Microbiability– new insights into (genetic) modelling methane emissions of cattle

G.F. Difford[1,2], P. Løvendahl[1], J. Lassen[1], Bernt Guldbrandtsen[1] & G. Sahana[1]

[1]Center for Quantitative Genetics and Genomics, Aarhus University, Blichers Allé 20, Box 50, DK-8830 Tjele, Denmark gareth.difford@mbg.au.dk (Corresponding Author)
[2]Wageningen University & Research Animal Breeding and Genomics, P.O. Box 338, 6700 AH, Wageningen, the Netherlands

Summary

Methane produced by methanogenic archaea in ruminants contributes significantly to anthropogenic greenhouse gas emissions. Inter-individual differences in methane (CH_4) emission are affected by the individual's genetics, environment (primarily feed and fodder) and also its rumen microbiome. Unlike other economic traits, controlling microbial CH_4 production in dairy cattle through genetic selection strategies is in formative stages. Here we adapt existing quantitative methods to quantify the microbial contribution to CH_4 emission and investigate the host genetics by microbiome interaction. The heritability (h^2) of CH_4 emissions was 0.19 ± 0.09 and the estimated proportion of rumen microbial variation to phenotypic variation (microbiability) was 0.15 ± 0.08. Estimating both effects jointly revealed a small interaction between the two sources of information. The moderate correlation (0.32) between estimates of individual's genetic component and rumen microbial components confirmed this interaction. However, the correlation between an index of CH_4 estimated breeding values (EBVS) with a combined index of CH_4 EBVS and microbial values was 0.87, demonstrating that naivety of the rumen microbiome does not result in sever re-ranking of animals for this trait.

Keywords: host microbe interaction, methane emission, microbiability, rumen microbes

Introduction

Cattle and other ruminants have co-evolved specialized structures (rumen) with complex communities of mutualistic microbes inhabiting them and can be viewed as macro-organisms i.e. 'holobionts' (Zilber-Rosenberg and Rosenberg, 2008). The rumen and its microbiota enable ruminants to derive energy from high cellulose diets which would otherwise not be possible for monogastrics like humans, pigs and poultry. However, by-products of fiber digestion by bacteria, protozoa and anaerobic fungi in the rumen, include H_2 and CO_2, which are converted to CH_4 by methanogenic archaea (Hungate, 1966). Consequently, dairy cattle and other ruminants contribute greatly to anthropogenic related CH_4. With the high climate change potential of CH_4 (~ 28 times) that of CO_2 and the calorie value of (2-12% of gross energy intake), reducing CH_4 losses from rumen to the environment is an attractive option towards mitigating environmental damage and concurrent improvement to energy utilization in dairy cattle production system.

Research into CH_4 mitigation strategies at the individual level have largely focused on improved dietary formulation and identification of low CH_4 emitting animals for selective breeding (Knapp et al., 2014). However, both strategies fail to take cognizance of the interaction between host genetics, environment (feeding practice) and the ruminal

microbiome. The effects of dietary formulations appear to be transient as ruminal microbes adapt to changes in substrate (Hristov et al., 2013). Selective breeding purely on the basis of additive genetics assumes the resemblance between relatives is independent of the rumen microbiome. An assumption violated under evolutionary holobiont theory, as coevolution of the rumen and rumen microbes necessitates some degree of vertical transmission from cow to calf (Rosenberg and Zilber-Rosenberg, 2011).

The objectives of this study were to 1) test and quantify the relative contributions of the rumen bacteria and archaea to host CH_4 emission, 2) investigate the interaction between host genome and microbiome and 3) evaluate the potential benefits from multilevel selection.

Material and methods

Animal Phenotyping and Rumen Sampling:

A total of 750 lactating Danish Holstein cows fed total mixed ration from six robotic milking herds were sampled for rumen contents by esophageal insertion of a rumen flora scoop. Phenotypes for CH_4 were obtained from installing sniffers inside the feed bin of the milking robot for a duration of one week and the ratio of CH_4 to CO_2 obtained as per (Difford et al., 2016a) and converted to CH_4 L/day as per (Madsen et al., 2010a). During the week of measurement milk samples were taken as per the national recording scheme to obtain fat and protein corrected milk yield (ECM) and body weight (BW) derived from the scales in the robotic milking stations.

