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Genetic parameters for daily milk weights in U.S. Holsteins using pen-based contemporary groups

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Abstract

The availability of daily milk weights and pen location information provides an interesting opportunity to review how contemporary groups are defined for dairy cattle genetic evaluations. In the U.S., dairy cows in larger herds are grouped into pens according to various characteristics like parity, production level, reproductive status, lactation stage, and health status. Our dataset includes pen location information for each daily milk weight, and our goal is to more accurately model contemporary groups when estimating breeding values for daily milk production. Therefore, instead of using herd-year-season, we updated our contemporary group to herd-pen-milking date, thereby capturing the differences in daily milk production more precisely by modeling the true environmental effects cows experience at the pen level. Our dataset includes 21,000,951 aggregated daily milk weights from 114,243 first parity Holstein cows in 157 herds representing 29 U.S. states. Our phenotype is 305-d milk yield or daily milk weight, and both animal and repeatability animal models were used to estimate genetic parameters and breeding values. Age at first calving (6 levels) and days in milk (10 levels) were included as fixed effects and cow (114,243 levels) was included as a random effect. Contemporary group effects included a fixed or random herd-year-season of calving effect (1,492 levels) and/or a fixed or random herd-pen-milking date effect (285,592 levels). Genetic parameters (kg^2; posterior SD) were estimated using GIBBSF90+ software, and we found the additive genetic variance for 305-d milk yield was 842,500 (25,093), the herd-year-season variance was 878,960 (33,617), and the residual variance was 1,442,700 (20,438). Whereas for genetic parameters estimated using daily milk weights as the phenotype, the additive genetic variance ranged from 10.48 (0.60) to 24.12 (0.66) the herd-year-season variance was 10.34 (0.40), herd-pen-milking date variance ranged from 4.91 (0.02) to 4.96 (0.02), permanent environmental variance ranged from 10.65 (0.44) to 16.94 (0.30), while the residual variance ranged from 11.81 (0.01) to 14.60 (0.01). Heritability estimates ranged from 0.21 (0.01) to 0.47 (0.01), while repeatability estimates ranged from 0.51 (0.01) to 0.71 (0.01). Although further work is required to disentangle the relationships among contemporary groups, our results suggest value in using daily milk weights and pen-based contemporary groups for genetic evaluation of production traits in dairy cattle.

Key words: daily milk weights, contemporary groups, variance components

Introduction

The dairy industry has invested significantly in modern technology such as innovative sensors that collect high-frequency data that monitors animals at the individual or group level to inform management decisions. Consequently, precision livestock farming has advanced remarkably over time, generating an extensive volume of data (Lovarelli et al., 2020). Such high frequency data is currently predominantly used for management purposes, while production and management information collected on the test day by milk recording organizations is the gold standard data collection method used for genetic evaluations. Daily milk weights are one example of such high throughput data that are currently generated during each individual cows milking. Along with the daily milk weight, other

valuable information such as the pen location of each cow is also recorded during milking with either an automatic milking system or through in-line milk meters installed in conventional milking parlors. This information allows us to precisely identify which pen each cow belonged to on a specific date.

Currently, to estimate variance components and genetic parameters, contemporary groups are typically defined using the herd-year-season of calving. The concept is that a cow is part of a cohort within a herd that experienced similar environmental conditions based on the year and season of calving (Van Vleck, 1987). Therefore, each cow would have only one contemporary group per lactation. Given the unique nature of our novel dataset, our objective was to redefine the contemporary group definition to more precisely reflect the actual (or micro) environment each cow experiences based on the specific pen she occupies on any given day. In the U.S., cows are grouped in pens according to parity, milk production level, lactation stage, and reproductive status (Contreras-Govea et al., 2015). Managing cows at the group or pen level in the U.S. may be more labor efficient and, additionally, cows in different pens within the same farm may be fed different rations and may experience different housing or management conditions. In theory, albeit unlikely, following data edits, each cow could be part of up to 300 distinct contemporary groups throughout the lactation period, assuming a daily pen change. This indicates a substantial increase in data availability, allowing for more accurate estimation of genetic parameters and, consequently, increased reliability of sire predicted transmitting abilities (PTAs). We found that fitting the contemporary group, either herd-year-season or herd-pen-milking date as fixed or random impacts the estimates of relevant genetic parameters and also the reliabilities of sire PTAs. Additionally, we observed differences when the phenotype for milk production changed from 305-d milk yield to daily milk weights. Interestingly, when modeling herd-pen-milking date as a random effect for daily milk weight phenotypes, we found a large increase in the additive genetic variance, and thus the heritability. However, it appears that this specific model (i.e. model 3) cannot disentangle the relationships among highly correlated daily contemporary groups, possibly due to the correlated residuals between levels of herd-pen-milking date. Consequently, we opted to model herd-year-season as a fixed effect and herd-pen-milking date as a random effect (model 4). This approach better disentangled the previous relationship and yielded estimates more consistent with the other models we evaluated. The aim of this study was to investigate genetic parameters for milk production traits using herd-pen-milking date as the contemporary group. Four models were employed to assess the impact of changing the phenotype from 305-d milk yield to daily milk weight. Additionally, these models were used to examine the effects of modeling contemporary groups using either herd-year-season or herd-pen-milking date, considering both as fixed or random, on genetic parameters and sire PTA reliabilities.

Materials and Methods

Data were provided by Dairy Records Management Systems (Raleigh, NC) and were extracted from PCDART on farm management software. Detailed descriptions of the initial data edits can be found in Guinan et al., 2024. Additional edits include a minimum of 25 cows per herd-year-season of calving and at least 25 cows per herd-pen-milking date contemporary group. After the additional edits above, our dataset contained 114,243 cows from 157 herds in 29 U.S. states with 21,000,951 daily milk weights. To investigate the differences between using 305-d milk yield and daily milk weights, along with the differences between using herd-year-season (HYS) and herd-pen-milking date (HPM) as contemporary groups and as fixed or random we estimated variance components using four different models that are described in Table 1:

Table 1. Outline of the four different models used to estimate genetic parameters for i) 305-d milk yield (kg) and ii) daily milk weights (kg).

Model	
1	305-d Milk (kg) = AFC + **HYS** + cow + e
2	Daily Milk Weight (kg) = AFC + **DIM** + **HYS** + cow + pe + e
3	Daily Milk Weight (kg) = AFC + DIM + **HPM** + cow + pe + e
4	Daily Milk Weight (kg) = AFC + DIM + **HYS** + **HPM** + cow + pe + e

AFC = Age at first calving; DIM = Days in milk; HYS = Herd-year-season; HPM = Herd-pen-milking date; pe = permanent environmental; e = residual. The contemporary group(s) for each model are in bold.

Where **AFC** is the fixed effect of age at first calving (6 levels; < = 22, 23–24, 25–26, 27–28, 29–30, 30+), **DIM** is the fixed effect of days in milk (10 levels; 30 days each), **HYS** is the fixed or random effect of herd-year-season of calving (1,492 levels), **HPM** is the fixed or random effect of herd-pen-milking date (285,592 levels), **cow** is the random additive genetic effect using up to 5 generations of pedigree data with 114,243 levels distributed as $\mathbf{a} \sim N(0, \mathbf{A}\sigma_a^2)$, **pe** is the random permanent environmental effect distributed as $\mathbf{pe} \sim N(0, \mathbf{I}\sigma_{pe}^2)$, and **e** is the random residual effect distributed as $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$.

Model 1, which utilized 305-d milk yield (kg) as the phenotype, served as a baseline for comparison with the more complex models. Models 2-4 utilized daily milk weights (kg) as the phenotype. For models 1-3, the contemporary group (HYS or HPM) was fitted as either fixed or random to estimate variance components, while for model 4, HYS was fitted as fixed and HPM was fitted as random to estimate variance components. GIBBSF90+ software was used to estimate variance components and posterior standard deviations using a Bayesian approach employing the Gibbs sampling algorithm with 50,000 samples. A total of 10,000 samples were discarded as burn in, while every 1 in 10 samples was stored to estimate posterior means and standard deviations (Misztal et al., 2024). Convergence was determined by visual inspection of the trace plots. Heritabilities (h^2) were estimated using two formulas; h^2 estimates include the contemporary group variance (when calculated) in the denominator, whereas h^{2*} estimates do not include the contemporary group variance in the denominator. PTA reliabilities were approximated using the following formulas $PEV = (posterior\ SD)^2; \quad REL = 1 - \frac{PEV}{\sigma_a^2}$, where PEV is the prediction error variance, or the squared posterior standard deviation of the PTA estimate. The reliability (REL) was estimated by subtracting the PEV divided by the additive genetic variance from 1. This serves as an estimate for the REL of the estimated PTA.

Results & Discussion

1. Milk yield phenotype - Daily Milk Weights vs 305-d milk yield

The first analysis included modeling the phenotype for milk production using the standard Council on Dairy Cattle Breeding (Bowie, MD) method to serve as a comparison for more complex models. The current phenotype typically used to estimate variance components and PTAs is 305-d milk yield where test day information is used to first fit a lactation curve for each individual cow, and milk production is projected to 305-d. Model 1 included 305-d milk production as the phenotype (1 phenotype), whereas the remaining models (2-4) used daily milk weights as the phenotype (at least 100 phenotypes). Depending on whether HYS was fitted as fixed or random, Model 1 had a h^2 ranging from 0.27 to 0.37, whereas for the remaining models with the exception of model 3 when HPM was

random, the h^2 was lower whether the contemporary group was fitted as fixed or random. The use of daily milk weights as the phenotype introduced greater environmental (residual) variance, and therefore this decreased the heritability estimates for model 2-4.

2. Updating contemporary group definition

The primary objective of this research was to update the definition of contemporary groups to estimate genetic parameters for daily milk weight phenotypes by capitalizing on high frequency data not currently utilized for genetic evaluations. The current method to capture environmental effects is HYS of calving, which was developed during a period when herd sizes were smaller, and hence all cows were experiencing similar environmental effects. As herd sizes have increased, cows are grouped according to characteristics like parity, milk production levels, and reproductive status, among others. Consequently, our novel contemporary groups are now formed based on the phenotype throughout the lactation period, for example, high producing cows may be grouped together during the late lactation period based on their milk yield production in mid lactation. The effect HYS was used as a basic model for comparison with both 305-d milk (model 1) and daily milk weights (model 2). The contemporary group currently used to estimate genetic parameters for milk production in the U.S. is HYS (Wiggans et al., 1988). Our goal was to redefine the contemporary group for daily milk weights to more accurately model the true or "micro" environment the cow is experiencing. Therefore, we utilized the pen information that is attached to each individual daily milk weight to define contemporary groups as herd-pen-milking date with at least 25 cows per level. For this section of results, we will focus primarily on model 2-4 for comparison purposes, as the variance components are in the same range. For model 2, depending on whether HYS was fitted as fixed or random, the additive genetic variance ranged from 10.76 to 10.85, contemporary group variance (when HYS was fitted as random) was 10.34, permanent environmental variance ranged from 15.01 to 15.08, and the residual variance was 14.60. Model 3 had similar results for variance components, with the exception of when HPM was fitted as random, which will be discussed in the next section. Model 3 had a smaller residual variance than model 2, indicating that the environmental variance decreases when HPM is used as the contemporary group in comparison to HYS for daily milk weight phenotypes (Table 2). Finally, model 4 (HYS fixed; HPM random) had similar additive genetic variance (10.48) and permanent environmental variance (14.23) to models 2 and 3, with the exception of model 3 where HPM was fit as random, and comparable residual variance with model 3 (11.85).

3. Fitting contemporary group as fixed vs random

For models 1-3, we also investigated differences between fitting the contemporary group (HYS or HPM) as fixed or random. The question of fitting contemporary groups to estimate genetic parameters as fixed or random is not novel, however we were interested in understanding the differences in variance component estimates with 305-d milk yield, and more interestingly, daily milk weights. Given the size of our dataset, specifically herd size, we expect to observe minimal differences among variance component estimation methods. Additionally, as our dataset spans 5 years, we do not expect a genetic trend that we need to account for or major improvements in management practices which may indicate that contemporary group should be fit as a random effect to account for these trends. For model 1 and 2, when HYS was fit as fixed or random, we found minimal differences between variance components. Similarly, the h^2 estimates did not change, except the h^2 decreased as expected when the contemporary

Table 2. Variance components, heritability and repeatability estimates (posterior SD), and sire predicted transmitting ability reliabilities for 305-d milk yield and daily milk yield using contemporary group (herd-year-season or herd-pen-milking date) as fixed or random effects.

	305-d yield (kg)		**Daily milk yield (kg)**				
	Model 1 Fixed	Model 1 Random	Model 2 Fixed	Model 2 Random	Model 3 Fixed	Model 3 Random	Model 4 Fixed & Random
σ^2_{cg}	-	878,960 (33,617)	-	10.34 (0.40)	-	4.91 (0.02)	4.96 (0.02)
σ^2_a	837,300 (27,385)	842,500 (25,093)	10.76 (0.49)	10.85 (0.47)	11.96 (0.40)	24.12 (0.66)	10.48 (0.60)
σ^2_{pe}	-	-	15.08 (0.35)	15.01 (0.33)	16.94 (0.30)	10.65 (0.44)	14.23 (0.43)
σ^2_e	1,442,700 (20,438)	1,493,200 (19,145)	14.60 (0.01)	14.60 (0.01)	11.81 (0.01)	11.86 (0.01)	11.85 (0.01)
h^2	0.37 (0.01)	0.27 (0.01)	0.27 (0.01)	0.21 (0.01)	0.29 (0.01)	0.47 (0.01)	0.25 (0.01)
h^{2*}	0.37	0.36	0.27	0.27	0.29	0.52	0.29
r^2	-	-	0.64 (0.01)	0.51 (0.01)	0.71 (0.01)	0.68 (0.01)	0.60 (0.01)
REL	0.79	0.79	0.81	0.81	0.81	0.89	0.81

cg = contemporary group. Depending on the model, this represents herd-year-season or herd-pen-milking date. σ^2_{cg} = contemporary group variance; σ^2_a = additive genetic variance; σ^2_{pe} = permanent environmental variance; σ^2_e = residual variance; h^2 = heritability; h^{2*} represents heritability calculated where cg is random without σ^2_{cg} in the denominator of the h^2 calculation; r^2 = repeatability; REL = Predicted Transmitting Ability Reliability for sires with ≥10 daughters.

group variance was included in the denominator. Interestingly, we found large differences between variance components when HPM was modeled as random in model 3. The estimates for residual variance did not change, however the additive genetic variance increased from 11.96 to 24.12, while the permanent environment variance decreased from 16.94 to 10.65. Given the high number of levels in HPM, and the non-independent relationship among HPM levels, we assume that the correlations among the residuals are high, which is causing this partitioning of variance between the additive component and permanent environmental variance. Finally, in model 4, once HYS is fit as fixed along with HPM as random, we see comparable results to model 2 and 3 (HPM fixed) in terms of variance component estimates and heritabilities (Table 2).

4. Confounding effects among variables

For model 3, we found large differences among variance components when HPM was fit as fixed vs random. Our hypothesis is that there is a relationship among residuals within the HPM variable that is creating a challenge to disentangling the relationship between repeated records in HPM levels and additive genetic variance. For example, although in theory a cow could move pens every day and have 300 unique contemporary groups throughout a

lactation, this is highly unlikely. The practice of grouping cows is to homogenously manage groups of cows, and to avoid the management and additional labor of managing cows at the individual level. Therefore, it is likely that the same group of cows are in the same pen for multiple days or weeks and this correlation among residuals is not being captured by the permanent environmental effect. Additionally, there is a risk of confounding between the genetic effect, contemporary group effect and permanent environment effect. This is due to the fact that HPM groups are reassigned throughout the lactation depending on the phenotype expressed by the individual animal and for management purposes, whereas in the past HYS was strictly based on the calving year and season of the cow. As such, we decided to fit HYS as a fixed effect along with HPM as a random effect and we found comparable results to previous models. This is probably because HYS is capturing the additive genetic effect that HPM was not previously capturing due to the confounding effect and breaking this relationship between repeated records of the same group of cows in the same pen over longer periods of time.

5. Sire PTA Reliabilities

Sire PTA reliabilities were estimated to assess whether using large volumes of daily milk yield data and assigning contemporary groups using pen information would increase the accuracy of selection decisions. We found a 0.02 increase in mean REL of sire PTA when using daily milk weights as the phenotype in comparison to 305-d milk yield. Aside from model 3 when HPM was fit as random, we did not observe differences among reliabilities when fitting contemporary group as fixed or random (Table 2). We did not observe differences in sire PTA reliabilities when using HPM as the contemporary group instead of HYS, which could be attributed to the fact that an autoregressive structure may be more suitable for modeling the HPM variable to account for the high correlations among residuals of each HPM levels, as discussed previously.

Conclusions

Utilizing daily milk weights generated by on-farm sensors increases the reliabilities of sire PTAs. The advent of high frequency novel data sources for use in genetic evaluation purposes may require new definitions for contemporary groups. In the specific case of milk production, the reliabilities of sire PTAs increased when using daily milk weights as opposed to 305-d milk production. Updating the definition of contemporary groups for genetic parameter estimation using herd-pen-milking date as a fixed or random effect impacts the reliabilities of sire PTAs, perhaps due to the high correlations among residuals for contemporary groups. Additional research is required to optimize genetic parameter estimation with high frequency data generated by sensors for genetic evaluation purposes. Including herd-year-season as a fixed effect along with herd-pen-milking date as a random effect may account for the non-independent relationships among residuals while also increasing sire PTA reliabilities in comparison to the current model utilized in the U.S. which uses herd-year-season along with 305-d milk yield as the phenotype.

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Heritable variation in gene expression is the key to maximizing genetic gain and preserving genetic diversity with a properly designed breeding program.

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Abstract

A hierarchical organization of molecular phenotypes provides a biological system of genes and pathways which can lead to different genotypes (redundancy) being selected in different subpopulations for the same phenotype. Heritable variation in transcription and translation is the key driver of genetic change. Redundancy in the regulatory code allows for genetic diversity amongst subpopulations. Gene expression is regulated by transcription factors (TF) ensuring that the right genes are active in the right tissue at the right time. A large amount of standing genetic variation is available from the many potential TF-TF interactions and TF interactions with other regulatory elements. Further diversity is possible in that each TF can target hundreds to thousands of different genes; and many of these genes through exon splicing, can produce functionally diverse transcripts and protein isoforms. These complex interactions form gene regulatory networks controlling specialized metabolic pathways. Which pathway is enriched is dependent upon the epistasis created by different founders, i.e., the ancestral makeup of the subpopulation. Selection on these epistatic effects leads to gametic disequilibrium between replicate populations causing them to differentiate. Different genetic architecture results in varied allele frequencies between subpopulations and intermediary allele frequencies in the global population. Genetic diversity within the Holstein breed can be preserved or increased with a proper population structure. This includes having multiple lines of Holsteins; utilizing multiple reference and target populations; genomic predictions for an overall global population and each separate subpopulation; avoidance of pooling SNPs; and analysis of transcriptomes for possible grouping of animals.

Key words: gene expression, redundancy, subpopulations, genetic variation

Introduction

Increasing rates of inbreeding is a concern amongst all the major Holstein breeding countries. Surveillance of undesirable monogenetic conditions along with genetic testing and selective purging of carriers has minimized the negative impact of an increase in homozygosity at undesirable individual loci. However, over the long-term, a reduction in genetic variation could be a bigger problem.

Rapid genetic change, population differentiation and maintenance of genetic variation has fascinated researchers for a long time. Charles Darwin reported that different species of finches lived on the different Galapagos islands. He speculated that population differentiation was a fundamental component of evolution. Sewall Wright spoke frequently and forcibly about the importance of population structure in maintaining genetic variation. In his 1950 paper on “The Genetic Structure of Populations”, he wrote “the subdivided population maintains more alleles at each locus and more at moderately high frequencies”. That is, selecting for different alleles and different genes in different subpopulations helps maintain genetic diversity across the entire population.

Those scientists would have been greatly aided in their understanding of the changes in the genetics of subpopulations by today's advancements in molecular biology. Much of the genetic variability observed in different polygenic traits originates from differences in gene expression. A meta-study in cattle estimated that 69% of the heritability of polygenic traits was due to variants associated with gene expression (Xiang et al., 2023).

Genes are regulated by transcription factors (TF) ensuring that the right genes are active in the right tissue at the right time. Given that there are thousands of TF, within a population a large amount of genetic variation is created during transcription from specific TF having the ability to interact with many other TF, TF interacting with other regulatory elements, and by each TF having the ability to target hundreds to thousands of different genes. Further variation is created during translation where many genes, through exon splicing, can produce functionally diverse transcripts and protein isoforms. The amount of standing genetic variation within a population can be very large and there can be many different combinations of the genetic variants controlling gene expression that can lead to the same phenotypic change. The phenomenon of multiple genetic solutions leading to the same phenotypic change is known as genetic redundancy (Barghi et al. 2019). That is, there are more variants segregating in the whole population than are needed to achieve a specific phenotypic change.

Genomic testing of populations that have been divided and selected for the same phenotypic goal frequently show non-parallel changes in allele frequencies (Barghi et al. 2019) along with different transcriptomic changes, different gene networks being formed, and different biological pathways being emphasizes (Lai et al., 2023). While the subpopulations differed in which genetic variants were favored or disfavored, the resulting change in metabolites were similar leading to the same overall phenotypic change. This hierarchical organization of molecular phenotypes provides a biological system of genes and pathways which can lead to different genotypes (redundancy) being selected in different subpopulations for the same phenotype.

The biological system of different genes and pathways are known as a gene regulatory network (GRN). Often described as being modular, different GRNs can be used in a similar way. Having redundancy of different GRNs has several benefits. For an individual, the most obvious benefit is that one GRN can compensate for mistakes in other pathways. For a population, different genetic changes in a GRN can lead to an improved function or an evolutionary change.

The ancestral makeup, i.e., the original founders, of the different subpopulations is important for multiple reasons. Given the large number of possible combinations of genetic variants involved in gene expression, different subsets of founders will possess different genotypes, different genes will be enriched, leading to different GRNs and pathways. Another important component is epistasis. Whereby a certain gene has a positive effect in one subpopulation and the opposite effect in another. The value of an epistatic gene differs across subpopulations because its value depends upon what other genes are in that subpopulation. With epistasis, different subpopulations are selecting for different gene combinations.

Integrating new molecular biology information along with quantitative genetic theory provides us with a more accurate prediction of how divided populations change over time. With selection, subpopulations should diverge and become more differentiated over time as different genotypes, i.e., different gene-gene interactions, are favored in different subpopulations. This process is known as gametic disequilibrium (Tomoko, 1982). Rapid changes in genetic architecture in divided populations, when selecting for the same phenotypic goal has been observed in both

plants and animals. The variants changing the most tend to be associated with gene expression. Comparison between subpopulations indicate changes occurring in both shared and unique pathways. In dairy cattle, a decline in predictability of future performance is observed as the time between the animals in the reference and target populations increases.

Materials and Methods

The population structure of U.S. Holsteins was investigated for two different time periods, 2014 and 2022, using the CDCB's National Cooperator Database. The 2014 data set has been discussed by Steyn et al 2023. K-means clustering on the genomic relationships of animals born between 2010 and 2014 identified five subpopulations. Four of the clusters were composed primarily of the descendants of four prominent sires that had been used extensively during that time period. The genetic contribution of the prominent sire for each cluster; Planet, Goldwyn, Shottle and O Man were 28.1%, 18.8%, 19.8% and 21.6%, respectively. The fifth and largest cluster was composed primarily of the offspring of many different sires, with no individual bull having a genetic contribution exceeding 4.3%. Trajectory of allele frequency change for 58,990 SNP markers was calculated across 10 generations for each of the subpopulation.

The combination of genomic selection with sexed semen, advanced reproductive technology and restricted access to young genetics has led individual breeding organizations and countries to genetically diverge from one other. By 2022, population structure was no longer determined by the heavy usage of individual bulls but more by the breeding program of large organizations. Young bulls with a minimum TPI value of 3000 were selected from the December 2021 official genomic evaluations of CDCB. Almost all the 713 young bulls were sired by a bull controlled by the same breeding organization. Four breeding companies controlled 91% of these bulls. Between 83% and 94% of the mothers of these top young bulls were also controlled by the same breeding organization that had control over the sire and his sons. Each of these breeding organizations has created their own subpopulation. To measure genetic differentiation Wright's Fixation Index (F_{st}) was calculated as follows:

$$F_{ST} = (F_{IT} - F_{IS}) / (1- F_{IT})$$

where F_{IT} is the inbreeding within total population and F_{ST} is the inbreeding within subpopulation.

An important component of genomic architecture is the size of the SNP effects or substitution effects which includes the effects due to additive and dominance gene action, inter-locus interactions.

$$\alpha_i = a_i + (1 - 2p_i)d_i + \sum \alpha_{ij}^i$$

Our primary interest in this paper was a researcher's ability to identify inter-locus interactions when data from all subpopulations are pooled together.

Results & Discussion

New information from molecular biology provides valuable insight on the vast amount of redundancy available with respect to alternative genetic solutions to achieve a common phenotypic change. Extensive use of individual bulls or control of access to top genetics are two ways that subpopulations have been created within the U.S. Holstein population. Existence of subpopulations is beneficial in that it helps preserve genetic diversity.

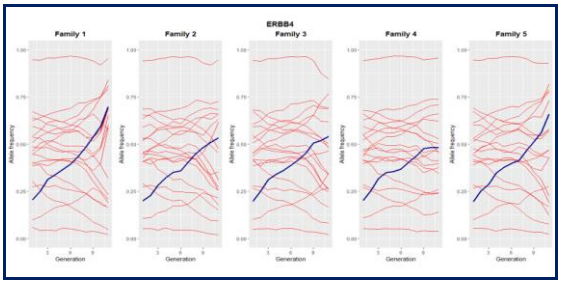
Steyn et al. 2023 reported that different sets of SNPs were changing over time in different subpopulations of U.S. Holsteins. Heterogeneity in SNP frequency changes across subpopulations indicates that different SNPs are being targeted in different subpopulations. While as many as 59 SNPs went to fixation in one of the subpopulations,

fixation of SNPs was infrequent across the whole population (3 alleles).

Much of the genetic variability observed in different polygenic traits originates from differences in gene expression. These heterogenous genomic changes in different subpopulations lead to differences in transcriptomic response, development of distinct GRNs and enrichment of different biological pathways. So why are gene interactions so frequently ignored by quantitative geneticists?

The pooling of all data together into a single national or global evaluation causes us to miss important gene-gene interactions that are important for maintaining genetic variation. Figure 1, adapted from Steyn et al, 2023, provides an illustration of this point. By pooling all data together, genetic interactions which have a heterogeneous effect in different subpopulations are ignored.

A. SNP marker identifying the gene ERBB4



B. SNP marker with no known function

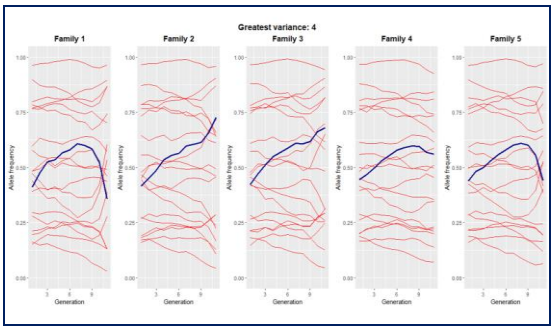


Figure 1. SNP A has a consistent effect in all families resulting it a high SNP effect. SNP B has an inconsistent effect across families, its SNP effect would be low due to being averaged across all families.

This means that our current genomic selection programs are selecting for certain type of gene actions and ignoring other genetic options. For a SNP to have a consistent or large effect across all subpopulations the SNP's action must be direct and largely independent of other genes, e.g., a protein coding gene or using terminology from the omnigenic model a "core" variant involved in gene expression (Mathieson, 2021). SNPs with an inconsistent effect across subpopulations are referred to as "peripheral" genes, affecting the phenotype through a network of interactions with other peripheral genes and core genes.

The important message for our Interbull community is that pooling data sets together for the sake of obtaining higher accuracy of prediction does so by focusing on a limited type of genes, i.e., core genes while ignoring the more numerous peripheral genes. The solution would be to recognized multiple subpopulations within the Holstein breed with separate reference populations and separate genomic evaluations. This allows for the selection of more peripheral gene action, enriching different GRNs in different subpopulations, and preserving genetic diversity across the entire breed.

Combining genomic selection with sexed semen, embryo transfer and restricted access to young genetics has led individual breeding organizations and countries to genetically diverge from one other. Figure 2 presents a measure of genetic differentiation (Fst) or population structure of the U.S. Holstein population in 2022. Each of the different breeding organizations are focusing on a slightly different group of animals. Current genetic differences between breeding organizations approach one quarter to one half of the genetic differences found between dairy breeds.

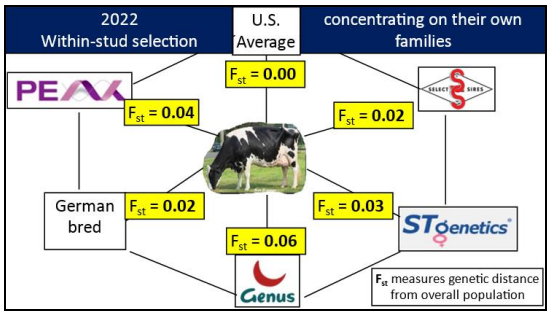


Figure 2. Wright's Fixation Index of major breeding organizations in U.S. Holsteins in 2022.

Within-stud selection has led to the assembly of breeding units made up of slightly different families. This is the start of our breed having different lines of Holsteins to choose from. AI breeding companies can further expand this concept by having multiple lines available within each organization. Farmers could then rotate between lines and continue to make rapid genetic gain while maintaining low inbreeding within their own herd and high genetic diversity across our breed.

Having multiple lines of Holstein does not mean that we all go off in different directions. Quite the contrary. It means that we must use our genetic resources more wisely. Breeding organizations will need to be committed to the program. National genetic evaluation centers will need to provide multiple genetic evaluations, which includes a national overall ranking as well as separate evaluations, with its own genomic reference population, for each domestic line. Our international organizations, such as Interbull, will need to develop genetic tools that routinely monitor the genetic distances between lines and the overall change in inbreeding in our global population. And we will all need to be heavily involved in the educational process of the benefits of this new breeding design and how to properly use multiple lines within a herd.

Conclusions

In our current genetic evaluations, all animals are pooled together causing the unique gene-gene interactions from the different subpopulations to cancel one another out. Rather than selecting for unique and/or epistatic combinations of genes, we select for those genes that have a similar or additive effect across the breed. The highest genetic merit animals are those with the highest total of high average effect SNPs. Genetic diversity within the Holstein breed can be preserved or increased with a proper population structure. This includes having multiple lines of Holsteins; utilizing multiple reference and target populations; and providing genomic predictions for each separate subpopulation as well as the overall global population.

Acknowledgments

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Revision of random regression test-day model has improved genomic prediction for Nordic Red dairy cattle

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Abstract

In 2006, Denmark, Finland, and Sweden have introduced across-country genetic evaluations for yield traits for their Holstein, Red dairy cattle (RCD) and Jersey populations. The implemented breed-specific random regression test-day models (RRM) were the outcome of an intensive research cooperation among the countries. Especially developing the RRM for the RDC population presented unique challenges due to its heterogeneous population structure spanning across Finland, Sweden, and Denmark. As genomic prediction became a key tool for breeding decisions, it became evident that the reliability of genomic enhanced breeding values (GEBV) for the RDC breed was lower than expected when compared to the Holstein and Jersey breeds. Several factors contributed to this discrepancy. Notably, during the last two decades, changes in herd and population structures were most pronounced within the RDC breed where the original RDC country-subpopulations have become much more alike. Thus, revision of the RRM is crucial in enhancing the reliability of GEBV for the selection of breeding candidate animals. First important improvements were the revision of modelling automated milking system data and a newly estimated set of variance components with lower h^2. The updates made so far indicate considerable improvement in the genomic predictions. The LR regression coefficient (b_1) values increased for example for milk yield from 0.85 of the original model to 0.92 of the revised model, indicating that the bias decreased with the revised model. Also, the coefficient of correlation (R^2) increased for all production traits on average 4.5%. In a next step, we will truncate the phenotypic data, optimize the pedigree information, and study whether modelling metafounders for the heterogeneous RDC population will result in further improvements for the genomic prediction.

Key words: Nordic Red dairy cattle, yield evaluation, automatic milking system

Introduction

In 2006, the Nordic countries, Finland (FIN), Denmark (DNK), and Sweden (SWE), introduced across-country genetic yield evaluations for their dairy cattle populations. The outcome of intensive research cooperation was the random regression test-day models (RRM) for Holstein, Red dairy cattle (RDC), and Jersey. In 2010 the models were updated to replace the Swedish lactation yield observations by test-day observations and to apply a common set of variance components instead of country-specific variance components (Lidauer et al., 2015) and continue to serve as the basis for predicting genomic enhanced breeding values (GEBV).

From the beginning, the model for the RDC breed proved to be the most challenging due to the heterogeneous population structure of RDC cattle across the Nordic countries. After genomic predictions for the three breeds were built, it was observed that the reliability of GEBVs for RDC was lower than expected, especially when compared to the reliability of GEBVs for Holstein and Jersey. Potential reasons for this discrepancy include changes in herd and population structures, which may have made the applied RRM suboptimal for genomic prediction. Revising the RRM for RDC

evaluation was found to be crucial for improving the reliability of GEBVs and for the optimal selection of candidate animals.

Materials and Methods

Model

The applied multiple-trait RRM describes test-day milk, protein, and fat yields for a cow's first three lactations using nine model equations. Each trait has a random regression function for random genetic and permanent environmental effects. The model includes fixed effects nested within countries, some of them also nested within breed, heterosis and recombination loss adjustments, and adjustments for heterogeneous variance (HV). Due to modelling of covariance functions, 15 cow-specific coefficients define all nine breeding value curves. For more information, see Lidauer et al. (2015).

Revising the variance components

In the old RRM (in use until November 2023), heritability was based on variance components estimated from Swedish data and were considered too high, especially for later parities. Most of the RDC test-day data come from Finland, where earlier studies found lower heritability (h^2) values compared to those used in the RRM. Specifically, variance components for permanent environment and genetic effects in later lactations were too high. This became even more critical after assigning higher weights to later lactations in the Nordic Total Merit Index. The updated variance components are now based on Finnish data, resulting in lower heritabilities, particularly for protein and fat yields in later lactations (Figure 1). Consequently, now the variance components better fit the data.

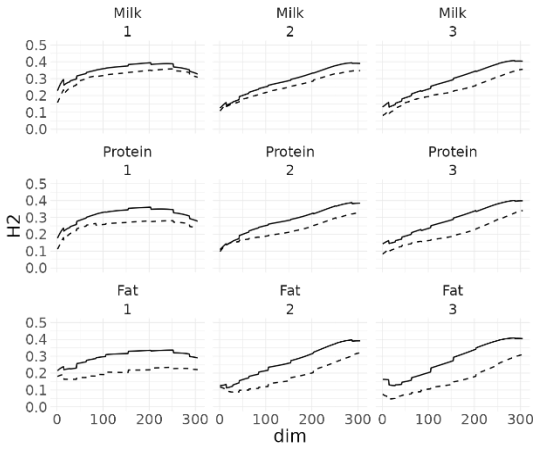

Figure 1. Heritabilities in old (solid line) versus updated (dashed line) model for milk, protein, and fat in lactations 1-3

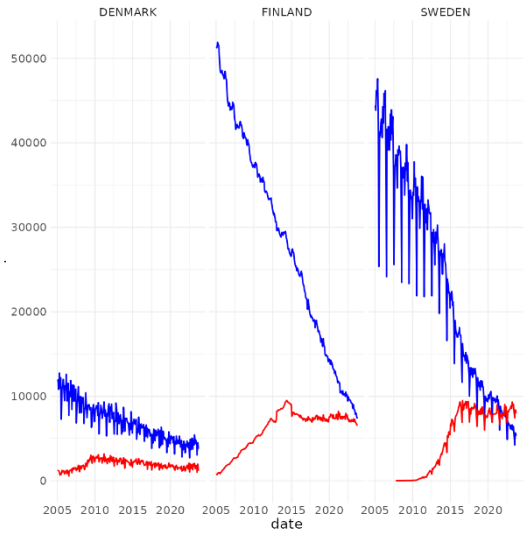

Figure 2. Number of test-day observations for first lactation milk yields by country and milking system. Blue lines from conventional milking system (CMS) and red lines from automatic milking system (AMS).

Updated modelling of automatic milking system data

Over the last two decades, there has been a rapid decrease in conventional milking system (CMS) observations. Meanwhile, automatic milking system (AMS) observations have increased slightly, and currently, approximately half of the test-day records in Finland, Sweden, and Denmark come from AMS (Figure 2).

The old RRM assumed the same residual variance for all AMS observations. However, in Finland, the measurement protocol has changed twice over the years, with the latest methods based on a smaller number of AMS milkings. The residual variances differ between measurement. This caused issues in simultaneously adjusting for HV, as it overcorrected observations made using the new measurement protocols due to incorrect

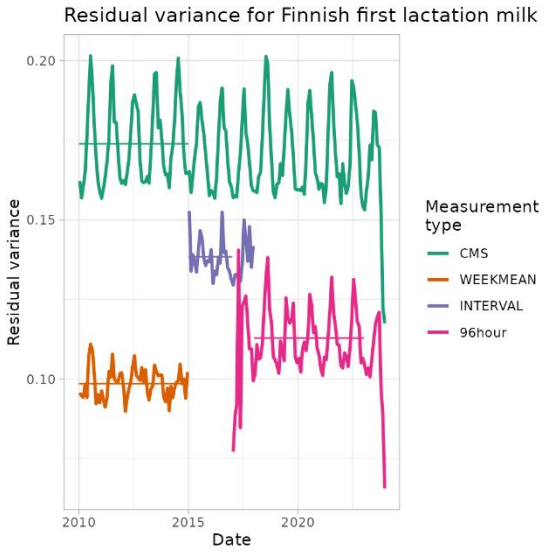

Figure 3. The level of residual variance in different milking system protocols by months from 2010 to 2023. Green line conventional milking system (CMS).

baseline variances. To address this, the model was updated to provide separate residual variances for the different methods to calculated 24h yield from AMS.

The updated milking protocols in Finland are defined 1) as sum of morning and evening milking yields in CMS for whole data collection period. For AMS there are three measurement types: 2) the average of one weeks milkings during years 2010-2015 (weekmean), 3) sum of two successive milkings scaled to 24h yield during 2015-2017 (Interval), and 4) four days average milkings (96hour) from 2017 onwards.

The Figure 3 shows the level of residual variance in different daily milk yield measurements. It shows that the residual variance for CMS is always higher than that for different AMS systems. The correct measurement information for AMS observations was included also in the data, and the HV adjustment was updated to handle the different AMS recording protocols.

Holstein observations in the Finnish test-day data

In the past, Finnish herd sizes were small. As a result, test-day records for Holstein cows were included in the model to increase contemporary group sizes, although the results of the Holstein evaluations were not used from this model. However, herd sizes in Finland have increased and this is no longer as relevant, so Holstein observations were removed from the Finnish test-day data. This removal also required revising model effects that included breed interactions.

Testing with updated model

All test-day data and genotype data available in February 2023 were obtained from the NAV. The data included 4.7 million cows with observations and 6.2 million animals in the pedigree. Genotype data included 229,706 genotyped animals.

The animal model and single-step RRM were solved by preconditioned conjugate gradient method (Strandén and Lidauer, 1999). The genomic evaluation was realized by ssGTaBLUP (Mäntysaari et al., 2017). For setting up the genomic relationship matrix (**G**) the VanRaden method 1 and a 10% residual polygenic proportion (RPG) were used, and diagonal of **G** was scaled to be on average equal to the pedigree-based relationship matrix of the genotyped animals ($\mathbf{A}_{22}$) (Vanderplas et al., 2023). The pedigree inbreeding coefficients were accounted for both in $\mathbf{A}^{-1}$ and $\mathbf{A}_{22}^{-1}$.

Model comparisons were based on forward prediction validation, utilizing solutions from both full-data and reduced-data evaluations. The reduced data were derived by removing the last four years of observations from the full data set. The linear regression validation (LR) method (Legarra and Reverter, 2018) was employed for validation. This method compares predictions based on reduced and full data, yielding estimates of accuracy and bias.

Danish, Finnish, and Swedish bulls born between 2014 and 2018, each having at least 20 daughters in the full-data set but no daughters in the reduced-data set, were defined as candidate bulls. This criterion resulted in a total of 222 candidate bulls.

Results & Discussion

The updated model changed the estimated breeding values (EBV) and the GEBV of the animals, with the correlation between the old and new EBVs averaging 0.97 and 0.98 for GEBVs. Consequently, the RRM update caused some reranking of the bulls. This was expected, as the incorrect AMS protocol had caused issues, particularly for Finnish cows, which also affected bull evaluations. With the corrected milking system data, the (G)EBVs for some bulls changed accordingly.

The comparison of EBVs and GEBVs from the updated model showed that validation of the bulls improved with corrections (Table 1). In genomic animal model bias decreased considerably and both regression coefficient (b_1) and coefficient of determination (R^2) improved compared to the old RRM.

Table 1. Linear regression (LR) results for the validation bulls' breeding values based on the BLUP (EBV) or ssGTaBLUP (GEBV) evaluations applying the old (old) or the updated (new) model. The values in the table are: b0= mean(Full_((G)EBV–reduced_(G)EBV) ±SD, b1 regression coefficient and R2 coefficient of determination.

