Multi-breed multi-trait single-step genomic predictions for Holstein and Jersey including crossbred animals

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Abstract

Crossbreeding exploits heterozygosity and is increasingly adopted in dairy cattle. However, genomic selection for crossbred animals is challenging due to difficulties in establishing suitable multi-breed reference populations and modelling missing pedigree information. This study aimed to investigate the benefits of multi-breed multi-trait single-step genomic evaluations that jointly analyse New Zealand data from two purebred populations (Holstein and Jersey) and a derived crossbred population (XBD). We also investigated the impact of modelling missing pedigree information using genetic groups (GG) or metafounders (MF). Pedigree (1.1M), genotypes (127K), and individual phenotypes for calving season days (deviation between planned and actual calving date, CSD; ~370K records) and 305-days milk yield (MY; ~538K records) were available for purebred and crossbred animals. Six scenarios were implemented: A) a single-step evaluation per breed, each using phenotypes of all breeds treated as a single trait, but only genotypes of the respective breed, and 255 GG; B) a joint evaluation using the genotypes of all breeds, with phenotypes and GG as in A; C) as B but grouping all GG into only 4 GG; D) as B but replacing all GG by MF; E) as B but replacing all GG by only 4 MF; F) as B but with phenotypes from different breeds treated as separate correlated traits. CSD and MY were jointly analysed in a multi-trait model in all scenarios. Validation statistics were computed for both purebred and XBD genotyped cows and bulls born in recent years. Scenarios using all purebred and XBD genotypes had higher accuracies than the scenario analysing each breed separately. Using all genotypes and modelling traits across breeds as different traits showed the highest accuracy among all scenarios for MY but the lowest for CSD. Reducing the number of GG gave similar results to using all GG. Moving from GG to MF had limited benefits. Overall, results showed that combining Holstein, Jersey, and the derived XBD data into multi-breed single-step evaluations can enhance the accuracy of genomic predictions for both purebred and crossbred animals.

Key words: multi-trait, multi-breed, genomic predictions, single-step, crossbreeding, dairy cattle

Introduction

In dairy cattle, high emphasis on functional traits, such as fertility and health, and longevity-related traits have contributed to an increase in the number of crossbred animals (Sørensen et al., 2008; Winkelman et al., 2015; VanRaden et al., 2020; Harris, 2022). Crossbreeding is increasingly adopted as it allows to take advantage of heterosis and breed complementary (Sørensen et al., 2008), next to reducing issues connected to inbreeding and loss of genetic diversity, which are increasing in different cattle breeds such as in Holstein-Friesian populations (e.g., Doekes et al., 2018; Makanjuola et al., 2020; Ablondi et al., 2022).

Single-step genomic prediction approaches allow combining pedigree, genomic, and phenotypic data into a single evaluation (Legarra et al., 2014). Multi-breed genomic evaluations that combine data from different populations and crossbred animals may allow

for more efficient use of collected data and the simultaneous prediction of genomic estimated breeding values (GEBVs) of both purebred and crossbred animals. However, genomic predictions including different populations and crossbred are challenging due to difficulties in establishing a suited reference population (Khansefid et al., 2020; van den berg et al., 2020; Cesarani et al., 2022). Single Nucleotide Polymorphism (SNP) and Quantitative Trait Loci (QTL) effects may differ between purebred and crossbred animals due to differences in their genetic background, environmental conditions (which can lead to genotype-by-environment interactions), and differences in linkage disequilibrium between SNP and QTL (Vandenplas et al., 2016). Thus, designing and validating multi-breed genomic predictions is crucial to ensure that data from different populations are efficiently combined into a single-step approach.

Multi-breed single-step evaluations combine genomic information from purebred and crossbred animals next to complex pedigree information in which individuals may have missing parental information. Unknown parents of individuals with missing parental information are assumed to come from the base population and therefore assumed to be unselected, unrelated, and having the same genetic level (Schaeffer, 2019). Due to selection, these assumptions are violated, especially when animals originate from different populations, countries, or breeds, as different genetic levels among individuals are expected. Genetic groups can be used in single-step models to model differences in the genetic levels of unknown parents (Masuda et al., 2022). An alternative approach to genetic groups is the use of metafounders, as proposed by (Legarra et al., 2015), which can also be implemented in multi-breed single-step evaluations. In addition to genetic groups, the concept of metafounders can model the relationships within and across different base populations of different breeds.