Rumen DNA extraction and 16s rRNA bacterial and archaeal amplicon sequencing:

DNA from rumen fluid samples were prepared following (Johnson et al., 2005). 16S rRNA libraries were constructed for bacteria and archaeal using primer sequences targeting the V1 – V3 and V4-V6 regions of the 16s rRNA gene by illumina Mi-seq / Hi-seq (GATC Biotech, Constance, Germany). Sequences with lengths longer than 300 bp were converted to operational taxonomic units (OTUs) using LotuS pipeline (Hildebrand et al., 2014) and clustered based on 97% sequence similarity. Cows with samples containing less than 50000 sequence reads were omitted as poor quality.

Variance co-variance matrices:

The rumen microbial relationship matrix (M) among cows was constructed as per (Ross et al., 2012), using the OTU tables. The matrix was computed as follows:

$$\mathbf{M} = \mathbf{XX}' / n \quad (1)$$

where **X** is the matrix of natural log transformed bacterial and archaeal relative abundance for all animals and n is the number of bacterial and archaeal OTUs within the population. Matrix **X** is derived from OTU tables after filtering out OTUs, which were absent from >50% of the samples and had no variation across individuals. The matrix **X** was subsequently scaled and centered (mean = 0, SD = 1) prior to the calculation of **M**.

The pedigree was traced back 7 generations to 1926, containing ~16 000 animals, with phantom parent groups assigned using DmuTrace and used to estimate additive genetic relationships between animals (Madsen, 2012).

Statistical Models:

The linear mixed model used to estimate additive genetic variance and microbial variance is as follows:

$$y_{ijkl} = \mu + h_j + p_k + b_1(dim_l) + b_2(e^{-0.065 \times diml}) + a_i + e_{ijkl} \quad (2)$$

where y_{ijkl} is methane production; μ is the model intercept; h_j is the herd fixed effect (j = 6 levels); p_k is the parity fixed effect (k = 4 levels); b_1 is days in milk fixed regression coefficient (dim_l = [1 - 350]); and b_2 is the Wilmink term fixed regression coefficient generated on *dim* to account for non-linearity in early lactation (Wilmink, 1987). Term a_i is individual animal's additive genetic effect NID(0, $\mathbf{A}\sigma^2_a$), where σ^2_a is the additive genetic variance and **A** is the pedigree relationship matrix; or alternatively the microbial effects NID(0, $\mathbf{M}\sigma^2_m$), where σ^2_m is the rumen microbial variance and **M** the microbial relationship matrix, and e_{ijkl} is the random residual NID(0, σ^2_e), where σ^2_e is the error variance. The analyses were performed using the DMU software (Madsen et al., 2010b).

Results and discussion

The heritability ($h^2 = \sigma^2_a / \sigma^2_p$) of CH_4 was 0.19 ± 0.09 as was consistent with previous reports in Danish Holstein cattle (Lassen and Løvendahl, 2016). The total phenotypic variance explained by rumen microbial content was estimated in analogy to heritability, as microbiability ($m^2 = \sigma^2_m / \sigma^2_p$) and was 0.15 ± 0.08 (Difford et al., 2016b). This result is similar to that of Ross *et al*., (2013) who first proposed the use of microbial relationships matrices using meta-genomic sequence data. They found a cross validation prediction accuracy of 0.47 for a small number of cows with CH_4 records, which would equate to a m^2 of 0.22. Thus the amount of phenotypic variation that can be explained by rumen microbial content is not trivial.

Microbiability is a useful metric for quantifying and contrasting microbial contributions to host phenotypes. Here we find that the microbial contribution due to rumen bacterial and archaeal microbiota is low to moderate but still comparable to that of the h^2. It is anticipated that for some host traits the microbial contribution maybe negligible and for others it may exceed that of additive genetics. For instance, in a study quantifying m^2 and h^2 for complex traits in pigs using fecal microbiota Camarinha-silva *et al*., (2017) found that for daily gain, h^2 exceeded that of m^2. However, for feed intake and feed conversion ratio m^2 exceeded that of h^2, although not significantly different. This highlights the importance of quantifying microbial contributions to complex traits.

Here we further quantified the relative change in h^2 and m^2 when estimated simultaneously to investigate the interactions between both determinants. When estimated simultaneously the m^2 decreased to 0.13 and the h^2 had a corresponding increase to 0.21. Moreover, the correlation between CH_4 EBVs and estimated microbial values (EMVs) was 0.32. These two metrics would indicate that for CH_4 emission in cattle there is some host genetic by rumen microbe interactions, likely due in part to the presence of a small proportion of microbes which are themselves heritable and some which are independent of host genetics. Such interactions were postulated under holobiont theory, where the phenotype is explained by the interaction between host genetics, microbial genetics and host environment (Rosenberg and Zilber-Rosenberg, 2011). Under holobiont theory, phenotypes affected by such interactions would require multilevel selection for both microbiome and host within specific

environmental conditions. However, in the present sample the correlation between the EBV and the EBV + EMV (multilevel selection) is 0.87, which exceeds the threshold of 0.8 for differences between breeding goals and genotype by environment interactions (Robertson, 1959).

