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Can microbiome improve genetic evaluations? Comparison between GBLUP and GO-BLUP in commercial beef populations

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Introduction

Genetic improvement in cattle depends on the accurate prediction of estimated breeding values (EBVs) for economically important traits. Genomic selection has substantially increased EBV accuracy relative to pedigree-based methods, particularly for traits that are difficult to measure or recorded at slaughter (Pimentel and König, 2012). The increasing availability of intermediate omics traits, such as microbiome data, has prompted the development of strategies to integrate this information into genetic evaluation models. These include multi-trait best linear unbiased prediction (BLUP) models using a subset of microbial abundances as correlated traits (Martínez-Álvaro *et al.*, 2024), which is computationally demanding and requires the subset preselection. An alternative is the use of the microbiome as an additional random effect in BLUP, assuming a structured covariance matrix between individuals defined by distances between their full microbiome profiles (Lakamp *et al.,* 2025). As formulated, these models ignore the portion of the microbiome variation that is under host genetic control. To address this limitation, Weishaar *et al.* (2019) proposed decomposing EBVs into direct host genetic and microbiome-mediated components, later generalized by the genomic-omic BLUP (GO-BLUP) framework (Christensen *et al.*, 2021), which models the genetic component captured by omics traits in a two-step strategy. The rumen microbiome supplies ~70% of energy via volatile fatty acids and 50–80% of amino acids, thereby closely impacting feed conversion efficiency, growth, as well as health, and body composition. Incorporating metagenomic data into genetic evaluations of beef can increase EBV accuracy of growth traits as shown by Martínez-Álvaro *et al.* (2024) using the multi-trait BLUP model, although an alternative implementation and validation using novel GO-BLUP methodology has not yet been tested. The objective of this study was to evaluate the EBVs accuracy improvement for carcass and feed efficiency traits when microbiome information is included, relative to genomic information alone, in ~1,450 commercial crossbred calves with phenotypes, genomic and whole metagenome data using GO-BLUP.

Materials & Methods

Animals. Data were obtained from 1,458 British Blue x Holstein crossbred calves (855 females and 603 males) balanced along 26 batches and two farms: Warwickshire, UK (n=1073, batches 1-21) or Hereford, UK (n=385, batches 22-26) during 2018-2021. All diet components were determined by calorimetric and chemical methods. Animals were genotyped using the GGP Bovine uLD 35K array (Neogene, Cambridgeshire, UK) and imputed up to 70K. Haplotype phasing was performed with Eagle (v2.4.1) and imputation was performed using Impute (v2). SNPs were filtered when their call rates were <95% or minor allele frequency <0.05, and animals were removed when call rates were <90%, keeping 1,458 genotypes and 65,782 SNPs for the analyses.

Performance traits record. Of 1,458 genotyped calves, performance traits were available for 1,421 of them. At 212±33 days of age, cattle were assessed for feed intake during 39±3.8 days using Growsafe (GrowSafe Systems Ltd., Airdrie, Canada) and Integrado (Betim, Brazil) electronic feeding equipment. Daily dry feed intake (DFI) was estimated by correcting fresh intake for the % of dry matter intake in the diet. Animals were weighed at the beginning (average weight=258±50 kg) and at the end (316±54 kg) of the feeding trial. Their average daily weight gain (ADG) was calculated as the slope from a regression model of weight on day. Feed conversion ratio (FCR; kg dry matter/kg weight) was calculated as the ratio between DFI and ADG. Residual feed intake (RFI) was estimated by correcting DFI by the metabolic weight (mid weight between the beginning and the end of the test to the power of 0.75), ADG, and fat depth (mm) at 12^{th}-13^{th} rib (SFT). Eye muscle area (EMA), SFT and intramuscular fat (IMF, %) were measured by ultrasound scan at the end of the test; and carcass weight was recorded at slaughter (CW).