	Model	b_0	b_1	R^2
Milk	EBV_{old}	129.46 (±540.0)	0.68	0.22
	EBV_{new}	-81.18 (±569.9)	0.69	0.23
	$GEBV_{old}$	-473.88 (±363.7)	0.85	0.66
	$GEBV_{new}$	-291.34 (±354.9)	0.92	0.70
Protein	EBV_{old}	-0.72 (±16.2)	0.62	0.21
	EBV_{new}	1.24 (±17.0)	0.63	0.21
	$GEBV_{old}$	-17.78 (±12.3)	0.75	0.60
	$GEBV_{new}$	-12.41 (±11.9)	0.84	0.63
Fat	EBV_{old}	-1.77 (±21.4)	0.77	0.28
	EBV_{new}	3.20 (±27.9)	0.76	0.27
	$GEBV_{old}$	-22.91 (±15.6)	0.84	0.63
	$GEBV_{new}$	-18.61 (±19.6)	0.91	0.65

The protein genetic trends for bulls are shown in Figure 4A. After the introduction of genomic selection, the genetic trend in single step evaluation started to be much higher in the

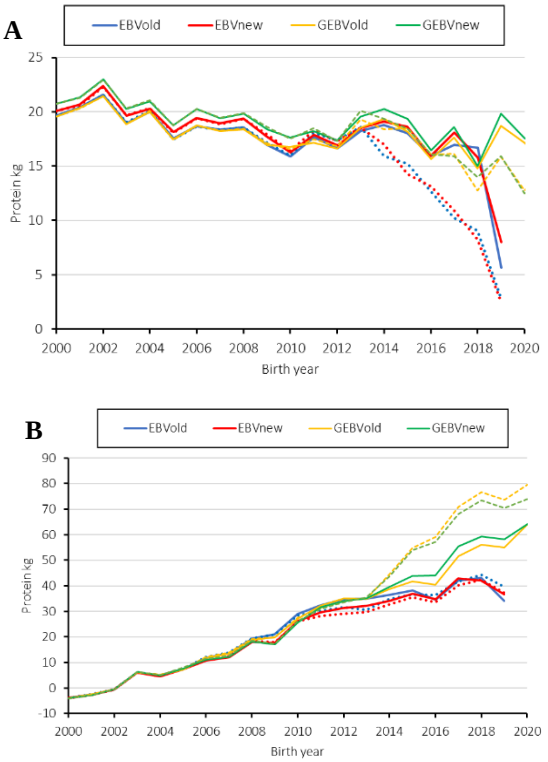

Figure 4. **A**) Genetic trends for protein yield (kg) by birth year averages. (**B**) SD for protein yield breeding values (kg) by birth year. EBVold the old RRM model, GEBVold the single step with old RRM, EBVnew the updated RRM and the GEBVnew the single step with updated RRM. Solid lines are for full data and dashed lines for reduced run.

reduced data compared to that in the full data run with the old RRM, whereas with the updated RRM the difference between the genetic trend in the reduced run and full run is reduced and thus bias is decreased. As expected, the SD of the EBVs and GEBVs slightly increased with the updated model due to new AMS protocol (Figure 4B).

The updates made to the RRM so far have already enhanced the genomic predictions for the RDC. Specifically, the bias has decreased, and the b_1 value has increased. Further changes are underway, including data truncation to exclude test-day records prior to 2005 and optimization of pedigree. Additionally, work is ongoing to refine the definitions of calving age, heterosis, and recombination effects. Once all effects in the RRM have been updated, we will investigate replacing unknown parent groups

with metafounders to assess whether this approach further improves genomic predictions.

Conclusions

As a final remark, changes made so far in the model improved validation results. These changes included updating variance components, improved handling of residual variance of Finnish AMS records, as well as removing Holstein observations.

Acknowledgements

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Genetic trend in milk fat percent is highly responsive to the relative economic value of milk fat and milk protein in the New Zealand dairy sector

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Abstract

Milk components are important traits within Breeding Worth (BWSI), the national selection index of the New Zealand dairy industry. Breeding Worth is an economic index, and trait weightings are calculated based on the economic value (EV) that each trait contributes to a dairy farm business. The EVs are updated annually, but key parameters like the absolute and relative values of milk solids, and the relative value of milk fat and milk protein are included as a 5-year rolling average to mitigate against volatility in animal rankings. In 2015, the global price for milk fat began to increase, and between 2015 and 2022 the value of milk fat relative to milk protein rose rapidly year on year. The increase in the dollar value of milk fat relative to milk protein was gradually mirrored in BWSI weightings, and although the response in milk fat yield was modest, a clear inflection in the genetic trend for milk fat percent was observed from 2018 onwards. We sought to understand the drivers for this change in the rate of genetic gain, especially given the multi-breed composition of the NZ dairy herd. For a trait like milk fat percent, which differs between the Jersey and Holstein-Friesian breeds, NZ farmers can use both breed substitution and within-breed selection to alter the performance of their herd. We report that both breed substitution and within-breed selection appear to have contributed to the genetic trend in milk fat percent. The use of Holstein-Friesian sires declined between 2015 and 2020 in favor of Holstein-Friesian cross Jersey sires. Similarly, a 15-year decline in the use of Jersey sires was reversed in 2015, with a small increase recorded between 2015 and 2020. The within-breed response to the change in relative weightings on milk fat and milk protein is also notable, demonstrating the power of coordinated selection towards a breeding objective. Our findings highlight the importance of carefully considering the approach used for determining weighting factors within a selection index, especially for traits as responsive to selection as milk fat.

Key words: *Genetic Trend, Milk Production, Jersey, Holstein-Friesian*

Introduction

The national breeding objective for the New Zealand (NZ) dairy sector is to breed cows that efficiently convert feed into profit. DairyNZ, and its subsidiary New Zealand Animal Evaluation Limited (NZAEL) are responsible for designing the national selection index 'Breeding Worth' (BWSI), which ranks animals according to their ability to meet this objective. The BWSI incorporates a range of economically important traits, each weighted according to its economic value to farmers. These economic values vary over time, and the weighting factors applied to each trait are updated annually in December to reflect current market conditions. The final BWSI assigned to each animal represents its ability to breed profitable replacement heifers for an NZ dairy herd, relative to other potential parents. The BWSI is widely used by farmers in NZ, and changes to the weightings and/or traits included in the BWSI have a noticeable effect on trait-specific genetic trends. For example, the fertility trait was introduced into BWSI

around 2002, resulting in an obvious increase in the rate of genetic gain for this trait (Pryce et al., 2014).

The national herd in NZ is a highly admixed population, comprised of two major breeds, Holstein-Friesians (HF) and Jerseys (J), and their crosses. The BWSI is produced using across-breed estimated breeding values (EBVs), meaning that it is designed to provide farmers with an objective ranking of animals, irrespective of breed. Trait means differ between HF and J cattle for several traits that are economically relevant to farmers, and so it could be expected that the breed composition of the national herd might change in response to changes to the weighting factors in BWSI. Furthermore, where trait means differ by breed, the availability of more than one breed could be strategically used as a tool to more quickly respond to changing market conditions.

Holstein-Friesians and Jerseys differ in milk fat percent (DairyNZ & LIC, 2022), and between 2015 and 2022 the value of milk fat relative to milk protein rose rapidly year on year. The structural shift in the market value of milk fat was reflected in the weighting factors within the BWSI and, consequently, an increase in the rate of genetic gain for milk fat percent. Given that NZ farmers can use both breed substitution and within-breed selection to modify the milk fat percent of their animals, we sought to understand the drivers for this change in the rate of genetic gain.

Materials and Methods

Data

All animal data and EBVs presented in this analysis were provided by NZAEL.

Breed categories

Animals were assigned to breed categories based on pedigree-derived breed percentages. Animals that were 87.5% or greater HF or J were categorized as those breeds. Animals that were not 87.5% or greater HF or J, but whose HF and J percentage summed to at least 87.5% were categorized as HF cross J (HFJ). The all-breeds category included all recorded animals, including minority breeds such as Ayrshires.

Weighting factor of milk fat and milk protein over time

The current weighting factors for traits included in the BWSI are publicly available on the DairyNZ website at the following URL: https://www.dairynz.co.nz/animal/breeding-decisions/economic-values/. We obtained historic weighting factors from NZAEL directly (Figure 1). The weighting factors are presented in economic terms, and no attempt has been made to adjust for inflation.

Genetic trends for milk fat and milk protein

The genetic trend plots for milk protein percent (Figure 2) and milk fat percent (Figure 3) represent the mean EBVs of all recorded dairy cows, by birth year and breed. Animal counts increased over time and varied by age and breed. The number of animals contributing to the all-breeds category ranged from around 700,000 to around 1,200,000. The number of animals contributing to the HF breed category ranged from around 300,000 to around 360,000. The number of animals contributing to the J breed category ranged from around 80,000 to around 100,000. The number of animals contributing to the HFJ breed category ranged from around 250,000 to around 750,000.

Breed composition of the national herd

The breed composition plot (Figure 4) represents the proportion of all recorded cows in each birth year sired by either Holstein (H), HF, HFJ, or J sires. The number of animals represented in each birth year increased over time, ranging from around 640,000 to around 1,100,000 in more recent years.

Results & Discussion

Weighting factor of milk fat and milk protein over time

The weighting factor applied to milk fat in BWSI rapidly increased from 2017 to 2021 (Figure 1). Conversely, between 2014 and 2020, there was a decrease in the weighting factor applied to milk protein. Between 2016 and 2022, the negative weighting on milk volume increased (data not shown), further incentivizing selection for dairy cattle with higher milk fat and protein percentages.

The findings of this study have important implications for the formulation and adjustment of BWSI weightings. The clear inflection in the genetic trend in milk fat percentage following the adjustment of weightings suggests that even modest economic signals can lead to significant genetic responses if selection pressure is sustained, especially for traits like milk fat percent, which exhibit moderate to high heritability (Ahlborn & Dempfle, 1992; Jayawardana *et al*., 2023). The study underscores the importance of considering long-term structural changes in market signals when setting selection index weightings, as powerful changes in animal performance can result from changes in both within- and between-breed selection decisions.

Genetic trends for milk fat and milk protein

The genetic trend for milk protein percentage has been consistently positive since 1995 (Figure 2). Conversely, the genetic trend for milk fat percent remained relatively flat until around 2017, at which point a positive inflection is clearly observed (Figure 3).

The study highlights the significant within-breed response to the changes in BWSI weightings. Successful within-breed selection for increased milk fat percentage demonstrates the potential for genetic improvement even within a single breed, provided that selection pressure is appropriately applied. This result emphasizes the importance of a national breeding objective and the need for continuous monitoring of selection indices. When clear new market signals are identified, indices should be adjusted to ensure they remain aligned with economic realities.

A second avenue for genetic progress in breed-divergent traits like milk fat percent is breed substitution. The breed composition of the NZ national dairy herd has changed dramatically in the past 20 years (Figure 4). Most notably the proportion of cows sired by HFJ sires has risen from a starting point of nearly 0 in year 2000 to around 35% in year 2022. Initially the increase in use of HFJ sires corresponded to a decline in the use of J sires, while the use of HF sires either remained stable or increased. During this period, breed substitution was likely to be limiting the all-breeds genetic trend for milk fat percent. Around 2015 J sire use stabilized, and then began to increase slightly around 2017. At the same time, the use of HF sires began to decline in favor of HFJ sires, further increasing the proportion of J genetics contributing to the national herd. From 2017, breed substitution may have supported the positive genetic trend for milk fat percent in both the all-breeds and the HFJ category.

The all-breeds genetic trend for milk fat percentage has likely been influenced by both breed substitution and within-breed selection. The recent decline in the use of HF sires in favour of J cross HF sires, coupled with the reversal of the long-term decline in J sire usage, indicates a potentially strategic shift among NZ dairy farmers in favour of J genetics. The ability to utilize breed substitution to increase milk fat percentage of the national herd highlights the importance of maintaining genetic diversity and the availability of multiple breeds with differing trait characteristics. The strengths of each breed represent a resource that can be exploited should market conditions require it.

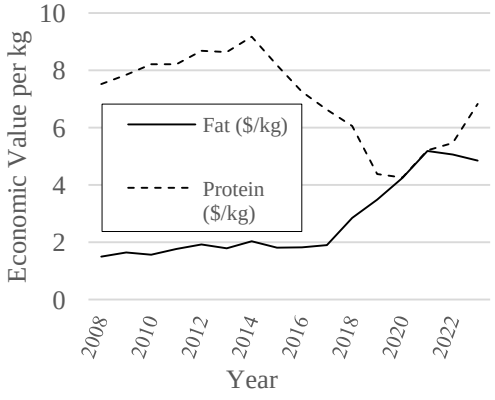

Figure 1. Weighting factor applied to milk fat and milk protein in the Breeding Worth Selection Index (BWSI) from 2008 to 2023.

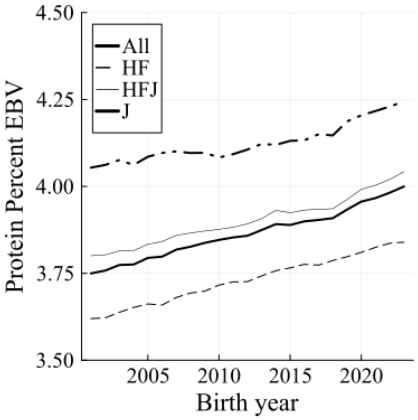

Figure 2. Genetic trend for milk protein percent by birth year for all female cows recorded in the New Zealand dairy sector (All), Holstein (H), Holstein-Friesian (HF), Holstein-Friesian cross Jersey (HFJ) or Jersey (J) cows.

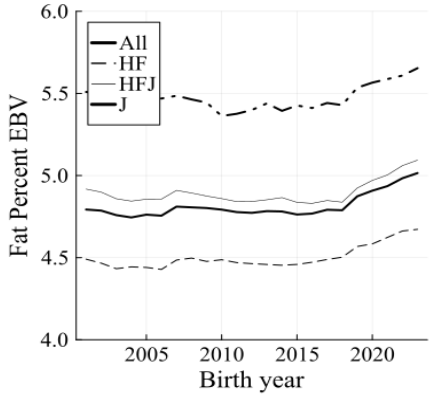

Figure 3. Genetic trend for milk fat percent by birth year for all female cows recorded in the New Zealand dairy sector (All), Holstein (H), Holstein-Friesian (HF), Holstein-Friesian cross Jersey (HFJ) or Jersey (J) cows.

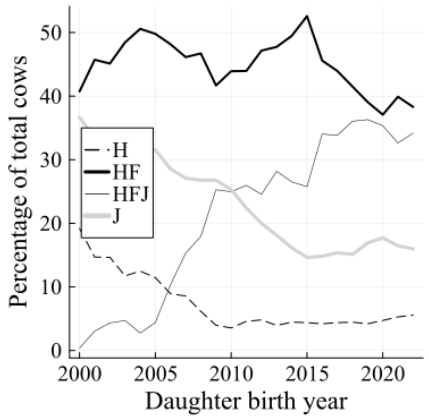

Figure 4. Proportion of cows in each birth year sired by either Holstein (H), Holstein-Friesian (HF), Holstein-Friesian cross Jersey (HFJ) or Jersey (J) sires.

Conclusions

In conclusion, this study provided valuable insights into the drivers of recent genetic gain in milk fat percentage in the NZ dairy herd. Although the genetic trend in milk fat percent appears to be primary driven by within breed selection, the occurrence of breed substitution highlights the opportunities that genetically diverse, mixed-breed populations can offer. This study contributes to a deeper understanding of the mechanisms driving genetic improvement in milk components in the NZ dairy sector.

Acknowledgments

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Next-Level Genomic Selection: Mitigating Inbreeding

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Abstract

Analysis of 88,068 autosomal or X-linked SNPs in the ANAFIBJ Holstein genomic database including males and females showed declining SNP heterozygosity over time. In 1990, average SNP heterozygosity was 0.3620. During the pre-genomics period (1990-2010), the annual decline was -0.0003, reaching 0.3558 in 2010. However, in the genomics period (2010-2024), the average SNP heterozygosity declined to 0.3191 in 2024, with an annual decline of -0.0027, over 7 times higher than before. So far, this trend has been highly linear (R^2=0.987), which would extrapolate (in the absence of other sources of genetic variation than selection and inbreeding) to result in a complete loss of genetic variation in ~130 years. We developed measures to estimate genomic expected future inbreeding (Gefi) based on Runs-Of-Homozygosity (ROH). A comparison with CDCB genomic future inbreeding (GFI) based on the genomic relationship matrix (GRM) was done using 38,280 genotyped proven males covering 70 years (1951-2020), which resulted in a Pearson correlation of 0.959. The CDCB GRM **G** was computed as $\mathbf{G} = \mathbf{ZZ}'/\Sigma 2p(1-p)$ using p=0.5. Gefi estimates had a mean of 6.9% with a standard deviation of 2.6%. Minimum Gefi was 0.1% and maximum was 15.3%. GFI estimates had a mean of 7.2% with a standard deviation of 2.6%. Minimum and maximum GFI were -3.1% and 13.5%. The correlation was quite high even though Gefi is an identity-by-descent (IBD) measure in the probability space of [0,1], whereas GFI measures identity-by-state (IBS) in the correlation space of [-1,1]. Comparison of the inbreeding depression across traits showed that the depression is largest on yield traits followed by contents, somatic cell score (SCS) and fertility at around 20% of the depression on yield traits. ANAFIBJ aims to reduce the increase in future inbreeding by giving a premium to male and female animals which are less related to the recent population, while penalizing those that are more related.

Key words: Inbreeding, Holstein, effective population size, genetic diversity, SNP, Runs-Of-Homozygosity, genomic selection

Introduction

If a population has no exchange of genes with other populations, then mutations become the only way for new genetic variation to arise, and they arise slowly. Over time, breeding within such a closed population reduces overall genetic variation unless mutations happen frequently enough. This results in individuals becoming genetically more similar and gradually leads to inbreeding. Choosing just a few breeding parents who are genetically alike speeds up the inbreeding process. This can have serious consequences; animals might

become less fertile and less healthy. Modern breeding techniques using genomic information (genomic selection) can worsen this decline, especially due to shorter generation intervals and stronger selection pressure. Unfortunately, low genetic variation makes populations less able to adapt to changing environments, increasing their extinction risk.

The Holstein reigns supreme in the dairy world. However, a hidden threat lurks beneath its success: a remarkably narrow genetic base. Despite the vast number of Holsteins globally, the breed's ancestry is surprisingly limited. Less than 10,000 Friesian animals were imported into North America over 130 years ago, and today, only two male lines effectively remain. This history of restricted gene flow, coupled with multiple genetic bottlenecks, has resulted in a population with low genetic diversity. While this focus has yielded high milk production, it could leave Holsteins vulnerable in the long run.

This paper and the presentation from the 2024 annual Interbull meeting in Bled are a follow-up of the paper and the presentation at the 2023 annual Interbull meeting in Lyon (Van Kaam et al., 2023). Estimates of genomic diversity were updated. Additional work was done to check genomic inbreeding coefficients from imputed data, to estimate the correlation between genomic future inbreeding measures, and to compare the size of inbreeding depression estimates per trait.

Materials and Methods

Issue of declining genetic variation

The ANAFIBJ genomic databank was used to analyze annual trends in genetic variation of Holstein SNP genotypes. After imputation, the annual average SNP heterozygosity of 88,068 autosomal or X-linked SNPs from male and female animals born between 1990 and 2024 was computed, excluding non-genotyped animals and animals without pedigrees. The year 2010 marked the transition from pre-genomic to genomic selection.

The analysis included both males and females. The average inbreeding coefficient per year was computed as (homozygosity this year - homozygosity first year) / (1 - homozygosity first year). Average generation intervals were calculated per year for the four pathways separately and then averaged. The relative year since 1990 was divided by the annual generation interval to estimate the number of generations that had passed since 1990. No Hardy-Weinberg equilibrium was assumed.

Genomic inbreeding coefficients from imputed data

A verification was done to check if genomic inbreeding coefficients from imputed data were reliable. This was done by comparing genomic inbreeding coefficients from imputed and genotyped SNPs. In the verification a set of high-density (139K/778K) genotypes were downgraded to either the GeneSeek Genomic Profiler 3 (26K) or to the Labogena MD (62K) SNP set. The downgraded set of 329 animals was also split into 2 subsets:

- Subset 1 of 266 animals with information on both parental sides i.e., S+D or S+MGS or S+D+MGS (S: Sire, D: Dam, MGS: Maternal grandsire)
- Subset 2 of 63 animals without information on one or both parental sides

Spearman rank, Pearson and concordance correlation coefficients were computed for the full downgraded set as well as the two subsets. Three genomic inbreeding coefficients were used. Fgrm0.5 was based on the GRM using p=0.5. Froh coefficients were based on 27 or 80 SNP segments, which correspond to 0.95 and 2.8 Mb, respectively. In the case of ROH, longer SNP segments indicate more recent inbreeding. The three types of correlation coefficients were computed for each of these three genomic inbreeding coefficients.

Correlation between Gefi and GFI

At Anafibj, a procedure was developed to estimate an Runs-Of-Homozygosity (ROH) style genomic inbreeding coefficient using an output file of our imputation software pedimpute.f90 (Nicolazzi et al., 2013), where haplotype segments are numbered. Using numbered haplotype segments, we could directly compare the segments by their number rather than using time-consuming SNP-by-SNP comparisons. The fraction of autosomal haplotypes with the same number (i.e., identical) on a pair of homologous chromosomes is an estimate of the Froh. A Froh is an identity-by-descent (IBD) type of inbreeding coefficient. We computed the own as well as the future inbreeding coefficients. Our genomic expected future inbreeding coefficient was named Gefi. An advantage of an IBD type of inbreeding measure is that values are within the probability space [0,1], which avoids negative values that are more difficult to understand.

A comparison was undertaken between the Gefi estimated by Anafibj and the genomic future inbreeding coefficients (GFI) estimated by CDCB using the diagonal from the genomic relationship matrix, which is an identity-by-state (IBS) measure with values within the correlation space [-1,1]. This Pearson correlation was computed based on 38,280 genotyped proven bulls covering 70 years (born 1951-2020), which had both estimates available.

Inbreeding depression standardized effect size across traits

In the literature, a number of articles showed results of genomic inbreeding depression using ROH-based inbreeding coefficients (Froh) in Holsteins. In order to compare traits and to understand which traits had a larger inbreeding depression, we standardized the inbreeding depression effects so that they became comparable across traits. We computed the standardized effect size as the inbreeding depression estimate (b) multiplied by the standard deviation of the genomic inbreeding coefficient (SD(F)) used to estimate the inbreeding depression. We then divided this by the observed standard deviation of the trait (SD(y)). Estimates from the following papers were included: Ablondi et al., 2023; Bjelland et al., 2013; Doekes et al., 2019; Makanjuola et al., 2020; Mugambe et al., 2023. Also, new unpublished results from Ablondi et al. were included.

Results & Discussion

Issue of declining genetic variation

Figure 1 shows the annual trend in SNP heterozygosity before and during the genomics era.

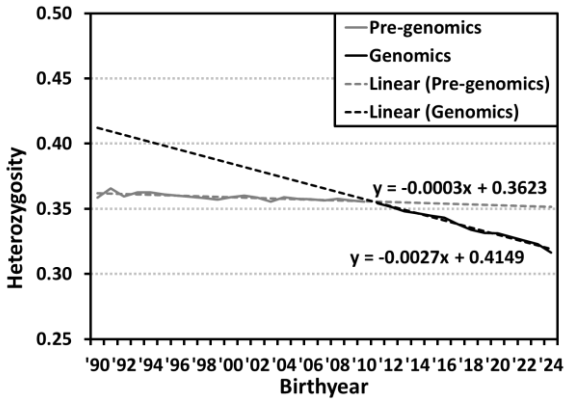

Figure 1. Pre- and post-genomic trends of SNP heterozygosity by birth year

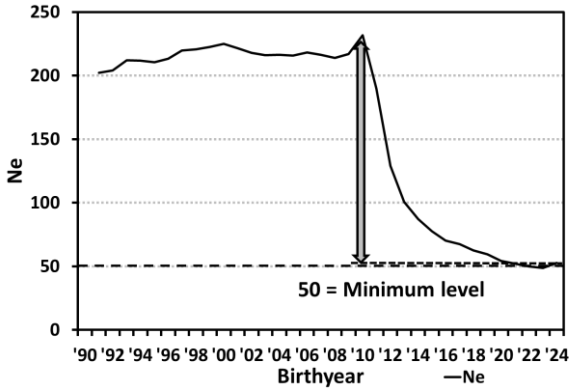

Figure 2. Decline of effective population size (Ne) during the most recent 16 birth years

Genomic inbreeding coefficients from imputed data

Table 1 shows different types of correlation coefficients between the genomic inbreeding

coefficients of the samples in the imputed downgraded set with their fully genotyped (i.e. not downgraded) samples.

In the same manner Table 2 and 3 show the correlation coefficients for the subsets 1 and 2.

Table 1. Correlations of inbreeding coefficients entire downgraded set (N=329)

Downgrade	Correlation	GRM	ROH 27	ROH 80
GGP3	Spearman	0.85	0.85	**0.90**
(26K)	Pearson	*0.43*	*0.48*	0.89
	Concordance	*0.26*	*0.39*	0.87
Labogena	Spearman	**0.93**	**0.96**	**0.96**
MD (62K)	Pearson	0.65	**0.97**	**0.98**
	Concordance	*0.55*	**0.97**	**0.96**

Table 2. Correlations of inbreeding coefficients downgraded set (N=266) with info on both parental sides

Downgrade	Correlation	GRM	ROH 27	ROH 80
GGP3	Spearman	**0.93**	**0.96**	**0.96**
(26K)	Pearson	0.89	**0.96**	**0.96**
	Concordance	0.82	**0.96**	**0.94**
Labogena	Spearman	**0.98**	**0.99**	**0.98**
MD (62K)	Pearson	**0.97**	**0.99**	**0.98**
	Concordance	**0.95**	**0.99**	**0.97**

Table 3. Correlations of inbreeding coefficients downgraded set (N=63) without info on one or both parental sides

Downgrade	Correlation	GRM	ROH 27	ROH 80
GGP3	Spearman	0.68	*0.54*	0.67
(26K)	Pearson	0.65	*0.34*	0.65
	Concordance	*0.14*	*0.10*	*0.55*
Labogena	Spearman	0.83	0.77	0.83
MD (62K)	Pearson	0.72	0.82	**0.90**
	Concordance	*0.27*	0.79	0.84

Correlation between Gefi and GFI

Summary statistics from Gefi and GFI are presented in Table 4 based on 38.280 proven bulls. The Pearson correlation between Gefi and GFI was 0.959.

Table 4: Summary statistics of Gefi and GFI

	Gefi	GFI
Average	6.9	7.2
Standard deviation	2.6	2.6
Maximum	15.3	13.5
Minimum	0.1	*-3.1*

Inbreeding depression standardized effect size across traits

Table 5 shows the average standardized effect size of the inbreeding depression per trait based on the estimates in literature. For all traits the effect was in the undesirable direction.

Table 5. Standardized effect size of the inbreeding depression

Trait	Standardized effect size
Milk kg	-.068%
Fat kg	-.063%
Protein kg	-.084%
Fat %	-.015%
Protein %	-.013%
SCS*	.020%
SCS5-150	.006%
SCS151-400	.017%
Age at 1st calving	.007%
Heifer interval 1st-last insemination	.022%
Heifer NR56	-.009%
Heifer conception rate	-.022%
Cow interval 1st-last insemination	.016%
Cow NR56	-.019%
Cow conception rate	-.028%
Interval calving to 1st insemination	.008%
Days open	.060%
Calving interval	.018%

*SCS: Somatic Cell Score

A further condensed overview of the standardized effect size of the inbreeding depression is given in Table 6. Here the average value per trait group is given.

Table 6. Average across trait standardized effect size of the inbreeding depression per trait group

Trait	Standardized effect size
Yields	-.072%
Contents	-.014%
SCS*	.014%
Fertility	-.019%

*SCS: Somatic Cell Score

Conclusions

Regarding genomic inbreeding coefficients from imputed data, we can conclude:

- The 62K chip outperforms the 26K chip.
- Froh exhibits stronger correlations than Fgrm0.5.
- Longer Froh segments demonstrate higher correlations than shorter segments.
- Spearman rank correlations > Pearson correlations > Concordance correlations
- Results are satisfactory when both parental sides have genotypes.
- In 2023, >97.3% of animals have genotypes on both parental sides, so for recent animals the results based on imputed data should be fine.

The high correlation between Gefi and GFI shows that they are both measuring future inbreeding producing very similar results.

For the inbreeding depression per trait group, we can conclude that by far the largest impact is on the yield traits. Fertility, contents and SCS all have an undesirable inbreeding depression of around 20% from the inbreeding depression on yields.

Overall take-home messages are:

- There has been a rapid increase in inbreeding since the advent of genomic selection.
- Inbreeding detrimentally affects nearly all traits, with the most pronounced impact seen in yield traits.
- Anafibj intends to introduce a premium/penalty for expected future inbreeding later this year.
- We will use genomic estimates when possible and otherwise pedigree-based estimates on a comparable scale.
- It is important to give a signal regarding the impact of inbreeding.

Acknowledgments

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Breeding programs compared across countries, continents, and breeds

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Abstract

Breeding programs differ in generation intervals, pedigree completeness, genomic merit, and relationships to recent US animals as measured by expected future inbreeding (EFI) using pedigree or by genomic future inbreeding (GFI). Properties were examined using December 2023 files for proven bulls born 2016-2017 with milk-recorded daughters in ≥10 herds from Interbull, and genotyped females born 2018-2023 from the Council on Dairy Cattle Breeding (CDCB). Those genotypes included 3,709,707 females from USA and Canada, 498,480 from 13 countries in Asia, 378,650 from 17 countries in western Europe, 125,849 from 17 countries in eastern Europe, 153,362 from 12 countries in Latin America, 53,235 from 3 countries in Oceania, and 4,082 from 5 countries in Africa. Percentages of bulls with a foreign sire averaged 43% in Holsteins (HOL) and Brown Swiss, 12% in Red Dairy Cattle, and 9% in Jersey. The sire's age at son's birth averaged 2.2 to 2.9 years in 11 of the 20 countries for HOL, indicating rapid use of young sires, whereas other countries and breeds chose older sires of sons. Numbers of first crop daughters per selected young bull averaged 771 in HOL and 201-676 daughters in other breeds. Percentages of proven HOL bulls with genotypes used in the USA reference population differed widely by country from 0-100% and averaged 66%. Average pedigree completeness for HOL females ranged from 64.2% for Latin America to 86.1% for western Europe but was much higher and averaged 98% for proven bulls due to the Interbull exchange. Pedigree inbreeding, EFI, and GFI showed that proven bulls and genotyped females in many countries and continents are almost as related to US animals as US animals are to each other, but relationships are lower in other breeds and with wider ranges due to less genetic exchange. Other populations have higher genetic merit bulls for some breeds, but North American HOL had higher merit than all other regions.

Key words: genomic prediction, inbreeding, global breeding

Introduction

Breeding programs changed quickly after genomic selection began more than a decade ago (Garcia-Ruiz et al., 2016). Over a million foreign animals now have US genomic predictions. Their genotypes and pedigrees allow comparing breeding programs and relatedness within breeds around the world, including many countries that do not participate directly in Interbull services. Bull evaluations, pedigrees, and genotypes from countries that participate in multi-trait across-country evaluation (MACE) also allow directly comparisons. The 69 countries providing genotypes were grouped into 7 continental regions, with numbers for breeds Holstein (HOL) and Jersey (JER) shown in Tables 1 and comparing the sires and selection methods used in each country. Goals were to examine relationships and use of foreign sires across countries, inbreeding, pedigree completeness, generation intervals, and genetic merit across countries and continents for several breeds.

Materials and Methods

The National Cooperator Database used for December 2023 official evaluations of CDCB included 3.3 million domestic and 1.4 million foreign genotyped females born 2018-2023 to provide recent

2. Individual countries providing the most foreign genotypes were 320,350 from Canada, 186,499 from Saudi Arabia, 160,558 from China, 135,971 from Japan, 114,501 from Italy, and 102,000 from Brazil.

Proven bulls born 2016-2017 with milk-recorded daughters in ≥10 herds from Interbull were examined using December 2023 official data from MACE on USA scale. Counts for breeds HOL, JER, Brown Swiss (BSW), and Red Dairy Cattle (RDC) are shown in Tables 3-6 for countries that had at least 10 domestic proven bulls during those 2 years, with Denmark-Finland-Sweden (DFS) treated as 1 country. German Simmental bulls (DEA) and French Montbeliard bulls (FRM) are reported on the HOL base because those breeds do not have a separate base in USA. Their evaluations include the expected 100% heterosis boost when mated to HOL females. Statistics for bulls included pedigree completeness (Ped%), percent of genotypes in USA reference (Gen%), percentage with foreign sires (ForSire%), and sire generation interval in years (SireGI).

Results & Discussion

For the recent genotyped HOL females, average Net Merit (NM$) was $480 in North America, $335 in Latin America, $381 in Western Europe, $370 in Eastern Europe, $317 in Africa, $366 in Asia, and $211 in Oceania (Table 1). Corresponding properties for 19,566 JER are in Table 2.

The average pedigree completeness for recent genotyped HOL females ranged from 64.2% for Latin America to 86.1% for western Europe. The average pedigree inbreeding for HOL females ranged from 8.7% for Africa and Oceania to 9.6% for North America. Oceania also had the lowest EFI of 9.0% and GFI of 9.4% compared to the highest EFI of 9.5% and GFI of 10.4% in North America. For JER females, pedigree completeness ranged from 57.3% in Latin America to 87.9% in North America. Western Europe had the lowest averages of 7.6% for pedigree inbreeding, 7.4% for EFI, and 5.7% for GFI compared to highest averages of 8.9% pedigree inbreeding, 9.0% GFI, and 7.7% EFI in North America.

For proven bulls, pedigree completeness was much higher and averaged 98% due to the Interbull exchange. HOL bulls had > 90% foreign sires in 7 of the 20 countries having at least 10 domestic proven bulls (counting DFS as 1 country), whereas NZL had 1% and USA had 11% foreign sires. The average was 43% foreign sires in HOL and BSW, 12% in RDC, and 9% in JER. The sire's age at son's birth averaged 2.2 to 2.9 years in 11 of the 20 countries for HOL, indicating rapid use of young sires, whereas other countries and breeds chose older sires of sons. Several populations average > 1,000 first crop daughters per young bull selected.

Percentages of proven bulls with genotypes used in the USA reference population differed widely by country from 0-100% and averaged 66% in HOL. For proven bulls, 11 of 20 countries had GFI of 10.0 to 11.3%; EFI patterns were similar. All other countries had GFI above 8.1% except ISR (3.2%) and NZL (2.4%). Compared to 19 years ago (VanRaden, 2005), pedigree completeness has improved a little but inbreeding levels for MACE bulls have more than doubled.

Tooker et al. (2015) summarized the first 200,000 foreign genotypes in Table 7. For recently genotyped HOL females, pedigree completeness decreased slightly compared to those born before 2015 on all continents except a small increase in Asia. Inbreeding levels have increased quickly, but foreign females are still almost as related to US animals as US animals are to each other (EFI and GFI). North American average NM$ remains higher than for all other continents but NM$ averages are not directly comparable to means from 2015 due to a base change in 2020 and other index formula changes. Other than the consistent increases in

inbreeding, properties in Tables 1 compared to 7 show good progress on all continents after 9 years of rapid growth in genotyping.

Conclusions

Most foreign HOL bulls and genotyped females are highly related to the USA reference population. Sire generation intervals were < 3 years in many HOL breeding programs but longer in other breeds and smaller populations. Almost half of HOL and BSW bulls had foreign sires but fewer in other breeds. HOL genetic merit was higher in North America than in all other continental regions but varied more in other breeds. Breeders in many countries are choosing genomic predictions from USA. Predictions for foreign animals should be almost as accurate as for domestic animals, but genetic correlations are unknown in many new markets.

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Table 1. Average pedigree completeness, inbreeding, expected future inbreeding, and genomic future inbreeding by continental region for genotyped **Holstein** females born 2018-2023.

Continent	Genotypes (N)	Pedigree completeness (%)	Net Merit ($)	Pedigree Inbreeding (%)	Expected future inbreeding (%)	Genomic future inbreeding (%)
North America	3,082,090	84.2	480	9.6	9.5	10.4
Latin America	141,679	64.2	335	9.1	9.4	9.9
Western Europe	288,475	86.1	381	9.1	9.2	10.2
Eastern Europe	96,940	74.5	370	8.8	9.2	9.7
Africa	3,964	70.5	317	8.7	9.1	9.8
Asia	491,371	76.5	366	8.8	9.2	9.7
Oceania	23,764	75.2	211	8.7	9.0	9.4

Table 2. Average pedigree completeness, inbreeding, expected future inbreeding, and genomic future inbreeding by continental region for genotyped **Jersey** females born 2018-2023.

Continent	Genotypes (N)	Pedigree completeness (%)	Net Merit ($)	Pedigree Inbreeding (%)	Expected future inbreeding (%)	Genomic future inbreeding (%)
North America	441,024	87.9	304	8.9	9.0	7.7
Latin America	8,902	57.3	75	8.5	8.7	7.0
Western Europe	4,948	75.8	118	7.6	7.4	5.7
Eastern Europe	519	79.4	284	7.8	8.0	6.2
Africa	107	85.0	90	8.0	8.4	7.8
Asia	1,430	74.6	41	8.1	8.6	7.1
Oceania	3,497	63.9	1	8.1	8.1	7.1

Table 3. Properties by country of ID for **Holstein** bulls and Simmental breed group bulls (DEA and FRM) expressed on HOL base.

Country	Bulls	Daughters	Ped%	Gen%	NM$	EFI	GFI	ForSire%	SireGI
USA	1754	1085	100	99	543	10.0	11.1	11	2.3
DEA	*1005*	*348*	*97*	*0*	*202*	*1.1*	.	*38*	*4.1*
DEU	590	871	100	88	378	8.6	10.1	79	2.4
NLD	444	1012	99	66	326	7.8	9.8	58	3.0
NZL	444	811	99	1	-61	2.8	2.4	1	5.9
CAN	332	1125	100	100	479	10.0	11.3	65	2.2
JPN	309	61	97	22	276	9.0	10.5	81	3.7
FRA	282	1289	94	52	257	8.0	10.1	79	2.6
FRM	*194*	*591*	*92*	*0*	*233*	*1.3*	.	*1*	*3.6*
ITA	186	329	99	75	315	7.6	10.7	90	2.8
DFS	162	1593	99	41	353	7.5	8.8	47	2.3
CHE	145	245	100	10	-23	5.0	9.3	50	4.0
POL	96	307	93	22	274	8.2	10.2	100	2.9
SVN	82	107	91	1	31	4.6	9.4	98	5.2
ISR	79	266	85	5	269	5.5	3.2	29	4.9
KOR	71	36	94	0	31	8.9	.	100	8.0
AUS	64	234	92	39	125	8.3	9.9	70	3.5
ESP	60	185	97	42	247	9.4	10.5	98	2.6
GBR	42	642	98	93	267	7.3	8.1	74	4.4
CZE	23	473	100	78	430	9.7	11.2	100	2.5
LUX	14	343	100	86	442	9.0	10.6	100	2.6
BEL	13	476	99	85	306	7.7	9.4	85	2.6

Globe	6412	771	98	54	324	7.0	10.7	43	3.2

Table 4. Properties by country of ID for **Jersey** bulls.

Country	Bulls	Daughters	Ped%	Gen%	NM$	EFI	GFI	ForSire%	SireGI
USA	359	594	95	100	279	9.1	7.4	4	3.1
NZL	207	455	98	4	-7	2.8	2.6	6	5.9
DFS	56	941	99	63	326	4.9	3.1	7	2.4
AUS	21	229	89	24	-6	8.1	7.7	57	6.2
CAN	19	176	100	100	69	8.4	8.1	47	2.5
Globe	673	551	96	65	177	6.7	7.0	9	4.0

Table 5**.** Properties by country of ID for **Brown Swiss** bulls.

Country	Bulls	Daughters	Ped%	Gen%	NM$	EFI	GFI	ForSire%	SireGI
DEA	157	213	96	93	436	5.4	6.3	37	4.4
CHE	100	287	99	39	123	5.2	0.3	32	3.7
ITA	57	116	99	93	289	6.2	6.8	63	3.8
USA	49	130	99	100	223	7.8	7.5	39	4.3
FRA	13	228	98	100	359	6.4	7.2	77	4.5
SVN	12	54	98	50	226	5.6	6.1	92	4.9
Globe	394	201	96	93	297	5.8	5.9	43	4.1

Table 6. Properties by country of ID for **Red Dairy Cattle** bulls.

Country	Bulls	Daughters	Ped%	Gen%	NM$	EFI	GFI	ForSire	SireGI
DFS	149	708	98	20	786	3.2	2.0	1	2.4
NOR	92	1177	97	0	652	2.2	.	4	4.4
NZL	27	56	95	0	438	2.1	.	33	8.3
CAN	18	99	100	94	412	7.3	5.8	33	5.3
AUS	15	90	76	0	432	3.2	.	60	6.5
GBR	11	147	83	64	57	6.1	4.2	27	9.4
USA	7	94	97	100	129	7.6	5.6	43	6.6
Globe	329	676	96	19	638	3.2	3.7	12	4.1

Table 7. Averages from Tooker et al. (2015) for pedigree completeness, inbreeding, expected future inbreeding, and genomic future inbreeding by continental region.

