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Selection response for reduced methane emissions through microbiome-driven breeding in a divergent selection experiment

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Introduction

Methane (CH4) is a highly potent greenhouse gas and ruminant livestock are among the major contributors to anthropogenic CH4 emissions. Consequently, recent efforts have focused on breeding strategies to reduce CH4 emissions, which we and others have reported to be a heritable trait (Roehe *et al*., 2016; Martínez-Álvaro *et al*., 2022a). The major challenge in reducing CH4 emissions in beef cattle is the high costs of measuring this trait accurately at scale. To address this limitation, we developed a cost-effective selection strategy for CH4 mitigation based on ruminal microbiome profiles. Within the microbiome, the abundances of the microbial genes (MGs) were identified as the most informative selection criterion for predicting directly in respiration chambers measured CH4 emissions (Roehe *et al*., 2016). By selecting the most informative MGs based on the heritability, genetic correlation with CH4 emissions and functional relevance, we developed the microbiome-driven breeding (MDB) strategy (Martínez-Álvaro *et al*., 2022a). This study showed that abundances of these MGs are under host genetic control and, according to Lima *et al.* (2024), are stable over time. The MDB approach includes the abundances of the selected MGs within a multiple-trait model to estimate the genomic estimated breeding values (GEBVs) for CH4 emissions, without the need to directly measure CH4 at scale. Simulated selection using MDB-derived GEBVs in beef cattle from SRUC's Beef and Sheep Research Centre showed substantial potential for reduction of CH4 yield (CH4Y; g CH4 per kg dry matter intake; DMI) of up to 17% per generation. To validate the effectiveness of MDB, divergent selection of British Blue sires for HIGH versus LOW estimated CH4Y GEBVs was initiated, based solely on the most informative ruminal MG profiles of their first progeny cohort. Direct measurement of CH4 emissions in the second cohort of progeny from these sires will provide a proof of principle that selection based on MG abundances is effective and can more directly target the underlying biological cause of CH4 emissions, namely excess hydrogen production, likely arising from inefficient ruminal microbial fermentation of feed.

Materials & Methods

Divergent selection was carried out based on MDB-derived GEBVs for CH4Y of British Blue sires to validate the effectiveness of this approach under practical breeding conditions. This divergent selection project was approved by the Animal Experiment Committee of SRUC and was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

Animals. The first cohort of progeny (n = 1,421) from crosses between British Blue sires and Holstein cows was performance tested for feed efficiency and growth by Genus plc on two farms. Following the performance test, rumen fluid samples were collected from the progeny using an oesophageal tube. As these offsprings were not measured for CH4 emissions, we

estimated the genetic parameters on a multi-breed population measured for CH4 emissions using respiration chambers at SRUC's Beef and Sheep Research Centre. This dataset comprised 359 beef cattle, of which 287 were measured for CH4Y and has been shown to be highly informative for estimating heritabilities of MG profiles and their genetic correlations with CH4Y (Martínez-Álvaro *et al*., 2022a). These cattle were bred and raised at the Research Centre, with individual feed intake recorded in trials balanced for breed and diet. Rumen samples were collected at slaughter. The second cohort of progeny, bred from 4 sires with HIGH and 4 sires with LOW GEBVs for CH4Y, estimated solely based on the rumen MG profiles of their first-cohort progeny, is planned to comprise 240 British Blue x Holstein crossbred cattle. These cattle are born and raised on commercial dairy farms until weaning, after which they are grouped together on a rearing farm before being moved to SRUC for CH4 emissions measurement using respiration chambers. To date, 126 cattle have been measured for CH4 emissions, on which the preliminary estimation of the selection response in this study is based.

Host genotypes and microbiome. The genetic parameter estimation using the multi-breed SRUC population was based on genotypes of 359 cattle using the 50K SNP array and 3,631 MG profiles identified from whole metagenomic sequences of microbial DNA from rumen samples collected at slaughter. The GEBVs of the British Blue sires were estimated using the genomic relationship matrix, developed from genotypes of a 35K SNP array imputed up to 70K, including 1,421 progeny with 3,594 MG profiles identified using whole metagenomic sequences of their ruminal DNA. Common 2,553 MG profiles in both datasets were used for the analysis. To address the compositionality of MG abundances, these were additive log-ratio transformed using the ribulose-phosphate 3-epimerase gene (K01783) as reference.

Genetic analyses. The heritabilities of the MGs and their genetic correlations with CH4Y were estimated by fitting a bivariate genomic model using MG abundances and CH4Y from cattle of the multi-breed population at SRUC. This Model 1 included as fixed effects the combination of breed, diet, and experiment (17 classes) as well as the random animal genomic effects (36,780 SNPs after quality control) and residual effects. Heritabilities of the abundances of 2,553 ruminal MGs in the first cohort of British Blue crossbreds were estimated using a univariate model (Model 2) including fixed effects of batch (test station entry) and sex, and random animal genomic effects (68,207 SNPs after quality control and imputation), along with residual effects.

To identify MGs with similar features in the SRUC multi-breed and British Blue crossbred population, the following criteria were applied: (i) relative abundance >0.01%; (ii) MG occupancy >70%; (iii) heritability of the MG abundances in the multi-breed population >0.20 and British Blue population >0.12; and (iv) in the multi-breed population, the sign of genetic correlation between MG abundances and CH4Y matching the phenotypic correlation. MGs meeting these criteria were ranked according to their expected correlated responses (CR) in CH4Y, predicted based on the heritability of the MG abundances, its genetic correlation with CH4Y and the genetic standard deviation of CH4Y. The 33 MGs with highest CR and putative functional relevance for CH4Y were selected for estimation of CH4Y GEBVs of sires based on data from progeny of the first cohort of British Blue crossbreds.