Collecting and sequencing ruminal microbial DNA samples. Ruminal microbial DNA was available for 1,398 of the 1,458 genotyped animals. Five mL of rumen fluid were collected through an esophageal tube at the end of the feeding trial and DNA was extracted as described in Martinez-Alvaro *et al.* (2024). 383 samples were sequenced at a depth of 85M average reads/sample, and 1,015 at 18M reads/sample. DNA Illumina TruSeq libraries were prepared from genomic DNA and sequenced on NovaSeq by Edinburgh Genomics (Edinburgh, UK). Paired-end reads (2x150 bp) resulted in 15.7-58.9 Gb/sample.

Taxonomic assignation. Metagenomic reads were quality trimmed using Fastp, and host-derived sequences were removed by mapping against the *Bos taurus* reference genome from Ensembl (ARS-UCD2.0) using Bowtie2 v2.5.4. The filtered reads were subsequently processed with Sylph (Shaw and Yu, 2025; parameter c = 200) for taxonomic assignment. Taxonomic classification was performed using the GTDB v-R226 and the RefSeqFungi databases (fungi-refseq-2025-10-11-c200-v0.3), and complemented with a custom protozoal database specifically developed for ruminal microbial studies (available at github.com/santisaeztorillo/Rumen_Protozoa_Ref). In total, 1,122 microbial genera (MGs) were identified. After applying a 70% occupancy threshold, we kept 131 MGs for downstream analyses, considered the core microbiome (Tovar-Herrera *et al.* 2025).

Estimation of genetic parameters of performance traits. ADG, DFI, IMF, FCR, RFI, SFT and CW were fitted with GBLUP and GO-BLUP univariate models. All performance traits models included sex and batch as fixed effects, diet composition variables (acid and neutral detergent fibre, starch, crude protein, and gross energy) and age as covariates (Saez-Torillo *et al.* 2026). GBLUP model included a genomic random effect distributed as $N(0, \mathrm{GRM}\sigma^2_{\mathrm{gGBLUP}})$, with GRM being the genomic-relationship matrix and $\sigma^2_{\mathrm{gGBLUP}}$ the genomic variance. GO-BLUP model was fitted in two steps. First a GBLUP with a genomic and a microbial random effect (Christensen *et al.* 2021) was fitted, distributed as $N(0, \mathrm{GRM}\sigma^2_{\mathrm{gdirect}})$ and $N(0, \mathrm{MRM}\sigma^2_{\mathrm{m}})$, with $\sigma^2_{\mathrm{gdirect}}$ being the non-microbiome mediated or direct genomic variance, σ^2_{m} the microbiome variance and MRM the metagenomic relationship matrix built as the cross-product of the chi-squared-transformed relative abundance matrix divided by the mean of its diagonal. Estimated microbial values were fitted as a phenotype in a second model including sequencing depth as a fixed effect and a genomic effect distributed as $N(0, \mathrm{GRM}\sigma^2_{\mathrm{gomics}})$, with $\sigma^2_{\mathrm{gomics}}$ being the microbiome-mediated genomic variance, to disentangle their genetic and environmental parts. As model variance components from GO-BLUP we provide the direct ($h^2_{\mathrm{d}} = [\mathrm{GRM_d} - \mathrm{GRM_{all}}]\sigma^2_{\mathrm{gdirect}}/\sigma^2_{\mathrm{y}}$) and microbiome-mediated ($h^2_{\mathrm{m}} = [\mathrm{GRM_d} - \mathrm{GRM_{all}}]\sigma^2_{\mathrm{gomic}}/\sigma^2_{\mathrm{m}}$) heritabilities, microbiability ($c^2 = [\mathrm{MRM_d} - \mathrm{MRM_{all}}]\sigma^2_{\mathrm{m}}/\sigma^2_{\mathrm{y}}$) and total heritability ($h^2_{\mathrm{t}} = c^2h^2_{\mathrm{m}} + h^2_{\mathrm{d}}$); and compared them with regular GBLUP heritability