Continent	Pedigree completeness (%)	Net Merit ($)	Pedigree Inbreeding (%)	Expected future inbreeding (%)	Genomic future inbreeding (%)
North America	86.1	191	6.5	6.4	6.9
Latin America	67.7	9	5.9	6.0	6.0
Western Europe	97.6	146	6.1	6.1	6.7
Eastern Europe	88.0	111	5.5	5.8	6.2
Africa	87.4	64	6.3	6.6	7.3
Asia	71.9	48	5.8	6.0	6.3
Oceania	93.6	44	6.0	5.8	6.2

Analysis of Factors Affecting Daily Milk Yields: An Initial Case Study in an Automatic Thrice-Milking Farm

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Abstract

The methodologies and parameters for estimating daily milk yields in the United States were mainly developed from the 1960s through the 1990s. A recent initiative by the Council on Dairy Cattle Breeding, USDA Animal Genomics and Improvement Laboratory, and the National Dairy Herd Improvement Association aims to update these methods and parameters for estimating daily yields by collecting and analyzing milking data from dairy farms. This study, serving as an initial case study, examined the factors influencing daily milk yield estimation at a dairy farm in New York State and compared the performance of the existing method with a recently proposed one. In total, 63,562 milking data were extracted from approximately 2,200 cows milked thrice daily in this farm. Data cleaning eliminated incomplete or missing records, retaining 47,670 entries from 1,869 cows for subsequent analyses. The average partial yields in kilograms (milking interval time in hours) of the three milkings were 14.6, 16.5, and 13.8 (7.88, 8.79, and 7.25), respectively. Analysis of variance based on an extended version of the Wiggans (1986) model revealed significant effects of milking interval time and months in milk on proportional daily milk yields. The lactation effects on proportional daily yields were significant for the first two milkings but not for the third milking. Nevertheless, the relative importance of milking interval time and lactations was very low. The polynomial-interaction-regression model analysis showed significant effects from partial yields and significant interactions between partial yields and milking interval times on daily yields. The latter model gave more accurate estimates than the Wiggans (1986) model. Regarding the relative predictability of the three milkings, the 2nd milkings, having the longest average milking interval time, gave more accurate estimates than the 1st and 3rd milkings. The calculated multiplicative correction factors in this farm increased slightly for the 1st milkings and remained roughly comparable (or slightly decreased) for the 2nd and 3rd milkings compared to the Wiggans (1986) assessment. These results revealed only minor changes in daily yield correction factors over the past four decades.

Keywords: accuracy, dairy cattle, milking interval time, interactions, lactation, test-day

Introduction

The 1960s witnessed a significant shift in milk testing in the United States. Previously, farms followed a rigorous schedule of twice-daily milk tests conducted under supervision every month. This system then shifted towards more economical sampling methods to reduce the costs associated with supervisory visits by the Dairy Herd Improvement Association (**DHIA**). Test frequencies are often adopted to align with varied herd management practices. On a test day, a cow is usually milked two or more times daily, but not all milkings were recorded. One prevalent technique is the morning and evening (**AM-PM**) method, which alternates between morning and evening milking throughout the lactation period (Porzio, 1953). Then, the total daily milk yield (**DMY**) was estimated by doubling the yield of a single milking,

assuming equal length and rate of milk production on both sessions, each lasting precisely 12 hours. In the case of unequal morning and evening milking intervals, the biases are assumed to be offset by complementary unevenness between AM and PM milkings. However, these assumptions do not hold in reality. Morning milking intervals tend to be longer than afternoon milking intervals. Hence, AM milk yields are usually higher than PM milk yields (Putnam and Gilmore, 1970).

Various statistical approaches have been developed to estimate daily milk yields from incomplete milking data (reviewed by Wu et al., 2023a,b). The methodologies and parameters for estimating DMY in the United States were primarily developed from the 1960s through the 1990s. A recent initiative by the Council on Dairy Cattle Breeding, USDA-AGIL, and the National DHIA seeks to update these methods and parameters for estimating DMY by collecting and analyzing milking data from dairy farms. This study represented an initial case study amid ongoing or planned data collection at other locations. We examined the factors influencing DMY estimation at a specific site, Farm 1 in New York State, and compared the performance of the existing method with a recently proposed one for estimating daily DMY.

Materials and Methods

Milking data

We extracted 63,562 milking data from Farm 1, representing thrice-milkings daily for around 2,200 Holstein cows. Milkings were collected and weighed at all three milkings for 18 weeks, starting May 5 and ending September 1, 2023. After that, three-day monthly milking data collections were carried out up to 305 days of milk and beyond. Milking times are 4am-12pm (1st milking), 12pm-8pm (2nd milking), and 8pm-4am (3d milking). Milk yields and timestamps were extracted from BouMatic parlor software (https://boumatic.com/us_en/). Records with incomplete and missing data were removed. Milking records with prolonged lactation beyond 305d for up to one more month were retained. Records with days in milk greater than 335 days, approximately accounted for 0.6% of the milking records, were excluded. After data cleaning, we retained 47,670 milking records representing 1,869 cows. The cleaned data represented up to nine lactations (Figure 1), with 64.0% from the first two lactations and 97.1% from the first five lactations. Milking records from lactation six and beyond, accounting for 2.9%, were pooled. Around 74.1% of the cleaned milking records were collected before 156 days in milk, and around 95.5% were collected before 250 days.

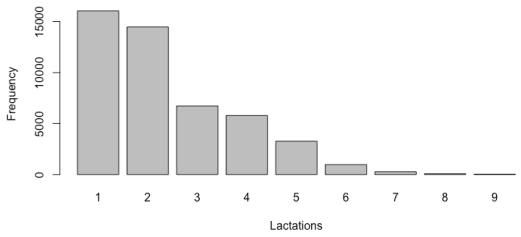

Figure 1. Distribution of milking records by lactation

Statistical methods

Two statistical models are defined. Firstly, for the *i*-th animal, a proportional DMY ($\frac{x_{ijl}}{y_{ijl}}$) is assumed to be a linear function of milking interval time (t_{ijl}), months in milk (m_j), lactations (γ_l), and a residual term (ε_{ijl}).

$$\frac{x_{ijl}}{y_{ijl}} = \alpha + \beta t_{ijl} + m_j + \gamma_l + \varepsilon_{ijl} \qquad (1)$$

The above model expands the Wiggans (1986) model by additionally including the categorical effects due to lactations and months in milk.

MCF are derived for milking interval classes, each spanning 30 minutes while accounting for the average months in milk and lactation effects:

$$F_k = \frac{1}{\hat{\alpha}+\hat{\beta}\bar{t}^{(k)}+\bar{m}+\bar{\gamma}} \qquad (2)$$

where $\bar{t}^{(k)}$ is the average milking interval time for the k-th milking interval class, and $\bar{m}$ and $\bar{\gamma}$ are weighted averages for estimated months in

milk and lactation effects, respectively. Omitting these two effects in (1) reduces the model to the original Wiggans (1986) model, with MCF calculated as follows:

$$F_k = \frac{1}{\hat{\alpha}+\hat{\beta}\bar{t}^{(k)}} \quad (3)$$

Hence, a DMY is estimated as follows:

$$\hat{y}_{ijl(k)} = F_k x_{ijl(k)} \quad (4)$$

The second model accounts for the interactions between partial yields and milking interval time in linear linear and quadratic terms, as follows:

$$\begin{aligned} y_{ijl} &= \left(b_0 + b_1 t_{ijl} + b_2 t_{ijl}^2\right) x_{ijl} + m_j + \gamma_l + \epsilon_{ijl} \\ &= b_0 x_{ijl} + b_1\left(t_{ijl} x_{ijl}\right) + b_2\left(t_{ijl}^2 x_{ijl}\right) \\ &\quad + m_j + \gamma_l + \epsilon_{ijl} \end{aligned} \quad (5)$$

This model is referred to as the polynomial-interaction regression (PIR) model. MCF are derived pertaining to a specific milking interval time *t*,

$$F_t = \hat{b}_0 + \hat{b}_1 t + \hat{b}_2 t^2 \quad (6)$$

In the above, the MCF at time t can be viewed as a baseline MCF, $F_0 = \hat{b}_0$ and adjusted according to the milking interval time, $\Delta_t = \hat{b}_1 t + \hat{b}_2 t^2$.

Then, a DMY is estimated as follows:

$$\hat{y}_{ijl} = F_{t=t_{ijl}} x_{ijl} + \hat{m}_j + \hat{\gamma}_l \quad (7)$$

Here, $F_{t=t_{ijl}}$ stands for a MCF on specific milking interval time t, assigned to all animals satisfying $t_{ijl} = t$.

Accuracy measures

The accuracy of estimated DMY was evaluated based on two criteria: correlation and R^2 accuracy. The former is the correlation between estimated and actual DMY. The R^2 accuracy is the following:

$$R^2 accuracy = \frac{Var(y)}{Var(y)+MSE} \quad (8)$$

where $Var(y)$ is actual phenotypic variance, and MSE stands for mean squared errors.

Analysis of variance (ANOVA) was conducted based on each of the two models separately. The importance of predictor variables was assessed by the Lindeman, Merenda, and Gold (LMG) metric of R squared (Lindeman et al., 1980), which measures the contribution of each predictor to the R-squared value, averaged over all possible orders of entering the predictors into the regression model. The confidence intervals for relative importances were obtained via 1000 bootstrap samples of the LMG R^2.

Results & Discussions

Milking data summary statistics

Overall, the mean (95% Confidence interval) of test-day milk yields was 45.0 (28.6 ~ 62.8) kg. Across lactations, the average test-day milk yield increased from 38.1 kg on the first lactation to 47.2 kg on the second lactation 2, peaked (49.9 kg) on lactation 3, and then began to drop on lactation four and beyond, from 49.7 kg (lactation 4) to 48.8 kg (lactation 6+) (Figure 2; upper).

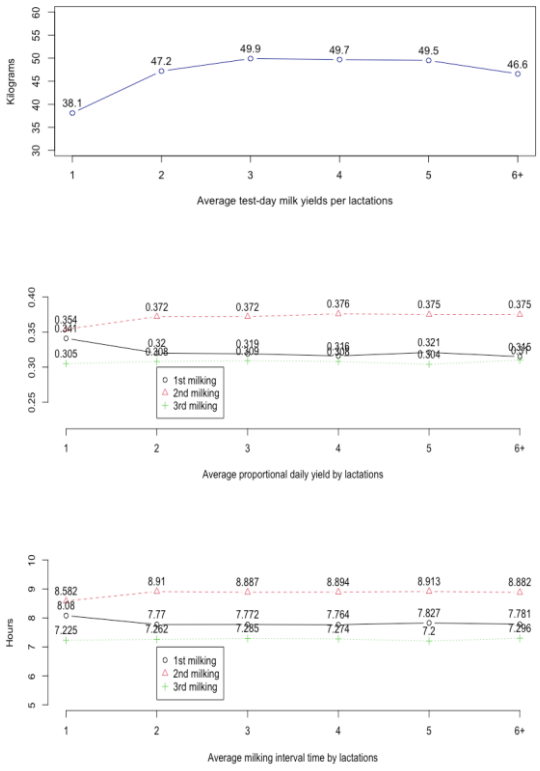

Figure 2. Trends of changes by lactations in average test-day milk yield (upper), average proportional daily yields (middle), and average milking interval time (bottom).

Average proportional daily yields showed slight variations between lactations, except lactation 1 (Figure 2; middle). The pattern agreed with the changes in the average milking interval times for the three milkings across the

lactations (Figure 2; bottom). Proportional daily milk yields are primarily determined by the milking interval time. Assuming consistent milking interval time across lactations, common yield correction factors are arguably plausible.

Overall, average proportional daily milk yields varied substantially between the three milkings (Figure 2; middle). The first milkings had the largest average proportional daily milking yield across lactations (0.35 – 0.38), followed by the third milkings (0.32 – 0.34); the second milkings had the least average proportional daily milk yield (0.31). The substantial differences in proportional daily yields were attributed to varied milking interval times for the three milkings (Figure 3; bottom). The average (95% confidence interval) of milking interval time was 8.79 (7.84-9.75) hours, 7.25 (6.39-8.07) hours, and 7.88 (7.06-8.81) hours, respectively, for the three milkings. On average, the first milking interval time was approximately 1 hour longer than the third and 1.5 hours longer than the second. Nevertheless, the average milking interval time varied very slightly between lactations, except for lactation one. Approximately the first milking interval time was 8.6 hours for lactation 1 and 8.9 hours for lactations 2 through 6+; the second milking interval time was 8.1 hours for lactation 1 and 7.8 hours for lactations 2 through 6+; the third milking interval time was 7.3 hours for lactation 1 and 7.3 hours (Figure 3; bottom). In accordance with the lengths of milking interval time, the first milkings had the largest average DMY (16.5 kg), followed by the third milkings (14.6 kg); the third milkings had the lowest average DMY (13.8 kg).

Relative importance of predictor variables

Analysis of variance based on model (1) showed significant effects of milking interval time (Pr <2.20e-16 for all three milkings), months in lactation (Pr = 0.0008 for 1st milkings; Pr = 2.52e-10 for 2nd milkings; Pr = 0.0001 for 3rd milkings), parities (Pr <2.20E-16) on proportional DMY. ANOVA based on the PIR model (5) revealed significant effects from partial milk yields (Pr < 2.20e-16), months in milk (Pr < 2.20e-16), and parities (Pr < 2.20e-16) on DMY. The results also showed significant interactions between partial yields and linear milking interval times (Pr < 2.20e-16) on DMY and significant interaction effects between partial yields and quadratic milking interval time for 1st milkings (Pr = 9.42e-08) and 3rd milkings (Pr = 1.03e-11) but not significant for the 2nd milkings (Pr = 0.1785) on DMY. These significant interaction effects justified using PIR models in the present study.

Table 1 presents the relative importance of predictor variables for two models in estimating daily milk yields across three different milkings (1st, 2nd, and 3rd). The values provided are the means and 95% confidence intervals of the LMG R^2, which measure the proportion of variance explained by each predictor. For the proportional DMY Model (1), milking interval time was the most significant predictor, with relatively high mean importance values across all milkings (0.157, 0.135, 0.159); months in milk had very low importance, indicating it contributes minimally to explaining the variance in DMY (0.002, 0.004, 0.002); Lactations also had a minor contributor, with slightly higher values than months in milk but still low (0.040, 0.032, 0.001). The low importance of months in milk and lactations agrees with the Wiggans (1986) model, which ignores these variables. Nevertheless, the total relative importance sums to around 0.199 for the 1st milking, 0.170 for the 2nd milking, and 0.148 for the 3rd milking, suggesting that the predictors in this model together explain only a low to modest portion of the variance in daily milk yields. There may be other significant variables influencing proportional DMY that have not yet been identified.

For the PIR model, partial yields were the most significant predictor, with consistently high importance across all milkings (0.285, 0.280, 0.274). The interactions between partial yields and linear and quadratic milking interval time also had a major contributor, with

substantial mean importance values (0.226, 0.244, 0.225) for the interaction with a linear milking interval time and also notable mean importance values (0.158, 0.199, 0.172) for the interaction with quadratic milking interval time. Months in milk showed higher importance in the PIR Model (5) compared to the proportional DMY Model (1), but still relatively low (0.022, 0.021, 0.020). The relative importance of lactations varies more across milkings, with higher values in the 1st and 3rd milkings compared to the 2nd (0.129, 0.083, 0.101). The total relative importance sums to 0.820 for the 1st milking, 0.830 for the 2nd milking, and 0.790 for the 3rd milking, indicating that the PIR Model predictors together explain a much larger portion of the variance in daily milk yields compared to the proportional DMY model. However, both results are not directly comparable because they modeled different quantities. The dependent variable in the former model was proportional DMY, whereas it was DMY in the latter model.

Table 1. Relative importance (mean and 95% of IMG R^2) of predictor variables in two models [1]

Predictors	1st milking			2nd milking			3rd milking		
	Mean	Q2.5%	Q97.5%	Mean	Q2.5%	Q97.5%	Mean	Q2.5%	Q97.5%
Model 1									
MIT	0.157	0.145	0.171	0.135	0.121	0.149	0.159	0.146	0.172
MIM	0.002	0.001	0.004	0.004	0.003	0.007	0.002	0.002	0.005
LACT	0.040	0.035	0.046	0.032	0.026	0.037	0.001	0.001	0.003
Sum	0.199			0.170				0.148	
Model 2									
PY	0.285	0.280	0.290	0.280	0.276	0.284	0.274	0.269	0.279
TAR1	0.226	0.222	0.230	0.244	0.240	0.247	0.225	0.222	0.229
TAR2	0.158	0.154	0.162	0.199	0.196	0.202	0.172	0.168	0.175
MIM	0.022	0.020	0.025	0.021	0.019	0.024	0.020	0.018	0.023
LACT	0.129	0.124	0.133	0.083	0.080	0.086	0.101	0.096	0.106
SUM	0.82			0.83			0.79		

[1] MIT = milking interval time; MIM = months in milk; LACT = lactations; PY = partial yields (1st, 2nd, or 3rd); TAR1 = interaction between PY and linear MIT; TAR2 = interaction between PY and quadratic MIT.

Accuracy of estimated daily milk yields

Table 2 compares the accuracy of estimated daily milking yields using two models, each under two scenarios. The scenarios differed based on whether the effects of months in milk and lactation were accounted for. GW1 and PIR1 did not include the variables for months in milk and lactations, whereas GW2 and PIR2 accounted for their effects. The accuracy is measured by the correlation between estimated and actual daily milk yields, the R^2 accuracy, and the K value, which is the ratio of the estimated daily milk yields over the variance of actual daily milk yields.

The Wiggans (1986) models, GW1 and GW2, showed roughly similar performance with slight differences in correlations, R^2 accuracies, and K values. Both models tend to overestimate the variance (K > 1). The PIR1 and PIR2 models generally had a higher correlation and R^2 accuracies than GW1 and GW2, indicating they provide more accurate estimates of daily milk yields than the current method. Compared to the GW models, PIR1 had around 1-2% increase in R^2 accuracy, and PIR2 had around a 4-6% increase in R^2 accuracy. The PIR models derived continuous yield correction factors, which remedies the biases with discrete yield correction factors, and consider possible interactions. Nevertheless, these two PIR models performed differently on the variance of estimated DMY. PIR1 gave an overestimated variance of estimated DMY, whereas PIR2 led to a smaller variance of DMY than the actual daily milk yield variance. Generally speaking, the estimates from a linear regression tend to have a smaller estimate variance than the actual variance because the residuals are excluded. However, PIR1 was defined without intercept.

When fitting linear regression models, the inclusion or exclusion of an intercept has a significant impact on the variance of the predicted values. The intercept in a regression model captures the average expected value of the dependent variable when all predictor variables are at zero (assuming zero is within the range of normal values for these predictors).

Table 2. Accuracy metrics of estimated daily milking yields using the Wiggans (1986) (GW) and the polynomial-interaction-regression (PIR) models [1,2]

Methods	1st milking			2nd milking			3rd milking		
	Corr	R^2	K	Corr	R^2	K	Corr	R^2	K
Before variance rescaling									
GW1	0.880	0.781	1.237	0.901	0.809	1.253	0.875	0.769	1.285
GW2	0.879	0.791	1.152	0.902	0.801	1.3207	0.875	0.769	1.283
PIR1	0.883	**0.800**	1.205	0.903	**0.815**	1.2277	0.877	**0.777**	1.249
PIR2	0.906	**0.847**	0.821	0.909	**0.852**	0.8278	0.889	**0.827**	0.792
After variance rescaling									
GW1	0.880	0.806	1.000	0.901	0.835	1.000	0.875	0.800	1.000
GW2	0.879	0.806	1.000	0.902	0.836	1.000	0.875	0.800	1.000
PIR1	0.883	**0.811**	1.000	0.903	**0.837**	1.000	0.877	**0.803**	1.000
PIR2	0.906	**0.841**	1.000	0.909	**0.847**	1.000	0.889	**0.819**	1.000

[1] Corr = correlation; R2 = R2 accuracy; K = ratio of estimated versus actual daily milk yield variance.
[2] GW1, PIR1 = Omitting months in milk and lactations; GW2, PIR2 = These models included the effects of months in milk and lactations.

Including an intercept typically reduces the sensitivity of the model to fluctuations in the data by adjusting the baseline level of the response. This often leads to smaller coefficients for the predictors because the intercept absorbs much of the average outcome, reducing the variability that each predictor needs to explain. Hence, the variance of the predicted values generally reflects more closely the natural variability in the data centered around the mean.

For a model without an intercept, each predictor variable must account not only for the variability related to its specific influence on the dependent variable but also for its overall mean. This often requires larger coefficients, as each predictor must scale more significantly to fit the data points. Because the model without an intercept is overly sensitive to changes in the predictor variables and tends to have larger coefficients, the range of predicted values can be significantly wider. This amplifies the variance of the predictions because the model tries to compensate for the lack of a baseline adjustment by stretching the effect of the predictors to cover all data points. Table 3 shows model parameters for the PIR models. Without accounting for the effects of months in milk and lactations (PIR1), the regression coefficients for partial yields were between 5.19 and 8.36. In contrast, the regression coefficients were substantially smaller (2.78 – 5.97) with the PIR2 model when accounting for the effects due to months in milk and lactations.

PIR2 had a higher R2 accuracy than PIR1 because it accounted for the effects of months in milk and lactation. This is often the case when one or more secondary variables are not randomized in the experimental design, such that deviates due to these differences are not zero. Otherwise, PIR and PIR2 would perform similarly. In contrast, GW1 and GW2 performed similarly, which may suggest that simply accounting for secondary variables by their averages in the Wiggans (1986) is inefficient.

It should be noted that, in PIR2, the effects of the months in milk were estimated for each category, which is inherently related to the overall mean. In other words, though the overall mean was not present in the PIR2 model equation, it was presented via the months in milk effects. Therefore, PIR2 gave a smaller estimate variance than the actual variance.

Variance rescaling brought all K values to 1, indicating that the variance of estimated daily milk yields now matches the actual yields perfectly. Thus, variance rescaling effectively adjusted the variance of estimated yields to match the actual yields, improving the overall accuracy of the models except for PIR2. For PIR2, because the estimated daily yield variance was smaller than the actual variance and because the months in milk and lactation effects were adjusted additively, variance rescaling led to a slight decrease in the accuracy.

Table 3. Model parameters for the polynomial-interaction-regression with and without accounting for the effects due to months in milk and lactations in a thrice-milking dairy farm [1]

Model parameters	1st Milking		2nd Milking		3rd Milking	
	Estimate	SE	Estimate	SE	Estimate	SE
M1a: Excluding the effects due to months in milk and locations						
b_0	8.358	0.353	5.185	0.288	7.554	0.535
b_1	-1.003	0.088	-0.326	0.066	-0.832	0.147
b_2	0.042	0.005	0.005	0.004	0.032	0.010
M1b: Including the effects due to months in milk and lactations						
b_0	5.973	0.290	2.781	0.254	5.313	0.457
b_1	-0.754	0.02	-0.014	0.057	-0.605	0.126
b_2	0.034	0.004	-0.008	0.003	0.025	0.009
m_1	10.49	0.172	10.51	0.170	11.75	0.184
…..						
m_{11}	9.650	0.316	7.892	0.314	10.70	0.340
γ_2	3.563	0.083	1.438	0.084	2.559	0.089
…..						
γ_6	3.897	0.188	1.136	0.186	2.476	0.203

[1] M1a: $y_{ijl} = (b_0 + b_1 t_i + b_2 t_{ijl}^2) x_{ijl} + \epsilon_{ijl}$; M1b: $y_{ijl} = (b_0 + b_1 t_i + b_2 t_{ijl}^2) x_{ijl} + m_j + \gamma_l + \epsilon_{ijl}$

Table 4. Comparison of 3X multiplicative correction factors (MCF) obtained for every 30 minutes based on the present milking dataset and the reference (Ref) MCF for trice-milkings[1,2]

Milking interval time, hrs	1st milking			2nd milking			3rd milking		
	Ref.	GW	PIR	Ref.	GW	PIR	Ref.	GW	PIR
5.75	3.76	4.11	3.98	3.89	3.74	3.48	3.92	3.94	3.83
6.25	3.54	3.81	3.73	3.65	3.53	3.34	3.68	3.69	3.60
6.75	3.34	3.55	3.50	3.45	3.33	3.21	3.47	3.46	3.40
7.25	3.17	3.32	3.29	3.26	3.16	3.08	3.28	3.26	3.20
7.75	3.01	3.11	3.11	3.10	3.01	2.96	3.12	3.08	3.03
8.25	2.87	2.94	2.94	2.95	2.87	2.84	2.96	2.92	2.87
8.75	2.74	2.78	2.80	2.81	2.74	2.72	2.83	2.78	2.72
9.25	2.62	2.63	2.67	2.69	2.62	2.60	2.70	2.64	2.60
9.75	2.51	2.51	2.57	2.57	2.51	2.48	2.59	2.53	2.48
10.25	2.41	2.39	2.49	2.47	2.42	2.37	2.48	2.42	2.39

[1] GW = MCF according to Wiggans (1986); PIR = polynomial-interaction-regression; both models did not account for the effects due to months in milk and lactations.

[2] Reference MCF (Wiggans, 1986): $F_{1st} = \frac{1}{0.077+0.0329t}$; $F_{2nd} = \frac{1}{0.068+0.0329t}$; $F_{3rd} = \frac{1}{0.066+0.0329t}$

In Table 4, multiplicative correction factors (MCF) for three milkings were derived from a historical reference (Wiggans, 1986), and compared to the current results derived by two models (GW and PIR) across milking intervals between 5.75 and 10.25 hours. For the 1st Milkings, the GW and PIR models consistently show higher MCF values than the historical reference across all intervals. For the 2nd and 3rd milkings, MCF derived from the GW and PIR models are slightly lower than the reference. These results indicate minor changes

in MCF over the past decades. The PIR model shows a trend towards slightly lower MCF values across all milkings compared to the GW model. The average (range) of the reference MCF (Wiggans, 1986) was 3.00 (2.41 – 3.76) for the 1st milking, 3.08 (2.47 – 3.89) for the 2nd milking, and 3.10 (2.48 – 3.92) for the 3rd milking. Based on the recent milking dataset analyzed by the Wiggans (1986) model, the average (range) of MCF was 3.11 (2.39 – 3.98) for the 1st milking, 2.99 (2.42 – 3.74) for the second milking, and 3.07 (2.39 – 3.83) for the 3rd milking.

Conclusions

In conclusion, this initial case study demonstrated that modeling proportional DMY as a linear function of milking interval time is a valid strategy. The present results have shown that milking interval is the primary predictor for proportional DMY, whereas the effects of months in milk and lactations are considered secondary. Still, other major variables that have not yet been discovered can influence proportional DMY. Still, we have also shown that the polynomial-interaction-regression model can provide more accurate yield estimates than the Wiggans (1986) model. A primary reason was that discrete MCF introduces biases. Besides, precisely adjusting secondary variables with the Wiggans (1986) model is not straightforward. Instead, the new model captures linear and quadratic interactions between partial yields and milking interval times and can naturally accommodate secondary predictor variables. In Farm 1, the second milking had the longest interval and offered the most precise estimates. The calculated MCFs showed only minor deviations over the past four decades despite the significant genetic improvement in daily and lactation yields in the past decades. This result suggests that the proportional daily yields, reciprocal to MCF, remain relatively comparable over the past decades. Finally, this study represents an initial case study, and all the conclusions are subject to large-scale validation.

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Genetic evaluation of differential somatic cell count in Italian Holstein cattle

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Abstract

Mastitis is a prevalent inflammatory condition affecting udder tissue in dairy cows. It leads to reduced milk production, increased veterinary costs and potential culling of affected animals, impacting both animal welfare and economic outcomes in dairy farming. Recently introduced as a supplementary measure to somatic cell count (SCC), the differential somatic cell count (DSCC) is an innovative indicator for intramammary infection. DSCC quantifies the proportion of polymorphonuclear neutrophils plus lymphocytes (PMN-LYM) within milk somatic cells, providing enhanced insights into udder health status and infection severity. The aim of this study was to estimate genetic parameters and develop a genetic evaluation of DSCC in Italian Holstein. An innovative, new categorical phenotype, named state of infection (SI), was created from each test-day, combining SCC and DSCC records. Values from 1 to 4 were assigned to the different test-day records based on two thresholds related to the parity order: 100,000 SCC and 60% DSCC for first parity cows; 200,000 SCC and 65% DSCC for later parity cows. Observations with both SCC and DSCC below the respective threshold were assigned a value of 1; SCC below and DSCC above were assigned to category 2; both above to category 3; SCC above and DSCC below to category 4. A multiple-trait repeatability linear animal model was applied to the two traits, with year-month-parity-region of recording, herd-parity of recording, parity-age at calving-year-region and parity-days in milk-year-region as fixed effects. Random effects included herd-test-day-parity of recording, herd-year-month-parity of calving, animal additive genetic, and permanent environment. The posterior mean (PM) for heritability was 0.13 for SCS (posterior standard deviation, PSD: 0.01) and 0.09 (0.01) for SI. The genetic correlation between SCS and SI was 0.94, highlighting the strong relationship between the two traits but also their differences. A SNPBLUP model was applied for estimating genomic breeding values (GEBV) using either a reference population composed of bulls or of both bulls and cows (mixed reference population). For the validation of GEBV, a three-year back cutoff date for phenotypes was used: the results highlighted the positive impact of a mixed reference population on dispersion and accuracy. The genetic trend based on bulls' GEBV indicates that the undergone selection for SCS indirectly improved the population also for SI. In conclusion, this study confirmed the possibility to select for SI in Italian Holstein population and provided the bases for the implementation of a routine genetic evaluation for this innovative udder health trait.

Key words: mastitis, dairy cattle, genomic selection, infection, health, genetic parameters

Introduction

Mastitis is one of the most relevant diseases in dairy farming, with negative consequences on farm net profit and animal welfare (Seegers et al., 2003): its subclinical form (subclinical mastitis, SCM), when there are no visible signs of inflammation, can lead to an undetectable spread of mastitis, resulting in significant economic loss (Halasa et al., 2007).

Early detection of an ongoing inflammatory process can strongly mitigate the adverse outcomes of mastitis. Historically, somatic cell count (SCC) or its log-transformation (somatic cell score, SCS) has been the main indicator of SCM, with 200,000 cell/ml as threshold (Sharma et al., 2011). SCS is used as a proxy for mastitis resistance due to: i) its high genetic correlation with clinical mastitis, ii) its higher heritability, and iii) its possibility to be routinely measured within the national milk recording system on a large scale and at a cost effective.

Nowadays, a novel indicator of inflammation is available, differential somatic cell count (DSCC) (Bobbo et al., 2018). DSCC is the percentage of polymorphonuclear neutrophils and lymphocytes (PMN-LYM) within milk somatic cells. A high level of DSCC indicates an active immune response in the mammary gland (Damm et al., 2017).

Using DSCC independently from SCC can be misleading; indeed, an animal with low DSCC but high SCC cannot be safely classified as healthy. For this reason, a new phenotype was analyzed in this study: the state of infection (SI), regarded as the relationship between DSCC and SCC, as proposed by Bobbo et al. (2020).

The objective of this study is to evaluate the feasibility of selecting for SI, the genetic trend occurring in Italian Holstein and the possibility of including females in the reference population for SNP effects estimation.

Materials and Methods

Data editing

Test-day data came from the official national milk recording system within the LEO project (PSRN mis 16.2, AIA, 2023).

Records from parity 1 to parity 5 and from 5 to 405 days in milk (DIM) were considered. Minimum age at first calving was set to 18 while the maximum admitted value for age at calving was 100 in parity 5.

Regarding TD records, the first recording of the lactation had to be within 60 DIM while the maximum allowed distance between consequent TD records was 70 days.

For the phenotypes, DSCC records out of the range 25 to 95% were deleted. SCC was log-transformed to somatic cell score (SCS) following Ali & Shook (1980) but adding 4 instead of 3 as in Martins et al. (2010), in order to have less records below 0: allowed values for SCS ranged from 0 to 10, with the lower bound not included in the range. From the relationship between SCC and DSCC a new phenotype was derived: two different parity-dependent thresholds were identified for both SCC and DSCC. For SCC, the thresholds were 100,000 cells/ml for first parity cows and 200,000 cells/ml for later ones. Regarding DSCC, the threshold for primiparae was 60% while for pluriparae was 65% (Bobbo et al., 2019a, Bobbo et al., 2019b, Zidi et al., 2019). Four categories were then identified, from best (1) to worst (4):

- Category 1, healthy: both parameters below the respective thresholds
- Category 2, at risk: SCC below while DSCC above the threshold
- Category 3, ongoing mastitis: both parameters above their respective thresholds
- Category 4, chronic: DSCC below while SCC above the threshold

The minimum number of contemporaries in contemporary groups (CG) was set to 5 and other constraints were applied in order to maintain a consistent numerosity per level of the fixed effects included in the model.

Finally, to be included in the analysis, TD records had to have both SCS and DSCC recorded.

The dataset after edits was composed of 8 million records.

Statistical model

A multiple trait repeatability linear animal model was applied, with SCS and SI as correlated dependent variables.

The model for both traits was the following:

$$Y_{ijklmnopqr} = htdp_i + hymp_j + S_k{*}Y_l + H_m + DIM_n{*}PARC_o{*}Y_l + AGEC\_PAR_p{*}Y_l + a_q + pe_q + e_{ijklmnopqr}$$

with $Y_{ijklmnopqr}$ as the r*th* phenotypic observation of DSCC or SI. Fixed effects were $S_k{*}Y_l$ as the crossed effect of season k by year l, H_m as the m*th* herd of recording, $DIM_n{*}PARC_o{*}Y_l$ as the n*th* days in milk class (10 classes of 40 days) by parity class o (3 classes: 1, 2, 3+) and year l, $AGEC\_PAR_p{*}Y_k$ as the p*th* age at calving by parity class (9 classes: 3 age at calving classes for every parity class) by year k. Random effects were $htdp_i$ as the i*th* contemporary group for herd-parity_class-date_of_recording, $hymp_j$ as the j*th* contemporary group for herd-parity_class-month_of_calving, a_q as the additive genetic effect of the q*th* animal, pe_q as the permanent environmental effect of the q*th* animal and $e_{ijklmnopqr}$ as the residual of observation r.

Variance components estimation, genetic and genomic evaluation

Variance components estimation was performed with the software THRGIBBS1F90 (Misztal et al, 2002) on a sample of 279,896 records on 26,168 animals located in 200 herds. The pedigree was traced back to 4 generations and was composed of 74,037 individuals. Convergence was assessed with R package BOA, Bayesian output analysis (Smith, 2007). Conventional estimated breeding values (EBVs) were obtained with MiX99 software (MiX99 Development Team, 2012). Genomic evaluation was performed with a SNPBLUP model using GS3 software (Legarra et al, 2011). For estimated deregressed proofs (EDPs), the method from Degano et al (2009) was applied. A conventional quality control was applied to SNP data. For the imputation process, PedImpute software was used (Nicolazzi et al, 2013).

Table 1. Results of variance components estimation.

	SCS	SI
SCS	0.13 (0.01)	0.94
SI	0.77	0.09 (0.01)

Genomic validation

Genomic validation followed the method described in Finocchiaro et al (2012) and Galluzzo et al (2022). Briefly, two datasets were used for EBVs estimation: one full (with records up to the 2404 run) and one reduced (with a 3-years back cutoff date). For both sets of EBVs, EDPs were calculated and used as pseudo-phenotypes for SNP effects estimation. Bulls with daughters in the full datasets but without in the reduced one were selected as validation bulls. Finally, a linear regression with EDPs of validation bulls from the full run as dependent variable and their direct genomic values (DGVs) from the reduced run as the independent one was fitted. The validation process was performed either using a training population composed of bulls only and of bulls and cows. Parameters considered for the comparison were the dispersion coefficient and the reliability of the linear regression model.

Results & Discussion

The dataset after edits was composed of 8 million records with phenotypes averaging 3.73% for SCS, 1.59% for SI, and 62.79% for DSCC, respectively. The results of variance components estimation are listed in Table 1. The posterior mean for heritability was moderate for both SCS and SI: the genetic correlation between the two traits was high reflecting also the phenotypic one.

Posterior means of heritability on diagonal with posterior standard deviations in parentheses, genetic correlation above diagonal and phenotypic correlation below.

The results of genomic validation for SI are listed in Table 2. Including females significantly increased the number of animals in the reference population and improved

reliability. Furthermore, its inclusion reduced the deviation of the dispersion coefficient from the expected 1. The mixed training population performed better than the one composed only of bulls for both parameters: dispersion coefficient and model reliability.

Table 2. Results of genomic validation.

	Training	Animals	b	r^2
SI	B	3,030	1.272	0.30
	M	136,763	0.950	0.60

B=bulls only; M=mixed; b=dispersion coefficient; r^2=model reliability.

Using a mixed reference population decreased the distance from 1 by 82% while doubling reliability. These results suggest that a mixed reference population composed by both bulls and cows would be beneficial for SI and thus was applied for the subsequent analyses.

The genetic trend of bulls' GEBVs by birth year is represented in Figure 1: an increasing trend is evident and may be due to the undergone selection for correlated traits like SCS.

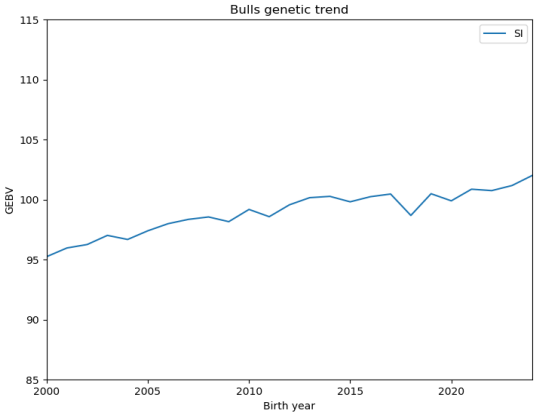

Figure 1. Bulls' genetic trend by birth year. GEBV=average GEBV.

Conclusions

In conclusion, this study demonstrated the possibility of genetically improving Italian Holstein for SI. It underscored the benefits of using a mixed reference population for SNP effects estimation. Moreover, selecting for correlated traits like SCS was effective to indirectly improve the population for SI. Based on these results, a routine genetic evaluation for SI in Italian Holstein will be developed and implemented. The SI is a powerful tool to help farmers make better decisions at the management and genetic level, thereby reducing the use of antimicrobials on the farm.

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Breeding for feed efficiency in German Holsteins: the new RZFeedEfficiency

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Abstract

A Genomic evaluation has been developed for feed efficiency for German Holsteins and the first official release was in April 2024. As of the release date, more than 327,000 weekly phenotypes of dry matter intake (DMI), body weight (BW) and energy-corrected milk (ECM) were obtained from 14,774 cows from six countries through a collaboration in the resilient dairy genome project. Lactations 1, 2 and 3+ are considered genetically distinct traits. Variance components were estimated with a multi-trait repeatability model, where each of the first three parities was divided into four equal lactation stages. (Co)variance matrices for the random regression model were derived from this multi-trait estimation using the covariance function approach. These are used to obtain genomic estimated breeding values (GEBVs) for DMI, BW and ECM with a single-step random regression model in the routine genetic evaluation. Fixed effects are herd-test-week, inbreeding depression (as a covariate), and calving age by lactation week as a fixed curve (2nd-order Legendre polynomials). The permanent environmental and additive genetic animal effects are fitted as random effects in the model. The averages of heritability estimates for parities 1 to 3, respectively, were 0.19, 0.17, 0.16 for DMI, 0.30, 0.22, 0.20 for ECM and 0.48, 0.45 and 0.50 for BW. The average genetic correlation between parities was 0.79 for DMI, 0.71 for ECM and 0.89 for BW. GEBV for body weight change (BWC) were derived from BW. GEBV correlations of DMI with ECM and BWC were 0.15 and 0.74, respectively. The GEBV correlation between ECM and BWC was -0.07. GEBV for feed saved (FS; expressed in kg DMI), which represents feed efficiency, is then computed from the traits' GEBV as 0.4×ECM + 4.5×BWC - DMI. GEBV correlations of FS with the milk production index RZM and other main indices in the total merit index are close to zero. The genetic standard deviation of FS is 247 kg per 305 days in milk, which is roughly 3.5% of total DMI per 305 d. Starting in April 2024, the new GEBV for feed efficiency, RZFeedEfficiency, will be published routinely, expressed on a scale with a mean of 100 and a genetic standard deviation of 12.

Key words: Feed efficiency, dairy cattle, genetic evaluation, Germany, covariance function

Introduction

As feed expenses form the largest single part of the operating costs of dairy farms, enhancing the feed efficiency (FE) of dairy cattle is a major priority for dairy farmers (Connor, 2015). Reducing the environmental impact of dairy production can also be achieved through improving feed efficiency. Animals with higher feed efficiency could generate less greenhouse gas emissions and manure (Bell et al., 2012; Connor, 2015). Furthermore, animals with lower feed requirements use less land (Connor, 2015).

Compared to many other dairy cattle traits, defining FE is particularly challenging. This is because selecting for FE requires knowledge of how much feed has been used for production. Consequently, several approaches (e.g., Pryce et al., 2015 and VandeHaar et al., 2016) have been developed to account for all components involved and to obtain a phenotype that most accurately identifies animals with favorable genetic merit for FE.

Data on FE is scarce because obtaining feed intake records is both costly and challenging. Hence, the amount of available data is still a limiting factor for achieving highly reliable genetic predictions for FE. Therefore, an international collaboration became essential to expand the reference population (van Staaveren et. al., 2024).

Random regression models (RRM) are well-suited for analyzing longitudinal data with varying variation of traits across the different lactation stages. However, with limited data size, estimation of the variance parameters of an RRM is difficult. Thus, an indirect estimation approach, based on a covariance function, has been proposed (Kirkpatrick et. al., 1994 and Liu et. al., 2000). We describe the German genetic evaluation of FE including variance component estimates, genetic correlations, and the development of the target selection index RZFeedEfficiency.

Materials and Methods

Data

The current data set used in the German genetic evaluation consisted of 327,408 weekly phenotypes of dry matter intake (DMI), body weight (BW), and energy-corrected milk (ECM) from 14,774 cows. The data was obtained through a collaboration with six countries in Europe and North America within the resilient dairy genome project (van Staaveren et al., 2024). These countries are Canada (CAN), Switzerland (CHE), Germany (DEU), Denmark (DNK), Spain (ESP) and the United States of America (USA; Figure 1).

In addition to the phenotypes of the three traits mentioned above, the pedigrees along with the genotypes (50K) of these cows were also obtained. As per quality control for the phenotypes, for each parity, a record was excluded if it exceeded 3 standard deviations either above or below the mean. Animals that had a conflict in the pedigrees and/or the genotypes, had less than 4 records per parity, or had no DMI records were also excluded.

