Rumen bacteria do not provide improved genetic evaluation of dairy traits in sheep

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Abstract

It is now widely accepted that animals' phenotypes are determined by the host genetic and the microbiome composition. For the genetic evaluation purpose, our objectives are to estimate the heritability and microbiability, and the additive metagenomic effect, assuming that these two components have additive effects. In this study were included, as dairy traits, milk production, fat and protein content; and 16S rRNA gene of rumen bacteria of 795 ewes. Two genomic models were used, a first model to predict individual microbiome contribution to phenotypes; and a second model to predict genetic effects on this predicted microbiome contribution. Results showed that the microbiabilities for milk traits were close to zero, and the residual heritabilities were not different from the trait heritabilities. By disentangling the milk traits' variance explained by the microbiome, we conclude that rumen bacteria abundances do not provide improved genetic evaluation of dairy traits in Lacaune sheep.

Introduction

The animal phenotypes are determined by the genetic component, the environmental conditions, and also by the digestive microbiome which is partially controlled by the host (Pérez-Enciso *et al*., 2021). To disentangle the holobiont impact on phenotypes, Christensen *et al*. (2021) and Weishaar *et al*. (2020) proposed models to account for intermediate -OMICs data (i.e. metabolomics, transcriptomics, and metagenomics) in genetic evaluation. In polygastrics, the rumen microbiome is crucial, as it allows ruminants to digest plant fibre. Bacteria are able to degrade and ferment fibrous feedstuffs into nutrients, directly usable by the animal for maintenance, growth and lactation. Considering the relevance of rumen fermentation products in the mammary gland, and having demonstrated that rumen bacteria are controlled by host genome, we propose to apply the Christensen *et al*. (2021) methodology on a Lacaune dairy sheep dataset including metabarcoding (16S rRNA gene) as intermediate -OMIC trait, and dairy traits as phenotypes. The objectives are to estimate variance components, as heritability (h^2) and microbiability (m^2), and the additive part of the predicted metagenomic effect.

Materials & Methods

Animals phenotyping. Data from 795 multiparous Lacaune dairy ewes were obtained from INRAE Experimental Unit of La Fage (UE 321 agreement A312031, Roquefort, France) between 2015 and 2019. All ewes (weighing 77 kg on average) raised indoors, and fed 93% meadow hay and silage plus 7% of concentrates diet (on dry matter basis). Daily milk production (MP), milk fat and protein contents (FC and PC, respectively) as weighted averages, were recorded as part of one official milk recording of the flock. In brief, two milk samples per animal for morning and afternoon milking were sent for analysis at the Interprofessional Milk Analysis Laboratory (Aurillac, France). Milk FC and PC were recorded for 777 ewes, and analysed with mid-infrared techniques with a Milko-Scan™ FT6000 instrument (Nanterre,

France). Rumen sampling was performed within 3 days on morning or afternoon around the official milk recording of the flock. Ruminal content was sampled from each ewe using a vacuum pump and a medical gastric tube. The protocol received approval from the French Ministry of Higher Education, Research and Innovation – Animal Ethics Committee (approval number: APAFIS#6292-2016080214271984 v8). After extraction, the DNA strands of the 795 samples were sequenced using v3-v4 region of 16S rRNA gene and processed with the FROGS 3.0 pipeline (Escudié *et al*., 2018), removing operational taxonomic units (OTUs) with average abundances below 0.005%. As result, we obtained an abundance table with 2,059 OTUs.

Animals genotyping. DNA extraction from blood samples and genotyping were performed for 795 Lacaune ewes. From those ewes, 743 were genotyped using a medium-density SNP chip (Illumina Ovine SNP50 BeadChip, 54,241 SNPs), 314 at Laboratoire d'Analyses Génétiques pour les Espèces Animales (Jouy-en-Josas, France) and 429 at Aveyron-Labo (Rodez, France). The remaining 52 ewes were genotyped with a low-density SNP chip (Illumina Ovine SNP15, 16,681 SNPs) at Neogen (Lansing, USA) and imputed to a medium-density SNP chip genotypes in the framework of the Lacaune dairy sheep genomic selection program (Larroque *et al*., 2017). SNPs were removed using a call rate <0.99 for SNPs, and <0.95 for individuals, and minor allele frequency <0.05. In total, 35,825 SNPs remained for the analyses.

Statistical analyses. The methodology used was that proposed by Christensen *et al*. (2021).

1) The first model for predicting individual contributions of metagenomics to phenotypes:

$$\mathbf{y} = \mathbf{Wb} + \mathbf{m} + \mathbf{a_r} + \mathbf{e}$$ ~~$\mathbf{y=Wb+Z_1m+Z_2a+e}$~~ (1)

where; **y** is the vector of observations for the dairy traits; **b** is the vector of fixed effects; **m** is the vector of random rumen bacteria effect (metagenomic effect); $\mathbf{a_r}$ is the vector of random additive effects; and **e** is the vector of random residual effects. **W** is the incidence matrix for **b**.