For estimation of GEBVs of the British Blue sires, Model 2 was extended to a multivariate model (Model 3) with 34 traits (33 MGs data and the unobserved CH4Y) and genetic and residual co(variance) of order 34 x 34 calculated based on estimated genetic and residual parameters of the MG abundances from the British Blue population and the genetic and residual correlations with CH4Y from the SRUC multi-breed population.

Preliminary selection responses were estimated as phenotypic differences between HIGH and LOW groups, or among sire progeny groups based on 126 second-cohort British Blue crossbreds with CH4 emissions measured using respiration chambers. These responses were estimated using a linear model (Model 4) including fixed effects of batch (3 classes) corresponding to entry into the four-week quarantine and adaptation period at SRUC's Research Centre, sex (two groups), respiration chamber (six classes), and alternatively either selection group (HIGH vs. LOW) or sire (four classes) of the progeny tested.

Results & Discussion

The genetic analyses based on Models 1 and 2, together with the defined requirements and the expected selection response based on the abundances of each MG, resulted in the identification of a set of MGs expected to be informative for the British Blue crossbred population in which CH4 emissions have not been measured.

Microbial genes. Based on all these analyses and requirements, 33 MGs were identified as most informative for predicting GEBVs for British Blue sires. The abundances of 12 MGs were negatively genetically correlated with CH4Y, ranging from -0.20 to -0.37, while 21 were positively genetically correlated (0.20 to 0.37). The MGs negatively correlated with CH4Y were mainly involved in amino acid transport and metabolism, indicating increased microbial growth as a hydrogen sink. Since excess hydrogen is the energy source for methanogenic archaea, its reduction by enhancing microbial growth would address directly the root cause of CH4 production while simultaneously increasing microbial protein, the main source of protein for the animal's growth and milk production. In contrast, abundances of MGs positively genetically correlated were directly related to methanogenesis, such as ferredoxin-type protein. The use of MGs rather than microbial taxa offers the advantage of capturing biological processes across the entire microbial ecosystem, including archaea, bacteria, fungi and protozoa. This is particularly important given that ruminal eukaryotic microbes remain poorly classified.

Realised selection response. The selection of British Blue sires was based on GEBVs estimated using Model 3, including the abundances of the 33 MGs and unobserved CH4Y as traits. Genetic and residual (co)variance among the abundances of the 33 MGs and the genomic relationship matrix were from the first cohort of British Blue crossbreds, while their covariances with CH4Y and its variances were estimates from the multi-breed SRUC population using Model 1. Average GEBVs for LOW and HIGH sires were -1.74 ± 0.55 and 2.54 ± 0.71 g CH4/kg DMI, respectively. Preliminary results using Model 4 based on the second cohort of 126 progeny (~50% of the total expected) showed a difference in measured CH4Y using respiration chambers between HIGH and LOW selection groups of 1.93 g CH4/kg DMI ($P = 0.09$), equivalent to 8.62% of the mean CH4Y, associated with a 4.87% ($P = 0.31$) increase in CH4/day and a 2.58% ($P = 0.61$) reduction in DMI/day. In one sire comparison, progeny from a HIGH sire produced 3.36 g CH4/kg DMI ($P = 0.04$; 15% of the mean) more than those from a LOW sire. This difference was associated with 16.3 g higher daily CH4 emissions and 0.75 kg lower DMI. These preliminary results suggest that MDB can reduce CH4Y without direct CH4 measurements by specifically targeting ruminal microbial metabolism. Although knowing the undesirable features (e.g., unpredictable responses in its components) of ratio traits such as CH4Y, at the time of starting the project, this trait was chosen to ensure that the selection response in mitigation of CH4 was not driven solely by reduced feed intake. To avoid using CH4Y, Nguyen *et al*. (2026) developed a novel MDB strategy based on newly identified MGs showing simultaneous negative genetic correlations with CH4 emissions and positive genetic correlations with growth rate. The use

of these MGs ensured that selection for CH4 mitigation did not reduce growth rate. Because this approach directly influences ruminal biological processes to reduce daily CH4 emissions, it is expected to avoid adverse effects on microbial fermentation involved in metabolism of volatile fatty acids, the main energy source of ruminants, and on microbial protein synthesis, their main protein source. In the present study, the diet was standardised; however, in practice this is often not the case. Recent findings from Saez-Torillo *et al*. (2026) demonstrates that dietary effects can be accounted for by predicting the basal diet and even its individual components from ruminal microbiome profiles.

Implementation. One of the main challenges of implementing MDB is the practical scalability of rumen samples, considering cost, logistic and adaptation. However, Lima *et al.* (2024) showed that a single rumen sample is sufficient to accurately predict CH4 emissions, with similar predictability using live or slaughter samples. In addition, these samples can be used to select MGs associated with feed efficiency (Auffret *et al*., 2020) and meat omega-3 fatty acids (Martínez-Álvaro *et al*., 2022b). Furthermore, microbial taxa abundances can be obtained and used to identify heritable biomarkers for nematode resilience (Lima *et al*., 2026) and for reduction in ruminal pathogenic bacteria (Roehe *et al*., 2024). Additionally, numerous novel metagenome-assembled microbial genomes (Stewart *et al*., 2019) provide a vast amount of information with the potential to improve ruminal and animal health.

Conclusions

The preliminary selection response in measured CH4Y of the second cohort of cattle, from sires selected based on GEBVs estimated solely from the abundances of 33 MGs in their first progeny cohort, is promising for the practical use of MDB. These results also provide preliminary, indirect insights into two fundamental questions, whether selection on MG abundances changes methanogenesis and whether these changes are heritable.

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