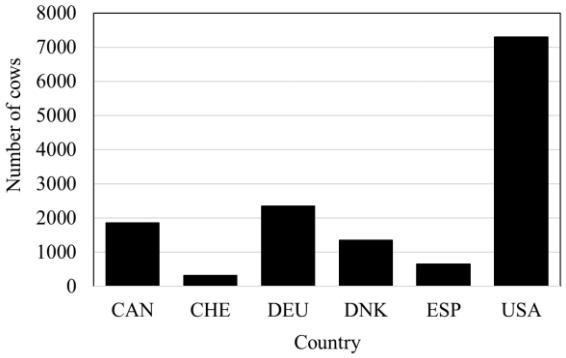

Figure 1. Number of cows with dry matter intake records per country.

Records from higher than the 3rd parity were considered repeated measurements of 3rd parity records. The quality control for the genotypes to clean the genotypic data was the same as for all other German Holstein routine genomic evaluations. For the variance component estimation, the pedigree was limited to the previous five generations of animals with phenotypes. A summary of the final data set is shown in Table 1.

Table 1. Number of records, means and standard deviations for DMI, ECM and BW within the first three parities.

Trait*	Parity	Number of records	Mean	SD
DMI	1	163,833	20.47	3.89
	2	110,419	24.06	4.50
	3+	92,813	24.78	5.06
ECM	1	127,599	32.51	6.25
	2	876,88	39.60	8.38
	3+	759,91	40.68	9.08
BW	1	124,535	605.59	62.84
	2	842,11	669.33	66.86
	3+	721,58	713.03	72.01

*DMI: Dry matter intake.
*ECM: Energy-corrected-milk.
*BW: Live body weight.

Model

The traits DMI, BW, and ECM were analyzed separately, but within trait, the first three parities were analyzed jointly, treating them as genetically distinct traits. Higher parities were considered as repeated measurements of the

third parity. We first fitted a repeatability animal model by dividing each parity into 4 equal stages (11 weeks). Herd-test-week was fitted as a fixed effect, animal as an additive genetic effect, as well as a permanent environmental effect, were fitted as random. The analysis was carried out using WOMBAT (Meyer, 2007).

The variance components obtained from this multivariate model were then used to derive the (co)variances of second-order random regression coefficients (RRC) for the additive genetic, the permanent environmental and the error effects. This was done based on the covariance function approach (Kirkpatrick et. al., 1994, Tijani et. al., 1999 and Liu et. al., 2000). The residual variance was assumed to be homogeneous throughout lactation. Subsequently, a single-step RRM was fitted to estimate the breeding values for each phenotype.

In the single-step random regression model, three fixed effects were fitted: herd-test-week, a fixed curve (2^{nd} order) of calving age by lactation week and a regression on inbreeding. Random effects were animal additive genetic and permanent environmental effects, and the analysis was implemented in MiX99 (Vuori et. al., 2006). In the April 2024 routine genetic evaluation run, the number of genotyped animals was 1,518,447 and the number of animals in the pedigree was 3,839,445. Genomic estimated breeding values (GEBV) for BWC were calculated as the weekly change in GEBV of BW, using the derivative of the genetic Legendre function. GEBV from the three parities were aggregated with an equal weight (1/3) into a single GEBV per trait.

Feed efficiency index (RZFeedEfficiency, short: RZFE)

The expected GEBV for DMI was calculated from ECM and BWC. It was assumed that an average ration's energy density is 7.0 MJ NEL per kg DMI. Additionally, it is assumed that 0.4 kg DMI are required to produce 1 kg ECM, and 4.5 kg DMI are required to produce 1 kg BWC. Hence, feed saved was calculated as follows:

$$GEBV_{Feed\ saved} = 4.5 \times GEBV_{BWC} + 0.4 \times GEBV_{ECM} - GEBV_{DMI}$$

The resulting EBV for feed efficiency is the feed saved, expressed in kg DMI, compared to the average cow. It represents a measure of feed efficiency over the first three lactations, which is roughly the average longevity of Holsteins in Germany. This feed saved GEBV is then expressed as a relative GEBV with a mean value of 100 in the female base population and a genetic standard deviation of 12. The female base population is defined as 4 to 6-year-old genotyped Holstein females at the time of the routine genetic evaluation.

Results and Discussion

The bar chart in Figure 1 shows the number of cows for the six countries whose data is used in the German genetic evaluation of feed efficiency from the resilient dairy genome project database. The United States have the highest number of cows by a significant margin, followed by Germany while Switzerland has the lowest number. Collaboration in the resilient dairy genome project (van Staaveren et al., 2024) with international partners provided the largest possible reference population for feed intake. Modelling the underlying component traits of feed efficiency (DMI, ECM, and BWC) offers flexibility in defining the target trait on the genetic side (based on GEBV), which could be, if necessary, easily adjusted. We found that applying the covariance function approach (Kirkpatrick et. al., 1994, Tijani et. al., 1999 and Liu et. al., 2000) provides stable random regression coefficients, especially as the phenotypic data is limited. Additionally, since feed efficiency varies at different periods of lactation, using an RRM has the advantage of fitting the genetic curves to capture the changes over time. Moreover, switching to daily measurements in this case would be

straightforward, and future improvements can be, if needed, easily implemented.

The heritability estimates obtained from the RRM over the 44 weeks in milk in the first three parities are depicted in Figure 1. In the first parity, heritability starts around 0.1, peaks around 0.25 between 15 to 20 weeks, then slightly decreases and stabilizes around 0.2. In the second parity, it starts around 0.1, peaks slightly below 0.2 around the 15th week, then gradually decreases and stabilizes around 0.15. Finally, in parity 3 heritability estimates were also around 0.1 at the beginning of the lactation and slightly increased to reach 0.2 while remaining relatively stable towards the end of the lactation.

It can be seen that heritability estimates of DMI change over time along the lactation cycle with slight differences between parities showing distinct patterns for each parity group. Heritability for DMI has been intensively reported in the literature based on different methods and in general, it averages between 0.08 and 0.34 (e.g., Berry et. al., 2014, Khanal et. al., 2022 and Stephansen et. al., 2023). Our estimates for the three parities were highly similar and generally slightly lower at the beginning of the lactation, compared to mid-lactation and after. Nevertheless, these estimates are consistent with the most recent reported estimates.

Total heritability estimates for each parity for the three traits are presented on the diagonal of Table 2. Highest estimates were observed in the first parity for all three traits, while the lowest were observed in the third parity for DMI and ECM. The heritability for DMI ranged from 0.30 to 0.39, with an overall total of 0.38. The weekly and the cumulative heritability over parities indicates substantial genetic variation, allowing for better discrimination of genetic differences between animals.

The heatmap in Figure 3 illustrates the correlations between weeks in milk across the first three parities. As expected, adjacent weeks had the highest positive correlations.

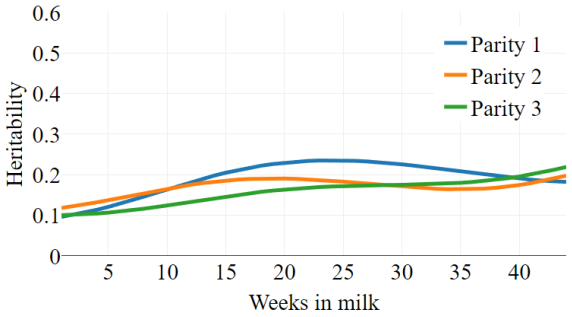

Figure 2. Heritability of DMI over the weeks in milk in the first three parities.

Although genetic correlations between different weeks are all moderately to highly positive, our results show that DMI is genetically not the same trait within and across lactations with minimum genetic correlation estimates within parities of 0.64 (parity 1), 0.52 (parity 2), and 0.70 (parity 3). The minimum genetic correlation between parity 1 and 2 was 0.50, between 1 and 3 was 0.39, between 2 and 3 was 0.49.

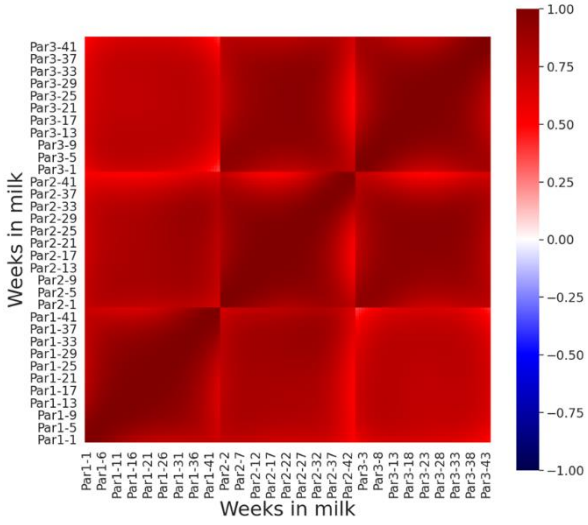

Figure 3. Genetic correlation of DMI over the weeks in milk in the first three parities (Par1, Par2, Par3). Each cell of the matrix represents the correlation between two weeks.

Numerous studies (e.g., Pech et al., 2014) have reported lower genetic correlations for DMI than our estimates, and even negative between mid-lactation and both the start and end of the lactation. It is well known that dairy cattle traits do differ over the course of lactation, but the vast shifts from high positive

to negative correlations could be essentially attributed to the way that RRM estimates are obtained when the data used is small (Kirkpatrick et al., 1994; Liu et al., 2000). For this reason, we implemented the covariance function to reliably estimate random regression coefficients. Genetic correlations within and between entire parities for the three traits are listed below the diagonal in Table 2. Genetic correlation estimates between parities are highly positive, but many values, especially between first and higher parities are below 0.90, indicating a somewhat distinct genetic background of the same trait at different times in the life of the cow.

Table 2. Estimates of heritability (on diagonal) and genetic correlations (below diagonal) for DMI, ECM and BW across the first three paraties.

Trait*	Parity#	1	2	3	1/2/3
DMI	1	0.39			
	2	0.88	0.31		
	3	0.79	0.95	0.30	
	1/2/3	0.93	0.99	0.96	0.38
ECM	1	0.43			
	2	0.79	0.31		
	3	0.63	0.97	0.29	
	1/2/3	0.86	0.99	0.94	0.40
BW	1	0.58			
	2	0.94	0.54		
	3	0.88	0.94	0.61	
	1/2/3	0.96	0.99	0.97	0.63

*DMI: Dry matter intake.

*ECM: Energy-corrected-milk.

*BW: Live body weight.

#1/2/3: Combination of parities 1, 2 and 3.

The variance component parameters were then used with the genetic evaluation model to obtain GEBV for the three traits based on a single-step approach, including all available genotyped and pedigree animals.

GEBV of the different traits were then combined to the overall index representing feed efficiency, expressed as feed (DMI) saved. For this index combination, we do not only consider the economically most relevant output of dairy cows, milk (ECM), but also BWC. This is, because body weight is an important storage of energy in the body of the cows. Not respecting BWC could therefore lead to a wrong estimation of the energy balance of the cows, e.g., when a cow uses energy from her body weight within a lactation, but regains weight between lactations. Additionally, slaughter weight is a secondary output from dairy cows that has also an economic value for the farmers. On average, Holstein cows gain more than 200 kg body weight over the first three parities. Mean genomic reliability is 0.4.

The correlation between GEBV of the different traits was 0.74 (DMI with ECM), 0,15 (DMI with BWC), and -0.07 (ECM with BWC). The values suggest that DMI is genetically highly correlated with ECM and mostly genetically independent of BWC. Many studies have reported a positive genetic correlation of DMI with ECM and typically ranging from moderate to high (e.g., Hüttman et. al., 2009 and Li et al., 2018). As selection increases milk production, DMI also tends to increase due to the higher energy demands. The genetic standard deviation of RZFE is 247 kg per 305 days in milk, which is roughly 3.5% of total phenotypic DMI. Correlations of RZFE with GEBV of other trait complexes calculated for 352,692 genotyped females born in 2021 and 2022 are shown in Table 3. Overall, GEBV correlations among RZFE and other trait complexes were low, close to zero. This suggests that genetic improvement for feed efficiency can be targeted without significantly affecting other main dairy traits, including health traits.

To find out how RZFE characterizes the more and the less efficient animals, we obtained the differences in GEBV between the 25% top and the 25% bottom RZFE genotyped females, born in 2021 or 2022 (N = 352,692 per quartile; Table 4). Clear differences exist between the two subgroups in feed efficiency (16.4 for RZFE and 1,264 kg feed saved) while the level for ECM and BWC is very similar between the top and bottom animals. It can be noted that selection for feed efficiency will not decrease ECM and will also not decrease the weight of the cows, because even the top 25% cows for RZFE have a slightly positive breeding value

for body weight. Therefore, most of the difference in RZFE between top and bottom animals stems from the difference in DMI.

Table 3. Correlations of RZFE and GEBVs of other trait complexes

Breeding value	Trait complex	Correlation to RZFE
RZG	Total merit	0.02
RZ€	Total merit (€)	0.05
RZM	Production	-0.07
RZN	Longevity	0.05
RZE	Conformation	-0.11
RZR	Reproduction	0.02
RZHealth	Health	-0.03
RZKm	Calving, maternal	0.03
RZKd	Calving, direct	0.10
RZCalffit	Young stock survival	0.06

Table 4. Differences between the top and the bottom 25% females for feed efficiency (352,692 per quartile) born between 2021 and 2022.

Item	Top 25%	Bottom 25%	Difference
RZFE[#]	107.9	91.5	16.4
FS[*]	607	-657	1,264
DMI[*]	-307	917	-1,224
ECM[*]	743	625	118
BWC[*]	0.6	2.2	-1.5
BW[+]	4.1	27.1	-23.0

RZFE: Feed efficiency index (RZFeedEfficiency).
FE: Feed saved.
DMI: Dry Matter Intake.
ECM: Energy Corrected Milk.
BWC: Body Weight Change.
[#] On a relative scale (Mean of 100±12)
[*] Sum of first three parities in kg.
[+] Mean of first three parities in kg

Conclusions

The German feed efficiency index (RZFeedEfficiency, short: RZFE) was first introduced in April 2024. The international data exchange enables a sufficient data basis for the genetic evaluation of feed efficiency. However, with 0.4, the genomic reliability of the RZFE is at the lower range of genomic reliabilities when compared to other traits under routine genetic evaluation in Holsteins. Applying the covariance function approach facilitated the estimation of variance components for the random regression model. Our results show that underlying traits are heritable with reasonable estimates of the genetic parameters, allowing for considerable genetic selection. The feed efficiency trait definition considers dry matter intake, energy-corrected milk and body weight change as main energy sources/sinks and refers to three lactations, the average productive life of Holstein cows in Germany. RZFE is mostly independent of production level and health traits. The genetic standard deviation of RZFE is 247 kg per 305 days in milk, which is roughly 3.5% of phenotypic DMI.

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Alternative Residual Feed Intake (RFI) expressions in dairy cattle

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Abstract

Residual Feed Intake (RFI) is commonly defined as residuals from linear regression of feed intake on energy sinks, expressed on the phenotypic scale. Estimates of partial regression coefficients are obtained by Least Squares, and RFIs are subsequently used as phenotypes in a genetic evaluation model. Alternatively, regression coefficients for RFI can be derived directly from phenotypic co-variances among feed intake and the energy sinks, and EBVs for RFI can be formulated as reparameterizations of EBVs for feed intake and energy sinks from a multiple-trait (MT) model. This is equivalent to the recursive model (RM) approach, with EBVs calculated as system parameters. Using RM as operational tools, RFI can be defined and the respective parameters calculated, for overall and any individual source of random variation covered by the MT model for feed intake and energy sinks, i.e., genetic, PE, residual. Different definitions of RFI result in independence of RFI from energy sinks on different levels of variability. These concepts are illustrated by application of the genetic evaluation model for feed efficiency of Canadian Holsteins. A six-trait MT model for Dry Matter Intake (DMI), Energy Corrected Milk (ECM) and Metabolic Body Weight (MBW) in two DIM intervals of 1st lactation was fitted to approximately 100,000 weekly records on 5,000 cows, with 9,000 genotyped animals in the pedigree via MC-EM-REML and Single-Step GBLUP, for the purpose of co-variance component estimation and genomic evaluation. Four different expressions of RFI in 61 – 305 DIM in lactation (phenotypic = pRFI, genetic = gRFI, permanent environmental = eRFI and residual = rRFI) were defined and examined as potential selection criteria or as tools for optimizing management, with respect to estimates of genetic parameters and GEBV. Standardized regression coefficients of DMI on sinks differed among RFI definitions, but the relative impact of sinks was similar. Heritabilities of RFIs ranged from 0.05 (gRFI) to 0.15 (rRFI). Genetic and phenotypic expressions of RFI were genetically correlated at 0.84. Genetic correlations between pRFI and energy sinks were 0.62 for ECM and 0.04 for MBW (versus 0.00 for gRFI). Genetic correlations with DMI were 0.37 and 0.59 for gRFI and pRFI, respectively. Correlations between GEBV, for official sires (N = 298), ranged from 0.64 (gRFI and pRFI) to 0.99 (pRFI and eRFI). Results illustrate substantial differences among definitions of RFI in dairy cattle and consequences of using different definitions for genetic evaluation and selection. Generalizations to other traits are straightforward.

Key words: RFI, feed efficiency, single-step genomic evaluation

Introduction

Feed represents a significant proportion of dairy cattle production expenses. To reduce costs, genetic selection for feed efficiency has recently become more widely used across different dairy populations. Examples include the Canadian Holstein genetic evaluation for metabolic feed efficiency (Jamrozik et al., 2022), and US genetic evaluation for feed saved (Parker Gaddis et al., 2021). Both North American approaches are based on the concept of Residual Feed Intake, as a measure of feed efficiency independent of an animal's body size and production level. It is considered to

represent the inherent variation in metabolic processes to describe efficiency.

Residual Feed Intake (**RFI**) was initially proposed by Koch et al. (1963) as the residuals from linear regression of feed intake on various energy sinks, expressed on the phenotypic scale. For simplicity, let Energy Corrected Milk (**ECM**) and Metabolic Body Weight (**MBW**) be the only energy sinks acting on Dry Matter Intake (**DMI**).

The equation for linear regression can be represented as:

$DMI_i = \mathbf{x}_i\text{'}\mathbf{b} + c_M*ECM_i + c_W*MBW_i + e_i$,

with **b** being a vector of selected systematic (fixed) effects acting on DMI.

Estimates of the covariable regression coefficients c_M and c_W are obtained by Least Squares (**LS**) and phenotypes for RFI are defined as residuals (e_i) from the above model. These residuals are subsequently used as observations in genetic and genomic evaluation models for RFI.

Alternatively and equivalently, c_M and c_W can be derived as partial regression coefficients from phenotypic co-variances between DMI and the energy sinks. Define **C** = $[C_{ij}]$ (2x2) phenotypic co-variance matrix for ECM and MBW, **w** = $[w_{ij}]$ vector of phenotypic co-variances between sinks and DMI. Then $[c_M \;\; c_W]' = \mathbf{C}^{-1}\mathbf{w}$ (Kennedy et al., 1993).

The calculation of phenotypes for RFI from LS, to be used for further (i.e. genetic) analyses, faces challenges from conceptual, statistical, and practical perspectives (Lu et al., 2015):

1. RFI is not an observable trait and hence it may be difficult to explain to farmers,
2. Any regression analysis used to derive RFI implicitly assumes that all covariates (i.e., energy sinks) are recorded and known without any measurement error,
3. If any of the energy sink covariates are completely missing for a particular animal, none of the records on that animal can be used to derive the animal's RFI, and
4. The presence of non-zero genetic and residual correlations between DMI and the energy sink traits distorts heritability estimates for RFI (Kennedy et al., 1993) and interpretation of the inferences.

Materials and Methods

Use of mixed model methods for RFI

Genetic parameters and EBVs for RFI can be obtained without directly using phenotypes for RFI. The mixed linear model associated with the i-th multivariate record for ECM, MBW and DMI can be written as:

$$\mathbf{y}_i = \mathbf{X}\,\mathbf{b} + \mathbf{a}_i + \mathbf{p}_i + \mathbf{e}_i, \text{ where}$$

$\mathbf{y}_i$ is a vector of observations on subject i for DMI and the two energy sink measurements, **b** is a vector of fixed effects, $\mathbf{a}_i$ is a vector of animal additive genetic effects, $\mathbf{p}_i$ is a vector of permanent environmental (**PE**) effects, $\mathbf{e}_i$ is a vector of residuals, **X** is an incidence matrix. Assumptions are that: $v(\mathbf{a}_i) = \mathbf{G}$, a genetic covariance (3x3) matrix; $v(\mathbf{p}_i) = \mathbf{E}$, a covariance (3x3) matrix for the PE effects; $v(\mathbf{e}_i) = \mathbf{R}$, a residual covariance matrix. Phenotypic co-variance matrix (**P**) can be defined as $\mathbf{P} = \mathbf{G} + \mathbf{E} + \mathbf{R}$.

Let $\mathbf{a} = [a_1, a_2, a_3]'$ refer to EBV for ECM, MBW and DMI, respectively. To obtain phenotypic independence between an RFI variable (not yet defined) and the energy sinks, a linear re-parameterization of the EBV for ECM, MBW and DMI can be postulated as:

$$\mathbf{a}^* = \mathbf{\Lambda}\,\mathbf{a}, \text{ with}$$

$$\mathbf{\Lambda} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ -L_{31} & -L_{32} & 1 \end{bmatrix}$$

Non-zero elements of $\mathbf{\Lambda}$, L_{31} and L_{32}, can be expressed as functions of elements of phenotypic co-variance matrix **P** as:

$L_{31} = (p_{12}*p_{23} - p_{13}*p_{22})/(p_{12}*p_{12} - p_{11}*p_{22})$

$L_{32} = (p_{12}*p_{13} - p_{11}*p_{23})/(p_{12}*p_{12} - p_{11}*p_{22})$,

and they are partial phenotypic regression coefficients of DMI on ECM and MBW. The EBV of ECM and MBW remain unchanged, and EBV for DMI is transformed into an EBV for RFI:

$$a_3^* = a_3 - L_{31}\, a_1 - L_{32}\, a_2.$$

This definition for RFI can be interpreted as DMI phenotypically adjusted for energy sinks. Co-variance components involving this RFI can be obtained as:

$\mathbf{G}^* = \mathbf{\Lambda G \Lambda}'$,
$\mathbf{E}^* = \mathbf{\Lambda E \Lambda}'$,
$\mathbf{R}^* = \mathbf{\Lambda R \Lambda}'$, and
$\mathbf{P}^* = \mathbf{G}^* + \mathbf{E}^* + \mathbf{R}^*$.

The re-parameterization described above can also be derived using a recursive model approach (Jamrozik et al., 2017). Let Y_1, Y_2, and Y_3 refer to phenotypes for ECM, MBW and DMI, respectively, and let recursive equations for DMI in this model be:

$Y_1 = \text{fixed}_1 + \text{random}_1 + e_1$
$Y_2 = \text{fixed}_2 + \text{random}_2 + e_2$
$Y_3 = L_{31}* Y_1 + L_{32}* Y_2 + \text{fixed}_3 + \text{random}_3 + e_3$,

with L_{jk} denoting a recursive coefficient parameter for the effect of change in trait j caused by the phenotype of trait k. The mixed linear recursive model associated with the i-th record for ECM, MBW and DMI can be written as:

$\mathbf{\Lambda}\, \mathbf{y}_i = \mathbf{X}\, \mathbf{b}^* + \boldsymbol{a}_i^* + \mathbf{p}_i^* + \mathbf{e}_i^*$, with

$v(\mathbf{a}_i^*) = \mathbf{G}^*$, $v(\mathbf{p}_i^*) = \mathbf{E}^*$, $v(\mathbf{e}_i^*) = \mathbf{R}^*$, and $\mathbf{P}^* = \mathbf{G}^* + \mathbf{E}^* + \mathbf{R}^*$.

Imposing restrictions on phenotypic co-variances i.e. setting $p_{13}^* = p_{23}^* = 0$ of the phenotypic co-variance matrix $\mathbf{P}^*$ of the recursive model will yield the same $\mathbf{\Lambda}$ and expressions of co-variance components and EBVs on a recursive scale for RFI, as presented earlier using a simple re-parametrization of the EBVs to compute EBVs for RFI. Additionally, the recursive model parameters $\mathbf{G}^*$, $\mathbf{E}^*$, and $\mathbf{R}^*$ can be interpreted as system co-variances.

Given the estimates of partial regression coefficients and the known co-variance structure of the model, EBV for RFI can be derived using estimates of EBV for DMI and sinks from a regular multiple-trait model for these traits, due to the equivalency between recursive and multiple-trait models (Jamrozik et al., 2017). In addition, the EBVs for RFI can be interpreted as parameters of the recursive model from sinks to DMI, under the assumption of known recursive regression coefficients.

Alternative RFI definitions

So far, RFI has been discussed on the phenotypic level (**pFRI**), as feed intake phenotypically adjusted for, or independent of, energy sinks. In other words, we looked at RFI as feed intake on the same phenotypic level of ECM and MBW. This can be extended to other random variables affecting DMI, like genetic or permanent environment effects, which would lead to different interpretations with different definitions for RFI. Genetic RFI (**gRFI**) can be defined as feed intake genetically independent of energy sinks. Similarly, PE RFI (**eRFI**) can describe feed intake adjusted for (or independent of) systematic environmental effects on repeated measurements for an animal over time (e.g. affecting all daily or weekly DMI measurements throughout a lactation). Finally, residual RFI (**rRFI**) will refer to feed intake adjusted for all effects in the model or independent of all residual effects on the energy sink observations. For derivation of regression coefficients on any given sources of variation we can use the corresponding co-variance matrix of interest to compute L_{jk}, either as shown in the previous paragraph for a pair of energy sinks, or using the more generalized equation below, which accommodates any number of sinks.

Let vector **L** with order (n-1) be the vector of multi-variate regressions of variable n on variables 1 through (n-1). Partition the covariance matrix **V** of interest for recursions

(e.g. genetic, phenotypic, etc.) to separate the n^{th} row and column from all previous rows and columns, and **L** is then defined as follows:

$$\mathbf{V} = \begin{bmatrix} \mathbf{A} & \mathbf{C} \\ \mathbf{C}' & \mathbf{B} \end{bmatrix}$$

$$\mathbf{L} = \mathbf{A}^{-1}\mathbf{C}$$

$$\mathbf{L}' = [L_{n(1)} \quad \cdots \quad L_{n(n-1)}]$$

It is easily verified that the general equation above yields the same values for L_{31} and L_{32} as shown in the earlier example for phenotypic RFI with two energy sinks, where **V**=**P**, and the generalization by Kennedy et al. (1993) for genetic RFI, where **V**=**G**.

In the scope of recursive modelling, phenotypic restrictions on covariances (i.e. zeroing phenotypic co-variances between traits) are replaced by restrictions related to the definition of RFI.

An example of application

A first lactation feed efficiency model applied to Canadian Holsteins was used to illustrate the concepts presented above. The data and model descriptions, from Jamrozik et al. (2021), are as follows.

Traits

The model defined all traits in two periods of first lactation: 5-60 days and 61-305 days in milk (DIM). Traits were:

- MBW, calculated as (body weight)$^{0.75}$;
- ECM, calculated as 0.25*Milk + 12.2*Fat + 7.7*Protein; and
- DMI.

All traits were weekly averages expressed in kg/day (ECM and DMI) or kg$^{0.75}$ (MBW).

Data

The feed efficiency data available at Lactanet included data from seven herds in five countries within the EDGP project plus eight more US herds outside of EDGP.

The final data (after edits) for co-variance component estimation consisted of 99,713 weekly records on 4,952 first lactation cows from 1,101 sires. Pedigrees of cows with phenotypes were traced back four generations, for a total of 18,085 animals included in the estimation. More details on the data can be found in Jamrozik et al. (2021).

Model

The linear animal model used for co-variance components estimation was the same for each of the 6 feed efficiency traits (ECM, MBW and DMI, in 2 DIM intervals). Fixed effects in the model were: Age at calving, Lactation week, Year-Season of calving, and Herd-Year of calving. Random effects included Additive genetic, Permanent Environmental (**PE**), and Residual effects.

The multiple-trait model for 6 traits can be written as:

$$\mathbf{y} = \mathbf{X}\,\mathbf{b} + \mathbf{Z}_1\,\mathbf{a} + \mathbf{Z}_2\,\mathbf{p} + \mathbf{e}, \text{ where}$$

y is a vector of observations (traits within cows within DIM interval), **b** is a vector of all fixed effects, **a** is a vector of animal additive genetic effects, **p** is a vector of PE effects, **e** is a vector of residuals, **X** and $\mathbf{Z}_i$ (i =1, 2) are respective incidence matrices.

Assumptions were that:

v(**a**) =**A** ⊗ **G**, **A** is additive genetic relationship matrix, **G** is the additive genetic covariance (6x6) matrix;

v(**p**) = **I** ⊗ **E**, **E** is the covariance (6x6) matrix for the PE effects;

v(**e**) = $\sum_{i=1}^{N}{}^{+} \sum_{i=1}^{N}{}^{+} \mathbf{R}_i$, $\mathbf{R}_i$ is a residual covariance matrix (3x3) for either first or second DIM interval, N is the total number of weekly records. Residuals for traits collected in the same week of lactation were assumed correlated, and uncorrelated otherwise.

Co-variance components of the model were estimated with the Monte Carlo - Expectation Maximization - Restricted Maximum Likelihood (**MC-EM-REML**) algorithm (Matilainen et al., 2012) implemented in the MiX99 software package (MiX99 Development Team, 2017).

Recursive model matrix for the six-trait model **Λ** was defined as $\sum^{+} \mathbf{\Lambda}_i$, where $\mathbf{\Lambda}_i$ (i =

1, 2) corresponds to the i-th DIM interval of lactation.

Genomic evaluation

The Single-Step method was used to fit the multiple-trait linear animal model for 6 traits (ECM, MBW and DMI, in 2 DIM intervals) with genotypic information via MiX99 software. The same model as presented for co-variance component estimations was used for genomic evaluation, with **A** replaced by **H** (combined pedigree/genotypes relationship matrix).

The data included 111,857 weekly records on 5,325 cows (4,585 cows with DMI; 4,313 genotyped cows with data). There were 1,160 sires of those cows with data (934 genotyped sires). In total, there were 19,137 animals in pedigree, and the genomic reference population included 8,375 genotyped animals.

GEBVs for different expressions of RFI were derived as presented earlier. Sire evaluation for all traits was defined as 'Official' when the bull had at least 5 daughters with DMI data and a minimum reliability for GEBV for RFI of 50%. There were 298 Holstein sires with an official status.

Results & Discussion

Genetic RFI calculated in 61 – 305 DIM is the principal selection criterion for feed efficiency in Canadian Holsteins. Therefore, and also for illustration purposes of the proposed methods, only results pertaining to traits (including different expressions of RFI) defined in this part of lactation will further be presented and discussed in this paper. In addition, the most emphasis will be put on comparisons between gRFI and pRFI, as the most popular expressions of RFI.

Genetic parameters

Estimates of regressions coefficients of DMI on energy sinks for different definitions of RFI are in Table 1.

Table 1. Estimates of regression coefficients and relative impact (%) of energy sinks on DMI

		gRFI	pRFI	eRFI	rRFI
Regression coefficient	ECM	0.48	0.31	0.28	0.19
	MBW	0.14	0.13	0.11	0.15
Relative impact	ECM	63	62	63	62
	MBW	37	38	37	38

Regression coefficients differed among different RFI definitions, especially for ECM. Relative impact of energy sinks on RFI remained approximately the same (60:40) for different RFI, with a larger emphasis on ECM.

Estimates of heritability for ECM, MBW and DMI in 61 – 305 DIM were 0.29, 0.50 and 0.27, respectively. Corresponding repeatabilities were 0.67, 0.91 and 0.57. Estimates of heritability and repeatability for different definitions of RFI are in Table 2.

Table 2. Estimates of heritability and repeatability (x100) for different RFI expressions

	gRFI	pRFI	eRFI	rRFI
Heritability	5	9	11	15
Repeatability	38	40	42	45

Heritability of RFI ranged from 5% (gRFI) to 25% (eRFI). Estimates of repeatability were more similar across RFI definitions (38 – 45%), with the same pattern of changes between different RFIs as observed for heritability.

Estimates of genetic and phenotypic correlations between each definition of RFI and the other traits in the model (sinks and DMI) are in Table 3.

By definition, genetic correlations between gRFI and energy sinks were equal to zero. Similarly, pRFI and energy sinks were phenotypically independent. The same patterns applied to eRFI and rRFI, they were independent of energy sinks on PE and R scale, respectively (results not shown).

Table 3. Estimates of genetic and phenotypic correlations (x100) between RFI versus energy sinks and DMI, for different expressions of RFI

Correlation		gRFI	pRFI	eRFI	rRFI
Genetic	ECM	0	62	67	80
	MBW	0	4	11	-11
	DMI	37	82	88	83
Phenotypic	ECM	-33	0	6	23
	MBW	-4	0	3	-6
	DMI	59	81	85	88

Phenotypic RFI was strongly genetically correlated with ECM. It was also genetically and phenotypically more similar to DMI than was the case for gRFI.

Genetic correlations among different RFI expressions were on average smaller than corresponding phenotypic correlations (Table 4). Genetic correlation of 0.84 between pRFI and gRFI would suggest that phenotypic and genetic RFIs are genetically not the same traits.

Table 4. Genetic (above diagonal) and phenotypic (below diagonal) correlations (x100) between different expressions of RFI

	gRFI	pRFI	eRFI	rRFI
gRFI	-	84	72	68
pRFI	94	-	92	99
eRFI	84	92	-	92
rRFI	84	98	91	-

Genomic evaluation

Correlations between GEBVs for gRFI and other definitions of RFI were significantly smaller than 1 for a set of 'Official' bulls (Table 5).

Table 5. Correlations (x100) between GEBV of RFI for 'Official' sires (N = 298)

	gRFI	pRFI	eRFI	rRFI
gRFI	-	64	58	46
pRFI	-	-	99	96
eRFI	-	-	-	96

Significant re-ranking of animals can therefore be expected between genetic versus phenotypic RFI.

Correlations in Table 6 show that relative to gRFI ranking, pRFI ranking was more similar to ECM and DMI. Selecting for pRFI is to some degree like selecting for ECM.

Table 6. Correlations (x100) between GEBV of RFI and other traits for 'Official' sires (N = 298)

	ECM	MBW	DMI
gRFI	-1	-8	21
pRFI	75	14	83
eRFI	80	23	89
rRFI	88	1	82

General remarks

Using recursive modelling as operational tools (re-parameterization of multiple-trait model parameters) allowed for definition, derivation and interpretation of different expressions of RFI in dairy cattle.

No causal links between traits were imposed in the context of structural equation models discussed above. Recursive parameterizations served solely as operational tools, enabling inferences for traits (e.g. RFI) defined as linear combinations of correlated variables (ECM, MBW and DMI), and given certain assumption regarding correlations (i.e. imposing restriction on system parameters).

The presented RFI derivations, based on either the multiple-trait co-variance matrix or the recursive model machinery, can be easily extended for additional energy sinks, for example body weight change.

Similarly, we may contemplate other definitions of RFI. 'Producing Ability' RFI, derived from **G** + **PE** co-variance components, can serve as another management tool. We may also have 'Herd' RFI, derived from random 'herd' (if considered in the model) parameters. These again will have different, and possibly not always straightforward, interpretations.

Generalizations can also include an expansion of the model for multiple recursive traits of interest. For example, with lifetime feed efficiency being of interest, the first lactation RFI model was extended to a

multiple-lactations RFI model, with DMI and energy sinks all treated as different traits in first versus second lactation for genomic evaluation of Canadian Holsteins (Jamrozik et al., 2022). Finally, heterogeneity of RFI between and across lactations can be modeled using random regressions for DMI and energy sinks (Houlahan et al., 2024)

Recursive model approach to attain genetic independence between trait and energy sinks/sources has recently been applied to derive residual methane production that is genetically independent of milk production traits, for methane efficiency of Canadian Holsteins (Oliveira et al., 2023). Another application could be for functional herd life in dairy cattle, derived as length of productive life independent of production levels.

A similar approach can be used for analysis of traits expressed as ratios (Jamrozik et al., 2017). This relates, in particular, to possible application of this method for methane yield (g/kg DMI) or methane efficiency (g/kg milk).

Conclusions

Results indicate substantial differences among definitions of RFI, for estimates of genetic parameters and genomic evaluations of animals. It should be emphasized, that this could have serious consequences of using genetic vs. phenotypic RFI for genetic/genomic selection in dairy cattle. Phenotypic RFI is commonly used across the world for genetic evaluation of feed efficiency in dairy cattle. An exception is Canada, where gRFI is the genomic selection criterion in Holsteins.

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Simulating genetic progress for traits with expensive phenotyping

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Abstract

Phenotyping costs in dairy cattle breeding exhibit significant variability across traits. While milk production is recorded routinely at only low costs, traits such as feed efficiency and methane emissions pose challenges due to their expensive measurement requirements. This study leveraged the real-size digital twin of the Geno breeding program for the Norwegian Red dairy cattle breed to simulate genetic progress following ten years of selective breeding, particularly targeting traits demanding costly phenotyping. Multiple scenarios were simulated, varying in the number of phenotypes recorded, economic weight, and genetic correlation between the trait and total merit index. Our results highlight the importance of genetic correlation in achieving progress for traits with expensive phenotypes recorded at a limited scale. Increasing economic weight and the number of phenotypes increased genetic progress. Thus, there is an indirect indication that traits with low phenotyping costs and high correlation to expensive phenotypes should be prioritized when selecting for genetic improvement of a trait with expensive phenotypes. However, precise phenotypes are required for accurately estimating genetic correlations between traits with expensive phenotypes and traits with cheap phenotyping.

Key words: genomic selection, phenotyping, Norwegian Red Dairy Cattle, breeding program, digital twin, future genetic progress

Introduction

Since the introduction of genomic selection in the Norwegian Red Dairy Cattle (NR) breeding program in 2016, single step genomic prediction approach was used (Nordbø et al., 2019). This method integrates pedigree and genotype information into a single relationship matrix, allowing the inclusion of all the individuals with phenotype and genotype information in the reference (Christensen and Lund, 2010). Consequently, the reference population comprises progeny tested bulls and phenotyped cows, enhancing the accuracy of predicted breeding values (Legarra et al., 2014).

As of 2024, the reference population of the NR breeding program includes approximately 100 000 animals for production traits and 47 000 animals for conformation traits. While phenotype data for production traits are collected routinely at low costs, the recording of type traits has a long history and incurs intermediate cost. However, recording for enteric methane emission and feed efficiency began only recently, resulting in smaller reference populations for these traits (Heringstad and Bakke, 2023). Due to high cost recording these traits, it will take significantly more time to establish a reference population sufficient for predicting highly accurate genomic breeding values.

This study aims to demonstrate the potential of genomic selection in the NR breeding program for traits with expensive phenotyping by utilizing the digital twin of Geno's breeding program (Ehsani et al., 2022). We analysed the effect of the reference population size, different economic weights,

and the correlation between the selection index and the expensive trait.

Materials and Methods

A real-size breeding program was simulated beginning with fifty years of historical breeding (from 1971 to 2020), followed by ten years of alternative future breeding scenarios. The future scenarios differed in the number of phenotypes collected annually (1000, 2000, or 3000), the economic weight of an expensive trait in the future selection index (0.2 or 0.5), and the genetic correlation between the index trait and the expensive trait.

reflecting the estimated heritability of enteric methane emission and dry matter intake in NR (Heringstad and Bakke, 2023).

For the first 45 years of historical breeding, breeding values were predicted using only pedigree data. From 2016 onward, pedigree information was combined with genotype information into a single step genomic evaluation approach. Genomic breeding values were calculated using the singular value decomposition method (Ødegård et al., 2018). In this method, chromosome specific principal components explained 98% of genetic variance among the 20 000 core individuals. This core group included genotypes from all progeny

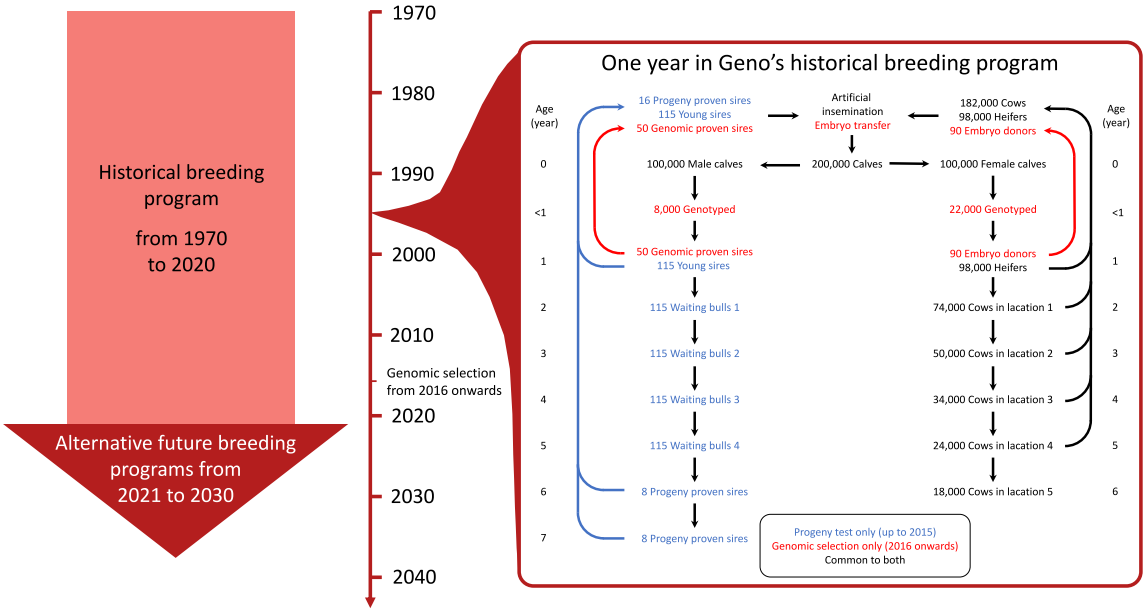

Figure 1. Fifty years of historical breeding program of Norwegian Red Dairy Cattle breed followed by ten years of future breeding as integrated in digital twin of Geno breeding program

During the historical breeding period, animals were selected based on the predicted breeding values for the index trait. For simplicity, this trait was represented milk yield (h^2=0.192), one of the most important traits in the history of NR breeding program. In the future breeding scenarios, animals were selected based on the future selection index, which combined the breeding values of the index trait and the expensive trait according to the specified economic weights. The heritability of the expensive trait was set at 0.3,

proven bulls while the rest were genotypes from the cows with available phenotype data.