In this study, we aimed to investigate the benefits of multi-breed multi-trait single-step genomic predictions that jointly analyse two purebred populations (Holstein and Jersey) and a derived crossbred population. In particular, we aimed to investigate the benefits of multi-breed genomic evaluations for both purebred and crossbred animals and to investigate the impact of modelling missing pedigree information using genetic groups (GG) or metafounders (MF).

Materials and Methods

Data available

Pedigree information was available for 1,151,801 dairy cattle animals from New Zealand. The population included purebred animals (≥87.5% of breed composition) for Holstein (HOL) and Jersey (JER) populations, and a derived crossbred population (XBD). The XBD population was composed of animals defined as having at least 50% of their breed composition as HOL or JER, and <87.5% HOL or JER. The pedigree had a total of 255 GG defined based on the breed and the year of birth of the animal.

Table 1. Pedigree size, number of phenotypes (for whole and partial datasets), number of genotypes, and number of validation animals per breed.

Breed [a]	Pedigree	Phenotypes [b] (whole)		Phenotypes (partial)		Genotypes	Validation animals	
		CSD	MY	CSD	MY		Cows	Bulls
HOL	341,215	140,441	207,905	118,911	169,911	46,610	8,953	353
JER	141,012	51,489	74,456	44,160	61,412	22,842	3,827	168
XBD	395,976	177,713	255,357	146,389	202,515	57,852	13,713	129
Other	273,598	-	-	-	-	-	-	-
Total	1,151,801	369,643	537,718	309,460	433,838	127,304	26,493	650

[a] HOL = Holstein, JER = Jersey, XBD = crossbred. [b] MY = Milk Yield, CSD = Calving Season days.

Individual phenotypes were available for first parity cows on one reproduction and one production trait: Calving Season Days (CSD) and 305-day milk yield (MY), respectively. CSD is defined as the (positive or negative) deviation in the number of days from the planned start of calving date to the actual calving date for a given herd-year. The number of phenotypes available in each breed is reported in
Table *1*. For both traits, most of the recorded phenotypes were available on XBD animals (~48% of the total), followed by HOL (~38%) and JER (~14%). All cows had a record for MY, and ~68% of them had a record for CSD.

A total of 127,304 genotypes were available at 85,394 SNP density.
Table *1* reports the number of genotypes available per breed. Overall, 45%, 37%, and 18% of the genotypes were from XBD, HOL, and JER, respectively.

Scenarios

Six scenarios were investigated to implement multi-breed single-step genomic predictions including both purebred and crossbred animals. All scenarios used the full pedigree and always analysed CSD and MY jointly with a multi-trait approach. The first 3 scenarios used 255 GG and are described below:

- **SINGLE**: three separate evaluations were conducted, each using the phenotypes of all breeds treated as a single trait, but only genotypes of the respective breed, i.e., only HOL, JER, or XBD.
- **ALL**: a multi-breed evaluation using phenotypes and genotypes from all breeds jointly and in which phenotypes of different breeds are treated as a single trait.
- **MBMT:** a multi-breed multi-trait evaluation using phenotypes and genotypes from all breeds jointly and in which phenotypes of different breeds are treated as different correlated traits.

Additional scenarios were implemented to investigate the impact of MF and of reducing the number of GG or MF. The last 3 scenarios are as follows:

- **ALL_4GG:** as ALL but replacing all GG by only 4 GG. The 4 GG were defined and assigned to individuals with unknown parents based on their breed composition and corresponded to HOL, JER, XBD, and OTHERS (for all other breeds).
- **ALL_255MF:** as ALL but replacing GG by MF.
- **ALL_4MF:** as ALL_4GG but replacing GG with MF.

Model and software

The following model was used:

$$\mathbf{y}_i \sim \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_i\mathbf{u}_i + \mathbf{e}_i,$$

where i is the trait (either CSD or MY), $\mathbf{y}_i$ is the vector of observations for trait i, $\mathbf{u}_i$ is the vector of random additive genetic effects for trait i, and $\mathbf{e}_i$ is the vector of random residual effects for trait i. $\mathbf{X}_i$ and $\mathbf{Z}_i$ are incidence matrices linking records of trait i to fixed effects and additive genetic effects, respectively. Fixed effects included heterozygosity, recombination, inbreeding, age at first calving (only for CSD), herd-year-season at first calving, and age at second calving (only for MY). It was assumed that:

$$var\begin{bmatrix}\mathbf{u}_{CSD}\\ \mathbf{u}_{milk}\end{bmatrix} = \mathbf{G} \otimes \mathbf{A} = \begin{bmatrix}\sigma^2_{u\,CSD} & Sym\\ \sigma_{u\,CSD,MY} & \sigma^2_{u\,\mathrm{MY}}\end{bmatrix} \otimes \mathbf{A},$$

where $\mathbf{G}$ is the genetic co-variance matrix, $\mathbf{A}$ is the numerator relationship matrix, $\sigma^2_{u_{CSD}}$ and $\sigma^2_{u_{MY}}$ are the additive genetic variances for CSD and MY, respectively, $\sigma_{u\,CSD,milk}$ is the additive genetic covariance between CSD and MY, and $\otimes$ indicates the Kronecker product. Residuals were assumed to be uncorrelated.

