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Novel microbiome-driven breeding strategies to reduce methane production while avoiding adverse effects on growth

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Introduction

Animal breeding has been proposed as a potential solution for methane mitigation from ruminant livestock. However, challenges remain regarding the costly large-scale measurement of methane production (CH4P) and limited understanding of its genetic correlations with economically important traits such as average daily gain (ADG). Microbiome-driven breeding (MDB) was developed as a cost-effective strategy for methane mitigation in beef cattle (Roehe *et al.*, 2016; Martínez-Álvaro *et al.*, 2022a). This strategy is based on the abundances of the most informative ruminal microbial genes (MG) that are heritable and genetically correlated with CH4P. Nguyen *et al.* (2025) reported that CH4P is highly heritable (h^2 = 0.56±0.12) but unfavorably genetically correlated with ADG (r_g = 0.58±0.29), indicating that selection to reduce CH4P would compromise ADG. In this study, we evaluated novel developed MDB strategies designed to avoid compromising effects of selection for CH4P mitigation on ADG. In the first strategy, selection was for an increase in abundances of MGs, which were favorably genetically correlated with both traits (negatively with CH4P and positively with ADG), referred to as MDB.FOR. In the second strategy, selection was against the abundances of MGs, which were unfavorably positively genetically correlated with CH4P and negatively with ADG (MDB.AGAINST). A third strategy was the combination of the highest informative MGs used in MDB.FOR and MDB.AGAINST. These novel strategies were compared with breeding on abundances of the most informative MGs genetically correlated to CH4P without considering their correlations with ADG (MDB.CH4P).

Materials & Methods

Animals. The experiments were approved by SRUC's Animal Experiment Committee under the UK Animals (Scientific Procedures) Act 1986. Data were available from 359 beef steers across five experiments conducted under identical farming conditions at SRUC Beef and Sheep Research Centre. The cattle were from different breed types (Aberdeen Angus-Limousin crosses, Charolais-crosses, and purebred Luing), with each experiment included two of these breed types. Numbers of cattle by breeds and diets (two basal diets of forage-to-concentrate ratios of 480:520 g and 80:920 g) were balanced across the experiments. Body weight (n = 352) was recorded weekly throughout each experiment and regressed over the 56-day performance test period to obtain ADG (kg/day). After the performance test, animals were individually kept in one of the six respiration chambers for 72 hours, during which the final 48 hours were used to measure CH4P (n = 287), expressed as grams of methane per day.

Microbiome data. At slaughter (4 to 25 days after leaving the six respiration chambers, timing varied to achieve the target batch size of 24 cattle), rumen digesta samples were collected from 359 steers immediately after opening the rumen for DNA extraction and sequencing. To determine the abundance of functional MGs from the rumen DNA sequences, we used the KOunt pipeline (Mattock *et al.*, 2023). Only microbial genes that were present in at least 70%

of the samples and had a mean relative abundance > 0.001% were kept for further analyses. Additive log-ratio transformation (alr) was then applied to the MG dataset to account for compositionality of microbiome data, resulting in 3,631 MGs alr-transformed abundances.

Genetic evaluation model. Bayesian bivariate genomic analyses (37K SNPs) between the alr-transformed abundances of MGs and CH4P or ADG were carried out to obtain the heritabilities, genomic (r_g) and residual correlations among them. The model was fitted as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zg} + \mathbf{e},$$

where **y** is a vector of observations of MGs, CH4P and ADG; **b** is a vector of fixed effects, including the combined breed-diet-trial effect (17 classes), and an age covariate (age at entering the chamber for CH4P and age at slaughter for MGs; for ADG, age at start of performance test was nonsignificant and therefore not included); **g** represents the random host genomic effects, and **X** and **Z** are incidence matrices relating **b** and **g,** respectively, to the observations. All analyses were performed using GIBBSF90+ program version 3.20 and POSTGIBBSF90 program version 3.15 (Misztal *et al*., 2002).

Identification of the most informative microbial genes. The informative MGs were required to have a mean relative abundance greater than 0.1% and were selected on expected correlated response in CH4P or ADG when selection targeting each MG, using the equation:

$$CR_{Trait.j} = (i * h_j * r_{gTrait.j} * \sigma_{gTrait}) / (\bar{P}_{Trait}),$$

where $CR_{Trait.j}$ is the correlated selection response in the traits CH4P or ADG, expressed as a percentage of their corresponding phenotypic population mean ($\bar{P}_{Trait}$), after selection based on the abundance of MG *j*. For simplicity, the selection intensity *i* is considered to be equal to one; h_j is the square root of the estimated heritability of MG *j*; $r_{gTrait.j}$ is the estimated host genomic correlation between CH4P or ADG and the abundances of MG *j*, and σ_{gTrait} is the genetic standard deviation of CH4P or ADG.