These results indicate that the level of interaction between the rumen microbiome and the host genome is not significantly large so as to disrupt traditional selective breeding for low CH_4 emitting cows. However, identifying superior microbiomes using MBVs for trans-faunation (rumen transplant) as donors, holds potential for horizontal acquisition of beneficial microbes and unlocking of higher progress in reducing CH_4 emission.

List of References

Camarinha-silva, A., M. Maushammer, R. Wellmann, M. Vital, and S. Preuss. 2017. Host genome influence on gut microbial composition and microbial prediction of complex traits in pigs. Genetics 206:1637–1644. doi:10.1534/genetics.117.200782/-/DC1.1.

Difford, G.F., J. Lassen, and P. Løvendahl. 2016a. Interchangeability between methane measurements in dairy cows assessed by comparing precision and agreement of two non-invasive infrared methods. Comput. Electron. Agric. 124:220–226. doi:10.1016/j.compag.2016.04.010.

Difford, G.F., J. Lassen, and P. Løvendahl. 2016b. Genes and microbes, the next step in dairy cattle breeding. Page in EAAP—67th Annual Meeting.

Hristov, A.N., J. Oh, J.L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H.P.S. Makkar, A.T. Adesogan, W. Yang, C. Lee, P.J. Gerber, B. Henderson, and J.M. Tricarico. 2013. SPECIAL TOPICS-Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045–5069. doi:10.2527/jas2013-6583.

Hungate, R.E. 1966. The Rumen and Its Microbes. Academic Press, New York.

Knapp, J.R., G.L. Laur, P. a Vadas, W.P. Weiss, and J.M. Tricarico. 2014. Invited review: Enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions.. J. Dairy Sci. 97:3231–61. doi:10.3168/jds.2013-7234.

Lassen, J., and P. Løvendahl. 2016. Heritability estimates for enteric methane emissions from Holstein cattle measured using noninvasive methods. J. Dairy Sci. 99:1959–1967. doi:10.3168/jds.2015-10012.

Madsen, J., B.S. Bjerg, T. Hvelplund, M.R. Weisbjerg, and P. Lund. 2010a. Methane and carbon dioxide ratio in excreted air for quantification of the methane production from ruminants. Livest. Sci. 129:223–227. doi:10.1016/j.livsci.2010.01.001.

Madsen, P. 2012. DMU trace, A program to trace the pedigree for a sub set of animals from a larger pedigree file.

Madsen, P., J. Jensen, R. Labouriau, O.F. Christensen, and S. G. 2010b. DMU - A Package for Analyzing Multivariate Mixed Models in quantitative Genetics and Genomics. Pages 2–4 in 10th World Congress on Genetics Applied to Livestock Production.

Robertson, A. 1959. The Sampling Variance of the Genetic Correlation Coefficient. Biometrics 15:469–485.

Rosenberg, E., and I. Zilber-Rosenberg. 2011. Symbiosis and development: The hologenome concept. Birth Defects Res. Part C - Embryo Today Rev. 93:56–66. doi:10.1002/bdrc.20196.

Ross, E.M., P.J. Moate, C.R. Bath, S.E. Davidson, T.I. Sawbridge, K.M. Guthridge, B.G. Cocks, and B.J. Hayes. 2012. High throughput whole rumen metagenome profiling using

untargeted massively parallel sequencing. BMC Genet. 13:53. doi:10.1186/1471-2156-13-53.

Ross, E.M., P.J. Moate, L.C. Marett, B.G. Cocks, and B.J. Hayes. 2013. Metagenomic Predictions: From Microbiome to Complex Health and Environmental Phenotypes in Humans and Cattle. PLoS One 8. doi:10.1371/journal.pone.0073056.

Wilmink, J.B.M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. Livest. Prod. Sci. 16:335–348. doi:10.1016/0301-6226(87)90003-0.

Zilber-Rosenberg, I., and E. Rosenberg. 2008. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. FEMS Microbiol. Rev. 32:723–735. doi:10.1111/j.1574-6976.2008.00123.x.