In the **MBMT** scenario, phenotypes of different breeds were modelled as different correlated traits. Thus, the model was adapted as follows:

$$\begin{bmatrix}\mathbf{y}_{H_{CSD}}\\ \mathbf{y}_{H_{MY}}\\ \mathbf{y}_{J_{CSD}}\\ \mathbf{y}_{J_{MY}}\\ \mathbf{y}_{X_{CSD}}\\ \mathbf{y}_{X_{MY}}\end{bmatrix}=\begin{bmatrix}\mathbf{X}_{H_{CSD}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{X}_{H_{MY}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{X}_{J_{CSD}} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{J_{MY}} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{X_{CSD}} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{X}_{X_{MY}}\end{bmatrix}\begin{bmatrix}\mathbf{b}_{H_{CSD}}\\ \mathbf{b}_{H_{MY}}\\ \mathbf{b}_{J_{CSD}}\\ \mathbf{b}_{J_{MY}}\\ \mathbf{b}_{X_{CSD}}\\ \mathbf{b}_{X_{MY}}\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_{H_{CSD}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{Z}_{H_{MY}} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{Z}_{J_{CSD}} & \mathbf{0} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_{J_{MY}} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_{X_{CSD}} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{Z}_{X_{MY}}\end{bmatrix}\begin{bmatrix}\mathbf{u}_{H_{CSD}}\\ \mathbf{u}_{H_{MY}}\\ \mathbf{u}_{J_{CSD}}\\ \mathbf{u}_{J_{MY}}\\ \mathbf{u}_{X_{CSD}}\\ \mathbf{u}_{X_{MY}}\end{bmatrix}+\begin{bmatrix}\mathbf{e}_{H_{CSD}}\\ \mathbf{e}_{H_{MY}}\\ \mathbf{e}_{J_{CSD}}\\ \mathbf{e}_{J_{MY}}\\ \mathbf{e}_{X_{CSD}}\\ \mathbf{e}_{X_{MY}}\end{bmatrix},$$

and it was assumed that:

$$var\begin{bmatrix}\mathbf{u}_{H_{CSD}}\\ \mathbf{u}_{H_{MY}}\\ \mathbf{u}_{J_{CSD}}\\ \mathbf{u}_{J_{MY}}\\ \mathbf{u}_{X_{CSD}}\\ \mathbf{u}_{X_{MY}}\end{bmatrix}=\begin{bmatrix}\sigma^2_{u\,H_{CSD}} & & & & & \\ \sigma_{u\,H_{MY},H_{CSD}} & \sigma^2_{u\,H_{MY}} & & Sym & & \\ \sigma_{u\,J_{CSD},H_{CSD}} & \sigma_{u\,J_{CSD},H_{MY}} & \sigma^2_{u\,J_{CSD}} & & & \\ \sigma_{u\,J_{MY},H_{CSD}} & \sigma_{u\,J_{MY},H_{MY}} & \sigma_{u\,J_{MY},J_{CSD}} & \sigma^2_{u\,J_{MY}} & & \\ \sigma_{u\,X_{CSD},H_{CSD}} & \sigma_{u\,X_{CSD},H_{MY}} & \sigma_{u\,X_{CSD},J_{CSD}} & \sigma_{u\,X_{CSD},J_{MY}} & \sigma^2_{u\,X_{CSD}} & \\ \sigma_{u\,X_{MY},H_{CSD}} & \sigma_{u\,X_{MY},H_{MY}} & \sigma_{u\,X_{MY},J_{CSD}} & \sigma_{u\,X_{MY},J_{MY}} & \sigma_{u\,X_{MY},X_{CSD}} & \sigma^2_{u\,X_{MY}}\end{bmatrix}\otimes\mathbf{A},$$

where *H*, *J*, and *X* refer to HOL, JER, and XBD, respectively. All other terms are defined as above. Residuals were fitted using block-diagonal variance matrices and were assumed to be uncorrelated across breeds.