(h^2_{GBLUP} = [GRM_d - GRM_{all}]σ^2_{gGBLUP}/ σ^2_y), where GRM_d and MRM_d denote the average diagonal of GRM and MRM matrices and GRM_{all} and MRM_{all} the average of GRM and MRM matrices. Additionally, a ridge regression GO-BLUP (RR-GOBLUP) was also fitted with MG as covariates in the first model and as traits in the second model to investigate the contribution of each MG to the final σ^2_{gomics} (Method 1, Christensen *et al.* 2021). The contribution was quantified by rescaling each single omic-mediated EBV by its corresponding regression coefficient estimated in the first model, calculating the resulting variance, and normalizing it by the total omics variance (σ^2_{gomics}). Residuals were assumed to be independent and normally distributed, and random effects uncorrelated. All models were fitted using Bayesian statistics in BGLR R package and MCMC chains had 12,000 samples, burn-in: 2,000 and 1/10 lag. The predictive ability (PA) of EBVs from GBLUP and GO-BLUP was compared using external validation with 100 training (80%)–testing (20%) data partitions. In each iteration, phenotypes of validation animals were masked and predicted. PA was calculated as the correlation between corrected phenotypes and final EBVs in the test set. For GO-BLUP, total EBVs were obtained as the sum of direct and microbiome-mediated EBVs.

Results & Discussion

Variance components for all traits were estimated with GBLUP and GO-BLUP (Table 1). The $\mathbf{h^2_t}$ ranged from 0.19 to 0.34 and were similar to the $\mathbf{h^2_{GBLUP}}$ (0.21-0.34). Under the GO-BLUP model, $\mathbf{h^2_m}$ ranged from 0.16 to 0.31, whereas microbiability estimates were low (0.01–0.06), indicating that most of the genetic variation was explained by non-microbiome-mediated genomic effects. In all cases, GO-BLUP and RR-GOBLUP produced similar variance components and solutions (results not shown).

Table 1. Mean and 95% confidence interval for variance components[1] of commercial traits.

Model	GBLUP	GO-BLUP			
Trait	h^2_{GBLUP}	h^2_d	h^2_m	c^2	h^2_t
ADG	**0.22**[0.14-0.30]	**0.18**[0.11-0.26]	**0.31**[0.21-0.42]	**0.04**[0.02-0.09]	**0.19**[0.13-0.28]
DFI	**0.33**[0.24-0.44]	**0.30**[0.21-0.40]	**0.24**[0.15-0.33]	**0.06**[0.02-0.14]	**0.31**[0.22-0.41]
EMA	**0.28**[0.19-0.39]	**0.25**[0.17-0.35]	**0.31**[0.22-0.43]	**0.03**[0.01-0.07]	**0.26**[0.18-0.36]
SFT	**0.24**[0.15-0.35]	**0.22**[0.11-0.33]	**0.16**[0.10-0.25]	**0.01**[0.00-0.03]	**0.22**[0.12-0.33]
FCR	**0.21**[0.14-0.31]	**0.18**[0.11-0.28]	**0.28**[0.18-0.39]	**0.02**[0.01-0.05]	**0.19**[0.11-0.29]
IMF	**0.28**[0.20-0.39]	**0.25**[0.16-0.35]	**0.21**[0.13-0.33]	**0.01**[0.01-0.03]	**0.25**[0.17-0.35]
RFI	**0.28**[0.19-0.39]	**0.26**[0.16-0.37]	**0.16**[0.10-0.23]	**0.03**[0.01-0.09]	**0.27**[0.17-0.38]
CW	**0.34**[0.24-0.45]	**0.32**[0.22-0.43]	**0.24**[0.16-0.34]	**0.05**[0.02-0.10]	**0.33**[0.24-0.44]

[1] $\mathbf{h^2_d}$ direct heritability, $\mathbf{h^2_m}$ heritability of the microbiome, $\mathbf{c^2}$ microbiability and $\mathbf{h^2_t}$ total heritability.