The estimated breeding values (rEBV) from the final year of future breeding were standardised (EBV) so that: EVB = m + k * (rEBV - mEBV) / sEBV, where m = 100, k = 12, mEBV is the mean breeding value of females born between 2023 and 2028, and sEBV is the standard deviation of the bulls breeding values born between April 2011 and March 2016. Each scenario was run in ten replicates, and the mean genetic progress was

calculated across the ten replicates for each year of selection. The different scenarios were compared based on the achieved genetic progress after then years of future breeding.

Results & Discussion

We present the achieved genetic progress in the final year of future breeding separately for the index trait and the expensive trait in each simulated scenario, as shown in Table 1. We will begin by analysing the effect of increasing the number of phenotypes. Next, we will examine the impact of assigning a higher economic weight to the expensive trait. Following this, we will explore the effect of varying the correlation between the index trait and the expensive trait. Lastly, we will compare the changes when two or three parameters are simultaneously adjusted

Table 1. Genetic progress (ΔG) in ten years of future breeding for index trait (IT) and expensive trait (ET) with different number of phenotypes (N) for ET, different economic weights (EW) for ET and IT in the future selection index and different genetic correlations between IT and ET (r_g)

N for ET	EW for ET*	r_g	ΔG for IT	ΔG for ET
1000	0.2	0	64.1	2.7
2000	0.2	0	63.9	3.7
3000	0.2	0	63.4	6.2
1000	0.5	0	58.7	11.6
2000	0.5	0	57.2	15.1
3000	0.5	0	56.0	17.0
1000	0.2	0.3	64.4	20.9
1000	0.2	0.6	64.2	38.6
1000	0.5	0.3	61.9	26.4
1000	0.5	0.6	63.3	40.7
2000	0.2	0.3	64.5	21.9
2000	0.2	0.6	64.4	39.5
2000	0.5	0.3	60.8	28.8
2000	0.5	0.6	62.6	42.6
3000	0.2	0.3	64.3	22.1
3000	0.2	0.6	64.4	39.9
3000	0.5	0.3	59.8	30.3
3000	0.5	0.6	62.1	43.4

*EW for IT is: 1 – EW for ET

Effect of increasing phenotype numbers

Collecting a higher number of phenotypes for the expensive trait slightly decreased the genetic gain for the index trait while increasing the gain for the expensive trait. When 1000 phenotypes were collected each year during the future breeding period, with the economic weight for the expensive trait set at 0.2 and no correlation between the traits, the genetic progress achieved was 64.1 for the index trait and 2.7 for the expensive trait. As the number of phenotypes increased to 2000 per year, the genetic progress for the index trait dropped slightly to 63.9, while the gain for the expensive trait rose to 3.7. With 3000 phenotypes per year, the genetic progress further declined to 63.4 for the index trait, but it increased to 6.2 for the expensive trait. This indicates that doubling the number of phenotypes for the expensive trait does not result in a proportional increase in its genetic progress.

Impact of economic weight

An increase in the economic weight of the expensive trait in the future selection index reduced the genetic gain of the index trait but enhanced the gain for the expensive trait. Specifically, when the economic weight of the expensive trait was raised from 0.2 to 0.5 in the future selection index, the genetic progress over ten years of future breeding fell from 64.1 to 58.7 for the index trait, while it rose significantly from 2.7 to 11.6 for the expensive trait. This indicates that increasing the economic weight by two and a half times leads to more than a fourfold increase in the genetic progress of the expensive trait, while the genetic progress for the index trait decreases by only 8.4%.

Effect of genetic correlation

A higher positive genetic correlation between the index trait and the expensive trait had no significant effect on the genetic improvement of the index trait, but it strongly enhanced the genetic progress of the expensive trait. When the genetic correlation between the two traits increased from 0 to 0.3 and then to 0.6, the genetic progress of the index trait remained

relatively stable, at 64.1, 64.4, and 64.2. In contrast, the genetic progress for the expensive trait saw substantial increases: from 2.7 with no correlation to 20.9 at a correlation of 0.3, and further rising to 38.6 when the correlation was 0.6.

Combined parameter adjustments

Increasing the number of phenotypes for the expensive trait, raising its economic weight, and having a higher genetic correlation between the index and expensive traits positively impacted the realized genetic gain of the expensive trait. The highest genetic gain of 43.4 for the expensive trait occurred in the scenario where 3000 phenotypes were collected, the economic weight was 0.5, and the genetic correlation with the index trait was 0.6. This gain is more than sixteen times greater than the baseline scenario, where only 1000 phenotypes were collected per year, the economic weight was 0.2, and there was no genetic correlation between the traits.

Among the three parameters analysed, the genetic correlation between the index trait and the expensive trait had the greatest impact on the genetic gain of the expensive trait. Therefore, identifying a phenotype with lower recording costs but a strong genetic correlation to the expensive trait could be a viable strategy for future trait improvement. Since building a reference population for the expensive trait requires many years, continuing to collect precise phenotypes is crucial. This allows for the accurate estimation of genetic correlations between traits with costly phenotyping and those with cheaper phenotyping. Thus, even on a smaller scale, ongoing phenotyping for the expensive traits is justified.

Conclusions

Our findings underscore the critical role of genetic correlation in enhancing genetic progress for traits with expensive phenotypes, especially when phenotyping is limited. Increasing the economic weight assigned to these traits in the selection index, along with the number of phenotypes collected, significantly boosts genetic gains. This suggests that traits with lower phenotyping costs but strong genetic correlations to expensive traits should be prioritized in breeding programs to achieve indirect genetic improvements in costly traits.

Moreover, the study highlights the necessity of obtaining precise phenotypes to accurately estimate genetic correlations between expensive and inexpensive traits. This precision is essential for developing cost-effective strategies in future breeding efforts aimed at enhancing traits with prohibitive phenotyping costs.

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Genomic Prediction of Genetic Residual Feed Intake Integrating a Novel Energy Sink for Change in Body Reserves.

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Abstract

Traditionally, a two-step modeling approach of residual feed intake (**RFI**) is incorporated into the Feed Saved index at dairy cattle genetic evaluation centers. Challenges have been identified in the 1st step on handling fixed effects in the statistical model and dealing with missing phenotypes. This could be solved using a multi-variate modelling approach for genetic RFI (**gRFI**). Most existing RFI models use changes in body weight, and therefore, likely inadequately account for changes in body reserves because energy density differs between mobilization and deposition, and between adipose and muscle tissue. Alternatively, energy balance can be estimated from body reserve changes ($\mathbf{EB_{body}}$). Therefore, this study aimed to explore a genomic evaluation of gRFI in Nordic primiparous cows using EB_{body} as energy sink for changes in body reserves. Weekly records were collected from 2,029 Jersey (**JER**) cows, 3,178 Red Dairy Cattle (**RDC**) cows, and 4,661 Holstein (**HOL**) cows. For JER and RDC, the feed intake data was obtained with the Cattle Feed InTake system (**CFIT**, VikingGenetics, Denmark). For HOL, feed intake data was collected from CFIT farms and a research farm (857 cows and 25,547 weekly records). The genotyping rate for cows with data were 92% for JER and RDC, and 81% for HOL. The gRFI model was a random regression multi-variate model with 2nd order Legendre polynomials for additive genetic and permanent environmental effects. The gRFI model was validated with an across-herd cross-validation scheme using the Legarra Reverter method and reporting bias, dispersion and correlation terms. Breeding values were predicted using the single-step approach for both genotyped and non-genotyped animals. The bias was close to 0 for all breeds. The dispersion coefficients were found in an acceptable range at 0.92 (DMI) and 0.87 (gRFI) for HOL and 0.96 (DMI) and 0.85(gRFI) for RDC, while overdispersion was observed for JER (DMI:0.75, gRFI:0.69). Correlations between genomic breeding values, estimated with whole and partial phenotypic information, were moderately high for all breeds (DMI: 0.51-0.68, gRFI: 0.46-0.59). In conclusion, it was possible to construct a genomic gRFI model for all three Nordic dairy cattle breeds and integrate EB_{body} as an energy sink indicator. We observed promising validation metrics for HOL and RDC, but JER models need further refinement. The results demonstrate selection for gRFI is expected to provide genetic gain of feed efficiency in dairy cattle.

Key words: feed efficiency, Feed Saved, multi-variate modelling, Nordic dairy cattle

Introduction

Improving feed efficiency through genetics poses an important part of enhancing economic viability and environmental sustainability in dairy cattle farming (VandeHaar et al., 2016). Several genetic evaluation centers have integrated the "Feed Saved" index, as selection criteria for feed efficiency in the national breeding goals. A significant component of this index lies in the residual feed intake (**RFI**) part, which traditionally is modelled in a two-step

process (Tempelman and Lu, 2020). Initially, a precorrection step generates a model-based residual for feed intake, serving as the phenotype for subsequent genetic evaluation. However, challenges arise concerning the handling of fixed effects and missing records within this initial step. To address these challenges, Tempelman and Lu (2020) proposed the genetic multi-variate approach to RFI (**gRFI**) based on the work by Kennedy et al. (1993). This model has not been tested within Nordic breeds.

Most existing RFI models address body reserve management using changes in body weight (**ΔBW**). However, this approach likely suffers deficiency because of significant variations in energy density between mobilization and deposition, as well as among different tissue types (adipose and muscle). An alternative is outlined by Thorup et al. (2018), who proposed to estimate energy balance from changes in body reserves (**EB_{body}**) by employing energy-specific coefficients tailored to tissue types and energy status. However, the effect of EB_{body} has yet to be investigated for RFI models.

Genomic prediction offers implementation of traits that have relatively few records due to expensive recording schemes (e.g. feed efficiency). Studies have demonstrated the feasibility of genomic prediction for dry matter intake (Berry et al., 2014, De Haas et al., 2015). As a limited number of records are available, the traditional forward prediction outlined in Mäntysaari et al. (2010) were not feasible for validation of genomic predictions. Alternatively, the Legarra-Reverter cross-validation method (Legarra and Reverter, 2018), using whole and partial datasets seems attractive. However, limited literature exists on genomic validation of gRFI.

This study aimed to explore the ability to establish a genomic evaluation of gRFI and perform herd cross-validation, using Nordic primiparous cows and incorporating the EB_{body} as energy sink trait for body reserve management.

Materials and Methods

The modelling of the multi-variate gRFI model is based on weekly means of dry matter intake (**DMI**), energy corrected milk (**ECM**), and body weight (**BW**) records for each individual cow. The phenotyping systems were the Cattle Feed InTake (**CFIT**) system installed on 19 commercial Danish farms and research data from the Danish Cattle Research Center (**DCRC**) at AU-Foulum. A detailed description of the 3D camera based CFIT system is outlined in Lassen et al. (2023) and for DCRC in Li et al. (2017) and Stephansen et al. (2023).

Feed intake data

The data compromised repeated records from one to 45 weeks in milk of 3,873 HOL cows with 161K weekly CFIT DMI records (2,564 primiparous), 2,068 JER cows with 93K weekly CFIT DMI records (1,505 primiparous), 3,235 RDC cows with 139K weekly CFIT DMI records (2,006 primiparous) and 878 HOL cows with 50K weekly DCRC DMI records from the Roughage Intake Control System (Insentec B.V., Marknesse, the Netherlands) (835 primiparous). A detailed description of the data and quality control can be found in Stephansen et al. (2024).

Energy balance from body reserves

We adapted the estimation method of EB_{body}, using frequent BW measurements from Thorup et al. (2013) as:

$$EB_{body}, MJ/day = z \times \Delta BL + y \times \Delta BP_{std}$$

where EB_{body} is the energy balance phenotype calculated from frequent BW measurements and expressed in changes of mega joule per day, z is the energy coefficient for lipid, being 39.6 MJ/kg mobilized and 56 MJ/kg deposited adipose tissue, y is the energy coefficient for protein, being 13.5 MJ/kg mobilized and 50 MJ/kg deposited muscle tissue, ΔBL is the change in body lipid and ΔBP_{std} is the predicted change in body protein outlined in Thorup et al.

(2013). Details on the modelling of EB_{body} can be found in Thorup et al. (2018) and in context of this data in Stephansen et al. (2024).

Pedigree and Genotypes

Breed-specific pedigrees used from the Danish cattle database and underwent a pruning process using the DMU trace software (Madsen, 2012) for cows with data. The pruned pedigrees consisted of 18,432 HOL animals, 7,294 JER animals, and 12,423 RDC animals. Phantom parent groups were assigned to animals with missing parents using combinations of sex (Male or Female), breed (breed in analysis or other breeds), country (HOL: Nordic, EU, North America & rest; JER+RDC: Nordic & rest), and birth year classes (HOL: <2000, 2000-2010, & >2010; JER+RDC: before and after 2000).

Imputed genotypes were provided by Nordic Cattle Genetic Evaluation (Skejby, Denmark). Most animals were genotyped with 50k Illumina Bovine SNP50 or imputed from the LD chip panels. The imputation was done by SEGES Innovation (Skejby, Denmark) and part of the routine genetic evaluations in Nordic Genetic Cattle Evaluation (Skejby, Denmark). For Holstein 46,342 single nucleotide polymorphisms (**SNP**) were available, which were 41,897 SNPs for Jersey and 46,914 SNPs for RDC. Genotypes from animals born before 2000 were omitted because genotypic information on distantly related animals contribute little to accuracy of prediction in focal animals, and because including genomic information across multiple generations can promote prediction bias.

To calculate the relationship matrix encompassing genotyped and non-genotyped cows for a ssGBLUP analysis, we calculated the inverse of **H** as (Aguilar et al., 2010, Christensen and Lund, 2010):

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & (\omega\mathbf{G} + (1-\omega)\mathbf{A}_{22})^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix},$$

where $\mathbf{A}^{-1}$ is the inverse of the pedigree relationship matrix, **G** the genomic relationship matrix, ω is the relative weight of the polygenetic effect (ω=0.8), $\mathbf{A}_{22}$ is the part of the pedigree relationship matrix with genotyped animals, and $\mathbf{A}_{22}^{-1}$ is the inverse of $\mathbf{A}_{22}$. The genomic relationship matrix was calculated according to VanRaden (2008) using method 1 and the *invgmatrix* software by Su and Madsen (2011).

Statistical model

Variance components for the multi-variate model were estimated using a Gibbs sampler in the RJMC module in DMU version 5.5 Madsen and Jensen (2013). For variance component estimation, the pedigree-based relationship matrix was used for following multi-variate model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Mh} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{e},$$

where **y** is the vector of phenotypes with sub-vectors for DMI, ECM, BW and EB_{body} in the different weeks of lactation; **b** is the fixed effects year x experimental diet at DCRC or version of CFIT system, a fourth order Legendre polynomial fixed regression on weeks in milk and nested within herd, and a second order Legendre polynomial fixed regression on age at calving; **h** is the vector of random effects for herd × year × test-week (record date); **a** is the vector of random regressions for random additive genetic effect of cows with sub-vectors for each of the traits; **pe** is the vector of random regressions for random permanent environmental effects of cows with sub-vectors for each of the traits. Weekly means were modelled across traits from one to 45 weeks in milk by a second Legendre polynomials (intercept, linear, quadratic) for both **a** and **pe**; **e** is the vector of random residual effects with sub-vectors of all traits included in the analysis. **X** is the design matrix for fixed effects, **M** is the design matrix for herd × year × test-week random effects and **Z** and **W** are the design matrices with covariable matrices containing

Legendre polynomial coefficients corresponding to week of lactation. Details on post-model processing of variance components to derive heritability, additive genetic correlations, and genetic regressors can be found in Stephansen et al. (2024).

Genomic herd cross-validation

We aimed to perform genomic validation by herd. Thereby, we assessed the expected value of genomic breeding values (**GEBV**) in herds which do not have the CFIT system. The estimated variance components of the multi-variate model and $\mathbf{H^{-1}}$ were applied to ssGBLUP models to estimate GEBVs using the DMU5 module with the preconditioned conjugate gradient computation method.

To set up the different datasets for the herd cross-validation, we first formed a whole dataset containing all phenotypic information, that was used to estimate GEBVs ($\mathbf{GEBV_{whole}}$). Hereafter, we formed three partial datasets for HOL and two for JER and RDC. In each of the partial datasets, we omitted all phenotypic information for 1-3 herds, and a herd could only appear as validation herd in one partial dataset. Assigning herds to be validation herds in the different partial datasets were done such that a group of validation herds consisted of herds that were geographically close and approximately 1,000 validation cows. These partial datasets (7 in total across breeds) were used to predict GEBVs ($\mathbf{GEBV_{partial}}$). A few of the CFIT herds were not used as validation herds and the DCRC herd were always included in the training population for HOL to avoid backward predictions in time. Using only validation animals, we created following linear model to assess herd cross-validation metrics according to Legarra and Reverter (2018):

$$GEBV_{whole} = \mu_{w,p} + ß_{w,p} \times GEBV_{partial} + \varepsilon$$

where $GEBV_{whole}$ was the GEBVs of validation animals with full phenotypic information, $\mu_{w,p}$ was the intercept (bias term), $ß_{w,p}$ was the slope (dispersion term), $GEBV_{partial}$ was the GEBVs of validation animals with no phenotypic information and ε was the residual. From the linear model we also reported the correlation ($\boldsymbol{\rho_{w,p}}$) for the lactation-sum GEBVs of DMI and gRFI. Detailed information on how lactation-sum results were calculated can be found in Stephansen et al. (2024).

Results & Discussion

Figure 1 presents the average phenotypic level of EB_{body} through first lactation. For all breeds, the cows undergo a period of negative energy balance in early lactation, which becomes positive between 5-10 weeks in milk. These phenotypic results of EB_{body} in terms of level and pattern throughout first lactation are in line with the findings in Holstein and Jersey with experimental data (Thorup et al., 2018).

Variance component from the tested gRFI model can be found in Stephansen et al. (2024). Genomic validation results, using the Legarra-Reverter method, are presented in Table 1. To the authors' best knowledge, no studies have conducted by-herd cross-validation of GEBVs for feed efficiency traits in dairy cattle. We observed limited bias for DMI and gRFI in all breeds, comparing $\mu_{w,p}$ to the lactation-sum additive variance level. Acceptable $ß_{w,p}$ values was found for HOL and RDC, but overdispersion was observed in JER. Further research is needed for JER on the observed overdispersion, when more data is collected. Moderately high $\rho_{w,p}$ were found across breeds and highest for DMI (0.51-0.68) compared to gRFI (0.46-0.59). The pattern across breeds shows the highest cross-validation correlations were obtained for HOL, the breed with most cows, while lowest for JER, the breed with

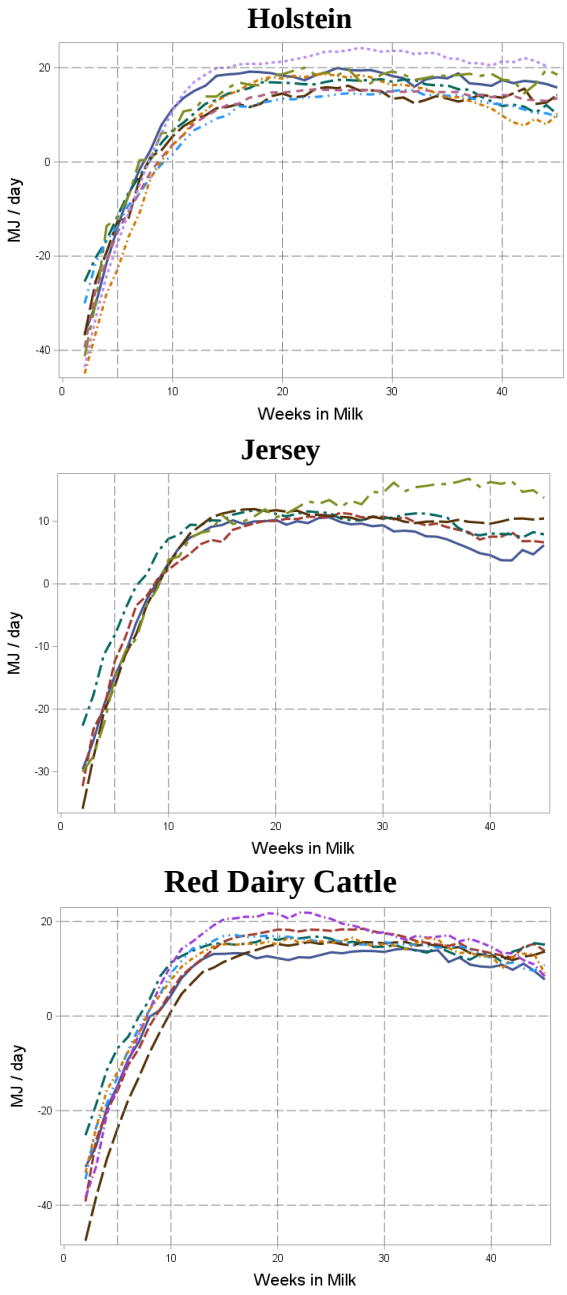

Figure 1. Lactation curves of energy balance calculated from changes in body reserves as MJ/day. Color and line pattern represents different herds.

smallest number of cows. The results suggest that genomic predictions of gRFI in herds with no phenotypic information can provide reliable GEBVs that can be used to generate genetic gain for feed efficiency.

This study aimed to validate the effect of GEBVs for gRFI in herds with no phenotypic information. However, it could be emphasized that not all phenotypic information would be missing, such as phenotypic information on milk production from a test-day recording scheme and BW records from some herds with milking robots or other measuring techniques (Lidauer et al., 2019). Future research should aim to investigate the effect of not having all phenotypic information missing in a herd cross-validation study, but as well validate the effect of missing information at different life stages, such as very young animals before first mating and only phenotypic information in very early lactation used for extension of the lactation. This can potentially be valuable information for management and breeding decisions on dairy farms, but as well for the breeding companies.

Table 1: Results from Genomic Legarra-Reverter validation using a herd cross-validation scheme for primiparous Nordic breeds. HOL = Holstein, JER = Jersey, RDC = Red Dairy Cattle, DMI = Dry Matter Intake, gRFI = genetic Residual Feed Intake, $\mu_{w,p}$ = intercept (bias term), $ß_{w,p}$ = slope of regression (dispersion term), $\rho_{w,p}$ = correlation between genomic breeding values with whole and partial phenotypic information for validation animals.

		Estimates		
Trait		HOL	JER	RDC
DMI	$\mu_{w,p}$	-0.36	3.64	-1.15
	$ß_{w,p}$	0.92	0.75	0.96
	$\rho_{w,p}$	0.68	0.51	0.66
gRFI	$\mu_{w,p}$	0.34	1.69	-2.00
	$ß_{w,p}$	0.87	0.69	0.85
	$\rho_{w,p}$	0.59	0.46	0.54

Conclusions

We aimed to evaluate gRFI genomically and test the feasibility of incorporating a novel energy sink trait for changes in body energy for Nordic primiparous cows, using data from the 3D camera-based system CFIT and the DCRC research herd. The genomic validation results show limited bias, and acceptable dispersion of predicted breeding values for HOL and RDC. However, overdispersion of predicted breeding values was observed in JER. Correlations between GEBVs from whole and partial datasets of validation cows, shows moderately high (0.46-0.59). These results show that selecting for gRFI GEBVs are expected to

provide genetic gain of feed efficiency in other dairy herds.

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Estimation of variance components for clinical mastitis and somatic cell scores for the Nordic dairy cattle populations

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Abstract

Clinical mastitis (CM) is a disease which causes great losses to the dairy industry. Due to the low incidence of CM and its discrete nature, somatic cell scores (SCS), which are measured on a regular basis, are often included in genetic evaluation. As such, determining the genetic architecture of udder health traits at different risk stages is important. Thus, the objectives of this study were to estimate variance components for (i) CM events at two risk stages (early and late lactation) and at three lactations, and for (ii) SCS at three lactations. Data consisted of CM and SCS records for Holstein (HOL), Jersey (JER), and Red dairy cattle (RDC) cows. For CM, each risk period of each lactation was considered as a correlated trait, and for SCS each lactation was considered as a separate trait modelled using random regression. The genetic component was modelled using sire information (sire model). Variance components were estimated using Monte Carlo expectation-maximization residual maximum likelihood. Mean CM incidence ranged from 3.5% to 13.2% for HOL, from 5.6% to 13.6% for JER, and from 3.10% to 10.9% for the RDC breed. Combined heritability of CM was 5.4%, 6.2%, and 6.3% for HOL, JER, and the RDC breed, respectively. Heritability estimates for individual lactations and periods ranged from 0.73% to 3.48% for HOL, 1.11% to 2.82% for JER, and 1.62% to 3.39% for RDC. In addition, genetic correlations among CM traits ranged from 0.28 to 0.95, from 0.32 to 0.98, and from 0.44 to 0.93 for the HOL, JER, and RDC breeds, respectively. On the other hand, the combined heritability of SCS of 305 days in milk and for 1st, 2nd, and 3rd lactations were 0.14, 0.17, and 0.19 for the HOL breed, 0.17, 0.18, and 0.16 for the JER breed, and 0.18, 0.19, and 0.20 for the RDC breed. Genetic correlations for SCS among lactations were high (> 0.80) for all breeds. Furthermore, genetic correlations between CM and SCS traits ranged from 0.41 to 0.78 for HOL, from 0.29 to 0.69 for JER, and from 0.27 to 0.66 for RDC. Overall, the heritability estimates for traits related to udder health, including CM and SCS was low or moderate for all the breeds considered. On the other hand, genetic correlations among CM traits, and among SCS traits were moderately high to high.

Key words: Udder health, heritability, genetic correlations

Introduction

Clinical mastitis (**CM**) is a costly disease affecting dairy cattle, causing not only direct losses due to a reduced production and early culling of affected cows, but also due to changes in management necessary for the treatment of affected cows (Rollin et al., 2005). The risk of clinical mastitis is not constant throughout the life of an individual, but instead is greater at the beginning of the lactation and increases for latter lactations, as compared to the first lactation (Valde et al., 2004). As such, trait definition should properly reflect these risks.

Due to the binary nature of CM, variance component estimation may be challenging. In

addition, depending on management and environmental conditions, the incidence of clinical mastitis may be low such that many records may be necessary for estimation of variance components. On the other hand, somatic cell scores (**SCS**) are regularly recorded and may provide additional information for estimation of variance components related to udder health (Nash et al., 2000).

Due to the interest in efficiently using the joint reference population, EuroGenomic member countries have agreed to harmonize traits and adopt the "gold standard" definition of CM traits which identifies early (up to 60d) and late (> 60d) risk periods for each lactation. Thus, the objective of the current work was to estimate variance components, including heritability and genetic correlations for (i) early and late risk periods for CM at three lactations, and for (ii) SCS at three lactations for the Holstein (**HOL**), Jersey (**JER**), and Red dairy cattle (**RDC**) breeds.

Materials and Methods

Data

Data for the HOL and RDC breeds were sampled from herds in Sweden, while for the JER breed were sampled from herds in Denmark. For all breeds phenotypic records were considered starting in 2010, and herds with a minimum of 20 and a maximum of 100 first year calves were included in the analyses.

Records corresponding to 68,422, 64,194, and 71596 HOL, JER, and RDC cows, respectively, were included. These cows were sired by 2159, 986, and 1258 bulls for the HOL, JER, and RDC, respectively. Mean CM incidence ranged from 3.50% to 13.20% in HOL, from 5.65% to 13.58% in JER, and from 3.11% to 10.86% in RDC. The largest incidence of CM was observed at the late period of the third lactation. As part of the EuroGenomic trait harmonization strategy, CM records were transformed into Snell scores (Snell, 1964). Mean SCS (in logarithmic scale) was 4.04, 4.40, and 4.70 for HOL, 4.19, 4.32 and 4.49 for JER, and 4.07, 4.41 and 4.71 for RDC.

Model

For CM, six traits were included consisting of the two risk periods defined earlier for each of three lactations. In addition, one SCS trait was defined for each lactation and analyzed with a multi-trait random regression model. All nine traits were analyzed using a multi-trait linear mixed model. For CM, fixed effects included herd-year and age while for SCS a fixed lactation curve was also included. Regression effects for both CM and SCS traits included heterosis, recombination loss, and inbreeding.

Random effects for both CM and SCS traits included a sire and a permanent environmental effect, both of which were modeled using random regression. For CM, only an intercept was fitted, while SCS was modeled using a quadratic Legendre polynomial (intercept, linear, and quadratic) plus an exponential (Wilmink) term. For the sire effect, pedigree information was pruned to four generations.

Variance components corresponding to the nine traits defined earlier were estimated using the Monte Carlo Expectation Maximization REML algorithm in MiX99 (Vuori et al., 2006).

Results & Discussion

Overall, estimates of heritability were low (Table 1), ranging from 0.74% to 3.48% for the early and late period of the first and third lactation, respectively, in the HOL breed. Heritability in the JER breed was slightly lower overall and remained consistent across periods and lactations ranging from 1.11% to 2.82%, while the RDC was similar to the HOL ranging from 1.62% to 3.39%. These estimates resemble those reported by Negussie et al. (2011) for first lactation in Finnish Ayrshire.

Table 1. Heritability (%) for CM traits at three lactations and two risk periods (early, late) in the HOL, JER, and RDC breeds.

Breed	Lactation	Early	Late
HOL	1	0.74	1.24
	2	1.38	2.08
	3	1.43	3.48
JER	1	1.11	1.22
	2	1.38	2.32
	3	1.51	2.82
RDC	1	1.62	1.13
	2	1.29	2.61
	3	1.61	3.39

Genetic correlations among CM traits ranged from 0.35 to 0.95 for the HOL, 0.33 to 0.98 for the JER, and from 0.47 to 0.91 for the RDC. Genetic correlations were, in general, larger for subsequent risk periods. On the other hand, phenotypic correlations were low for all breeds.

Heritability for SCS traits, as a function of days in milk, ranged from 0.05 to 0.12 for the HOL, from 0.04 to 0.13 for the JER, and from 0.07 to 0.14 for the RDC (Figure 1). Overall, the largest estimates of heritability were found in the third lactation. Genetic correlations among

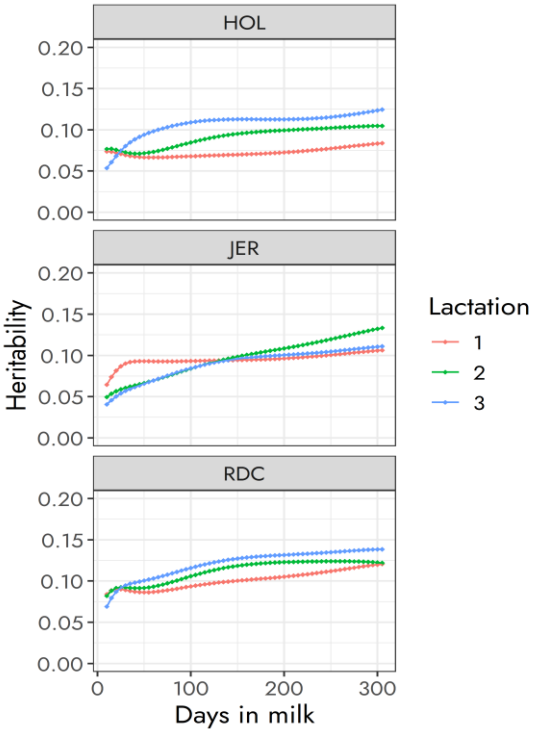

Figure 1. Heritability of SCS as a function of days in milk for HOL, JER, and RDC at three lactations.

daily SCS were high within a lactation, ranging from 0.79 to 0.99, and decreased slightly across lactations ranging from 0.59 to 0.84.

In general, combined CM heritability was about 6% for all three breeds, while the 305-d SCS heritability ranged from 14% to 21% for all breeds (Table 2). Genetic correlations among 305-d SCS traits were high and ranged from 0.84 to 0.98. On the other hand, phenotypic correlations for those traits were smaller, ranging from 0.18 to 0.51.

Genetic correlations between combined CM and 305-d SCS were moderate to large and increased with each lactation (Table 2). For the HOL these ranged from 0.61 to 0.74, for the JER they ranged from 0.43 to 0.63, and for the RDC they ranged from 0.47 to 0.63. Due to the magnitude of these genetic correlations, information from SCS can be useful for the estimation of CM traits.

Table 2. Heritability (diagonal), genetic correlations (upper triangle), and phenotypic correlations (upper triangle) for combined CM and 305-d SCS for HOL, JER and RDC breeds.

Breed	Trait[1]	CM	SCS1	SCS2	SCS3
HOL	CM	0.058	0.147	0.191	0.208
	SCS1	0.614	0.146	0.404	0.321
	SCS2	0.733	0.861	0.172	0.478
	SCS3	0.736	0.836	0.982	0.198
JER	CM	0.061	0.108	0.182	0.227
	SCS1	0.433	0.179	0.408	0.328
	SCS2	0.486	0.911	0.188	0.488
	SCS3	0.627	0.851	0.952	0.169
RDC	CM	0.064	0.128	0.175	0.191
	SCS1	0.474	0.182	0.443	0.336
	SCS2	0.564	0.913	0.201	0.506
	SCS3	0.632	0.845	0.960	0.215

1. Combined heritability for CM, and 305-d heritability for SCS at each lactation.

Conclusions

Overall, heritability for CM traits was low for all breeds but increased for the late period of each lactation and was larger for the third lactation. On the other hand, heritability for SCS traits was larger. Because of this, and due to the moderate to large genetic correlations

between SCS and CM traits, it would be beneficial to include both sets of traits to aid in the genetic evaluation of udder health traits in the Nordic evaluation.

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Genetic Correlations Between Daily Dry Matter Intake, Body Weight, and Enteric Methane in Norwegian Red

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Abstract

Selecting for reduced enteric methane emissions and improved feed efficiency in cattle is of interest. These are important traits both considering climate and utilization of feed resources on farm. Before genetic selection on new traits can be implemented, a base population with phenotypes on the desired trait(s) must be established. This study collected and analyzed data on feed intake, methane emissions, and body weight on Norwegian Red dairy cows in commercial dairy farms in Norway. Our goal was to estimate genetic parameters and breeding values for the three traits, and to estimate genetic correlations amongst them. We also calculated correlations to current breeding indices in Norwegian Red dairy cows. The relevant traits were daily dry matter intake (dDMI), average daily body weight (dBW), and average daily methane emissions (dCH4). There were 452 055 daily records on 2 074 cows in the dataset. Bivariate linear animal repeatability models were used. The model included fixed effects of parity week, age at calving, and herd in addition to permanent environmental effects of herd testday, additive genetic effects and residual as random effects. The pedigree was traced 8 generations back and contained 18 697 animals. The estimated heritabilities were 0.29, 0.39, and 0.57 for dDMI, dCH4, and dBW, respectively. Standard errors were low (0.04 to 0.05). Genetic correlations among all three traits were significant and strong, and ranged from 0.50 to 0.65. The strongest correlation (standard error) was found between dDMI and dCH4 of 0.65 (0.10). Positive and relatively strong genetic correlations imply that selection for lower level of one trait also will reduce the level of the other two traits. The correlation between the breeding values of the three novel traits to current indices from routine genetic evaluations of Norwegian Red ranged from (-0.26 to 0.16), and the fertility index had strongest favorable correlation to the three traits with -0.19, -0.22, and -0.26 for dBW, dCH4, and dDMI, respectively. This indicate that higher feed intake, larger cows and more methane emissions are associated with lower genetic merit for fertility. Further research is needed to investigate the consequences of selecting for reduced methane emissions or reduced body weight and how this will affect cows' ability to utilize grass. For Norwegian Red it remains to define the feed efficiency trait, but we started to analyze traits that are key ingredients of a future feed efficiency trait.

Key words: Novel traits, phenotype on farm, greenfeed, body size, index correlations

Introduction

Improving feed efficiency and at the same time reduce the methane produced by Norwegian dairy cows have gained increased focus in recent years. Norway is committed to reduce greenhouse gas emissions from agriculture by 55 % of 1990 levels before 2030 according to the agreement made between government and the farmers organization. Selection and breeding for climate friendly cows are suggested as one of the most important solutions to achieve the reduction goal in the climate agreement.

Norwegian Red (NR) dairy cattle has a broad breeding goal, where beef traits are part of the breeding goal and weighted in the total

merit index (www.norwegianred.com). Before we can implement new traits in the breeding goal we need knowledge on how feed efficiency and enteric methane can be improved and how this will affect current traits in the breeding program. Direct measurements of feed intake and methane phenotypes are costly, despite this collecting data on the actual traits are necessary as the cost of implementing new traits without considering the effect on current breeding goal can be more expensive in the long run. Hence, Geno have established a project collecting data both on daily feed intake, methane, and body weight for cows in 14 commercial herds, ongoing since 2021.

Some breeding organizations have recently published breeding values for feed efficiency traits based on feed intake records from commercial herds (CRV 2023, Manzanilla-Pech et al. 2023, Viking genetics 2021), while others publish breeding values based on phenotypes measured at research farms (Jamrozik et al., 2021). Heritability of feed intake and methane traits in Nordic Red dairy breeds range from 0.18 to 0.20 for feed intake (Bakke and Heringstad 2023, Manzanilla-Pech et al. 2023) and from 0.22 to 0.44 for enteric methane (Chipondro 2024, Wethal et al. 2022), respectively. Methane and feed efficiency are reported to be genetically correlated with estimates ranging from 0.05 to 0.76 (López-Paredes et al. 2021, Manzanilla-Pech et al. 2022). In the study by Manzanilla-Pech et al. (2022) they reported favorable and positive genetic correlations between two definitions of feed efficiency with methane intensity and methane production in Holstein cows. This suggests that genetic selection for both improved feed efficiency and reduced enteric methane at the same time, is feasible.

A relatively strong genetic relationship between methane production and body weight of 0.65 was reported for Holstein cows across-country (Manzanilla-Pech et al. 2021), and a genetic correlation of 0.69 was estimated for Nordic Red (Manzanilla-Pech et al. 2023), which imply more methane are produced with increasing cow size.

Body weight is accounted for in some definitions of feed efficiency. In RFI, a popular way to define feed efficiency in dairy cattle, energy sinks used for production, maintenance of body weight and loss or gain in body weight are usually accounted for (Stephansen et al., 2021). Hence, collection of longitudinal data on cows' body weight are important for calculating individual feed efficiency. Genetic correlations between body weight and methane are scarcely investigated, and previous studies have reported strength and direction of the correlations ranging from non-significant, negative, to a strong positive correlation (Breider et al. 2019, Lassen and Løvendahl 2016, Manzanilla-Pech et al. 2021). This suggests that more research are needed on how cows body weight might affect the other traits.

Limited research on genetic associations between methane, body weight and feed intake in Red dairy breeds is published. Therefore, the current study aimed to analyze these traits genetically based on data from commercial herds with NR and examine how they correlate with some of the traits in the current breeding goal of NR dairy cattle.

Materials and Methods

Data

Data was collected by Geno's feed efficiency project and included records from fourteen Norwegian dairy herds with equipment from BioControl for individual feed intake recording, as well as weight scales from BioControl. In addition, were methane data from 25 herds with GreenFeed included in the study. We had records from GreenFeed from more herds because these units were moved during the period of data collection. Feed samples of silage was collected weekly and feed analyses gave information for calculation of daily dry matter content of the feed consumed. The dataset had data from 2020 to 2024 and a total of 452 055 observations. The number of cows measured for one or more of the traits methane, feed intake, or dry matter intake was 2 074.

Edits of data

Before genetic analysis of the novel traits was performed, data was checked and edited for logical values. Average body weight for six parity and lactation stages was calculated, and observations within 3 standard deviations of the mean was considered a logical body weight record for the cow and used in further analyses. All records out of this range were excluded from the genetic analysis. Records from 6 to 350 days in milk were included. Cows had to have a minimum of eight days with feed intake data and information on both silage and concentrates intake in order to be included in the genetic analysis. Additional information on birth info and calving data was collected from the national herd recording system and pedigree information used to construct relationship matrix. The pedigree was traced eight generations back, and cows with a known NR A.I. sire was included in the analysis. Lastly, breeding values on current established traits from Genos database for the routinely breeding value estimation was collected.

Traits

Feed intake was defined as daily dry matter intake (dDMI) from both grass (silage) and concentrate. Methane production was measured as gram per day for each visit in GreenFeed. The phenotype for methane was calculated as daily average methane emission (dCH4) for each cow. Body weight was collected from each visit on the scale and the final phenotype included for further analysis was the daily average body weight (dBW). Descriptive statistics for the traits are given in Table 1. Daily dry matter intake (dDMI) ranged from 7 to 35.9 Kg, Methane (dCH4) from 100 to 799 gram per day and daily body weight (dBW) from 400 to 850 kg. The number of records for each trait combination were 3 162 for dDMI and dCH4, 40 284 for dDMI and dBW, and 49 290 for dCH4 and dBW (Table 1).

Table 1. Descriptive statistics of daily dry matter intake (dDMI), methane produced (dCH4), and body weight (dBW) after editing the data.