The same co-variance components were used for CSD and MY in all scenarios (heritability and genetic correlations between traits are reported in Table 2), except for the MBMT scenario in which pedigree-based co-variance components were estimated using GIBBSF90+ (Misztal et al., 2002). The data for variance component estimation was prepared as follows to reduce the size of the analysed dataset: i) animals with phenotypes deviating more than 3 standard deviations from the mean of each breed were removed; ii) only phenotyped animals born from 2010 onwards, with both parents known, and belonging to a contemporary group (i.e., herd-year-season) with a size of at least 5 individuals were retained; iii) a pedigree depth of six generations from the retained phenotyped animals was used. The genetic and residual co-variances used in other scenarios were used as starting values. Gibbs sampling was run for two hundred thousand samples, 2,000 samples were discarded as burn-in, and every 150th sample was saved. POSTGIBBSF90 (Misztal et al., 2002) was used to monitor convergence and to obtain estimates and standard errors.

In all the above models, a single-step SNP-BLUP (ssSNPBLUP) approach (Liu et al., 2014) assuming 30% of the additive genetic variance due to residual polygenic effects was used. A **J** covariate was added as a fixed effect in the model to ensure the compatibility between pedigree and genomic information (Hsu et al., 2017), except for the two scenarios using MF (i.e., ALL_255MF and ALL_4MF). **J** covariates were computed as described by (Tribout et al., 2019).

GEBVs were computed using the software MiXBLUP (Vandenplas et al., 2022). The computed GEBVs were rebased using HOL, JER and XBD animals born in 2000 with an available phenotype for MY as the base population. All validation results were obtained using the rebased GEBVs.

Table 2. Heritability (diagonal) and genetic correlation (below diagonal) for CSD and MY.

	CSD	MY
CSD	*0.05*	
MY	0.22	*0.31*

Validation

The Linear Regression (LR) validation method was used to compare the different scenarios implemented *(*Legarra and Reverter, 2018; Macedo et al., 2020*)*. For each scenario, a "*whole*" and a "*partial*" evaluation were carried out. In the *whole* evaluation, GEBVs (u_w) were obtained using all information (pedigree,

phenotypes, and genotypes). In the *partial* evaluation, GEBVs (u_p) were obtained using less information, i.e., by removing the phenotypes of animals born in the last 6 years (corresponding to a cut-off in the year 2016) while maintaining the same pedigree and genotypes as in the *whole* evaluation. Table 1 reports the number of phenotypes in the *whole* and the *partial* evaluations.

In each scenario, the following estimators from the LR method were computed:

- Level bias ($\hat{\Delta}_p$): defined as the difference between the mean GEBV of the partial evaluations and the mean GEBV of the whole evaluation as: $\hat{\Delta}_p = \overline{\hat{u}}_p - \overline{\hat{u}}_w$. In absence of level bias, $\hat{\Delta}_p$ is expected to be 0. Level bias was expressed in genetic standard deviations for easier interpretation ($\hat{\Delta}_p / \hat{\sigma}_u$).
- Dispersion bias ($\hat{b}_p$): defined as the slope of the regression of u_w on u_p and calculated as $\hat{b}_p = \frac{cov(\hat{u}_w, \hat{u}_p)}{var(\hat{u}_p)}$. In absence of dispersion bias, the expected value of $\hat{b}_p$ is 1. Values of $\hat{b}_p < 1$ indicate over-dispersion, while values of $\hat{b}_p > 1$ indicate under-dispersion. Values of $\hat{b}_p$ within 15% from the expected value were considered as acceptable similarly to other studies (e.g., Tsuruta et al., 2011; Bonifazi et al., 2022).
- Accuracy of partial GEBV ($\widehat{acc}_p$): computed as $\widehat{acc}_p = \sqrt{\frac{cov(\hat{u}_w, \hat{u}_p)}{(1-\bar{F})\,\sigma_u^2}}$, where $\bar{F}$ is the mean inbreeding coefficient of the validation group derived from pedigree and σ_u^2 is the additive genetic variance.

LR validation statistics were obtained for two validation groups within each breed and defined as follows:

- cows: genotyped cows phenotyped for MY and/or CSD and born after the cut-off.
- bulls: genotyped bulls with at least 20 daughters with phenotypes for MY and/or CSD born after the cut-off, and with no daughters with phenotypes for MY or CSD born before the cut-off.