In terms of PA in animals with masked phenotypic records, GO-BLUP outperformed GBLUP for ADG, DFI, and FCR. For ADG, PA increased from 0.16 to 0.21 (20% improvement; $p < 0.001$); for DFI, from 0.25 to 0.27 (8% improvement; $p = 0.003$); and for FCR, from 0.15 to 0.17 (11% improvement; $p = 0.015$). No relevant differences were observed for the remaining traits, with similar PA values between models: EMA (0.20–0.21), SFT (0.18), IMF (0.12–0.13), RFI (0.23), and CW (0.33–0.34) for GO-BLUP and GBLUP, respectively. The PA improvement in ADG of 20% agrees with the reliability-based accuracy improvements reported by Martínez-Álvaro *et al.* (2024) of 11-22%, when comparing a multi-trait GBLUP including ADG and a reduced set of microbial genes vs. only ADG, fitted with a multi-breed beef population of 359 animals and without data partitions. In another multi-breed beef population of 717 animals, Lakamp *et al*. (2025) reported higher prediction

accuracies for ADG (0.08–0.11 to 0.12–0.16) and DFI (0.15–0.21 to 0.22–0.28) when including microbiome effects in GBLUP model, consistent with our results. The improvement on FCR PA is expected given the improvements in ADG and DFI.

Contribution of each MG to the genetic prediction. Across traits, *UBA2810* (phylum Pseudomonadota) consistently accounted for the largest proportion of σ^2_{gomics}, explaining 24% for ADG, 10% for DFI, and 20% for FCR. *Dialister* was the second most relevant MG, contributing 6%, 4%, and 4.7% for ADG, DFI, and FCR, respectively. For ADG, an additional nine MGs (including *CAG-791*, *Succiniclasticum*, and *Prevotella*) each explained between 2 and 3.2% of the variance (Figure 1A). In DFI, other notable contributors included *Colimorpha*, *UBA6985*, *UBA2897*, *Intestinibaculum*, *UBA629*, and *Physcousia*, each accounting for 3–4%. Similarly, for FCR, MGs such as *Physcousia*, *Weimeria*, and *Prevotella* explained between 3.2 and 4.7% of the variance. *UBA2810* belongs to the family Succinivibrionaceae, a group of amylolytic, propionate-producing bacteria previously associated with variation in growth-related traits in calves (Omontese *et al*., 2022).

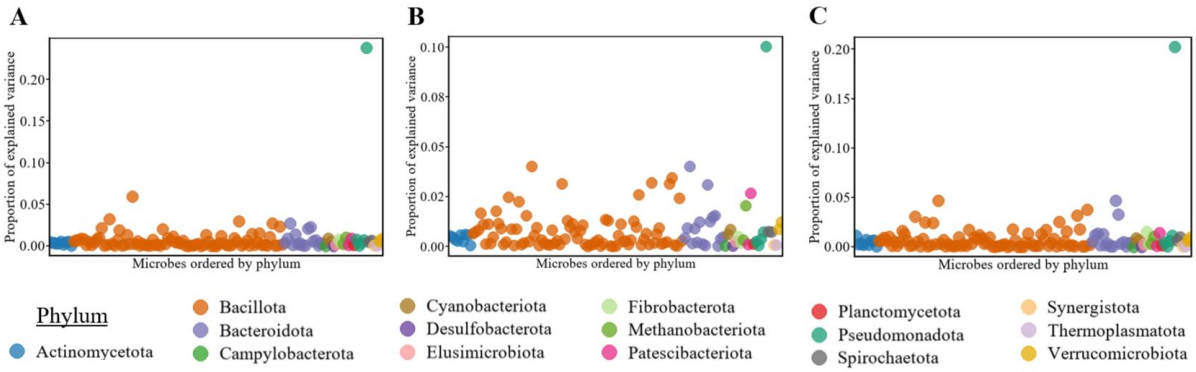

Figure 1. Proportion of genetic variance explained for each MG coloured by phylum for ADG (A); DFI (B); FCR (C).

Conclusions

Including microbiome information in genomic evaluations using a GO-BLUP strategy increased PA for ADG, DFI, and FCR by 20%, 8%, and 11%, respectively, compared with GBLUP under a masked-phenotype validation scheme. The MGs explaining the largest proportion of omics-mediated genetic variance were *UBA2810* and *Dialister* for ADG, DFI and FCR. No clear advantage of GO-BLUP was observed for CW, RFI, EMA, SFT, or IMF, indicating trait-specific benefits of this approach.

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