**Application**

The identified animal genetic effects on ruminal metabolites concentrations and on the abundances of microbial genes together with their genetic correlations with methane emissions are expected to improve the accuracy of microbiome-driven breeding to reduce this highly potent greenhouse gas efficiently and cost-effectively by reliable selection of low emitting cattle.

**Introduction**

Volatile fatty acids (VFAs) in the rumen are the primary energy source for cattle and are known to be phenotypically related to CH4 emissions. Our research aimed to investigate how these ruminal metabolites are animal genomically influenced and genetically correlated with CH4 emissions. Additionally, we were interested in identifying microbial genes that are closely genetically correlated with both metabolites and CH4 emissions. The identification of the most informative biomarkers (VFAs, microbial genes) is essential for the microbiome-driven breeding strategy (Roehe et al., 2016; Martinez-Alvaro et al., 2022) and for improving our understanding of the functional regulation of the ruminal metabolite metabolism and CH4 production.

**Materials and Methods**

The animal trials were conducted following the UK Animals Act 1986 and were approved by the Animal Experiment Committee of SRUC. The data comprised of 363 steers that were deeply phenotyped (including CH4 emissions measured using respiration chambers) and genotyped using a 50k SNP chip. The animals were tested at SRUC’s Beef Research Centre across five trials and represented four breed types, with two basal diets (480:520 and 920:80 forage:concentrate ratios). In two of the trials, the feed additives nitrate and rapeseed oil were investigated. In addition, whole metagenome sequencing data of microbial DNA from rumen fluid samples taken at slaughter were available. Aligning the ruminal metagenomic sequence reads to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database resulted in the identification of 3362 microbial genes. For a subset of these animals (n = 137), VFA concentrations in the rumen fluid, collected at slaughter, were determined using HPLC. Bayesian genomic analyses were applied to estimate the heritabilities of the ruminal metabolites and their genetic correlations with CH4 emissions as well as with functional microbial KEGG genes. The genomic model included fixed environmental effects and the animal’s random genomic effects, considering 36780 SNPs. Fixed effects in the model for metabolites included trial, breed, basal diet, feed additives and as a covariable age at slaughter, whereas for CH4 emissions and microbial KEGG genes, fixed effects were the combined trial-breed-diet effects with additional consideration of a covariable, either the age entering the respiration chambers or age at slaughter, respectively.

**Results**

Estimated heritabilities of molar proportion of (iso)butyrate were at high magnitude, whereas those of acetate, propionate and (iso)valerate were at moderate level, indicating a host genomic influence on the ruminal microbial metabolism of VFAs (Table 1). The genetic correlations of the main VFAs with daily CH4 emissions (CH4d) were moderate in magnitude and associated with probabilities (*P*0) of more than 80% to be different from zero. The direction of the correlations indicates that higher molar proportions of acetate and butyrate in the rumen were genetically associated with increased CH4d. In contrast, higher proportions of propionate and valerate were genetically correlated with decreased CH4d. The genetic correlations of isobutyrate and isovalerate were close to zero, indicating that they did not relate to CH4 metabolism. The magnitude of the genetic correlations between VFAs and CH4 emissions per kg dry matter intake (CH4y) were similar to those emissions obtained on a daily basis.

One interesting microbial gene group that was moderately to highly genetically correlated with the concentration of the main ruminal metabolites was anaerobic sulphite reductase (*asr*) subunits. The abundance of the microbial KEGG gene *asrC* showed genetic correlations with acetate, propionate, butyrate, and valerate of -0.74, 0.87, -0.67 and 0.77, respectively, which were associated with *P*0 in the range of 0.95 to 0.98. Genetic correlations of equal direction and similar magnitude were also found for *asr* subunits A and B. The abundance of the *asr g*enes were negatively genetically correlated with CH4d between -0.23 to -0.41 with *P*0 ranging from 0.77 to 0.90. These results indicate that selection for increased abundances of the *asr* genes will decrease CH4 emissions by favouring ruminal propionate and valerate metabolism compared to acetate and butyrate production.

**Table 1**

 Heritabilities (h2) of ruminal metabolites and their genetic correlations (rg) with daily CH4 emissions and CH4 yield

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Trait |  | h2 | SD1 | rg with CH4d2 | *P*0 rg with CH4d | rg with CH4y3 | *P*0 rg with CH4y4 |
| Acetate |  | 0.22 | 0.18 | 0.51 | 0.84 | 0.37 | 0.75 |
| Propionate |  | 0.34 | 0.24 | -0.57 | 0.88 | -0.62 | 0.89 |
| Butyrate  |  | 0.51 | 0.26 | 0.43 | 0.82 | 0.52 | 0.87 |
| Isobutyrate |  | 0.46 | 0.27 | -0.09 | 0.58 | 0.24 | 0.67 |
| Valerate  |  | 0.36 | 0.26 | -0.50 | 0.83 | -0.41 | 0.77 |
| Isovalerate |  | 0.32 | 0.24 | -0.01 | 0.50 | 0.26 | 0.68 |

1Standard deviation of the posterior distribution of h2 (SD); 2daily methane emissions (g/d) (CH4d); 3methane yield (g/kg dry matter intake) (CH4y); 4probability that the genetic correlation is different from zero (*P*0). Heritabilities of CH4d and CH4y were 0.46 (±0.19) and 0.43 (±0.20), respectively.

**Conclusions**

The molar proportions of ruminal VFAs were found to be heritable and genetically correlated with CH4 emissions. These VFAs could be combined with the microbial gene-based microbiome-driven breeding strategy to improve its accuracy to estimate breeding values for CH4 emissions. In addition, key microbial genes (*asr* subunits), genetically correlated with both VFAs and CH4 emissions were identified, which are of high value to be directly included into microbiome driven breeding to mitigate CH4 emissions. The *asr* genes are involved in the reduction of sulphite to sulphide and might compete with methanogenic archaea for molecular hydrogen (H2).

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