The estimators of the LR method were computed using the "compute_LR_stats" R function available in Bonifazi (2023). Standard errors (SE) of LR estimators were obtained using bootstrapping with replacement of individuals within each validation group. A total of 10,000 bootstrap samples were utilized for all analyses.

Results & Discussion

Hereafter, we first report results on the population structure and the relationship between the breeds analysed. We then present the validation results and discuss the findings of this study.

Population structure and estimated genetic parameters

Figure 1 reports the three principal components from a Principal Component Analysis (PCA) using genotypes from all three breeds. The PCA shows that HOL and JER clustered separately and that the XBD is an unstructured cross which is genetically linked to both purebred populations. This pattern was expected as the XBD population is derived from HOL and JER crossing (Khansefid et al., 2020).

Table 4 reports estimated heritabilities and genetic correlations for the MBMT scenario. For CSD, estimated heritabilities were similar

for all breeds (ranging from 0.03 to 0.04), while for MY they ranged from 0.24 for HOL to 0.27 for JER. For CSD, across-breed genetic correlations were the lowest between JER and other breeds (≤0.66), while a high genetic correlation (0.93) was estimated between XBD and HOL. For MY, across-breed genetic correlations were high, ranging from 0.82 between JER and HOL to 0.96 between XBD and HOL. Within-breed across-traits genetic correlations ranged from 0.24 for JER to 0.46 for XBD. Across-breed across-traits genetic correlations ranged from 0.34 for CSD in XBD and CSD in JER to 0.70 for MY in JER and CSD in XBD (Table 4). Across-breed across-traits genetic correlations not significantly different from zero were estimated between

CSD in JER and MY in HOL, and between CSD in JER and MY in XBD. Overall, the estimated genetic correlations indicate that XBD is genetically closer to the HOL than to the JER for both CSD and MY. The closer genetic link between XBD and HOL than with JER was also reflected in the estimated **Γ** matrix representing the relationships within and between MF for the ALL_4MF scenario. A higher relationship was estimated between the XBD MF and the HOL MF than with the JER MF (Table 3). As expected, the OTHER MF showed the lowest relationships between MF since it included all other breeds.

Validation results

Level bias

Overall, larger level bias was observed for CSD than MY and, for both traits, standard errors were larger for bulls than for cows (Table 5).

For CSD, larger $\hat{\Delta}_p$ were observed for bulls compared to cows in all scenarios, with XBD bulls showing the largest $\hat{\Delta}_p$. Scenario SINGLE showed $\hat{\Delta}_p$ for CSD of -0.05 GSD and 0.00 GSD on average across breeds for bulls and cows, respectively. Scenario ALL showed similar level bias to SINGLE: $\hat{\Delta}_p$ for CSD of -0.04 GSD and 0.02 GSD on average across breeds for bulls and cows, respectively. Likewise, ALL_4MF showed similar bias to ALL: $\hat{\Delta}_p$ of -0.04 GSD and 0.01 GSD on average across breeds for bulls and cows, respectively. Finally, $\hat{\Delta}_p$ for CSD under the MTMB scenario was of -0.06 GSD and -0.01 GSD on average across breeds for bulls and cows, respectively.

For MY, no large differences were observed across the different scenarios for level bias (Table 5): on average across breeds, $\hat{\Delta}_p$ ranged between -0.04 GSD for cows for the ALL_255MF scenario to 0.02 GSD for bulls for the ALL_4GG scenario (results not shown).

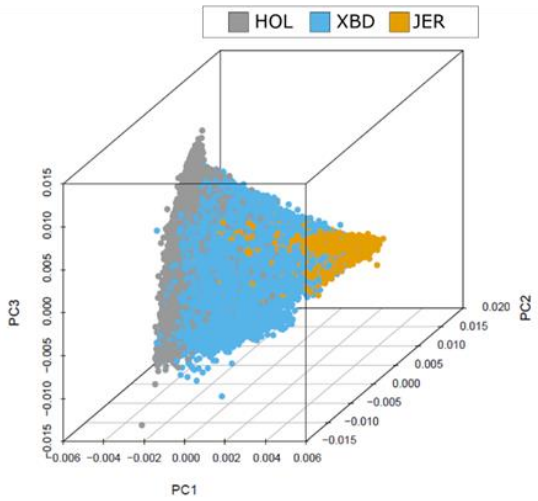

Figure 1. Plot of the first three principal components (PC). Colours indicate the breed associated with the genotype (HOL = Holstein, JER = Jersey, XBD = crossbred).

Table 3. Estimated **Γ** matrix for the ALL_4MF scenario.

