, Germany, pp. 1-6.

Roehe R., Dewhurst R.J., Duthie C.A., Rooke J.A., *et al*. (2016) PLoS Genet 12(2):e1005846. https://doi.org/10.1371/journal.pgen.1005846

Ross E.M., Moate P.J., Marett L.C., Cocks B.G., and Hayes B.J. (2013) PLoS ONE 8(9): e73056. https://doi.org/10.1371/journal.pone.0073056

VanRaden P.M. (2008) J. Dairy Sci. 91:4414-4423. https://doi.org/10.3168/jds.2007-0980

Wall E., Simm G., and Moran D. (2010) Animal 4(3):366-376. https://doi.org/10.1017/S175173110999070X

Zetouni L., Kargo M., Norberg E., and Lassen J. (2018) J Dairy Sci 101:2273-2280. https://doi.org/10.3168/jds.2017-13402