The MGs were ranked by their absolute $CR_{Trait.j}$ (i.e. $|CR_{Trait.j}|$, %). For the MDB.CH4P strategy, the trait considered was solely CH4P. For the MDB.FOR and MDB.AGAINST strategies, only MGs with $|CR_{Trait.j}| > 1\%$ for each of the traits (CH4P and ADG) were considered and they were further ranked according to the sum of $|CR_{Trait.j}|$ (%) of CH4P and ADG. For each MDB strategy, the top 35 MGs were selected. In addition, we developed MDB.COMBINED, which used a combination of the highest informative MGs selected from both MDB.FOR (n = 18) and MDB.AGAINST (n = 17).

Selection responses.

Host genomic and residual (co)variances between MGs and traits obtained from the bivariate analyses were used to construct the 37 x 37 (CH4P, ADG and 35 MGs) genomic and residual matrices for each MDB strategy. To estimate genomic breeding values (GEBVs), CH4P and ADG observations were treated as missing and their GEBVs were solely predicted based on their genetic correlations with the abundance observations of MGs. In all strategies, selection was based solely on the lowest GEBVs for CH4P. Selection response in CH4P and correlated responses in ADG were estimated as the difference between mean GEBVs of selected animals and the population mean using selection proportions of 10%, 20%, and 30%.

Results & Discussion

Among the MGs used in MDB.CH4P, none overlapped with those selected for MDB.FOR or MDB.AGAINST. Considering all 105 MGs across the three selection strategies, MGs in MDB.CH4P showed $r_{gCH4P.MG}$ in the range from -0.75±0.29 to 0.80±0.19. The MGs in MDB.FOR had negative $r_{gCH4P.MG}$ values ranging from -0.71±0.27 to -0.13±0.46, whereas the

MGs in MDB.AGAINST were positively genetically correlated with CH4P ranging from 0.14±0.42 to 0.73±0.24. The heritability of these MGs varied from 0.12±0.06 to 0.50±0.15. Selection of the best animals based on $GEBV_{CH4P}$ using MDB.CH4P resulted in the largest reduction in CH4P, expressed in percentage of its mean per generation, ranging from -10.36% ($GEBV_{CH4P}$ accuracy = 0.71; 95% highest density posterior interval, HPD95%: -13.07% to -7.81%) at i = 1.16 to -17.40% (HPD95%: -21.06% to -7.81%) at i = 1.76 (Fig. 1). However, corresponding correlated responses in ADG decreased significantly from -4.12% ($GEBV_{ADG}$ accuracy = 0.69; HPD95%: -6.69% to -1.78%) to -8.34% (HPD95%: -12.14% to -5.13%) (Fig. 2).

In contrast, selection using MDB.FOR, and particularly MDB.AGAINST, led to lower CH4P reductions of -14.08% ($GEBV_{CH4P}$ accuracy = 0.59; HPD95%: -19.10% to -10.19%) and -10.56% ($GEBV_{CH4P}$ accuracy = 0.50; HPD95%: -14.97% to -5.80%), respectively, at i = 1.76 (Fig. 1), however, without any significant adverse responses in ADG (+1.81%,$GEBV_{ADG}$ accuracy = 0.55; HPD95%: -2.40% to 6.12% and +0.44%, $GEBV_{ADG}$ accuracy = 0.49; HPD95%: -3.56% to 4.65%, respectively) (Fig. 2). Combining informative MGs from MDB.FOR and MDB.AGAINST into MDB.COMBINED did not enhance methane mitigation. Surprisingly, for this strategy, the selection responses were similar to the low CH4P reductions obtained using MDB.AGAINST, at -6.65% ($GEBV_{CH4P}$ accuracy = 0.58, i = 1.16) to -10.13% (i = 1.76). The correlated response in ADG using this strategy was +1.48% ($GEBV_{ADG}$ accuracy = 0.49) at i =1.76.