	HOL[a]	JER	XBD	OTHER
HOL	*0.93*	0.78	0.83	0.57
JER		*0.72*	0.75	0.54
XBD			*0.78*	0.56
OTHER				*0.77*

[a] Four metafounders: HOL = Holstein, JER = Jersey, XBD = crossbred, OTHER = other breeds.

Table 4. Estimated heritabilities (on the diagonal) and within- and across-breeds genetic correlations (lower diagonal) for CSD and MY (standard errors between brackets).

		CSD [b]			MY		
		HOL[a]	JER	XBD	HOL	JER	XBD
CSD	HOL	*0.03 (0.00)*					
	JER	0.59 (0.09)	*0.04 (0.01)*				
	XBD	0.93 (0.03)	0.66 (0.06)	*0.03 (0.00)*			
MY	HOL	0.41 (0.05)	-0.02 (0.10)	0.47 (0.05)	*0.24 (0.01)*		
	JER	0.55 (0.06)	0.24 (0.07)	0.70 (0.06)	0.82 (0.05)	*0.27 (0.02)*	
	XBD	0.34 (0.04)	-0.03 (0.10)	0.46 (0.04)	0.96 (0.01)	0.87 (0.03)	*0.26 (0.01)*

[a] HOL = Holstein, JER = Jersey, XBD = crossbred. [b] CSD = Calving Season Days, MY = Milk Yield.

Dispersion bias

Overall, CSD showed mostly over-dispersion ($\hat{b}_p$ < 1 in all scenarios, except for JER bulls and cows) while MY showed both over- and under-dispersion (Table 5). For both CSD and MY, $\hat{b}_p$ were within the 15% acceptable range for all validation groups and scenarios, except for CSD-HOL bulls in the SINGLE scenario and for MY-XBD bulls in the MTMB scenario (Table 5). For both CSD and MY, cows showed less dispersion than bulls, with $\hat{b}_p$ closer to 1 on average across breeds and scenarios. Scenarios ALL_255MF and ALL_4GG showed similar dispersion bias to ALL (results not shown). Larger standard errors of $\hat{b}_p$ were observed for bulls compared to cows, likely due to the smaller number of validation animals available.

For CSD, SINGLE showed the most dispersion across all scenarios: $\hat{b}_p$ of 0.90 and 0.94 on average across breeds for bulls and cows, respectively. Scenario ALL showed less dispersion for CSD than SINGLE, with values closer to 1: $\hat{b}_p$ of 0.94 and 0.95 on average across breeds for bulls and cows, respectively. Scenario ALL_4MF showed the least dispersion for CSD among all scenarios analysed: $\hat{b}_p$ of 0.96 and 0.97 on average across breeds for bulls and cows, respectively. Finally, dispersion for CSD under the MTMB scenario was in between that of SINGLE and ALL: $\hat{b}_p$ of 0.91 and 0.95 on average across breeds for bulls and cows, respectively.

For MY, SINGLE and ALL gave overall similar results, with $\hat{b}_p$values ranging between 0.99 and 1.01 on average across breeds for bulls and cows, respectively. Scenario ALL_4MF gave slightly higher dispersion than ALL for MY: $\hat{b}_p$ of 1.04 and 1.03 on average across breeds for bulls and cows, respectively. Finally, MTMB had the highest dispersion among all scenarios, albeit within the acceptable range: $\hat{b}_p$ of 1.06 and 1.05 on average across breeds for bulls and cows, respectively.

Accuracy of partial GEBV

Overall, for both CSD and MY, higher $\widehat{acc}_p$ were obtained for HOL validation groups, followed by XBD and JER (Table 5).

For CSD, $\widehat{acc}_p$ in scenario SINGLE was 0.49 and 0.44 on average across breeds for bulls and cows, respectively. Scenario ALL gave the highest accuracies for CSD: $\widehat{acc}_p$ of 0.53 and 0.49 on average across breeds for bulls and cows, respectively. MTMB showed the lowest accuracies for CSD among all scenarios: $\widehat{acc}_p$ of 0.44 and 0.42 on average across breeds for bulls and cows, respectively. Finally, accuracies for ALL_4MF were close to those of scenario ALL (Table 5). ALL_4GG and ALL_255MF scenarios gave similar accuracies as scenario ALL (results not shown).