Trait			
	dDMI	dCH4	dBW
Cows, n	557	1 370	1 011
Mean	20.4	418.4	607
(SD)	(4.4)	(104)	(77)
Maximum	35.9	799	850
Minimum	7.0	100	400
N obs. dDMI	61 321		
N obs. dCH4	3 162	220 932	
N obs. dBW	40 284	49 290	260 132

Statistical model

Bivariate mixed linear repeatability animal models were used to estimate (co)variance components and breeding values (EBV). Variance components were estimated with DMUAI (Madsen and Jensen, 2013). The following mixed model was used for all 3 traits:

$$Y = Herd + Week + Parity/CAge + htd + a + pe + e$$

where the effect of herd, lactation week, and parity and age at calving were fixed effects, while the effect of testday within herd (htd), additive animal genetic effect (a), permanent environment of animal (pe), and residual (e) were random effects.

Days in milk ranged from 6 to 350 and was grouped in 50 classes according to week after calving. Age at first calving and parity were merged in contemporary groups due to limited records in the tails of the dataset. For dDMI we grouped first parity cows in six contemporary groups according to their age in months at calving: ≤ 22, 23, 24, 25, 26, and ≥ 27, while second parity and third or later parities were in two separate groups. For dCH4 age at first calving were grouped as ≤ 21, 22, 23, 24, 25, 26, 27, and ≥ 27 while parity 2 and ≥ 3 was in a separate group. For dBW age of calving in first and second parity was in different groups, while cows in parity ≥ 3 were in one group. The pedigree was traced 8 generations back and contained 18 697 animals. A relationship matrix

(A) was constructed assuming no inbreeding between animals and without genetic groups for animals with unknown parents.

Correlations between breeding values

We calculated the spearman correlations between EBV for the novel traits in this study to other traits in the breeding goal of NR. For the cows with phenotypes on dDMI, dCH4, and dBW correlations between their estimated EBV's and indexes included in routine genetic evaluations of NR were calculated.

Results and Discussion

The phenotypic mean (standard deviation, SD) for dDMI and dBW was 20.3 (4.4) and 607 (77) kg per day, respectively. For dCH4 the mean (SD) was 418.4 (104). The phenotypic distribution for dBW (Figure 1) did not follow a perfect normal distribution. This can be explained by different mean weights for cows in first and second parity compared to older cows.

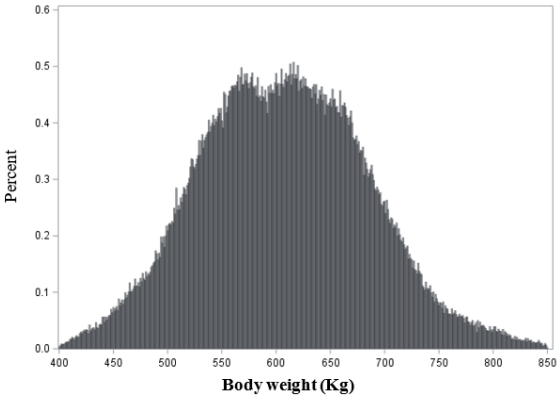

Figure 1. Phenotypic distribution of daily body weight for 1 011 Norwegian Red cows in commercial herds.

Body weight can vary a lot between two visits and high standard deviation is expected. Body weight is largely influenced by time from eating, milking, and drinking before weighing. The cows were either weighed in the concentrate feeding station, or before they entered or after they left the milking unit. Current results are the first to report longitudinal records on feed intake, body weight and methane in commercial herds with NR. The average dBW was higher than the average body weight reported to be 557 kg for NR cows in a previous study by Wallén et. al (2018). However, their study was from one research facility at the Norwegian University for Life Sciences and might not be representative for the population as a whole.

Variance components

There were significant genetic variation for all three traits, and the variance components differed from zero. Estimated variance components are given in Table 2.

Table 2. Variance components (standard errors), heritability and repeatability for daily dry matter intake (dDMI) (kg), daily methane (dCH4) (gram), and daily body weight (dBW) (kg)

Variance components	dDMI	dCH4	dBW
	Estimate (SE)	Estimate (SE)	Estimate (SE)
Herd-testday (σ^2_{htd})	4.4 (0.2)	1891.7 (28.5)	836.5 (17.2)
Additive (σ^2_a)	4.7 (0.9)	3971.9 (419.3)	3057.5 (374.6)
Permanent (σ^2_{pe})	1.5 (0.6)	522.6 (272.3)	911 (245.5)
Residual (σ^2_e)	5.6 (0.02)	3707.4 (11.5)	540.8 (1.5)
Repeatability*	0.38	0.45	0.74
Heritability**	0.29	0.39	0.57

* $r = (\sigma^2_a + \sigma^2_{pe}) / (\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e)$
** $h^2 = \sigma^2_a / (\sigma^2_a + \sigma^2_{pe} + \sigma^2_{htd} + \sigma^2_e)$

Heritability of dDMI was 0.29 (0.05) which is larger than the estimate from previously analyses of the trait in a univariate model (Bakke and Heringstad, 2023). The heritability of dry matter intake is in line with results reported in Holstein (Li et al. 2016). Heritability of daily methane production on 0.39 (Table 2) corresponds with what Wethal et al. (2022) estimated based on a subset of the data used here. Body weight had the highest heritability of 0.57, and this is comparable to heritability

estimates for body weight in Holstein from another study on using electronical weight measurements from scales (Toshniwal et al., 2008).

Our results confirms that significant genetic variation for dry matter intake, body weight, and methane production in NR dairy cattle exists. The relatively high heritabilites of dDMI, dCH4, and dBW are promising for the further work of defining feed efficiency in NR. The repeatabilities for dDMI and dCH4 was low compared to dBW, we need to find good solutions to improve repeatability for these traits. Good quality controls of data are important, and filtering and editing of data at feed bin level, testday level or cow level is important in order to improve the repeatabilities.

Genetic correlations

The genetic correlations between the traits were strong and positive, ranging from 0.50 to 0.65 (Table 3). The highest correlation was between dDMI and dCH4.

Table 3. Estimated genetic correlations between daily dry matter intake (dDMI), average daily methane (dCH4), and body weight (dBW) in Norwegian Red cows. Standard errors of correlations in parenthesis.

Genetic correlations		
Trait	dDMI	dBW
dCH4	0.65 (0.10)	0.50 (0.09)
dBW	0.59 (0.11)	

Our results for dDMI and dBW are comparable with what Manzanilla-Pech et al. (2023) reported in Nordic Red and Holstein cattle who estimated correlations from 0.58-0.65 with phenotypes from 3D-cameras. Genetic correlation between dCH4 to dBW and dDMI are a little higher compared with what Breider et al. (2018) estimated. The direction and level of the genetic correlations was logical. Body weight (adult), methane production, and dry matter intake can be reduced when selection pressure is put on one of the traits. Our results support that the level of methane production and feed intake will be reduced if selecting for lower body weight.

Fixed effects

The significance of effects included in our models were tested. For all 3 traits there was a significant effect of weeks in milk, parity and age at calving. For dDMI and dCH4 the fixed effect solutions for lactation week followed a lactation like curve, as illustrated for dDMI in figure 1, with largest effect around peak lactation.

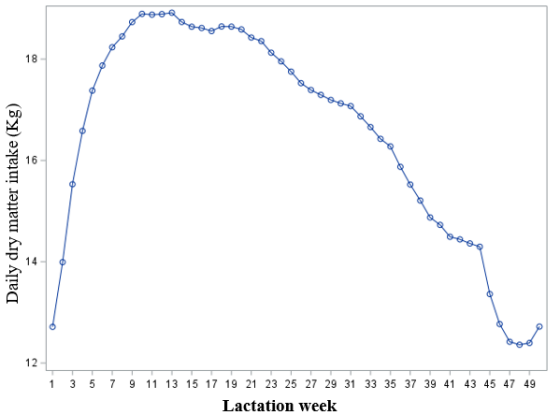

Figure 1. Best linear unbiased estimates of fixed effect of lactation week on daily dry matter intake for Norwegian Red cows.

For dBW we discovered the opposite pattern for the effect of weeks in milk (Figure 2). Here the body weight drops after calving, before increasing almost linearly throughout the lactation. Cows are losing weight after calving for biological reasons and on average 20 kg of weight loss are within the first 7 weeks of the lactation. The phenotypic change of body weight throughout the lactation showed a different curve for different parities (not shown).

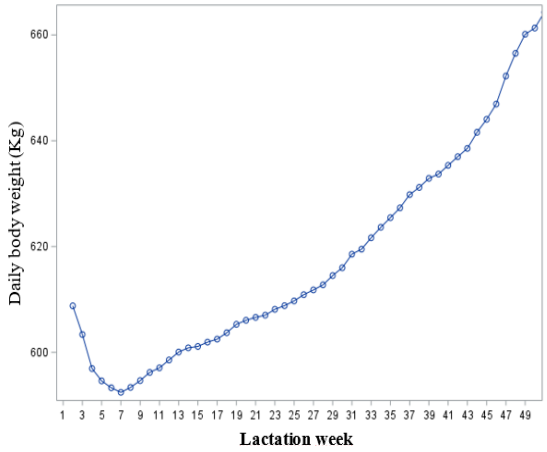

Figure 2. Solution of fixed effect of lactation week on body weight (Kg) for Norwegian Red cows.

Breeding values estimation

The EBVs for dDMI ranged from -4.01 to 6.49 kg dry matter per day (±1.5). For dCH4 the EBVs ranged from -124.5 to 150 gram per day (± 40.5), for dBW from -178. 5 to 190.9 kg of body weight. There was a large variation in EBVs for alle tree traits as illustrated for dBW in figure 3. This shows the differences between animals with high and low breeding values.

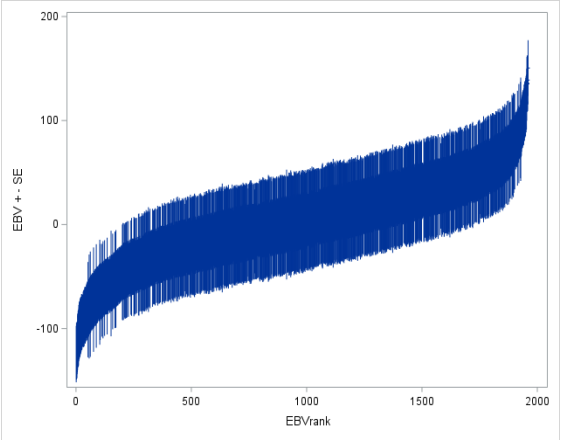

Figure 3. Breeding values (EBV) for body weight in kg for 1 960 Norwegian Red cows. Standards error (SE) illustrated with blue bars. EBVs are sorted from lowest to highest (x-axis) and illustrated with EBV +/- SE (y-axis).

Index correlations

Correlations between EBV for dDMI, dCH4, dBW and indexes for traits in the routine genetic evaluations of NR are given in table 4. In general correlations were low and ranged from -0.26 to 0.14 for dDMI, -0.22 to 0.14 for dCH4, and from -0.19 to 0.14 for dBW. Fertility came out as the sub-index with highest correlation to the EBVs for all the 3 analysed traits. The correlation was negative from -0.19 to -0.26, indicating higher EBVs are associated with lower genetic merit for fertility i.e. larger cows, with higher feed intake and more methane will have poorer fertility. The weak positive correlation between EBVs for dDMI and dCH4 to milk index (0.09-0.14) indicates that higher yielding cows tend to eat more and produce more methane.

Table 4. Index correlations to daily dry matter intake (dDMI), methane (dCH4), and body weight (dBW) in Norwegian Red cows.

Trait (index)	dDMI	dCH4	dBW
Milk yield	0.14 (<.001)	0.09 (<.001)	-0.00 (0.96)
Fertility	-0.26 (<.001)	-0.22 (<.001)	-0.19 (<.001)
Udder health	-0.10 (<.001)	-0.14 (<.001)	-0.13 (<.001)
Milk fever	0.13 (<.001)	0.14 (<.001)	0.16 (<.001)
Mastitis	-0.10 (<.001)	-0.08 (<.001)	-0.4 (0.05)
Claw health	-0.11 (<.001)	-0.11 (<.001)	-0.15 (<.001)
Ketosis	0.05 (0.03)	0.06 (<.01)	0.13 (<.001)
Carcass	0.00 (0.1)	0.07 (<.01)	0.14 (<.001)
Total Merit	-0.04 (0.07)	-0.07 (.001)	-0.13 (<.001)

Correlations between EBVs and the current total merit index (TMI) for NR were slightly negative for both dCH4 (-0.07) and dBW (-0.13). Negative correlation indicates lower TMI with increased genetic potential for more dry matter intake and more methane produced

because the EBVs were not standardized. For dDMI the correlation to TMI was not significant different from 0.

We need estimates of genetic correlations to other traits before we can start selecting for feed efficiency or methane. It is important to have a make good decisions when defining feed efficiency and methane in the breeding goal. More research is needed to understand the genetic relationship between methane and feed efficiency traits. A definition of feed efficiency considering body weight changes, energy intake (from grass and silage versus concentrate), and milk production will be investigated further for the NR population. We aim to balance feed efficiency, climate effects, production, health and fertility in a sustainable breeding goal for NR.

Conclusions

Genetic variation for traits considered important for feed efficiency in NR exists. The new traits dDMI, dBW, and dCH4 measured in commercial dairy farms are genetically correlated. The results are promising for the further work on feed efficiency as a new trait to be included in routine genetic evaluations of NR.

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Next steps towards the development of a collaborative genomic evaluation system for residual methane production in Walloon Holstein cows

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Abstract

Greenhouse gases emissions from ruminants are one of the causes of climate change. Methane (CH_4) from dairy cows is a major greenhouse gas and is also associated with the energy use efficiency in dairy cows. This study aimed to use data of CH_4 emissions (PME, g/d) predicted using the recorded milk mid-infrared (MIR) spectra to develop a genomic evaluation system for CH_4 of Holstein cows in the Walloon region of Belgium. The preliminary relationships among predicted CH_4 (PCH_4, defined as the estimated breeding value for PME), expected CH4 (ECH_4, estimated based on production traits), residual CH4 (RCH_4) [defined as PCH_4- ECH_4] and MACE traits and local indices were also investigated. The data of PME predicted between 2007 and 2023 on Walloon Holstein cows were used. The number of used test-day records (cows) was 2,129,225 (319,301), 1,675,056 (250,707), 1,184,377 (178,882) for the first, second, and third lactation, respectively. Genotypic data on 28,317 SNPs were available for 18,378 (3,887 sires) animals. The EM-REML method was employed to estimate the variance components. Mean (SD) daily PME per cow was 324 (68) g/d, 353 (71) g/d, and 367 (73) g/d for the first, second, and third lactation, respectively. Mean (SD) heritability estimates for daily PME were 0.13 (0.04), 0.13 (0.04), and 0.14 (0.04) in the first, second and third lactation, respectively. The average reliability of PCH_4 for the selected bulls was 70% and ranged from 51% to 98%. The corresponding value for RCH_4 was 71% and ranged from 50 to 98%. The ECH_4 was estimated for 1,170 selected international sires using available GEBV of milk, fat, and protein yields as: $ECH_4 = b_1GEBV_{MY} + b_2GEBV_{FY} + b_3GEBV_{PY}$. The Pearson correlation of PCH_4 and RCH_4 was 0.83. PCH_4 was correlated with production traits (from 0.16 to 0.51) while RCH_4 was independent of them. The Pearson correlation among PCH_4 with MACE traits and local indices ranged from 0.05 to 0.45, while the results of RCH_4 ranged from -0.01 to 0.14. Our results suggest that an efficiency CH_4 trait could be incorporated into our current genomic evaluation systems, but our results also showed that definitions of methane efficiency solely on production traits can be dangerous.

Key words: Methane production, mid-infrared spectroscopy, single-step genomic evaluation

Introduction

Emissions of greenhouse gases (**GHG**) such as carbon dioxide (**CO_2**), methane (**CH_4**), nitrous oxide (**N_2O**), and halocarbons are considered to have a considerable impact on climate change (Knapp et al., 2014). Dairy cattle production is a significant contributor to the global human-induced GHG emissions mainly in the form of CH_4 (De Haas et al., 2021). Each dairy cows emits between 60 and 160 kg of CH_4 per year (Hristov et al., 2013). The produced CH_4 is a part of feed energy that is not metabolized by the animal for productive, reproductive or maintenance purposes and the majority is eliminated in the atmosphere by

eructation and respiration. It has been reported that between 2 and 12% of the total gross energy intake in dairy cows is lost in the form of CH_4 (Johnson and Johnson, 1995, Boadi and Wittenberg, 2002, Benchaar and Greathead, 2011). Therefore, next to the environmental impact, methane production has a negative effect on energy use efficiency and may have therefore a direct, however not yet clearly established, economic value that may be a financial incentive for dairy farmers beyond carbon taxes or similar potential future developments.

Opportunities for nutritional and microbial manipulation to reduce enteric CH_4 emissions have been extensively investigated in dairy cows (Benchaar and Greathead, 2011, Tseten et al., 2022). However, genetic selection of lower CH_4 emitting cows should be added as an effective tool to any combination of strategies, making a permanent, cumulative over generations, and long-term contribution to reduce CH_4 production from dairy cattle (González-Recio et al., 2020, Manzanilla-Pech et al., 2022). However, conducting a successful genetic selection needs to establish a method to measure the trait of interest on many animals at low costs. Milk mid-infrared (MIR) spectra, currently used to predict various milk components, has been proven to be a fast and cheap method for predicting the amount of daily CH_4 produced by individual daily cows (Vanlierde et al., 2021) providing an opportunity for genetic studies and genetic evaluations (Kandel et al., 2017). Although MIR-predicted daily CH_4 production was found to be a heritable trait (Kandel et al., 2017), it also relates to traits of milk yield and milk composition beyond links between daily CH_4 production and other traits of interest. Moreover, the assessment of the best way how CH_4 should be reported in will need the collection of new information. Therefore, the primary aim of this report is to report the next steps towards the development of a collaborative genomic evaluation system for residual methane production in Walloon Holstein cows, the final aim being the development a genomic evaluation system using MIR-predicted CH_4 and MACE traits.

Materials and Methods

Data

Data of CH_4 emissions (PME, g/d) predicted between 2007 and 2023 on Walloon Holstein cows using the recorded milk MIR spectra were used. Records from days in milk (**DIM**) lower than 5 d and greater than 365 d were eliminated. The number of used test-day records (cows) was 2,129,225 (319,301), 1675056 (250,707), 1,184,377 (178,882) for the first, second, and third lactation, respectively.

Genomic data

Genomic data was available for 18,378 (3,887 sires) animals. Non-mapped SNP, SNP located on sexual chromosomes, SNP with Mendelian conflicts, and those with minor allele frequency (**MAF**) less than 5% were excluded. The difference between the observed and expected heterozygosity was estimated, and if the difference was greater than 0.15, the SNP was excluded (Wiggans et al., 2009). Finally, genotypic data used consisted in 28,317 SNPs located on 29 Bos taurus autosomes (**BTA**).

Model

Variance components and estimated (genomic) breeding values (**G(EBV**)) of the animals were estimated based on the integration of the random regression test-day model (**RR-TDM**) into the single-step GBLUP procedure (**SS RR-TDM**) using a multi-trait model (PME_1, PME_2, and PME_3), considering the fixed effects HTD and random effects of –herd-calving-year (**HY**), animal additive genetic (**a**), permanent environmental (**PE**), and residual. The genomic relationship matrix (**G**) is constructed by VanRaden Method I. (VanRaden, 2008), and **G** is blended with the additive relationship matrix (**A**) assuming that

60% of the total genetic variance was explained by SNP effects.

Because of computational demands, genetic parameter estimation was performed using six different subsets each representing 10% of the herds in the dataset. On average, the subsets contained 211,325, 162,385, and 113,551 records from 30,562, 23,932, and 17,002 cows in the first, second, and third lactation, respectively.

The EM-REML method was employed to estimate the variance components in REMLF90, with each of the subsets (Misztal et al., 2014). The average GEBV of PME was calculated by summing the daily GEBV divided by number of DIM. Subsequently, the mean of the average GEBV for the first three locations was computed (**PCH_4**). Then, sires having at least 30 CH_4-phenotyped daughters, and genomic reliability (**GREL**) for $PCH_4 \geq 0.50$ were selected for the next analyses (n = 1,170). As these sires were all also locally evaluated, Multiple Across Country Evaluation (**MACE**), respectively local (G)EBV for traditional evaluated and reported traits were also available for all these sires.

The expected CH_4 (**ECH_4**) was estimated for the selected sires using GEBV of milk, fat, and protein yields (collected from the Walloon genetic evaluations of dairy cattle (https://www.elinfo.be/indexEN.html) as: $ECH_4 = b_1GEBV_{MY} + b_2GEBV_{FY} + b_3GEBV_{PY}$ and the residual CH_4 (**RCH_4**) was defined as: $RCH_4 = PCH_4 - ECH_4$. In this study regression coefficient b_1, b_2 and b_3 were developed directly from the observed covariances between (G)EBV.

Polygenic reliability, calculated based on the Effective Daughter Contributions and computed using established procedures in routine genetic evaluations, was used as a prior to estimate GREL. Double-counting due to pedigree information was removed (Zaabza et al., 2022) and GREL computed implementing an approach based on Gao et al. (2023). The GREL of RCH_4 was calculated using the method (selection index) given by VanRaden et al. (2018). The Pearson correlations among the PCH_4 and RCH_4 with the selected MACE traits and local indices were calculated based on the selected sires. The MACE traits included udder health (represents the opposite SCS), longevity, fertility, direct calving ease (**DCE**), maternal calving ease (**MCE**), and local indices included production economic index (**V€L**), member economic index (**V€M**), capacity economic index (**V€C**), udder economic index (**V€P**), functional type economic index (**V€T**), functional economic index (**V€F**), global economic index (**V€G**) (https://www.elinfo.be/indexEN.html).

Results and Discussion

Mean (SD) daily PME per cow were 324 (68) g/d, 353 (71) g/d, and 367 (73) g/d for the first, second, and third lactation, respectively. Mean (SD) heritability estimates for daily PME were 0.13 (0.04), 0.13 (0.04), and 0.14 (0.04) in the first, second and third lactation, respectively.

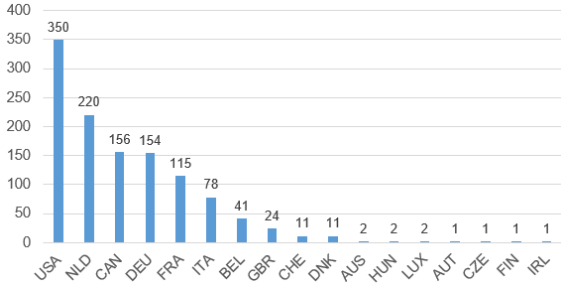

Figure 1: Distribution of 1,170 sires based on country of origin.

The distribution of the selected sires based on the country of origin is presented in Figure 1. As Wallonia is importing most of its Holstein semen and this from many countries over 100 bulls were present from major exporting countries like USA, NLD, CAN, and DEU. Sires from other exporting countries, especially including ITA and GBR were less

present, still one can speculate that many internationally important sires were evaluated.

The distributions of standardized PCH_4 and RCH_4 for the selected sires are shown in Figure 2. The average GREL of PCH_4 for the selected bulls was 70% and ranged from 51% to 98%. The corresponding values for RCH_4 were 50% and ranged from 50 to 98% (Figure 3). As GEBV for RCH_4 was computed combing CH_4 with relatively reliably evaluated production traits (in ECH_4), GREL for RCH_4 did not show the loss of reliability that maybe one could expect for a residual trait.

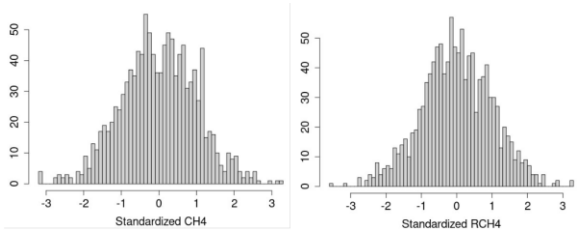

Figure 2: Distribution of standardized GEBV for PCH_4 and RCH_4 (n = 1,170 sires)

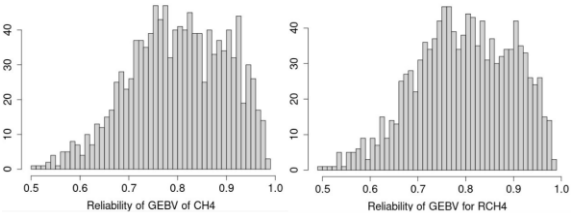

Figure 3: Distribution of reliability for PCH_4 and RCH_4 (n = 1,170 sires)

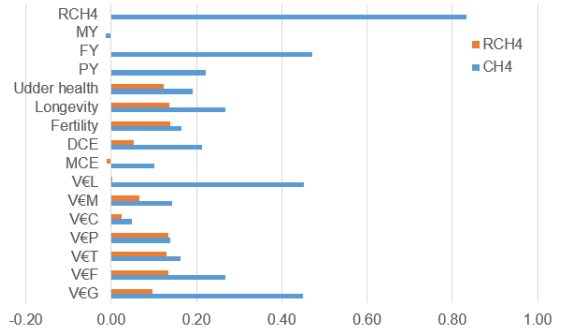

Figure 4: Pearson correlations of PCH_4 and RCH_4 with selected traits and indices (n = 1,170 sires).

The estimated Pearson correlations among PCH_4, RCH_4, MY, FY, and PY are presented in Figure 4. The correlation of PCH_4 and RCH_4 was 0.83 showing that a large part of variance of RCH_4 was not explained by ECH_4. PCH_4 was correlated with production traits while RCH_4 was independent of them as expected by its definition. These results were similar to those reported by Van Doormaal et al. (2023).

The Pearson correlations PCH_4 with other MACE and the local (G)EBV and indices ranged from 0.05 to 0.45, while the results of RCH_4 ranged from -0.01 to 0.14 (Figure 4). The correlations of PCH_4 with other traits and indices were bigger than those results of RCH_4. It is important to remind that positive correlations mean that in the case of a direct selection against PCH_4, but also RCH_4, we would lose production (because of its definition not for RCH_4), udder health, fertility, longevity, calving ease and all indices. Even if these results should be considered preliminary, they indicate that expression of CH_4 traits must be done be very carefully and the definition of methane efficiency solely on production traits (Van Doormaal et al., 2023) can be dangerous.

Conclusions

The RCH_4 has been defined as an efficient trait to be included into genetic selection programs for dairy cows. This trait is not associated to production levels and has the potential to decrease CH_4 emissions without impacting milk, fat, and protein yields. Our results showed that the Walloon genomic evaluation system can evaluate many foreign AI sires. However, our results also showed that definitions of methane efficiency solely on production traits can be dangerous.

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Single-step genomic prediction models for metabolic body weight in Nordic Holstein, Red dairy cattle, and Jersey

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Abstract

Nordic Cattle Genetic Evaluation (NAV) introduced a breed-specific index for Saved Feed in 2020, focusing on the maintenance and metabolic efficiency of cows. Maintenance efficiency is based on genomic breeding values for metabolic body weight (MBW), for which a multi-step (genomic) evaluation was implemented in 2019. The model utilizes body weight and conformation observations from Finland and Denmark, but only conformation observations from Sweden. This study aimed to enhance the MBW evaluation by including carcass weight (CARW) from all three countries and by developing a single-step genomic prediction model. The new model includes three MBW traits and two correlated traits: CARW and stature (STA). The data were collected from Danish, Finnish, and Swedish Red Dairy Cattle (RDC), Holstein (HOL), Jersey (JER) cows born between 1990 to 2020. After data editing, the RDC, HOL, and JER datasets comprised of 2.3 million, 4.3 million, and 0.4 million records, including 0.9 million, 0.5 million, and 11 thousand MBW observations, respectively. The pedigree of RDC, HOL, and JER included 3.9, 7.2 and 0.6 million animals, respectively. Among these, 84 232 RDC, 117 845 HOL, and 39 650 JER animals were genotyped since 2009 onwards. To develop single-step genomic best linear unbiased prediction (ssGBLUP) models, we applied VanRaden method I to construct the genomic relationship matrix, with a residual polygenic proportion of 30%. We utilized the ssGTaBLUP method to solve the models. Separate ssGBLUP models were developed for each breed, and these models were validated through forward prediction cross-validation, linear regression of full data breeding values on reduced data breeding values, and comparison of pedigree-based and ssGBLUP breeding values. The inclusion of carcass weight data substantially increased phenotypic information in all three breeds, resulting in enhanced reliability of MBW breeding values. The new ssGBLUP models showed higher validation reliability and better predictive ability than the pedigree-based BLUP models. Furthermore, the new models corrected the genetic trend of MBW, addressing a previous underestimation in all breeds. Including CARW records as correlated observations and applying ssGBLUP models offers a significant improvement for the Nordic metabolic body weight evaluations, thereby enhancing the Saved Feed index.

Key words: animal breeding, genomic predictions, carcass weight, saved feed index

Introduction

The Saved Feed index of the Nordic Cattle Genetic Evaluation (NAV) was integrated into the Nordic Total Merit index in 2020. It comprises two components: maintenance and metabolic efficiency. Metabolic body weight (MBW, $kg^{0.75}$) is the core trait for maintenance

feed requirement, while residual feed intake is the main trait for metabolic efficiency (Lidauer et al. 2019, Stephansen et al. 2021). Each breed, including Red Dairy Cattle (RDC), Holstein (HOL), Jersey (JER), has its own evaluation. The current multiple-trait model for maintenance efficiency includes six traits: MBW in the first, second, and third parity, and the conformation traits stature (STA), chest width, and body depth, as correlated indicator traits.

A current challenge is the decreasing number of body weight recordings in Denmark and Finland, and no body weight (BW) data available from Sweden. However, there is a substantial amount of slaughter information available across the Nordic countries. The correlations between carcass weight (CARW) and MBW are high, ranging from 0.77 to 0.85 in RDC (Mehtiö et al. 2021). Additionally, CARW has high heritability with estimates of 0.52 for RDC and 0.37 for Jersey (Mehtiö et al. 2021, 2023). These characteristics make incorporating CARW information highly valuable in the genetic evaluation of MBW.

The aims of this study were to incorporate CARW data into the evaluation of MBW, upgrade the current multiple-step genomic prediction model to a single-step genomic prediction model, and assess the prediction ability of the models through validation tests.

Materials and Methods

Data

Phenotypic data and pedigree were obtained from the February 2022 NAV Saved Feed evaluation. Breeding organizations Faba, Växa, and Seges extracted country-specific carcass weight data for this study. Observations were from the Danish, Finnish and Swedish RDC, HOL, and JER cows born between 1990 and 2020. The data included all available MBW observations (kg), the first parity STA observation (cm) from the NAV routine conformation evaluation (NAV, 2022) and CARW data from the year 2007 onwards. The CARW data were further restricted to: a) parities 1 to 5, b) 60-550 days after the last calving, c) animals aged 24–110 months at slaughter, and d) herds with more than three CARW records. CARW records deviating more than 3 SD from the mean were removed as outliers. The BW observations were pre-processed as described by Lidauer et al. (2019) to obtain one MBW observation per lactation. After editing, the RDC and HOL data consisted of 0.93 and 0.54 million MBW observations, respectively. The JER data had 11 thousand MBW observations. The number of phenotypic records is presented in Table 1.

Table 1. Number of records for metabolic body weight in the first three parities (MBW1, MBW2, MBW3), first parity stature (STA), and carcass weight (CARW) in Red Dairy Cattle (RDC), Holstein, and Jersey dairy cows.

	RDC	Holstein	Jersey
	N	N	N
MBW1	521 132	293 237	6 064
MBW2	318 764	173 686	3 458
MBW3	93 502	72 766	1 926
STA	349 329	740 521	301 844
CARW	686 946	1 740 589	175 636

Genotype data from February 2022 included 84k RDC, 117k HOL, and 39k JER animals. The genomic data were truncated, retaining only the most resent genotyped animals from the year 2009 onwards. The pedigrees of the RDC, HOL and JER cows with observations were pruned for five generations, including 3.9, 7.2 and 0.6 million animals, respectively. Genetic groups were formed by categorizing unknown parents within country and breed based on 5-year birth year classes, resulting in 182, 202 and 70 unknow parent groups (UPG) for RDC, HOL, and JER, respectively.

Models

The pedigree-based Best Linear Unbiased Prediction (BLUP) models developed for the NAV routine MBW evaluation (Lidauer et al., 2019) served as the foundation for building the single-step genomic prediction (ssGBLUP)

models. These multiple-trait models were updated by replacing the traits chest width and body depth with CARW, resulting in multiple-trait BLUP models with five traits: MBW in the 1st, 2nd, and 3rd parity, first parity STA, and CARW. Multiple-trait linear mixed animal models for the 1st, 2nd, and 3rd parity MBW and STA are detailed in Lidauer et al. (2019). The linear model for CARW was as follows:

$$y_{ijkln} = sageP_i + catsP_j + sym_k + shy_l + a_n + e_{ijkln},$$

where y_{ijkln} is a CARW observation, $sageP_i$ is the slaughter age × parity × 5–year period interaction, where year periods are constructed from the birth years; $catsP_j$ is the fixed effect of days from calving to slaughter × parity × 5–year period, again with periods based on birth years; sym_k is the fixed effect of slaughter year × month; shy_l is the fixed effect of slaughter herd × birth year; a_n is the random additive genetic effect of animal, and e_{ijkln} is the random residual.

Single-step models were solved with the ssGTaBLUP approach (Mäntysaari et al. 2017). The VanRaden method I (VanRaden 2008) was used for building the genomic relationship matrix by blending the **G** matrix with a 30% residual polygenic proportion. Pedigree inbreeding coefficients were considered in $\mathbf{A}^{-1}$ and $\mathbf{A}_{22}^{-1}$. Genetic groups were included in the single-step models using the partial QP transformation that omitted $\mathbf{G}^{-1}$ in QP (Koivula et al. 2021).

For each animal, combined MBW breeding values (BV), including estimated breeding value (EBV) and genomic enhanced breeding value (GEBV), were formed using the BVs from the 1st, 2nd, and 3rd parities with weighting coefficients of 0.30, 0.25, and 0.45, respectively.

BLUP and ssGBLUP BVs were validated using forward prediction cross-validation. For the evaluations with reduced data, observations from the most recent four years (2016-2020) were excluded. Candidate bulls for validation were chosen from genotyped bulls born between 2013 and 2018 that had an effective record contribution (ERC) >1 in the full data and ERC=0 (i.e., no daughters) in the reduced data. For the cow validation group, genotyped cows born between 2015 and 2020, with no records in the reduced data (ERC=0) and at least one record in the full data (ERC>0), were considered as candidate cows. In the validation cohort, we had 43 503 RDC cows and 290 RDC bulls, 75 707 HOL cows and 470 HOL bulls and 18 235 JER cows and 150 JER bulls. The same pedigree and genomic information were used in the reduced data as for the full data set evaluations to obtain BVs (either EBV or GEBV) for candidates (BV_c). Cross-validation reliability (r^2_{cv}) was calculated as:

$$r^2_{cv} = corr(DRP, BV_c)^2 / r^2_{DRP},$$

i.e., squared correlation between deregressed proofs (DRP) estimated from the full data and BV_c divided by the average reliability of the DRPs. The second statistic applied was the linear regression of full data breeding values on reduced data breeding values (Legarra and Reverter 2018).

Results and Discussion

Results showed that the genetic trend of combined MBW is increasing in each breed (Figure 1). The current BLUP models underestimate the genetic trend for MBW compared to the new BLUP or ssGBLUP models. The new ssGBLUP models give a slightly higher trend compared to new BLUP-models. This was an expected result because the ssGBLUP models incorporate genomic information directly, which increases the accuracy of estimated breeding values and allows to account for genomic pre-selection.

The cross-validation results are given in Table 2. The correlations between candidates' BV_c and their future DRP were the highest when BV_c were estimated with ssGBLUP for both bulls and cows in all breeds. On average, correlations between candidates' BV_c and their

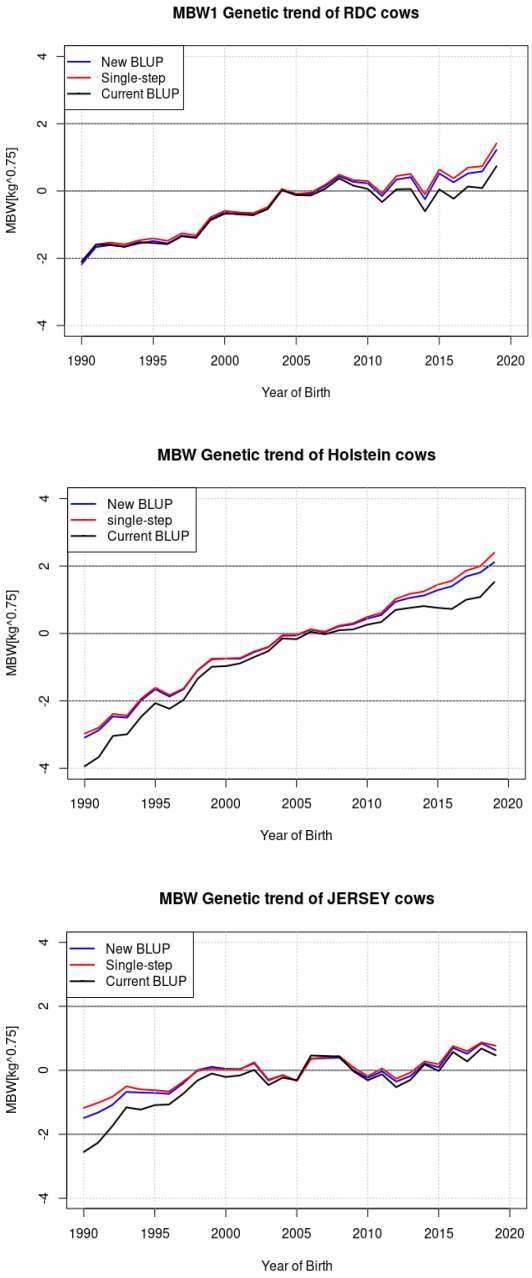

Figure 1. Genetic trends of MBW (G)EBVs by birth year in Red Dairy Cattle, Holstein, and Jersey cows. Trends of MBW from the single-step model (red line), new BLUP model (blue line), and current BLUP-model used by NAV (black line) are expressed as standardized breeding values for cows born between the years 2005-2007.

DRP were 20.0 and 14.0 percentage units higher for the single step models compared with BLUP models in cows and bulls, respectively.

The optimal prediction of genetic merit of young individuals should have a regression coefficient (*b1*) of one. In our cross-validation of bulls, the *b1* estimates obtained using the BLUP model were slightly better compared to those from the ssGBLUP model (Table 2). This difference is likely because our DRPs were based on the BLUP model. Using the Legarra-Reverter (LR) validation method, all *b1* values were close to one for both bulls and cows in both the BLUP and ssGBLUP models, except in HOL. The BLUP model appeared to slightly overpredict the future breeding values for HOL candidate cows and bulls.

Table 2. Cross-validation and Legarra-Reverter (LR) estimates: Correlation between DRP and BV of canidates ($r_{(DRP, BVc)}$), regression coefficient (b_1), validation reliability (r^2_{cv}), and coefficient of determination (R^2) for RDC, HOL and JER bull and cow candidate groups by different models.

		Cross-validation			LR	
		$r_{(DRP, BVc)}$	b_1	r^2_{cv}	b_1	R^2
	BLUP[1]					
RDC	cows	0.25	1.04	0.19	1.04	0.43
	bulls	0.63	1.03	0.43	1.03	0.39
HOL	cows	0.19	0.87	0.11	0.92	0.33
	bulls	0.60	0.88	0.40	0.88	0.36
JER	cows	0.14	0.97	0.09	0.94	0.28
	bulls	0.46	0.91	0.23	0.98	0.21
	ssGBLUP[2]					
RDC	cows	0.48	1.32	0.73	1.08	0.80
	bulls	0.76	0.92	0.61	1.06	0.71
HOL	cows	0.36	1.08	0.39	1.00	0.70
	bulls	0.74	0.85	0.60	0.98	0.67
JER	cows	0.34	1.39	0.51	1.03	0.59
	bulls	0.61	0.88	0.40	1.08	0.59

[1]BLUP = Best Linear Unbiased Prediction
[2]ssGBLUP = Single-step Genomic BLUP
BVc = Breeding Value for candidates

The validation reliabilities (r^2_{cv}) for the BLUP model varied between 0.23 and 0.43 for RDC, JER, and HOL bulls, and between 0.09 and 0.19 for cows (Table 2). In contrast, the r^2_{cv} for the ssGBLUP model varied between 0.40 and 0.61 for bulls, and between 0.39 and 0.73 for cows. This indicates that, across all breeds, the validation reliability was on average 18.3 percentage units higher for the single-step model in bulls and 41.3 percentage units higher in cows. Additionally, using the LR method, the coefficients of determination (R^2) were on average 33.7 percentage units

higher for the single-step model in bulls and 35.0 percentage units higher in cows. These results suggest a better predictive ability of the model with genomic data.

Conclusions

In this study we developed models that include carcass weight data as correlated information for predicting genomic breeding values for MBW. The CARW data significantly increased the amount of phenotypic information used for the genomic evaluation in all Nordic breeds. This, along with the development of single-step genomic prediction, contributes positively to the reliability and unbiasedness of predictions of breeding values for maintenance. As a result, animals will receive more accurate breeding values.

