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Establishing an across-country breeding metagenome for methane mitigation in cattle

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Introduction

Enteric methane emissions from ruminant livestock represent a major challenge for sustainable animal production by contributing substantially to global greenhouse gas emissions. Methane is produced as a by-product of anaerobic fermentation in the rumen, where the microbial community enables cattle to convert fibrous plant material into usable energy and nutrients. Reducing methane emissions from cattle has become a priority for climate change mitigation strategies in the livestock sector.

The rumen microbiome plays a central role in methanogenesis and is increasingly recognised as an intermediate phenotype connecting host genetics and methane emissions. Several studies have demonstrated associations between specific microbial taxa and methane production. Cow genetic effects on rumen microbial composition have been reported, suggesting that microbiome composition is heritable. Several studies have estimated moderate heritabilities for individual microbial taxa and microbial-derived traits (e.g. Gonzalez-Recio et al. 2023). These findings support the concept that methane emissions could be mitigated through selective breeding strategies that target the rumen microbiome composition (Sepulveda et al., 2025). Advances in whole-metagenome sequencing have substantially improved the characterisation of rumen microbial communities by capturing bacteria, archaea, protozoa, and functional genes directly involved in hydrogen metabolism and methane production. These have strengthened evidence for genetic correlations between microbial features and methane emissions, highlighting the potential of metagenomic traits as selection criteria in breeding programs.

So far, most studies integrating host genetics, rumen metagenomics, and methane phenotypes have been conducted within single countries or production systems. Environmental conditions, management practices, diets, host breeds, and sampling methodologies differ across regions, all of which can shape microbial composition. Sepulveda et al. (2025) showed that a rumen metagenomic core could connect methane emission from different cattle populations in Australia and Spain. However, validation of rumen core metagenomes is necessary before implementation in breeding programs.

In this study, we investigate the potential for establishing an across-country breeding metagenome for methane mitigation in cattle using long-read metagenomic sequencing data. We characterize rumen microbiomes from Holstein dairy cattle in the Netherlands (NL) and Spain (ES), as well as oral microbiomes from Brahman and Brahman-cross beef cattle in Australia (AUS) to quantify taxonomic diversity across countries and production systems. Further, we estimate genetic parameters for aggregated microbial traits within and across country and the genetic correlations with methane emission. The AUS data were included to characterise cross-country microbiome diversity but were not used in genetic analyses due to the absence of methane phenotypes.

Materials & Methods

Rumen metagenome data. In NL, rumen fluid samples were collected once per cow between 2022 and 2023 for a total of 955 Holstein cows. All farms applied comparable feeding strategies based on partial mixed rations supplemented with concentrates in the milking parlour. Only lactating cows, including primiparous and multiparous animals, were sampled. Rumen fluid was obtained via oral stomach tubing. In ES, rumen content per cow was collected using a custom-built mechanical device to elevate the cow's snout, inserting a tube connected to a mechanical pump via the esophagus for 445 Holstein cows. In AUS the oral microbiome was collected via buccal swabs of 218 Brahman-cross beef cattle. DNA was extracted using the PureLink™ Microbiome DNA Purification Kit (Thermo Fisher Scientific). Long-read sequencing was performed on GridION, MinION, or PromethION platforms using R10.4.1 Reads were quality-checked with FastQC and filtered using Chopper v0.7 (minimum length 500 bp; minimum quality score 10). Taxonomic annotation was performed using SqueezeMeta software (Tamames and Puente-Sanchez, 2019) and filtered for 90% within population prevalence. Principal components (PC) analysis was carried out on taxonomic level within the NL and ES data sets as well as in a combined dataset using the function prcomp of the R package stats (R Core Team, 2022) to derive aggregated microbial phenotypes within country. The first five PC (PC1-PC5) were further used as phenotypes for genetic analysis.

Methane phenotype. For NL and ES data a methane phenotype was available. In NL, methane concentration data were available for 644 lactating Holstein cows out of the 955 cows with rumen metagenomic information. Enteric methane and CO_2 concentrations were recorded non-invasively using infrared gas sniffers (WD-WUR v2.0; Carltech BV) installed in automatic milking system (AMS) feed bins. Raw sniffer data were edited and quality-controlled following Van Breukelen et al. (2024). Visit-level records were averaged to daily values per cow. Daily records were retained if measured within ±50 days of rumen sampling; records <30 ppm methane and cows with fewer than five valid daily records were excluded. Remaining daily values were averaged to derive a single methane phenotype per cow. In ES), methane concentration was recorded for 400 Holstein cows in first or second lactation from 14 commercial farms located in Cantabria, País Vasco, Navarra, and Gerona. Methane concentration (ppm) was measured using a nondispersive infrared methane detector (The Guardian NG; Edinburgh Sensors) installed in AMS feed bins, following Hammond et al. (2016). Cows were measured at each AMS visit (2–8 visits per day) over a 2–3 week period. Eructation events were averaged per visit and minute, and subsequently across the full recording period to obtain a single methane phenotype per cow. No methane data was available for the AUS animals. These were only used to quantify inter-country variation in microbiome composition.