For MY, $\widehat{acc}_p$ in scenario SINGLE was 0.44 and 0.50 on average across breeds for bulls and cows, respectively. Scenario ALL gave higher accuracies than SINGLE for MY: $\widehat{acc}_p$ of 0.48 and 0.56 on average across breeds for bulls and cows, respectively. The MTMB scenario showed the highest accuracies for MY among all scenarios: $\widehat{acc}_p$ on average across breeds of 0.56 and of 0.63 for bulls and cows, respectively. Finally, similarly to CSD, $\widehat{acc}_p$for MY for scenarios ALL_4MF, ALL_4GG and ALL_255MF were close to ALL (results not shown).

Impact of moving from single-breed to multi-breed evaluations

Our results show that a combined multi-breed evaluation improves the accuracy of GEBVs compared to a single-breed evaluation. LR validation results showed increased $\widehat{acc}_p$ when moving from single-breed genomic evaluations (scenario SINGLE) to multi-breed genomic evaluations, such as those of scenario ALL, for both CSD and MY and for both purebred and crossbred animals. The observed increase in $\widehat{acc}_p$ is likely due to the close genetic relationship among the three populations (Figure 1), which allows for (genomic) data

Table 5. Level bias in genetic standard deviations ($\hat{\Delta}_p$), dispersion ($\hat{b}_p$), and accuracy of partial GEBVs ($\widehat{acc}_p$) for CSD and MY from validation cows and bulls. Standard errors between brackets.

	CSD [b]						MY					
Scenario [c]	Bulls			Cows			Bulls			Cows		
	HOL [a]	JER	XBD	HOL	JER	XBD	HOL	JER	XBD	HOL	JER	XBD
$\widehat{acc}_p$												
SINGLE	0.58	0.38	0.50	0.49	0.39	0.45	0.49	0.36	0.47	0.55	0.38	0.59
	(0.03)	(0.03)	(0.03)	(0.00)	(0.01)	(0.00)	(0.03)	(0.03)	(0.04)	(0.00)	(0.01)	(0.00)
ALL	0.63	0.42	0.53	0.54	0.43	0.51	0.52	0.45	0.48	0.59	0.46	0.62
	(0.03)	(0.02)	(0.04)	(0.00)	(0.01)	(0.00)	(0.03)	(0.03)	(0.04)	(0.01)	(0.01)	(0.00)
ALL_4MF	0.62	0.44	0.52	0.52	0.43	0.47	0.54	0.47	0.47	0.60	0.49	0.62
	(0.03)	(0.03)	(0.04)	(0.00)	(0.01)	(0.00)	(0.02)	(0.03)	(0.04)	(0.00)	(0.01)	(0.00)
MBMT	0.52	0.30	0.49	0.46	0.32	0.47	0.59	0.55	0.55	0.65	0.55	0.70
	(0.02)	(0.02)	(0.04)	(0.00)	(0.00)	(0.00)	(0.03)	(0.04)	(0.05)	(0.01)	(0.01)	(0.00)
$\hat{b}_p$												
SINGLE	0.84	0.94	0.91	0.90	0.99	0.93	0.87	1.03	1.06	1.01	0.99	1.01
	(0.04)	(0.08)	(0.08)	(0.01)	(0.01)	(0.01)	(0.06)	(0.10)	(0.10)	(0.01)	(0.02)	(0.01)
ALL	0.87	1.03	0.92	0.91	1.01	0.94	0.91	0.96	1.11	1.01	0.99	1.02
	(0.04)	(0.06)	(0.08)	(0.01)	(0.01)	(0.01)	(0.06)	(0.07)	(0.11)	(0.01)	(0.01)	(0.01)
ALL_4MF	0.90	1.04	0.93	0.92	1.02	0.95	1.00	1.01	1.11	1.03	1.01	1.03
	(0.04)	(0.06)	(0.08)	(0.01)	(0.01)	(0.01)	(0.05)	(0.07)	(0.11)	(0.01)	(0.01)	(0.01)
MBMT	0.88	0.92	0.94	0.92	0.97	0.95	0.98	1.05	1.17	1.04	1.06	1.05
	(0.04)	(0.08)	(0.08)	(0.01)	(0.01)	(0.01)	(0.05)	(0.07)	(0.11)	(0.01)	(0.01)	(0.01)
$\hat{\Delta}_p$												
SINGLE	-0.03	0.07	-0.20	0.02	0.02	-0.04	0.01	0.04	-0.03	0.00	0.02	-0.04
	(0.03)	(0.03)	(0.04)	(0.00)	(0.00)	(0.00)	(0.03)	(0.03)	(0.04)	(0.01)	(0.01)	(0.00)
ALL	-0.04	0.08	-0.17	0.03	0.04	-0.01	0.02	-0.02	0.00	-0.02	-0.02	-0.03
	(0.03)	(0.03)	(0.04)	(0.00)	(0.00)	(0.00)	(0.03)	(0.03)	(0.04)	(0.00)	(0.01)	(0.00)
ALL_4MF	-0.03	0.06	-0.13	0.01	0.02	-0.01	-0.02	0.00	0.01	-0.03	-0.01	-0.02
	(0.02)	(0.02)	(0.03)	(0.00)	(0.00)	(0.00)	(0.02)	(0.02)	(0.03)	(0.00)	(0.00)	(0.00)
MBMT	-0.06	0.03	-0.16	0.00	0.01	-0.04	-0.02	0.01	-0.02	-0.04	-0.01	-0.05
	(0.03)	(0.03)	(0.04)	(0.00)	(0.00)	(0.00)	(0.03)	(0.04)	(0.05)	(0.01)	(0.01)	(0.00)