Acknowledgments

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Effect of heat stress on methane emissions of Dutch Holstein cows

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Abstract

Over the past decade, climate change has raised the importance of addressing heat stress in dairy cattle. The Temperature-Humidity Index (THI) is a key tool for assessing the animals' response to varying weather conditions, serving as an indicator of heat stress. Studies suggest that higher THI is linked to reduced milk production and compromised health. Despite this, little is known about the effect of temperature and humidity on methane emissions of dairy cattle. Our study aims to investigate the potential impact of temperature and humidity on methane emissions in the Dutch Holstein population. We analyzed 132,960 weekly methane concentration (CH_4c) records from 7,669 cows across 72 commercial farms in the Netherlands spanning from 2019 to 2023. Each methane record was paired with weekly THI data computed from meteorological records provided by the Nederlands Meteorologisch Instituut (KNMI). Weekly THI values were calculated using the National Research Council formula, resulting in indexes ranging between 28 and 72. At the population level, a repeatability animal model included fixed effects such as herd-year-season interaction, week of lactation, and parity-age of cow at calving interaction (parities = 1, 2, 3, ≥4). Random effects included animal additive genetic effects and permanent environment effects. Methane concentrations showed a significant increase starting at a THI value of 46. At the individual level, a reaction norm model focusing on THI values higher than 46 (THI_{46+}) was implemented. An interaction between animal additive genetic effect and THI46+ level using Legendre polynomials of first order was fitted, resulting in different aggregate Estimated Breeding Values (EBVs) at different THI46+ values per animal. Results demonstrated a significant THI effect ($P < 0.001$) on CH_4c at a population level. Estimated aggregated heritabilities at different THI_{46+} level for CH_4c ranged between 0.11 (at THI 55 and 56) and 0.50 at THI level 70 (SE=0.01). The permanent environment ratio ranged between 0.19 at THI level 70 to 0.35 at THI level 56. Based on the EBV for CH_4c at the THI value lower than 46 (that is, in a thermo-neutral environment), cows were ranked into top (high emitting animals, n=50) and bottom (low emitting animals, n=50) groups. The results revealed that aggregate EBVs for low-emitting cows tended to increase as THI levels rose, whereas high-emitting cows showed decreasing EBVs at higher THI_{46+} levels. This could potentially impact the selection of CH_4 emissions reduction strategies in a future affected by climate change (global warming) and/or in countries with different temperatures and humidity levels.

Key words: heat stress, methane emissions, temperature-humidity index.

Introduction

Climate change has intensified the need to address heat stress in dairy cattle over the past decade. The Temperature-Humidity Index (THI) serves as a critical indicator of heat stress, correlating with reduced milk production and compromised health in dairy cows (Hammami et al. 2015; Herbut et al. 2018). Despite these associations, little is

understood about how temperature and humidity specifically affect methane emissions in dairy cattle. Understanding these relationships is crucial for developing effective strategies to mitigate methane emissions in dairy farming, particularly in the context of climate change. This study investigates the potential impact of temperature and humidity on methane emissions within the Dutch Holstein population.

Materials and Methods

Data collection

Methane records

The data included 7,669 Dutch Holstein cows with 132,960 records of CH_4 concentration (in parts per million, ppm). These records were collected in primiparous and multiparous cows during 2019 to 2023 in 72 commercial farms in the Netherlands. Parities were grouped into categories of 1, 2, 3, and 4+, and only records up to lactation week 53 were included.

THI records

Each CH_4c record was associated with a corresponding weekly THI record computed from daily meteorological information measured in the closest meteorological station to each farm. Twenty-four meteorological stations were identified as the closest to the 72 farms. Daily meteorological records were provided by the Nederlands Meteorologisch Instituut (KNMI). Weekly THI were computed using the National Research Council formula as follows:

$$THI = (1.8*t+32) - (0.55-0.0055*rh) * (1.8*t-26) \quad \textbf{(1)}$$

where t is daily average temperature (in degrees Celsius), and rh is the daily average relative humidity (in %). Subsequently, THI were averaged weekly to match the weekly CH_4c records.

Population-level analyses

To evaluate the effect of THI at a population level we used the following repeatability animal model:

$$\mathbf{y= Xb + Z_a a + Z_p pe + e} \quad \textbf{(2)}$$

where **y** is the vector of phenotypes (CH_4c); **b** is the vector of fixed effects: herd, year-season interaction (n=312), week of lactation (n=54), age of cow at calving nested within parity (n=4), and THI (n=44); **a** is the vector of direct additive genetic effects; **pe** the vector of random permanent environment effects; and **e** is the vector of residual effects. The matrices **X**, $\mathbf{Z}_a$ and $\mathbf{Z}_p$ are the incidence matrix relating observations with the fixed effects, random genetic effects, and random permanent environment effects. Distributions of the random effects are var(**a**) = $\mathbf{A}\sigma^2_a$ where **A** is the pedigree relationship matrix and σ^2_a, and var(**pe**) = $\mathbf{I}\sigma^2_{pe}$, where I is an identity matrix of an order equal to the number of observations and σ^2_{pe} is the residual 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^2_e is the residual variance. The pedigree included 98,324 individuals, with maximum 14 generations. The estimation of the variances components and of the different effects was performed with ASReml 4.0.

Individual level analyses

Based on the results of the previous analyses at a population level, we determined that CH_{4c} increased from a THI value of 46. Therefore, a reaction norm model was used to evaluate the effect of THI values higher than 46 (THI_{46+}) at an individual (cow) level. To estimate variance components and EBV for CH_4c associated to heat stress the following model was used:

$$\mathbf{y = Xb + QZ_a a_T + Z_p pe + e} \quad \textbf{(3)}$$

where **b** is the vector of fixed effects as defined for Model (2), except that THI level classes have been replaced by three THI group

classes (that is, low, mid, and high, and defined below); $\mathbf{a}_T$ is a vector of random regression coefficients for additive genetic effects; **Q** is the covariate matrix for first-order Legendre polynomials for THI defined below; and other vectors and matrices are the same as for Model (2).

The group "low" includes THI values between 28 and 43 , the group "mid" between 43 to 58 and the group "high " between 58 to 72.

In the random terms an interaction between animal additive genetic effect and THI values ≥46) was modelled using first-order Legendre polynomials as followed:

$$T = \begin{bmatrix} 0 & \text{if THI} \leq \text{THI}_{\text{-TH}} \\ \text{THI-THI}_{\text{-TH}} & \text{if THI} > \text{THI}_{\text{-TH}} \end{bmatrix} \quad \textbf{(4)}$$

Where, THI is the THI value, and $THI_{\_TH}$ is the THI (heat) threshold (46). In our case this could be represented as:

$$\mathbf{THI_{46+} = THI_{46+n} - 46}$$

Genetic parameters estimation

Estimated (co)variance components of the random regression were used together with Legendre polynomial coefficients to calculate genetic variances and covariances for each THI_{46+} level using the methodology described in Fischer et al. (2004):

$$\mathbf{G = \Phi K \Phi'} \quad \textbf{(5)}$$

where ***G*** is the genetic variance-covariance matrix within trait per THI_{46+} level (matrix of size n × n), **Φ** is a matrix of order *l* × *n*, which contains *l* orthogonal polynomial coefficients for each of the traits through n THI_{46+} levels; **K** is a matrix of order *l x l* , which contains the estimated covariance function describing the genetic variance components for the random regression coefficients. Where *n* = 23 THI_{46+} levels (47-70) and *l* = 2 (2 coefficients, one for the intercept and one for the lineal regression).

Phenotypic variance, heritabilities and permanent environmental ratio were estimated as follows:

$$\boldsymbol{\sigma^2_{\ P}}^2 = \boldsymbol{G} + \boldsymbol{\sigma^2_{pe}} + \boldsymbol{\sigma^2_e} \quad \textbf{(6)}$$

$$\boldsymbol{h^2 = G / \ \sigma^2_{\ P}} \quad \textbf{(7)}$$

$$\boldsymbol{pe^2 = \sigma^2_{pe} / \ \sigma^2_{\ P}} \quad \textbf{(8)}$$

where $\boldsymbol{\sigma^2_{\ P}}$ is the phenotypic matrix of (co) variances, **G** is the genetic matrix of (co)variances, σ^2_{pe} is the (scalar) variance for permanent environment and σ^2_e is (scalar) the residual variance; h^2 is the heritability and pe^2 is the permanent environment ratio.

Estimated breeding values per THI unit

Based on the EBV for CH_4c in a thermo-neutral conditions (that is THI<46), cows were ranked into top (high emitting animals, n=50) and bottom (low emitting animals, n=50). The purpose of this ranking was to observe how low-emitting and high-emitting animals perform under heat stress (higher THI values). Subsequently, their EBV were plotted at different THI_{46+} levels.

Results and Discussion

Among the 72 herds and between 2019 and 2023, Temperature-Humidity indexes varied between 28 and 72 with an average of 50. Descriptive statistics for CH_4c (for all and per THI group) are presented in Table 1. The mean CH_4c was 572 ppm, and the mean per THI group was lower for the group with high THI compared with the group with low THI. However, the standard deviations (SD) were high. Methane concentration averages were higher than those reported by van Breukelen et al. (2022; in a subset of this population), Sypniewski et al. (2021) in Polish Holstein cows and Manzanilla-Pech et al. (2022) in Danish Holstein cows.

Table 1. Descriptive statistics for CH_4 concentration (ppm) for the whole population, and per group of THI value.

THI group	THI range	No. obs	No. animals	Mean	SD
Whole	28-72	132,960	7,669	572	294
Low	28-43	27,960	5,614	597	312
Mid	43-58	83,101	7,332	571	296
High	58-72	30,511	5,602	561	284

Population level analyses

Figure 1 shows the effects on THI level on CH_4c based on the solutions of Model 2. A positive effect on CH_4c is observed with increasing THI values starting at THI level of 46. Therefore, this level was used as a heat threshold (THI_{46+}) as suggested by McWhorter et al. (2023) for the individual level analyses. However, McWhorter reported a higher heat threshold (69 THI), this could be due to geographical and climate differences.

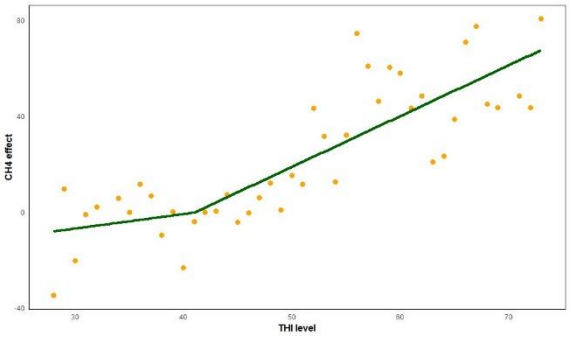

Figure 1. Effect of THI level on CH_4 concentrations.

Genetic parameters for CH_4c

Estimated (co)variance components of the random regression were used together with Legendre polynomial coefficients to calculate genetic variances and covariances for each THI level after the heat threshold (THI_{46+}). Heritabilities for CH_4c at different THI_{46+} level are presented in Figure 2. Heritabilities ranged between 0.11 (at THI 55 and 56) and 0.50 at THI level of 70. There h^2 agree with heritabilities for CH_4c in the literature and ranging between 0.1 to 0.3 (Difford et al. 2020, Sypniewski et al. (2021) and van Breukelen et al. (2022).

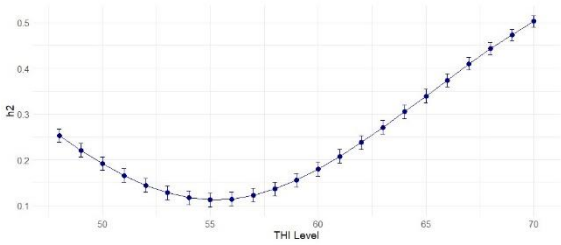

Figure 2. Heritabilities for CH_4 concentrations per THI level (from the heat threshold defined at a THI value equal to 46). Average standard error was 0.01.

Genetic correlations for CH_4c between different THI_{46+} levels are presented in Figure 3. Genetic correlations between consecutive THI_{46+} levels are high as expected. However, as the THI_{46+} levels become more distant from each other, the correlations can approach zero (e.g., between THI 47 and THI 59) or show moderate negative correlations (e.g., -0.5 between THI 46 and THI 70). This negative correlation could potentially impact the selection of CH_4 emissions reduction strategies in a future affected by climate change (global warming) and/or in countries with different temperatures and humidity levels (e.g., Mediterranean vs. Scandinavian countries).

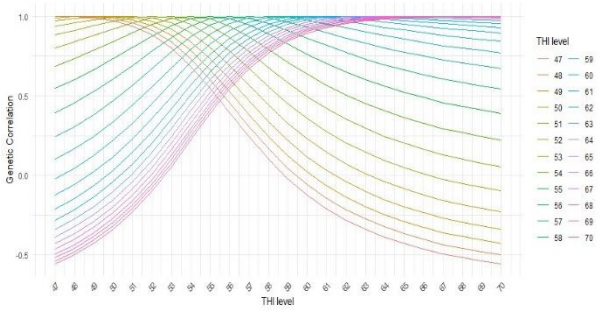

Figure 3. Genetic correlations for CH_4c between different THI_{46+} levels

Estimated breeding values per THI unit

When plotting aggregate EBVs of the 50 top animals and 50 bottom animals at different THI_{46+} levels (Figure 4), the bottom animals showed an increased CH_4c effect at higher THI levels, whereas the top animals reduced their CH_4c effect. McWhorter et al. (2023) reported a study where 100 animals with high genetic

merit for milk production and 100 animals with high genetic merit for heat tolerance were plotted at different THI levels. The first group experienced a slight decrease in production (Holstein) and a more drastic decrease in production (Jersey) at higher THI levels. In contrast, the second group increased their production at higher THI levels. However, in this study, the 50 top and bottom animals were chosen based on their EBV at the THI_{46+} level, which represents the intercept, and were further plotted at different THI levels.

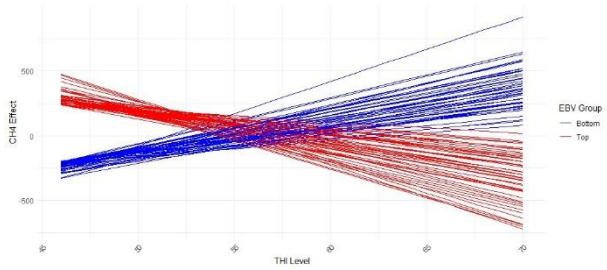

Figure 4. EBV for CH_4c for top 50 (high emitting) and bottom 50 (low emitting) cows at different THI_{46+} levels

Conclusions

This pilot study aimed to assess the impact of THI on CH_4 emissions (concentration), and revealed significant findings that is the bottom animals showed an increased CH_4c effect at higher THI levels, whereas the top animals reduced their CH_4c effect. This knowledge could be pivotal for future selection strategies aimed at reducing CH_4 emissions, taking into account genotype by environment interactions. Additionally, further validation through multi-trait analysis, including milk production, is recommended. This would allow us to form the groups with animals based on their methane emissions at the same production level e.g. high and low emitting animals at a high milk production level.

Acknowledgments

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Breeding for resilience in the Netherlands and Flanders

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Abstract

Dairy cows could face several environmental disturbances during a lactation like weather conditions or changes in roughage quality, resulting in reduced functioning. Cows which are minimally affected by disturbances and/or quickly recover are the preferred cows. Reduced functioning of dairy cows is measured as the difference in daily milk yield and the expected milk yield for that day. This is called deviation. The expected daily milk yield is estimated with polynomial quantile regression based on all milkings of a cow during a lactation. Based on the deviations, two resilience traits are calculated: stability and recovery. Stability is the natural logarithm of the variance (LnVar) from all deviations during a lactation, recovery is the autocorrelation (Rauto) between all deviations during a lactation. A lower LnVar indicates less affection by disturbances, thus more stability, a lower Rauto indicates quicker recovery. Breeding values are estimated for stability and recovery for lactation 1, lactation 2 and lactation 3 and higher (3+). Heritability (h^2) is 0.09, 0.06 and 0.09 for stability and 0.07, 0.04 and 0.04 for recovery for respectively lactation 1, 2 and 3+. Genetic correlations between different parities for stability ranges from 0.91 to 0.98 and for recovery it ranges from 0.84 to 1.00. Overall breeding values for stability and recovery are calculated based on traits in lactation 1, 2 and 3+. An overall index for resilience is calculated based on the overall breeding values for stability and recovery. Breeding on resilience results in cows that are less affected by environmental disturbances and recover quicker. The overall index and the two overall breeding values are the traits that are published in the Netherlands and Flanders since April 2024.

Key words: resilience, milk yield, variance, autocorrelation, automatic milking system, dairy cow

Introduction

Focus in dairy breeding is more and more on health. There are numerous health traits where breeders can breed for: fertility, udder health, claw health, reproduction disorders and metabolic disorders.

These health traits are all aiming at one specific health factor. However, dairy farmers are looking for cows with a good general health and which can cope with different circumstances and changes in the environment. This is called resilience. Resilience is the ability to be minimally affected by disturbances and/or to recover quickly when affected (Colditz and Hine, 2016).

Previous research has shown that fluctuations in milk yield are heritable and can be used as resilience indicator (Elgersma et al., 2018), and different resilience traits can be made from the fluctuations in milk yield (Poppe et al., 2020).

Since 2010, records on milk yield of individual cows during their visit in the automatic milking system (AMS) or milking parlour with electronic milk measure device (EMM) are automatically uploaded to the database of CRV when the Dutch or Flemish dairy farmer is willing to share the data. This makes it possible to estimate the lactation curve of the cow and to calculate fluctuations in milk yield based on 24h milk yield of the cow.

The availability of the data on individual milk yield observations and the previous research on resilience based on fluctuations of

milk yield (Poppe et al., 2020), made it possible for CRV to estimate and publish breeding values for resilience in the Netherlands and Flanders since April 2024.

Materials and Methods

Data for breeding value estimation

Individual milk yield data is available for the genetic evaluation from more than 6,850 Dutch and Flemish dairy farms. These farms are milking their cows with an AMS or milk parlour with EMM. Each week, around 14 million milk yield observations are added to the database.

Based on milk yield and milk interval with the previous milk event of the cow, the 24h milk production of the cow is calculated up to 350 days in lactation. If the milk interval with the previous milk event is more than 24 hours, the 24h milk production is not calculated for that day, and also not for the day before.

Special attention is needed to uncompleted milk events, because the milk yield will be less than expected based on milk interval, while the next milk event, if completed, will result in a higher milk yield than expected based on milk interval because the extra milk from the uncompleted milk event is still in the udder of the cow. Therefore, the milk yield of both milk events is summed up and the milk interval is calculated as the time between the milk event before and after the uncompleted milk event.

If the cow has at least 50 days with a known 24h milk yield between day 11 and 340 in lactation, and if at least 70 percent of the days between the first and last day with a known 24h milk yield has a known 24h milk yield, an optimal production curve will be estimated for the cow in that lactation. This optimal production curve will be calculated using a fourth order polynomial quantile regression (Koenker, 2005). A 0.7 quantile is used, what makes the assumption that the realized production of the cow (24h milk yields during the lactation) is already disturbed by environmental factors.

From day 11 to day 340 in lactation, for each day the deviation (realized production – optimal production) will be calculated. Two resilience indicators will be calculated based on the deviations: Rauto and LnVar. The Rauto is a lag-1 autocorrelation, and LnVar is the natural logarithm of the variance.

The Rauto is a measure of how quickly a cow can recover from a drop in milk production, so the trait is called recovery. The LnVar is a measure of how many drops in milk yield the cow has during a lactation, so the trait is called stability.

Data from all parities is used. In the April 2024 breeding value estimation, the number of individual milk events was 5,056,750,621 which can be reduced to 5,967,719 lactations. After some selection steps as described in E-chapter Resilience (CRV u.a., 2024), 2,818,469 lactations from cows milked by an AMS and 869,526 lactations from cows milked in a milking parlour with EMM are left. This data is from 6,857 different herds and 1,338,368 animals.

Parameters

Parameter estimation was based on 357,531 lactations from 172,981 cows with 149,275 lactations belonging to parity 1 (104,878 from AMS, 44,397 from EMM), 103,552 belonging to parity 2 (78,657 from AMS, 24,895 from EMM) and 104,704 belonging to parity 3 and higher (81,970 from AMS, 22,734 from EMM). All cows were at least 87.5% Holstein. Parameters were estimated using an animal model.

Model

The statistical model used for resilience based on fluctuations in daily milk yield is:

$$Y_{ijklmnopqrst} = HYS_i + DIL_j + AFC_k + PAR_l + DM_m + KGM_n + HET_o + REC_p + INB_q + A_r + PME_s + Rest_t$$

In which:

Y observation on resilience on heifers (parity 1), young cows (parity 2) and cows (parity 3+);
HYS herd x year x season of first calving;
DIL length of lactation;
AFC age at first calving;
PAR parity number;
DM difference in milk yield compared to herd mean;
KGM average daily milk yield during the lactation;
HET heterosis effect;
REC recombination effect;
INB inbreeding effect;
A additive genetic effect;
PME permanent environmental effect;
Rest residual term of that which is not explained by the model of Y.

The effects A, PME and Rest are random, the effects HET, REC and INB are covariables, the other effects are fixed. AFC is only added to the model for parity 1 and parity 2, PAR is only added to the model for parity 3+.

DIL consist of seven classes, divided into periods of three years. The first class is between 50 and 90 days, where the number of days reflects the amount of days with a known 24h milk yield during the lactation. Each class consist of 40 days, and the seventh class is from 290 days and higher with data.

The variance of the deviation in milk yield is sensitive for the level of milk production of the cow. High yielding cows have a higher variance by nature. Therefore, DM and KGM are added to the model to correct for the level of milk production of the cow and the herd.

Results & Discussion

The descriptive statistics for recovery and stability are given in Table 1. These numbers are based on the April 2024 breeding value estimation, and data selection was done as described in E-47 (CRV u.a., 2024). The descriptive statistics are based on cows milked by AMS.

Table 1: Descriptive statistics (mean, standard deviation (sd), minimum (min.) and maximum (max.)) of the autocorrelation (recovery) and LnVar (stability) for parity 1 (p.1), parity 2 (p.2) and parity 3+ (p.3) based on cows milked by AMS.

Trait	Mean	SD	min.	max.
autocorrelation p.1	0.56	0.20	-0.21	0.98
autocorrelation p.2	0.56	0.19	-0.26	0.99
autocorrelation p.3	0.56	0.19	-0.32	0.98
LnVar p.1	1.57	0.67	-1.01	3.95
LnVar p.2	1.85	0.69	-0.74	4.30
LnVar p.3	2.06	0.70	-0.98	4.64

The mean observation for autocorrelation is equal over the different parities, while for LnVar there is a clear increase over the lactations. Higher values for autocorrelation and LnVar indicates less resilience, so younger cows have better observations on LnVar than the older cows (1.57 *vs.* 2.06).

When a cow has a drop in daily milk yield, the deviation between realized and predicted milk yield becomes large and negative. Many large deviations during the lactation will result in a high LnVar (poor stability), and many large negative deviations in a row will result in a high autocorrelation (long recovery). So, lower values for LnVar and autocorrelation indicates better recovery and stability because the realized daily milk yield is close to the predicted milk yield.

Genetic parameters

Table 2 shows heritabilities of the resilience traits. Table 3 shows the genetic correlations between the same traits, but measured on two different milking systems. Table 4 shows genetic correlations over the different parities within the same traits measured on cows milked by AMS.

The heritabilities in table 2 shows that stability has a higher heritability compared to recovery. Traits retrieved from AMS data have higher heritability compared to traits retrieved from EMM data.

Table 2: Heritabilities of recovery and stability in parity 1 (p.1), parity (p.2) and parity 3+ (p.3) for AMS and EMM observations.

Trait	AMS	EMM
recovery p.1	0.07	0.04
recovery p.2	0.04	0.03
recovery p.3	0.04	0.02
stability p.1	0.09	0.05
stability p.2	0.06	0.05
stability p.3	0.09	0.04

An AMS will measure all milk events of the cow, even when the cow is sick and treated with, for example, antibiotics. An EMM will not measure the milk yield of a cow when the cow is treated with antibiotics, because this milk is not going into the milk tank. Cows are normally only treated with antibiotics when they are ill, so affected by an environmental disturbance. This makes that in the EMM dataset data is missing that will bring most variance in the resilience traits. As a result, traits based on EMM data have lower heritabilities.

Table 3: Genetic correlations for the resilience traits in parity 1 (p.1), parity (p.2) and parity 3+ (p.3) between AMS and EMM observations.

	recovery	stability
p.1	0.76	0.90
p.2	0.63	0.88
p.3	0.35	0.91

The difference between resilience based on AMS or EMM observations is visible in the genetic correlations for recovery. In parity 1, the genetic correlations between recovery for cows milked by AMS or milked by EMM is 0.76. In parity 2, this genetic correlation declined to 0.63. In parity 3+ it is even lower, 0.35. Lower genetic correlations are found for the later parities because there will be more use of antibiotics for older cows.

Table 4: Genetic correlations for recovery and stability between parity 1 (p.1), parity (p.2) and parity 3+ (p.3) for AMS and EMM observations.

	recovery	stability
p.1 – p.2	0.98	0.98
p.1 – p.3	0.84	0.91
p.2 – p.3	1.00	0.98

The genetic correlations between different parities of the same trait are all high. The lowest correlations are found for the parities that are most apart from each other, namely parity 1 and parity 3+. For recovery, this genetic correlation is 0.84, and for stability it is 0.91. These genetic correlations indicate less reranking of animals between the different traits.

Health

The aim of developing breeding values for resilience is to captures all health factors in one trait. Table 5 shows the genetic correlations with other health traits and production, since production is still one of the most important traits in dairy breeding. The resilience traits in table 5 are overall breeding values, and are composed of parity 1, parity 2 and parity 2+ with a weight of respectively 0.41, 0.33 and 0.26.

Table 5: Genetic correlations for the overall resilience traits with production and other health traits.

trait	recovery	stability
milk production	-0.14	-0.36
fertility	0.08	0.31
ketosis	0.16	0.49
longevity	-0.06	0.33
metabolic disorders	0.14	0.48
claw health	-0.03	0.14
reproduction disorders	0.06	0.15
udder health	0.22	0.50

The model is correcting for the level of milk yield of the cow on a phenotypic level. Despite this correction, there is still a negative genetic correlation between resilience and milk production. High yielding cows are less resilient.

The health traits have positive genetic correlations with resilience. Only recovery has slightly negative correlations with longevity and claw health, but they are not significant different from zero. Highest genetic correlations between resilience and health traits are found for stability with fertility (0.31), ketosis (0.49), longevity (0.33),

metabolic disorders (0.48) and udder health (0.50).

Resilience on farm

The performances of dairy cows with breeding values for resilience were analyzed to check the validity of the breeding values. For recovery, the time it takes to recover from a drop in milk production was counted during a lactation. For stability, the number of drops in milking production during a lactation were counted.

This was done for cows with breeding values two standard deviations below average (low resilience, EBV 92), cows with a mean breeding value (average resilience, EBV 100) and cows with breeding values two standard deviations above average (good resilience, EBV 108). The results of this check are shown in table 6.

Table 6: On farm performances for cows with low (EBV 92), average (EBV 100) or high (EBV 108) breeding values for recovery and stability.

		EBV		
trait	unit	92	100	108
recovery	days to recover	14.0	10.9	7.0
stability	number of drops	4.8	3.8	2.4

Cows with high breeding values for recovery recover two times faster than cows with low breeding values, 7.0 *vs.* 14.0 days.

For stability is the same pattern visible. Cows with high breeding for stability have half of the number of drops in milk production compared to cows with lows breeding values, respectively 2.4 *vs.* 4.8.

Conclusions

Fluctuations in milk yield, retrieved from AMS and EMM systems, can be used as indicator to derive resilience traits. In the Netherlands and Flanders, two resilience traits are derived based on individual milk event data: recovery and stability.

Heritabilities are low for both recovery and stability, ranging from 0.04 to 0.09. The heritabilities for the same traits based on EMM data are slightly lower. Between AMS and EMM data, recovery is genetically different, whereas stability is genetically more equal.

Between parities are the traits genetically almost equal.

The resilience traits are positively correlated with other health traits, especially stability. Genetic correlations of stability with ketosis, fertility, metabolic disorders, longevity and udder health were highest ranging from 0.31 to 0.50.

Cows which are genetically resilient have less drops in milk production during a lactation and recover quicker when having a drop. Breeding for resilience leads to trouble-free animals, which can be seen as animal welfare, and an easy to manage herd.

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Cross-Validation Assessment of Random Regression Specifications in a Single-Step Genomic Model for Dry Matter Intake

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Abstract

Selection on feed efficiency traits can help to reduce costs and improve sustainability in the dairy cattle industry. Recent advances propose to use a random regression to derive breeding values for dry matter intake (DMI) from longitudinal models. In this study, we conduct a forward cross-validation of different random regression specifications of an animal model for DMI. The specifications combine basis functions for regression over days in milk with varying numbers of factors used in variance component estimation via a factor-analytic approach. Data from 10,766 predominantly Dutch and Belgian Holstein cows, comprising 21,008 lactations and 1,026,192 DMI records from 10 farms, were analyzed. Estimates obtained from partial data (pre-2020) were compared to those from the full dataset (up to early 2024). Multiple sets of focal individuals were used to estimate prediction errors for the models, decomposing global error summaries into intercept bias, slope bias, and correlation; for early, middle, and late lactation stages. The validation results identify random regression specifications that outperform the accuracy of a conventional repeatability model for DMI, in particular on the early and middle stages of lactation. This provides valuable insights for genomic prediction modeling of feed efficiency in cattle.

Key words: cross-validation, random regression, dry matter intake, dairy cattle

Introduction

Feed efficiency is an important trait in dairy cattle breeding due to its significant economic and environmental implications. Until at least 2017, the genetic trend for feed efficiency was slightly negative (Pryce and Bell, 2017; de Jong et al., 2019), primarily due to increased body size and associated maintenance feed requirements (de Jong et al., 2019). While debate continues on whether to include dry matter intake (DMI) directly in breeding goals or consider it through traits like residual feed intake (RFI) (Veerkamp et al., 2013), individual DMI recording remains essential for genetic improvement of feed efficiency in dairy cattle.

Direct measurement of DMI is expensive and logistically challenging (Berry et al., 2014). Genomic prediction models help to address this issue by enhancing the value of phenotypes recorded from genotyped cows. Furthermore, single-step genomic models enable efficient use of information from both genotyped and non-genotyped animals connected through pedigree. Despite these advantages, genetic models for DMI have typically been focused on repeatability models due to limited data availability.

As more DMI records accumulate, there is an opportunity to explore more complex random regression models (RRMs) that can account for changes in genetic effects across lactation. These models provide dynamic predictions of breeding values depending on lactation stage and can be used in conjunction

with predictions for energy sinks and sources (milk production and liveweight changes) to estimate recently proposed traits such as genomic residual feed intake (gRFI; Islam et al., 2020). Even when obtaining a gRFI is not the objective, RRMs can improve the utilization of records from cows at different lactation stages by accommodating higher correlations between records taken close together in time, while allowing for lower correlations between early and late lactation and across lactations (Veerkamp et al., 2013). In contrast, repeatability models assume a genetic correlation of unity and can over-estimate the amount of information for cows with sparsely recorded data.

In this study, we assess the efficacy of these random regression models and compare them to each other with a forward cross-validation technique. This process involves comparing estimates from partial datasets to those from complete datasets, thereby evaluating the predictive accuracy of the models. Through this empirical validation, the study aims to identify the most accurate random regression specifications for estimating breeding values for DMI, offering insights for genomic prediction models in feed efficiency.

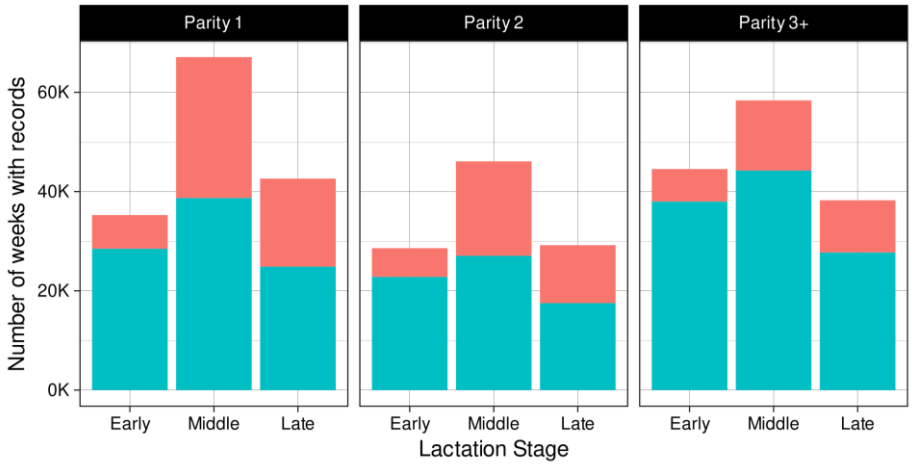
Materials and Methods

Feed Intake Data and Genetic Model

Data used in this study were routinely collected for the analysis of feed intake in dairy cattle in the Netherlands and northern Belgium. Individual feed intake was recorded at 10 facilities: the Dairy Campus of Wageningen Livestock Research, Schothorst Feed Research, ILVO Research Institute, feed companies Trouw Nutrition and AVEVE, and five commercial farms associated with CRV.

Cows with fewer than 3 DMI records and those with less than 50% Holstein breed composition were excluded. Records below 8 kg/day and above 55 kg/day were removed. The final dataset included 10,766 cows, 21,008 lactations, and 1,026,192 DMI records. Daily records were aggregated into 389,967 weekly averages (Figure 1).

Figure 1: Number of weeks with DMI records per parity and stages of lactation. In cyan, measurements recorded before 2020, in red measurements from 2020 onwards. Lactation divided into early (0-9 weeks), middle (10-24 weeks), and late (25+ weeks).



The following statistical model, based on research by Veerkamp et al. (2014), was used to calculate breeding values for DMI:

y = PAR + HM + HY + AGE + LS + B + PERM + A + Res

where:
y: Individual DMI (weekly average)
PAR: Parity, 3 levels (parity 1, 2, and 3+)
EXP: Experiment, a combination of farm and management/experiment effect
HM: Herd*month of calving
HY: Herd*year of calving
AGE: Age at calving per parity, quadratic polynomial
LS: Lactation stage (Days in milk), 4th order polynomial
B: Breed % of the second breed, intercept and slope
PERM: Permanent environment of animal
A: Breeding value of animal
Res: Residual

The random effects PERM and A were specified with random regression models, as described in the next section. For the animal effect A, the numerator relationship matrix was used for pedigree-based models (Henderson, 1976). For single-step genomic models, marker information was integrated following the method of Liu et al. (2014), using a ssSNPBLUP model fitted with the hpblup solver in MiXBLUP (Vandenplas et al., 2022).

Random Regression Specifications

Four random regression model structures were evaluated on days in milk:
1. Repeatability
2. Piecewise-constant
3. Linear
4. Cubic

The repeatability model structure is the simplest, similar to those currently used in genetic evaluations, with a single breeding value for each parity of the cow. The remaining models allow the breeding values of an individual animal to change over days in milk, within the same lactation. The piecewise-constant model can be considered a multi-trait model, where DMI is divided into six different traits depending on the stage of lactation. The linear and cubic models are typical random regression models, modeling the varying breeding value as a polynomial curve of the corresponding order.

All models can be formulated as random regressions, differing only in the basis functions used:
- The repeatability model uses a single constant basis function.
- The piecewise-constant model uses an indicator function for each stage of lactation.
- The linear and cubic models use Legendre polynomial basis functions of degree 1 and 3, respectively.

Variance components for each model were estimated using ASReml (Gilmour, 2019). Except for the repeatability model, variance component estimation was simplified using a factor-analytic approach, iteratively increasing the number of factors until model likelihood stopped improving.

Cross-validation Scheme

A forward cross-validation scheme was used to assess the predictive accuracy of the models. Data were split into partial (records before 2020) and whole (records up to early 2024) datasets. Breeding values for DMI were predicted from the partial dataset and compared to corresponding breeding values from the whole dataset (similarly to Legarra and Reverter, 2018). Each lactation was divided into early (weeks 5 and 10), middle (weeks 15 and 25), and late (weeks 35 and 45) periods for the validation.

To overcome the limitation of each model being validated against itself, a common reference model (piecewise-constant) was selected, and partial predictions of each model

were compared to the whole predictions of this reference model.

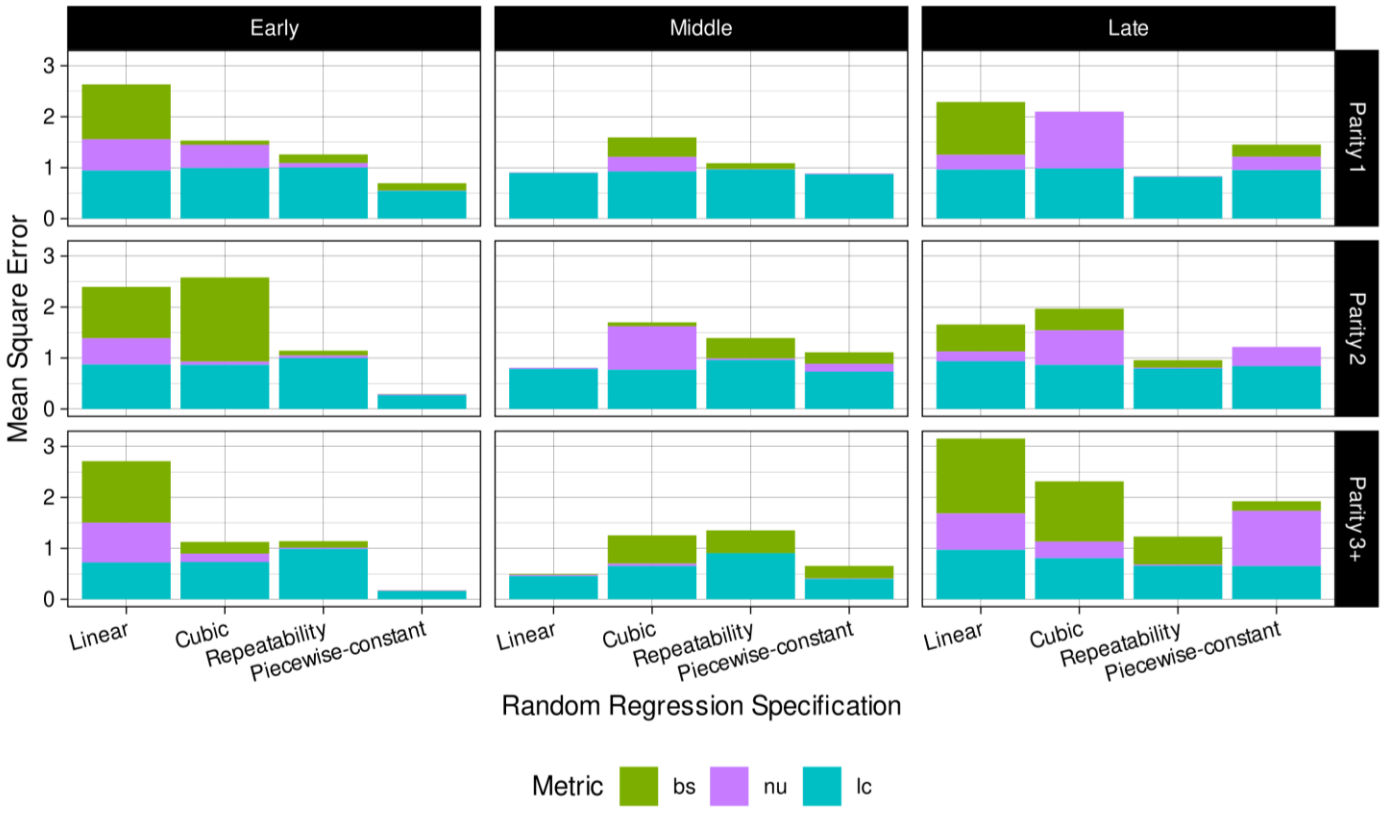
Validation was performed for multiple sets of focal individuals, with metrics reported here for validation cows (the largest focal group). Validation cows were defined as those with at least 3 DMI records after January 1st, 2020, and no DMI records before that date, consisting of 2,958 cows.

Following Gauch et al. (2003), the following validation metrics were calculated (Table 1): Bias Squared (BS), Non-unity of slope (NU), Lack of Correlation (LC), and their sum which equals the Mean Squared Error (MSE).

Table 1: Validation metrics used in this study. Where, 'a' are breeding values for DMI, subindices 'p' and 'w' indicate the partial and whole dataset, respectively.

Validation metric	Quantifies	Related Variable	Formula
Bias Squared (BS)	Level bias	Intercept (b_0)	$(\bar{a}_w - \bar{a}_p)^2$
Non-unity of Slope (NU)	Inflation/deflation	Slope (b_1)	$(1-b_1)^2 Var(a_p)$
Lack of Correlation (LC)	Accuracy	Correlation (r)	$(1-r^2)Var(a_w)$
Means Square Error (MSE)	All discrepancies	BS + NU + LC	$\Sigma_i (a_w(i) - a_p(i))^2$

Figure 2: Validation metrics for different random regression specifications with pedigree-based models. Lactation divided into early (0-9 weeks), middle (10-24 weeks), and late (25+ weeks).



Results and Discussion

Comparison of the four random regression models for predicting DMI breeding values, using pedigree-based models and the piecewise-constant model as a reference, revealed that the piecewise-constant model showed the best overall performance (Figure 2). The piecewise-constant model had the lowest MSE (0.48) and highest correlation (0.41) between predicted and observed values.

The linear model performed relatively well in mid-lactation but poorly in early and late lactation periods. Most of the MSE in early and late lactations was due to intercept and slope bias rather than lack of correlation, suggesting that true genetic effects on DMI are non-linear across days in milk, and the linear model lacks the flexibility to capture these changes.

The cubic model showed variable performance across lactation stages, with the highest MSE in early lactation for second parity and the lowest in middle and late lactation for 3+ parities. The general lack of improvement over the linear model suggests that pedigree information alone is insufficient to predict varying breeding values accurately with a cubic regression, given the current data availability.

The repeatability model showed low bias overall but low accuracy for early and middle lactation periods. The low bias indicates that the typical curve for the true genetic effects does not deviate greatly from the constant breeding value assumed in the repeatability model. However, the lack of correlation in early and middle lactation suggests that the absence of distinctions between different stages of lactation in the repeatability model impacts the predictive accuracy.

Prediction was generally most challenging in early lactation, with the piecewise-constant model showing the lowest MSE in this period. This may be due to metabolic changes during the transition period and reduced data availability in early lactation compared to middle lactation. Late lactation was easier to predict, though it is unclear whether this is due to the pattern of data available for validation cows or a more stable metabolic state at this stage.