Genotypes. Pedigree and genotype information was available for all cows in NL and ES with methane phenotypes. Animals were genotyped using a 10K SNP array and imputed to 50K density. After quality control, 52,162 SNPs remained for analysis. In ES, cows were genotyped using the EURO12K SNP chip (Illumina) and imputed to 54,609 SNPs using BEAGLE software with a national reference population provided by CONAFE (>200,000 genotypes). For analyses involving both populations, a common panel of 39,058 SNPs shared between countries and with minor allele frequency >0.05 was used. A genomic relationship matrix was built involving all cows in NL and ES.

Statistical genetic models. Heritabilities for and genetic correlations between methane concentration and the aggregated metagenomic variables (PC1-PC5) were estimated with

GBLUP animal model in ASReml v. 4.2.1 (VSN International Ltd.). The following model was used:

$$\mathbf{y}=u+\mathbf{Z_1b}+\mathbf{Z_2a}+\mathbf{e} \quad (1)$$

where **y** is the vector of phenotypes (methane emission in NL and ES, PC1-PC5 in NL and ES); *u* is the overall mean, **b** is the vector of fixed effects: herd-year-season-AMS interaction (n=28), lactation stage (n=3), parity (n=3); **a** is the vector of direct additive genetic effects; and **e** is the vector of residual effects. The matrices $\mathbf{Z_1}$ and $\mathbf{Z_2}$ in Eq. 1 are the incidence matrix relating observations with the fixed effects and random genetic effects, respectively. Distributions of the random effects were var(**a**) = $\mathbf{G}\sigma^2$a where **G** is the genomic relationship matrix and σ^2a is the animal additive genetic variance, and var(**e**) = $\mathbf{I}\sigma^2$e, where **I** is an identity matrix of an order equal to the number of observations and σ^2e is the residual variance. Bivariate analyses were conducted to estimate the genetic parameters for all phenotypes.

Results

Microbiome composition across countries. Across the NL and ES rumen microbiome datasets, 4794 genera of microbes were found. Of these 64 % were found in both datasets while 31 % and 5 % were unique to the NL or ES dataset , respectively. The AUS microbiome data contained 5162 genera, of which 63 % were unique genera, reflecting differences in host-breed, production system and sampling method (oral versus rumen). The predominant genus across the rumen samples from the NL and ES was *Prevotella* which made up on average 37.2 % (± 7.9%) of all genera present. In the AUS samples *Moraxella* was the most present genus which made up on average 13.7 % (± 6.4%) of the reads while *Prevotella* was only 1.6 %. The 20 most abundant genera were similar between the NL and ES. In AUS however abundances of these 20 most abundant genera was much lower and five of them were not found at all (Unclassified *Succinivibrionaceae*, Unclassified *Proteobacteria*, *Fibrobacter*, Unclassified *Kiritimatiellae* and *Treponema*).

Genetic parameters. Heritability estimates for methane concentration measured using sniffers were 0.18 (± 0.11) in the NL data and 0.25 (± 0.13) in the ES data, with a genetic correlation of 0.84 (± 0.73) between countries (Table 1).

Table 1. Estimates of heritability (h^2) and genetic correlations (r_g) with standard errors between methane concentration (CH4) the first five principal components based on taxonomic level (PC) for the Dutch (NL) and Spanish (ES) dataset.

	PC1	PC2	PC3	PC4	PC5
NL					
h^2	0.36 ±0.10	0.28 ±0.10	0.22 ±0.10	0.14 ±0.08	0.31 ±0.10
$r_{g\ CH4,PC}$	0.32 ±0.32	-0.11 ±0.35	-0.07 ±0.4	0.78 ±0.41	0.12 ±0.34
ES					
h^2	0.44 ±0.14	0.01 ±0.13	0.17 ±0.12	0.09 ±0.12	0.11 ±0.14
$r_{g\ CH4,PC}$	0.29 ±0.29	n.c.[1]	-0.15 ±0.44	-0.98 ±0.66	-0.56 ±0.6

[1] not converged

Microbial principal components (PCs) showed low-to-moderate heritabilities (0.14–0.36 in NL; 0.01–0.44 in ES) and moderate-to-strong genetic correlations with methane concentration (|0.07–0.78| in NL; |0.15–0.98| in ES). Preliminary cross-country genetic correlations for microbial PCs were encouraging and ranging between |0.07–0.65| (SE 0.35-0.68), supporting the potential for sharing rumen metagenomic information across countries.

Discussion

Our study included ruminal metagenome data of 1044 Holstein cows in NL and ES and oral microbiome data of 218 Brahman-cross beef cattle in AUS. As expected, clear differences were found between the NL/ES and AUS microbiome composition due to differences in breed, production system and recording technique.

The aggregated rumen metagenome variables (PC1-5) were heritable in NL as in ES and showed genetic correlations with methane concentration in both countries. This is in good accordance with Martínez-Álvaro et al., (2022) and Sepulveda et al. (2025). These results support the central premise that microbial profiles can function as intermediate traits linking host genotype to methane output. First genetic correlations between PCs in NL and ES are promising, however, given the small dataset, standard errors are large and one correlation did not converge, results require validation in a bigger dataset. This aligns with Sepulveda et al. (2025) highlighting the need for larger datasets and across country collaboration.

Conclusions

In conclusion, rumen microbiome–derived principal components are heritable and genetically associated with methane emissions in Holstein cattle, highlighting their potential use in breeding strategies aimed at reducing methane emissions. While further validation using larger datasets is required, the Global Methane Genetics microHub project will enable this by integrating rumen metagenomic data across countries to identify a core metagenome suitable for genetic selection of low-methane-emitting cattle.

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