[a] HOL = Holstein, JER = Jersey, XBD = crossbred. [b] CSD = Calving Season Days, MY = Milk Yield. [c] SINGLE = separate single-breed evaluations using the phenotypes of all breeds treated as a single trait, but only genotypes of the respective breed; ALL = multi-breed evaluation using all phenotypes and genotypes from all breeds and treating phenotypes of different breeds as a single trait; MBMT = a multi-breed multi-trait evaluation using phenotypes and genotypes from all breeds jointly and treating phenotypes of different breeds as different correlated traits; ALL_4MF = as ALL, but using four metafounders.

collected on one breed to contribute valuable information for the prediction of GEBVs in other breeds. Finally, no consistent pattern across scenarios and traits was observed for level bias and dispersion bias when moving from single-breed to multi-breed genomic evaluations for both purebred and crossbred.

The results of our study are in line with those of Khansefid et al. (2020) and Karaman et al. (2021), who reported increased accuracies for both purebred and crossbred animals when using a multi-breed reference population for genomic evaluations of both (small) purebred and crossbred populations. In contrast, Cesarani et al. (2022) reported a decrease in accuracy and an increase in inflation for breeds with a small reference population when included in a multi-trait evaluation next to other purebred but numerically dominant breeds. This reduction in accuracy was not observed in our study, likely due to the sizeable (genomic) data collected on both purebred and crossbred individuals (Table 1) and the inclusion of data from crossbred individuals in the multi-breed evaluation.

The MTMB scenario treated the same trait in different breeds as different correlated traits and showed the lowest $\widehat{acc}_p$ for CSD but the highest $\widehat{acc}_p$ for MY (Table 5). These results could be related to the higher genetic

correlations between MY in different populations compared to CSD (Table 4). Genetic correlations influence the degree to which information recorded in one population will influence the GEBVs in another trait and population. The results of this study suggest that a MTMB scenario may perform better for traits showing high correlations between populations and highlight the importance of genetic correlations in determining the optimal scenario for implementing multi-breed genomic evaluations. Nonetheless, further testing and validation of the multi-breed multi-trait approach on other traits should be conducted.

Impact of reducing the number of GG and implementation of MF

We observed no impact in reducing the number of GG on the accuracy of validation animals. Having a large number of GG with potentially few animals in each group may impact the performance of genomic evaluations (ten Napel et al., 2022). The results of this study suggest that the number of GG could be reduced for the studied population without negatively impacting the GEBVs of animals in recent generations. This observed lack of impact was likely related to missing parental information being related to mostly animals in older generations, resulting in limited to no impact on younger animals. Moreover, animals with missing parental information were mostly related to other breeds than the three validated ones. Out of the total number of animals in the pedigree with missing parental information, 19%, 6%, 23% and 52% were assigned to the HOL, JER, XBD, and "other breeds" GG, respectively. Therefore, reducing the number of GG did not have a large impact on the HOL, JER and XBD animals. Finally, results showed limited benefits in replacing GG with MF.

Conclusions

We implemented different scenarios to model data of two purebred and a derived crossbred population into a multi-breed single-step evaluation. First, moving from single-breed to multi-breed single-step evaluations improved the accuracy of genomic predictions for both purebred and crossbred animals. Multi-breed multi-trait evaluations that treated phenotypes of different breeds as different correlated traits showed the highest accuracy for MY but the lowest for CSD. Second, we observed no impact on the GEBVs of validation animals when reducing the number of GG in multi-breed evaluations likely due to missing parental information being mostly related to animals belonging to other breeds or older generations. Finally, there were limited benefits in replacing GG with MF.

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