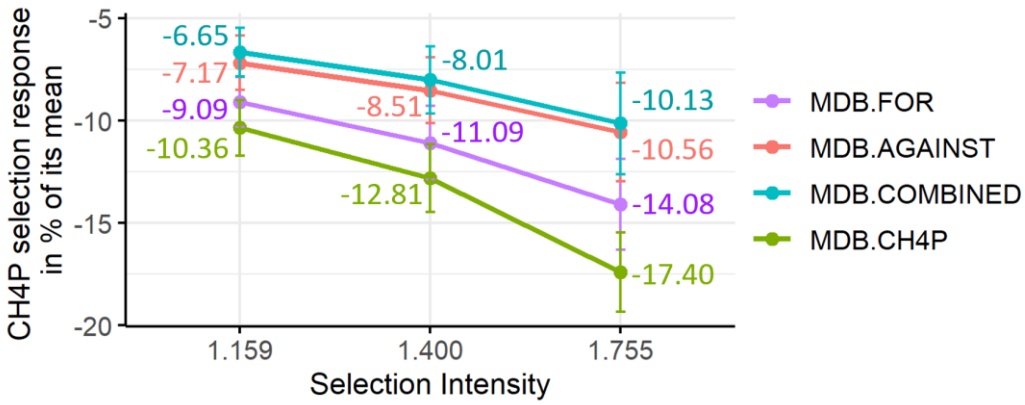

Figure 1. Selection responses (± SD) in methane production under different microbiome-driven breeding strategies, at selection intensities 1.16, 1.40, and 1.76 (selection proportion of 30%, 20%, and 10%, respectively).

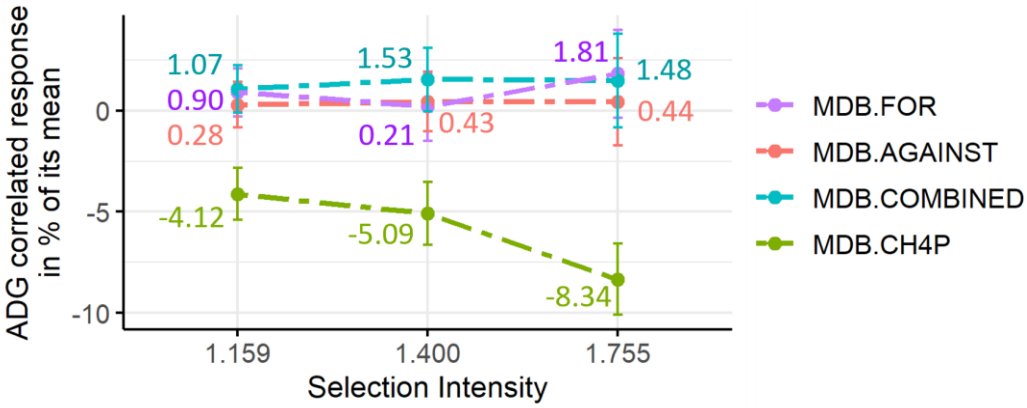

Figure 2. Correlated responses (± SD) in average daily gain under different microbiome-driven breeding strategies, at selection intensities 1.16, 1.40, and 1.76 (selection proportion of 30%, 20%, and 10%, respectively).

Although no MGs overlapped across selection strategies, shared functions were observed between MGs in MDB.FOR and those negatively associated with CH4P in MDB.CH4P. These MGs are involved in beneficial pathways that promote sufficient ruminal nutrient metabolism, including protein, amino acid and nitrogen metabolism (*amt*, *hisH*, *hisD*, *serC*, *serA*, *asnA*, *pepT*, *gltD*, *aroB*, *metA*, *gltD*, *sdaA*); carbohydrate metabolism (*amyA*, *gtrB*, *kdul*, *lplC*, *ldhA*); and vitamin biosynthesis (*cobU*, *cobT*). Similarly, common functions were identified between MGs positively associated with CH4P in MDB.CH4P and those used in MDB.AGAINST. These MGs were involvement in oxidoreductase processes (*ackA*, *coaD*, *CoaW*, *nqrA*, *nqrD*, *rnfC*, *rnfD*, *fold*, *korD*), and in the biosynthesis of essential cofactors, NADH and ferredoxin (*pnuC*, *sufE*), which promote dihydrogen formation used by methanogenic archaea to produce methane.

Among the evaluated MDB strategies, MDB.FOR, which targeted beneficial pathways toward more efficient ruminal nutrient metabolism to provide energy, protein, vitamins for animal growth, appeared to be superior to the other two strategies, which focused on selection against unfavourable MGs (MDB.AGAINST and, in part, MDB.COMBINED). Therefore, MDB.FOR is recommended for practical application of MDB to achieve sustainable genetic improvement in beef cattle by reducing CH4P without negatively impacting growth rate. The findings reinforce MDB's flexible applications, which besides others has also been shown to improve feed efficiency (Martínez-Álvaro *et al*., 2022b, Lima *et al*., 2019).

Conclusions

The newly developed MDB strategies successfully mitigated CH4P without compromising growth by targeting the abundances of specific MGs, which are involved in enhanced microbial growth as a hydrogen sink (MDB.FOR) or by selecting against abundances of MGs that predominantly contributed to methane biosynthesis (MDB.AGAINST and partly MDB.COMBINED). Among these approaches, MDB.FOR was the most effective in reducing CH4P without negatively affecting ADG. These findings indicate that selecting for beneficial pathways that improve ruminal microbial fermentation efficiency and thereby limit substrate availability for methanogenesis is more effective than directly targeting the methane metabolism pathways.

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