2) Second model used estimates of **m** to predict the additive part of the predicted metagenomic effect:

$$\hat{\mathbf{m}} = \mathbf{W\beta} + \mathbf{g} + \boldsymbol{\varepsilon}$$ (2)

where; $\hat{\mathbf{m}}$ is the predicted effects **m** in Equation 1; **β** is the vector of fixed effects; **g** is the vector of random additive effects; and **ε** is the vector of random residual effects. **W** is the incidence matrix for **β**. The same fixed effects (P<0.05) were included in both models: sampling year, days in milk (DIM), and litter size. The distributional assumptions are $\mathbf{m} \sim N(\mathbf{0}, \mathbf{M}\sigma^2_m)$, $\mathbf{a_r} \sim N(\mathbf{0}, \mathbf{G}\sigma^2_{a,r})$, $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_e)$, $\mathbf{g} \sim N(\mathbf{0}, \mathbf{G}\sigma^2_g)$, $\boldsymbol{\varepsilon} \sim N(\mathbf{0}, \mathbf{I}\sigma^2_\varepsilon)$, where **M** is the microbial similarity matrix, **G** is a genomic relationships matrix (VanRaden, 2008), **I** is an identity matrix; σ^2_m is the metagenomic effect variance, σ^2_a and σ^2_g are additive variances, and, σ^2_e and σ^2_ε are residual variances. The microbiability was estimated as $m^2 = \sigma^2_m/\sigma^2_y$, the residual heritability as $h^2_r = \sigma^2_{a,r}/\sigma^2_y$, and the heritability of metagenomic effect as $h^2_m = \sigma^2_g/(\sigma^2_g + \sigma^2_\varepsilon)$. These variance components were estimated using BLUPF90test (Misztal *et al*., 2002) with OPTION method VCE. Thus, following the formula $h^2 = m^2h^2_m + h^2_r$ presented by Christensen *et al*. (2021), the heritability of the phenotype is decomposed into a metagenomic mediated heritability ($m^2h^2_m$), and residual heritability (h^2_r).

3) **M** matrix construction

The matrix was computed as a variance-covariance matrix from rumen bacteria abundances as follows:

$$\mathbf{M=XX'/n}$$ (3)

where; **X** is the abundance matrix with **n**=2,059 OTUs with zeros corrected with the geometric Bayesian-multiplicative (GBM) method (Martín-Fernández *et al*., 2015), and centred log-ratio (CLR) transformed, for the 795 ewes. OTU abundances were pre-corrected for the significant fixed effects (for more than 10% of OTUs), such as lactation number, sequencing run, and

sampling time and order, all of them nested on sampling year, and total number of sequences per rumen sample. Then, the similarity among animals in rumen bacteria composition was estimated by constructing a microbial similarity matrix **M** as in Ross *et al*. (2013).

Results

The decomposition of the parameter estimation using the two proposed models is shown in Table 1, where we see that the microbiability is only different from zero for milk protein content. Although, the heritability of metagenomic effect is different from zero for MP and PC, but only the heritability of PC is slightly higher than the estimated residual heritability. For MP and FC, we obtained a null microbiability leading to heritability of phenotypes equal to residuals ones.

Table 1. Estimates of residual heritability (h^2_r), microbiability (m^2), heritability of metagenomic effect (h^2_m), and heritability of phenotype (h^2).

Trait	h^2_r (SE)	m^2 (SE)	h^2_m (SE)	h^2
Milk production (ml)	0.250 (0.068)	0.00	0.083 (0.058)	0.250
Fat content (g/ml)	0.533 (0.066)	0.00	0.00	0.533
Protein content (g/ml)	0.515 (0.070)	0.040 (0.037)	0.046 (0.052)	0.517

SE= standard error.

Discussion

The heritability estimates obtained were similar to Rupp *et al*. (2003) with 0.28 to 0.32 for annual MP, and 0.41 and 0.51 for annual FC and PC, using all Lacaune dairy ewes (up to 90,000 animals). This is the first estimate of the rumen microbiome contribution to the variability of milk traits, using the largest sample size from a single population of dairy ruminant. The variances explained by rumen bacteria (m^2) were close to zero for most dairy traits, and the genetic variances (h^2_m) were lower than 9%, which is consistent with the weak phenotypic correlations between milk traits and rumen bacteria demonstrated by Martinez Boggio *et al*. (2021). The higher m^2 of PC could be explained by the key role of rumen bacteria in the production of essential amino acids needed for milk proteins (Xue *et al*., 2019). Our estimates were similar to those presented for dairy cows by Buitenhuis *et al*. (2019) for milk fat (0.08 ± 0.07) and protein (0.08 ± 0.09) contents, and Zhang *et al*. (2020) for methane emissions (0.07). Despite this, it is interesting that with the method proposed by Christensen *et al*. (2021) we can decompose the heritability, and identify which part of the genetic variance was explained by the rumen microbiome, and which was directly explained by the host genetic (residual heritability). In our study, as shown by the low microbiabilities obtained, most of the variance is not explained by the rumen microbiome, even if the M matrix is constructed by omitting OTUs with zero, or considering those present in at least 50% of the animals. But we could further study other traits such as milk fatty acids and proteins, for which preliminary results showed positive microbiabilities. So far, we can conclude that with our dataset, and the low genetic variance explained by metagenomic data, the rumen bacteria abundances do not provide improved genetic evaluation for dairy traits in sheep.

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