Inclusion of genomic information in single-step random regressions (for linear and cubic models) improved both stability within models and consistency across models (Table 2). This improvement was more pronounced for the more complex cubic model (65% vs. 30% improvement in stability, 45% vs. 21% in consistency) compared to the linear model. This suggests that the more efficient use of available records with genomic information might allow for effective use of a polynomial model, contrary to observations with pedigree-based models.

Table 2: Correlations for pedigree and single-step genomic models, between estimated breeding values in partial and whole datasets.

		Validation Target	
Model	Regression	Linear	Cubic
Pedigree	Linear	0.39	0.28
	Cubic	0.29	0.26
Single-step	Linear	0.51	0.34
	Cubic	0.42	0.43

Conclusions

This study demonstrates the potential for improving genetic evaluation of DMI in dairy cattle using more flexible random regression models compared to simple repeatability models. The piecewise-constant approach appears promising, though it may be beneficial to further refine the lactation periods over which prediction is constant in this model. With single-step genomic models, a polynomial random regression model may sufficiently model genetic changes throughout lactations.

Future work could explore additional model structures such as splines, evaluate uncertainty in validation metrics, and combine results across different focal groups. As DMI data continues to accumulate, a reassessment of

model comparisons and optimal recording periods can be useful to ensure optimal use of available information for genetic improvement of feed efficiency.

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Using cow carcass weight to select efficient cows in UK

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Abstract

Breeding more efficient cows is important for both increased profitability and reduced environmental impact. Therefore, there is a need to estimate genetic merit for feed intake of cows. While direct measurement of feed intake is difficult, maintenance requirements which accounts for one third of the energy intake of a cow, can be adequately approximated using body weight. Mature cows are not usually weighed, but abattoirs do collect carcass weights of cull cows. Carcase weight varied between 268 kg and 400 kg. Heritability estimates of carcass weight, conformation and fat class of mature cows were calculated. Mature cows between 1 095 and 7 301 days of age were included in the study. A total of 4 721 cows with weight phenotypes were included, born between 1997 and 2020. A mixed linear animal model was fitted considering the cow, parity of the cow's dam, number of calvings per cow, breed and season of birth as fixed effects and coefficient of heterosis and recombination loss estimated from four breed groups as covariate effects. The study cows were traced back up to five generations in the pedigree that include 67 641 animals in total. The heritability estimates were generated using ASReml. The estimated heritabilities were 0.64 ± 0.01, 0.49 ± 0.10 and 0.44 ± 0.01 for carcass weight, carcass fat and conformation, respectively. The moderate to high heritability estimates observed in this study indicates there is cull cow carcass weight genetic variation to allow for genetic improvement and that when data for direct feed intake is limited, this trait in the meantime could be used as a proxy for cow feed intake and consequently, predicted methane emissions.

Key word: Carcass trait, Genetic parameter, Heritability, Mature cow

Introduction

Compared to historic breeding goals which focussed on increased production alone, increasing milk yield in dairy and growth rates in beef, modern breeding goals aim to increase overall efficiency and increasing production per unit of input. The impact of selection for increased production levels can be seen across dairy and beef across the globe. For example, in the past 50 years, the annual milk yield per cow increased from 1 000 kg to >11 000 kg in Canada and from 4 000 kg to ~12 000 kg in US (Cole et al., 2023). In the same period in the US, beef production increased by 25%, even while the number of cattle destined for beef production decreased by 6%, the latter percentage has been countered by a more than 30% increase in average cattle (mainly steers and heifers) weights (USDA, 2019).

However, the positive genetic correlations between both milk yield and growth rates with animal size (e.g. cow) (Ouataha et al., 2021) imply that selection focussed on production has increased the average mature cow size across beef and dairy (Rowan, 2022). Although these heavier cows have some benefits, including less ketosis, metabolic, infectious, and other diseases than lighter cows (Frigo et al., 2010), they also have increased feed requirements (Liinamo et al., 2012), meaning they cost more to feed and have a greater environmental impact (IPCC, 2019). For example, in dairy production, feed accounts up to 60% of the operating cost (European Commission, 2013). On the other hand, animals that consume more

feed tend to produce more methane (CM4) on a daily basis (Crompton et al., 2011; de Haas et al., 2014). These all imply that our previous selection goals may have led to less efficient cows.

In order to breed for efficiency, a measure of cow size can be included as a trait in a selection index. Various strategies are used to measure cow size. Both liveweight and linear body measurements have been also used as selection indices for beef production in different countries like New Zealand, Australia and US. The use of some measures of body size and other linear body measurements instead of liveweight are used whenever there is absence of liveweight data (Haile-Mariam et al., 2014) as there are situations where animals do not have either liveweight records or any linear body measurements. This absence of liveweight records is very common for cows. However, interestingly abattoirs in countries like UK collect records for carcass traits of the animals slaughtered. These records can be used as a proxy for cow feed intake and consequently to predict methane emissions, liveweight prediction, genetic parameters estimation to help understand the genetic merits for cow size for efficient cows and evaluate breeding values of the carcass traits that can be later utilized for selection and improvement purposes by considering the traits in the selection index. In this preliminary study we estimated heritabilities for carcass weight, carcass conformation and carcass fat for mature cows of different breeds combined in UK.

Materials and methods

In this study, cows that include multiple beef, dairy and cross breeds with age in days above 1 095 days were considered as mature cows (Figure 1). Carcass traits that include carcass weight, carcass conformation and carcass fat were evaluated. Carcass conformation and fat were scored as the EUROP carcass classification (EEC Regulation N.1208/81 and N.2930/81; details present at Englishby et al., 2016). Carcass weight varied between 268 kg and 400 kg, defined based on the mean and standard deviation (μ ± SD) of extracted data, were considered in the study (Table 1). This range of the carcass weight is equivalent to the liveweight between 487 kg and 727 kg in an assumed killing out percentage of 0.55. The box plot distribution of the carcass weight by breed, parity of the cow's dam and number of calvings per cow is presented at the figure below (Figure 2). Heritability estimates of carcass weight, conformation and fat class of the mature cows (n = 4 721) were evaluated. These animals were born between 1997 and 2020 (Figure 3). Mixed linear animal model was fitted as follows considering sources of the cows, parity of the cow's dam, number of calvings per cow, breed and season of birth as fixed effects. In addition, heterosis and recombination loss estimates generated from four breed groups were fitted as covariate effects.

$$Y_{ijklmnopqr} = \mu + S_i + P_j + C_k + B_l + Se_m + H_{1n} + \ldots + H_{6n} + R_{1p} + \cdots + R_{6p} + a_q + e_{ijklmnopqr}, \quad (1)$$

where, $Y_{ijklmnopqr}$= the analysed trait; μ = the overall mean; S_i is i^{th} source of the cows; P_j = j^{th}parity of the cow's dam (j = 1, …, 7; parities > 7 merged into the 7th parity); C_k is k^{th} number of calvings per cow (k = 1, …, 10; number of calvings > 10 were merged in to 10th calving); B_l is l^{th} breed (l = LIMX, CH, SMX, BRBX, AAX, HEX, BBX, BF, HF, LIM, HFX and HO); Se_m = m^{th} is season of birth of the cows (m = March–May, June–August, September–November, and December–February); H_{1-n} is estimates of coefficient of heterosis generated from four breed groups considered as covariate effect in the model; R_{1-n} is estimates of coefficient of recombination loss generated from four breed groups considered as covariate effect in the model; $a_q$is the random additive genetic effect of cow q with var (a), ~ ND (0, $\boldsymbol{A}\delta_a^2$), where ND is normally distributed, δ_a^2 is the additive genetic variance, $\boldsymbol{A}$ is the additive relationship

matrix using pedigree information that was traced back five generations for 67641 animals in total; and $e_{ijklmnopqr}$ is the random residual variance with var (e), ~ *IND* (0, δ_e^2), where δ_e^2 is the residual genetic variance. The variance components were estimated using ASReml (Gilmour et al., 2015), and used to evaluate heritability estimates as:

$$h^2 = \delta_a^2/(\delta_a^2 + \delta_e^2) \quad (2)$$

The coefficient of heterosis (*Het*) and recombination loss (*Rec*) were calculated for all animals using the formulae derived by VanRaden and Sanders (2003):

$$Het = 1 - \sum_{k=0}^{n} Sire_i \; x \; Dam_j \quad (3)$$

$$Rec = 1 - \sum_{k=0}^{n} (Sire_i^2 + Dam_j^2)/2 \quad (4)$$

where, $Sire_i$ and Dam_j are the proportion of breed i and breed j in the sire and dam, respectively

Table 1. Descriptive statistics of carcass weight by breed

Breed*	N	µ±SD	Max.	Min.
HF	738	319.26 ± 31.60	399.8	268.2
LIM	695	338.97 ± 33.84	399.9	269.5
HFX	673	320.48 ± 32.88	399.7	268.1
BRBX	736	348.20 ± 32.83	400.0	268.2
HEX	766	326.30 ± 33.57	399.3	268.3
AAX	734	330.38 ± 34.84	399.9	268.4
BBX	798	342.97 ± 32.62	399.9	269.3
HO	738	322.53 ± 32.62	399.8	268.3
CH	435	351.65 ± 34.23	400.0	268.1
BF	664	314.58 ± 29.78	398.1	268.1
LIMX	791	338.95 ± 33.14	400.0	268.1
SMX	796	334.82 ± 33.75	399.8	269.1

*LIMX=Limousin cross; CH=Charolais; SMX=Simental cross; BRBX=British Blue Cross; AAX=Aberdeen Angus Cross; HEX=Hereford Cross; BBX=Belgian Blue cross; BF=British Friesian; HF=Holstein-Friesian; LIM=Limousin; HFX=Holstein-Friesian cross; HO=Holstein; Max= maximum; Min=minimum

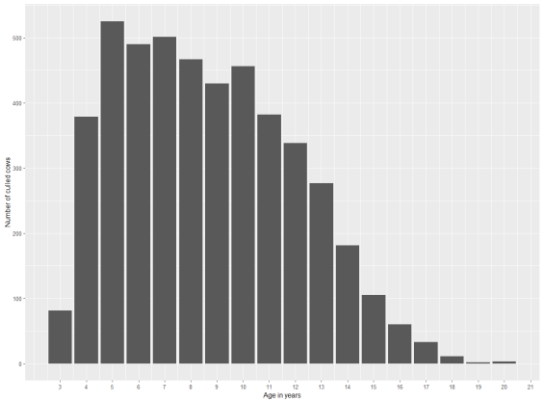
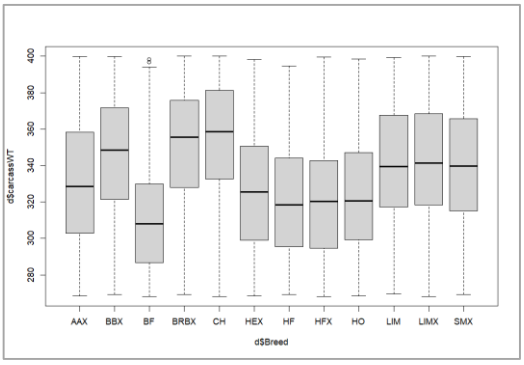
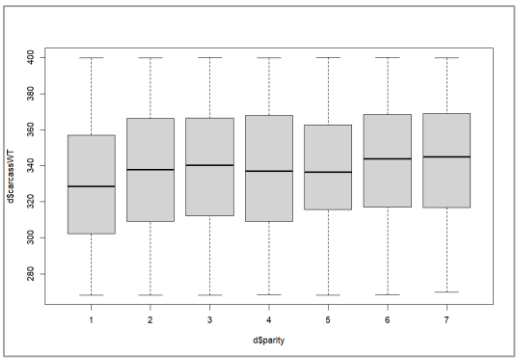


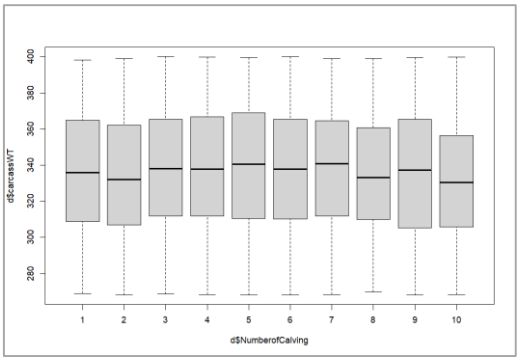
Figure 1. Distribution of culled mature cows by age at slaughter



a)



b)



c)

Figure 2. Distribution of carcass weight by: a) breed; b) parity of the cow's dam; c) number of calvings per cow

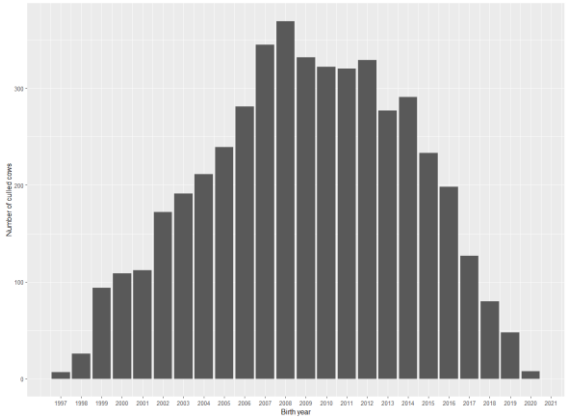


Figure 3. Distribution of culled mature cows by year of birth

Results and Discussion

The average carcass weight considering all breeds was 332.42 ± 33.0 kg. The breakdown of average carcass weight by breed is present in the table (Table 1). It is not a surprise that the beef origin mature cows showed heavier carcass weight over the dairy type, as carcass weight reflects lifetime growth (Pabiou et al., 2011a) and lifetime growth varies between breeds and breed types. Heritability estimates of the carcass traits are depicted in the table (Table 2). The result indicated high heritability estimate ($h^2 = 0.64$) for carcass weight of mature cows, followed by carcass fat ($h^2 = 0.49$) and carcass conformation ($h^2 = 0.44$). The genetic parameter estimates for carcass conformation and carcass fat obtained in the present study go in line with the previous studies reported for other cattle populations (Kause et al., 2015; Pabiou et al., 2009, 2011b). Similarly for carcass weight, comparable heritability estimate was reported for Black cattle in Japan ($h^2 = 0.70$) (Hoque et al., 2006) and Charolais sire groups in Ireland ($h^2 = 0.65$) (Hickey et al., 2007). Whereas, in breed specific evaluation of the beef breeds, moderate heritabilities were reported for carcass weight (h^2 = 0.39 to 0.48), for conformation (h^2 = 0.30 to 0.44) and carcass fat (h^2 = 0.29 to 0.44) in Finland (Kause et al., 2015).

Table 2. Heritability (h^2) and standard error (SE) estimates of carcass traits of mature cows

Trait	Variance components		$h^2 \pm SE$
	δ_a^2	δ_e^2	
CWT[1]	664.778	375.357	0.64 ± 0.01
CC[2]	30.9875	38.6982	0.44 ± 0.01
FC3	22.6136	23.5714	0.49 ± 0.10

[1]CWT=Carcass weight; [2]Carcass conformation; [3]FC=Fat class; δ_a^2= Additive variance; δ_e^2= Residual variance; h^2 = heritability; SE=Standard error

However, compared with the current study lower and wider range of estimates of heritability were reported for carcass traits (carcass weight: h^2 = 0.24 to 0.42; conformation: h^2 = 0.08 to 0.34; fat score: h^2 = 0.16 to 0.40) for Irish beef herds that included heifers, steers and young bulls (Englishby et al., 2016) where the highest heritability estimate was observed for heifers (age in days: 420 to 970 days) compared to steers and young bulls. Similar range of heritability estimates for carcass conformation and fat were previous reported for sire groups of eight beef breeds in Irland (Hickey et al., 2007) unlike to the highest heritability estimates (Carcass conformation: h^2 = 0.78; carcass fat: 0.63) for pooled data of dairy and beef breeds still in Irland in later study (Pabiou et al., 2009).

In the current study we observed that the carcass traits evaluated are highly heritable and this suggests in helping to improve and maximize the response to selection if the carcass traits are considered in the breeding program. However, we pooled the data set from different breeds together that may shadow to provide full picture of breed specific evaluation as there is huge variation between breeds on the heritability estimates of these traits (Hickey et al., 2007; Pabiou et al., 2009; Englishby et al., 2016).

Overall, to our knowledge evaluating genetic parameters for mature cows is the first work that could provide insights on the importance of this group of animals for efficient cow selection specially when data for direct feed intake is limited. Moreover, the carcass traits for this group of animals demonstrated

high heritability and may encourage to use for the purpose of genetic evaluation in the breeding programs. However, evaluating these traits for each breed separately could help to provide breed specific estimates for effective breed specific breeding management decision. It is also important to note that the carcass data collected in UK abattoirs can serve as a proxy of cow liveweight prediction and feed intake.

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A weekly genomic evaluation of newly genotyped selection candidates based on a single-step genomic model

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Abstract

A single-step SNP BLUP model was developed for routine genomic evaluation of German Holstein. The current weekly genomic evaluation of young selection candidates based on a multi-step SNP BLUP model needed to be upgraded to optimally use the effect estimates from the single-step model. For indirect genomic prediction of newly genotyped selection candidates, two alternative statistical methods were assessed, an exact GRV method and a summation method. Both methods calculated direct genomic values using the SNP effect estimates from the full evaluation in the same way, but they differed in the computation of residual polygenic effects for the young candidates. GEBV of the candidates from the two methods were then compared to those from a single-step evaluation using phenotypic, genotypic and pedigree data from April 2023. To investigate the accuracy and bias of the two weekly evaluation methods, all 1,318,720 genotyped Holstein animals were divided into a reference set containing 1,169,502 animals born before April 2022 and a validation set of 149,218 animals born after April 2022. For all 69 evaluated traits in the German dairy cattle evaluation, correlation of GEBV of the weekly evaluation with the full evaluation was unity for the exact GRV method and ranged from 0.996 to 1 for the summation method. The regression coefficient of GEBV the full evaluation on the weekly evaluation was 1 for the exact GRV method and ranged from 0.988 to 1.002 for the second summation method. The two statistical methods for the indirect prediction of young candidates were shown to be accurate and unbiased.

Key words: indirect prediction, genomic evaluation, single-step model, selection candidates

Introduction

An indirect prediction of genomic breeding values (GEBV) for newly genotyped selection candidates at a weekly basis provided a key service for routine genomic selection in German Holstein (Alkhoder et al. 2014). In contrast to a full genomic evaluation, based on either a multi-step model (MSM, Liu et al. 2011) or a single-step model (SSM, Liu et al. 2014), the weekly genomic evaluation does not have any new phenotypic records added to evaluate but only new genotypic data of typically young animals. Therefore, the SNP marker effect estimates from the latest full genomic evaluation can be used to calculate direct genomic values (DGV) of the newly genotyped animals. Under the model MSM, a parental average (PA) of conventional evaluation was estimated via a BLUP animal model and was then combined with DGV using the selection index approach to obtain genomic estimated breeding values (GEBV) of the young candidates. For the SSM model, Liu et al. (2014) showed that GEBV of a newly genotyped animal be equal to the sum of DGV and parental average of residual polygenic effect (RPG, Liu et al. 2011).

A full single-step genomic evaluation including genotypes of new animals provides the most accurate GEBV for the newly genotyped animals. However, it is infeasible to complete the full single-step evaluation with millions of genotyped animals for all trait groups during a weekend. Therefore, the single-step weekly evaluation must be

computationally fast while ensuring GEBV being as accurate as possible.

The aims of this study were 1) to compare two statistical methods for an indirect prediction of GEBV of newly genotyped animals; and 2) to investigate accuracy and bias of the indirect prediction methods via a validation study.

Statistical methods for indirect prediction of GEBV for candidates

For the single-step SNP BLUP (ssSNPBLUP) model with an RPG effect (Liu et al. 2014), GEBV of a genotyped animal is the sum of its two components DGV and RPG:

$$u = d + a \qquad [1]$$

where u is GEBV, d is DGV, and a is RPG of the animal. GEBV of a newly genotyped young candidate after the full single-step evaluation can be approximated based on estimates of all model effects from the latest full single-step evaluation (Liu et al. 2014):

$$u_c = d_c + a_c = d_c + \tfrac{1}{2}(a_s + a_d) \qquad [2]$$

where u_c is GEBV of the genotyped candidate, d_c is its DGV, a_c is its RPG, a_s a_d represent RPG of its sire and dam, respectively, that were evaluated in the latest full single-step evaluation with their own genotype data. Note that the models [1] and [2] are a univariate model or single-trait model, not like a multi-lactation random regression test-day model for a full single-step evaluation of milk production traits in German dairy cattle (Alkhoder et al. 2024).

The ssSNPBLUP model estimated RPG for all genotyped animals, with or without their own phenotypic data in the latest, full single-step evaluation. However, for the young, genotyped animal that was not included in the latest single-step evaluation, its RPG effect was assumed be equal to its expected value of parental average of RPG, $\frac{1}{2}(a_s + a_d)$.

An Exact Method for GEBV of New Animals

In contrast to the single-step genomic BLUP model (ssGBLUP, Aguilar et al. 2010), the ssSNPBLUP provided direct estimates of SNP marker effects that can be used to calculate DGV of all newly genotyped animals.

The RPG effects of the newly genotyped animals can be estimated using RPG effect estimates of all genotyped animals in the latest single-step evaluation (Liu et al. 2016):

$$\widehat{\boldsymbol{a}}_c = \mathbf{A}_{\mathrm{cg}}\,\mathbf{A}_{\mathrm{gg}}^{-1}\boldsymbol{a}_{\mathrm{g}} \qquad [3]$$

where $\widehat{\boldsymbol{a}}_c$ is a vector of estimated RPG effects of all new genotyped candidates, $\boldsymbol{a}_{\mathrm{g}}$ is a vector of RPG effects of all genotyped animals in the latest single-step evaluation, $\mathbf{A}_{\mathrm{gg}}^{-1}$ is the inverse of pedigree relationship matrix for all the genotyped animals of the latest evaluation and $\mathbf{A}_{\mathrm{cg}}$ is the pedigree relationship matrix between the new candidates and the old, genotyped animals. This statistical method for indirect prediction of the RPG, together with the calculation of DGV, was termed as an *exact GRV method* (Vandenplas et al. 2023).

The RPG effects for the new selection candidates via Equation [3] are estimated by setting up the following equations:

$$\begin{bmatrix} \mathbf{A}^{00} & \mathbf{A}^{0\mathrm{g}} & \mathbf{A}^{0\mathrm{c}} \\ \mathbf{A}^{\mathrm{g}0} & \mathbf{A}^{\mathrm{gg}} & \mathbf{A}^{\mathrm{gc}} \\ \mathbf{A}^{\mathrm{c}0} & \mathbf{A}^{\mathrm{cg}} & \mathbf{A}^{\mathrm{cc}} \end{bmatrix} \begin{bmatrix} \widehat{\boldsymbol{a}}_0 \\ \widehat{\boldsymbol{a}}_g \\ \widehat{\boldsymbol{a}}_c \end{bmatrix} = \begin{bmatrix} \mathbf{0} \\ \mathbf{A}_{\mathrm{gg}}^{-1}\widehat{\boldsymbol{a}}_g \\ \mathbf{0} \end{bmatrix} \qquad [4]$$

where $\widehat{\boldsymbol{a}}_0$ is a vector of RPG effects of for ancestors of the genotyped animals from the latest single-step evaluation and the new selection candidates. Solving the Equation [4] is technically equivalent to deregress the RPG effect estimates of the genotyped animals $\boldsymbol{a}_{\mathrm{g}}$ without using the inverse matrix $\mathbf{A}_{\mathrm{gg}}^{-1}$ but the Henderson's inverse of the pedigree relationship matrix for the three groups of animals:

$$\begin{bmatrix} \mathbf{A}^{00} & \mathbf{A}^{0\mathrm{g}} & \mathbf{A}^{0\mathrm{c}} \\ \mathbf{A}^{\mathrm{g}0} & \mathbf{A}^{\mathrm{gg}} & \mathbf{A}^{\mathrm{gc}} \\ \mathbf{A}^{\mathrm{c}0} & \mathbf{A}^{\mathrm{cg}} & \mathbf{A}^{\mathrm{cc}} \end{bmatrix} = \begin{bmatrix} \mathbf{A}_{00} & \mathbf{A}_{0\mathrm{g}} & \mathbf{A}_{0\mathrm{c}} \\ \mathbf{A}_{\mathrm{g}0} & \mathbf{A}_{\mathrm{gg}} & \mathbf{A}_{\mathrm{gc}} \\ \mathbf{A}_{\mathrm{c}0} & \mathbf{A}_{\mathrm{cg}} & \mathbf{A}_{\mathrm{gg}} \end{bmatrix}^{-1} \qquad [5]$$

The deregression process, without generating deregressed RPG effects for the genotyped animals g, estimates RPG effects of the ancestors denoted as group 0, that is equivalent to solving:

$$\widehat{\boldsymbol{a}}_0 = -(\mathbf{A}^{00})^{-1}\,\mathbf{A}^{0\mathrm{g}}\,\boldsymbol{a}_{\mathrm{g}}\,. \qquad [6]$$

From Equation [4], we can see that the RPG effects of the new candidates, $\boldsymbol{a}_c$, are estimated with the (deregressed) RPG effects of the genotyped animals via the pedigree [5].

A Summation Method for GEBV of New Animals

GEBV of the newly genotyped selection candidates are computed using the Model [1], as with the exact GRV method. However, a simpler method is assumed here for calculating the RPG effects of the new candidates, namely a linear summation of RPG effects of all genotyped ancestors from the latest single-step evaluation. When both parents of a new candidate *c* were evaluated with own genotype data in the latest single-step evaluation, $a_c = \frac{1}{2}(a_s + a_d)$. Should only male animals be genotyped in a population, then $a_c = \frac{1}{2}(a_s + \frac{1}{2}(a_{mgs} + \frac{1}{2}(a_{smgd} + \cdots)))$, where a_{mgs} is RPG effect of maternal grandsire of the candidate, and a_{smgd} is RPG effect of sire of maternal granddam of the candidate. In practice, the RPG of the candidate a_c is calculated by processing the pedigree from the youngest candidate to its oldest genotyped relatives for the summation. Ancestors having no genotype data in the latest single-step evaluation were assumed to have RPG effect being 0 in this process. The summation method for computing RPG effects of the new candidates may be described as:

$$\hat{\boldsymbol{a}}_c = \mathbf{A}_{\mathrm{cg}}\,\mathbf{I}\boldsymbol{a}_{\mathrm{g}} \qquad [7]$$

where **I** is an identity matrix.

Data materials for a comparison of the indirect prediction methods

Genotypic, phenotypic and pedigree data from the April 2023 single-step evaluation were used to investigate accuracy and bias of the two indirect prediction methods. A total of 1,318,720 genotyped German Holstein population were divided into two groups: 1,169,502 '*reference animals'* born in March 2022 and earlier, and 149,218 '*genotyped candidates'* born from April 2022 onwards. The pedigree for all animals of the two groups contained 3,427,852 animals, including 2,109,132 non-genotyped ancestors.

In the single-step evaluation for German Holstein, a total of 69 single traits or indices of evaluated traits were evaluated. For instance, a total of 9 random regression coefficients of a multi-lactation random regression test-day model (Alkhoder et al. 2024) were combined into a single value, 305-day milk yield on a combined lactation basis. The weekly genomic evaluation was conducted for milk yield on the 305-day combined lactation basis instead of the 9 random regression coefficients.

Results & Discussion

Estimates of SNP markers for the 69 traits or indices were obtained from the latest single-step evaluation with data from April 2023. The RPG effects for the genotyped candidates were computed using the two statistical methods: the exact GRV method and the summation method. The program *predict_GEBV* of the MiX99 software suite (Strandén and Lidauer 1999) was used to compute GEBV of the young candidates with the exact GRV method (Vandenplas et al. 2023). Our own software for the summation method was developed in python. For all the 69 traits or indices, the GRV method took 38 minutes on 46 cores simultaneously and the peak RAM usage was 15.5 Gb.

Table 1 shows correlations of GEBV with DGV and RPG for the reference animals as well as regression slopes of GEBV on DGV or RPG for the genotyped animals in the full single-step evaluation April 2023 for all the 69 traits or indices. Similarly, the correlations and regression slopes are given in Table 2 for the genotyped selection candidates. In general, GEBV is higher correlated with DGV than RPG for either group of the genotyped animals. Regression slope values of GEBV on DGV are close to 1, on average, for both groups of the animals, whereas the average regression slopes of GEBV on RPG deviate more from 1.

Table 1: Correlations and regressions of GEBV, DGV and RPG estimates of the reference animals for all 69 traits or indices

	Average	Minimum	Maximum
Correlation of GEBV with DGV			
	0.969	0.935	0.986
Regression slope of GEBV on DGV			
	1.05	0.990	1.138
Correlation of GEBV with RPG			
	0.406	0.248	0.650
Regression slope of GEBV on RPG			
	1.64	0.886	3.169

Table 2: Correlations and regressions of GEBV, DGV and RPG estimates of the genotyped selection candidates for all 69 traits or indices

	Average	Minimum	Maximum
Correlation of GEBV with DGV			
	0.976	0.944	0.991
Regression slope of GEBV on DGV			
	1.024	0.974	1.118
Correlation of GEBV with RPG			
	0.304	0.064	0.589
Regression slope of GEBV on RPG			
	1.434	0.324	3.102

To validate GEBV of the weekly genomic evaluation, GEBV of the new candidates from the full single-step evaluation were correlated with their GEBV from the weekly genomic evaluation. Figure 1 shows the GEBV correlations of the selection candidates between any of the three evaluations: the weekly genomic evaluations with the exact GRV and the summation methods, and the latest full single-step evaluation. It can be seen in Figure 1 that the exact GRV method has a unity correlation with the latest single-step evaluation for all the 69 traits or indices. As far as the summation method for the weekly evaluation is concerned, its GEBV correlations with the single-step evaluation ranged from 0.9970 to 0.9999 with a mean of 0.9995. The GEBV correlations between the two methods for the weekly evaluation have an average of 0.9994.

GEBV of the candidates from the latest single-step evaluation were regressed on those from the weekly genomic evaluation based on either of the method: the exact GRV or summation method. Figure 2 shows the regression slope values of the two weekly evaluation methods for all the traits or indices. The regression slope values of the exact GRV method ranged from 0.9987 to 1.0008 with an average of 0.9998. In comparison, the summation method has a regression slope value between 0.9872 to 1.0018 with a mean of 0.9981 for the 69 traits or indices.

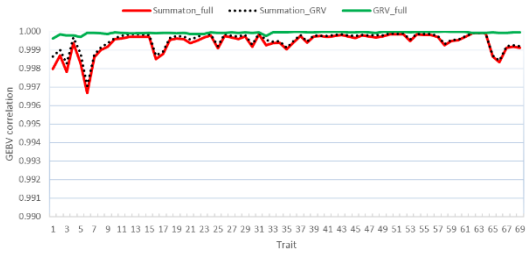

Figure 1. Correlations of GEBV of the candidates using the exact GRV and summation methods with the latest single-step evaluation for all the traits or indices.

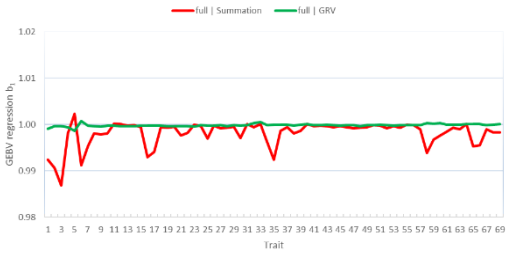

Figure 2. Regression of the latest single-step GEBV of the candidates on that of the exact GRV or summation method for all the traits or indices.

GEBV bias of the weekly genomic evaluation

In addition to the GEBV correlations and regressions of the weekly genomic evaluation methods, GEBV bias, defined as GEBV of the weekly evaluation minus that of the latest single-step evaluation, was investigated for all the selection candidates.

Figure 3 shows the frequency distribution of the GEBV biases of all the 149,218 candidates for trait no. 3 which was under the highest selection pressure among all the 69 traits or indices. A total of 87% or 67% of all the candidates had no bias, i.e., GEBV of the weekly evaluation being equal to that of the

single-step evaluation, for the exact GRV or the summation method, respectively. The distribution of the GEBV bias was symmetric around zero for both weekly evaluation methods. However, for the summation method about 5.4% of all the candidates had a downward bias of lower than -20% of genetic standard deviations of the trait no. 3. The downward bias was caused by the fact that some ancestors of the candidates did not have genotypic data in the latest single-step evaluation, and the summation method assumed RPG of those ancestors being zero. Due to the high selection pressure on this trait, those ancestors might have had an RPG greater than zero, if they had been genotyped.

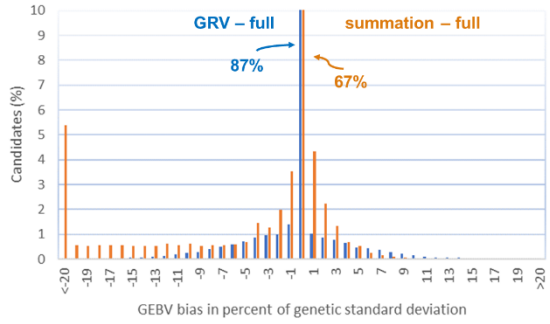

Figure 3. Distribution of GEBV bias of all the candidates using the two weekly evaluation methods for the trait no. 3 under the highest selection pressure.

To further investigate the impact of ancestors having no own genotype data in the full single-step evaluation, we selected a trait with little selection pressure on it, trait no. 4. The distribution of GEBV bias is shown for all the candidates in Figure 4. In contrast to Figure 3, no candidates have a downward GEBV bias for this trait as for the trait no. 3.

Compared to the summation method, the exact GRV method did not have the group of candidates showing a downward GEBV bias, because the GRV method estimated RPG of those ancestors based on RPG of all the genotyped animals in the full single-step evaluation.

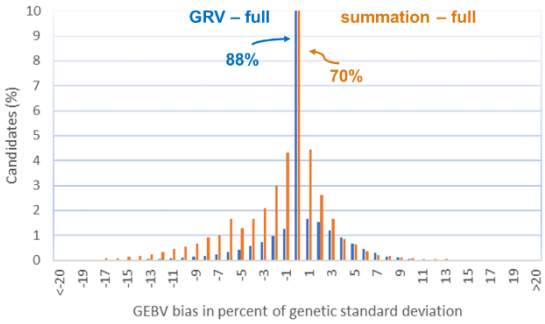

Figure 4. Distribution of GEBV bias of all the candidates of the two weekly evaluation methods for trait no. 4.

For the selection candidates of the weekly evaluation, GEBV differences between the exact GRV method and the full single-step evaluation were small but not non-existent. This may be contributed by several factors. Firstly, both weekly genomic evaluations assumed a single trait model, whereas a multi-trait model was used for all the 69 traits or indices in the full single-step evaluation. Secondly, the two weekly genomic evaluation methods estimated parental average of RPG effect for the selection candidates, while their RPG effects were estimated in the full single-step evaluation using all available genotypic and phenotypic data of all animals.

The same procedure of the Interbull genomic reliability method can be followed for approximating genomic reliabilities for the weekly genomic evaluation as for the full single-step evaluation, except that conventional reliability values of all the animals can be calculated from effective daughter contributions of bulls and effective record contributions of cows, which have been obtained from the latest, full single-step evaluation, instead of processing original phenotypic data.

Conclusions

Two statistical methods were assessed for the weekly genomic evaluation of newly genotyped selection candidates, based on the effect

estimates of the single-step model from a latest, full single-step evaluation. As a validation of the weekly genomic evaluation methods, GEBV of young selection candidates born in the last year were compared to the latest, full single-step evaluation containing those selection candidates. For all 69 traits or indices, GEBV of the selection candidates estimated using the two weekly genomic evaluation methods, the exact GRV and summation methods, were fully correlated with those from the single-step evaluation. Regression slopes of the single-step GEBV of the selection candidates on those of the weekly evaluation were all close to 1 for all the traits or indices. According to the distribution of GEBV bias to the single-step evaluation among the selection candidates, the exact GRV method resulted in equal GEBV as the full single-step evaluation. However, the summation method led to a downward bias for 5% of candidates whose partial ancestors had no own genotypic data in the latest, full single-step evaluation. Whenever possible, the exact GRV method should be preferred to the summation method for routine weekly evaluations. Both statistical methods of the weekly genomic evaluation were computationally efficient and feasible for a genomic evaluation of newly genotyped animals of German Holsteins during weekend.

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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).

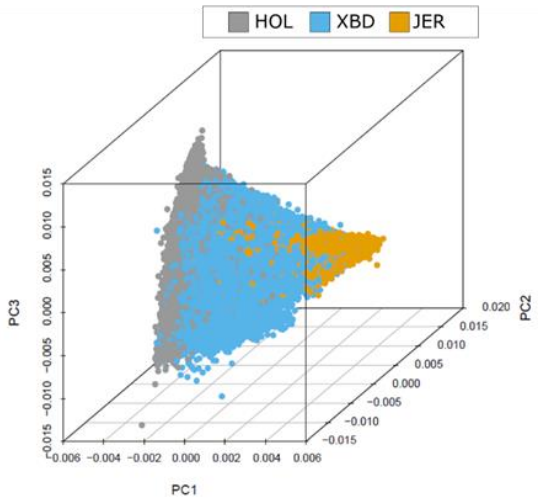

Figure 1. Plot of the first three principal components (PC). Colours indicate the breed associated with the genotype (HOL = Holstein, JER = Jersey, XBD = crossbred).

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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Improving single-step genomic prediction reliabilities for clinical mastitis in Nordic Red dairy cattle and Jersey by applying marker-specific weights

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Abstract

The standard single-step genomic prediction assumes that all single nucleotide polymorphism (SNP) markers explain an equal amount of genetic variance. The true state may deviate from this assumption, and it has been suggested to consider SNP marker-specific weights when predicting genomic enhanced breeding values (GEBV). We hypothesized that the benefit may be more pronounced in low heritable traits and investigated this hypothesis using the udder health evaluations for Nordic Red (RDC) and Jersey (JER) dairy cattle. In the first step, we develop a standard single-step genomic prediction (ssGBLUP) model based on the currently used multiple-trait evaluation models, and estimated GEBVs. The models included four clinical mastitis (CM) traits, and five correlated traits, namely test-day somatic cell score (SCS) in 1st, 2nd, and 3rd lactations, fore udder attachment and udder depth, and describes all additive genetic effects of an animal by one covariance function. Then, we investigated three alternative approaches, where we applied SNP-marker specific weights. The three approaches for SNP-marker weighting were: 1) a nonlinear method similar to BayesA, 2) the classical formula ($2pq\hat{u}^2$), and 3) the mean of SNP weights for every 20 adjacent SNP markers calculated based on $2pq\hat{u}^2$. To solve the models with SNP marker-specific weights, we applied the single-step SNPBLUP solver implemented in MiX99. We validated the models by forward validation where the last four years of the data were removed. The datasets for RDC and JER included 6.9 and 1.2 million animals of which 5.6 and 0.9 million cows had records, respectively. The number of genotyped animals was 125,789 and 64,777 for RDC and JER, respectively. We found a significant increase in prediction reliability for CM when applying SNP-marker specific weights. For instance, applying the $2pq\hat{u}^2$ weights compared to the standard ssGBLUP for SCS, the prediction reliability increased from 0.58 to 0.64 and from 0.61 to 0.56 for RDC and JER bulls, respectively. We found similar improvements in the prediction reliability for cows. In general, all weighing approaches improved prediction reliability, but the highest improvement was achieved by weighing the SNP-markers by $2pq\hat{u}^2$.

Key words: genomic prediction, SNP marker weights, single-step SNP-BLUP, udder health traits

Introduction

Clinical mastitis (CM) is the costliest disease affecting animal welfare and reducing profitability by lowering milk quality and quantity. Furthermore, it is a lowly heritable trait, which means it will take longer to genetically improve it. Fortunately, studies show that genomic selection can be especially beneficial for traits with high recording costs or traits with low heritability (Meuwissen et al., 2001; Schaeffer, 2006). In addition, it is possible to improve prediction reliability by employing a single-step genomic prediction

(ssGBLUP) model which combines all information from genotyped and non-genotyped animals (Christensen and Lund, 2010).

In a standard ssGBLUP model, the assumption is that all single nucleotide polymorphisms (SNP) are equally important in terms of the amount of genetic variance they explain. This may not be true as some SNPs are in the proximity of influential genes. Results of several studies indicate improvements in prediction reliability by applying SNP marker weights (Wang et al., 2012; Fragomeni et al., 2019). Different formulas have been used to calculate SNP weights ranging from Nonlinear which is a BayesA-like procedure (VanRaden, 2008) to square of marker effect size (Wang et al., 2012). There were some discrepancies between reports which may be due to the differences in the traits, population or breed, and weighing procedures between the studies.

The objective of this study was to investigate the possibility of improving prediction reliability for CM through applying marker weighting in a single-step genomic prediction framework.

Materials and Methods

Data

Records of udder health traits including CM, test-day somatic cell score (SCS) and two udder type traits namely fore udder attachment (UA) and udder depth (UD) from Nordic Red (RDC) and Jersey (JER) dairy cows collected since 1990 in Denmark, Finland and Sweden were used. There were 74.5 and 17.1 million records for 5.6 and 0.9 million RDC and JER, respectively. The number of genotyped animals used in this study was 125,789 and 64,777 for RDC and JER, respectively. The number of SNP markers was 46,914 for RDC and 41,897 for JER.

Observations for CM were grouped into four classes (CM11, CM12, CM2 and CM3) based on the lactation number and the days in milk in which the disease occurred. Also, SCS records were grouped into three classes (SCS1, SCS2 and SCS3) based on the lactation number.

Statistical model

The multi-trait model used in this study is the standard model currently used for the evaluation of udder health traits by Nordic Cattle Genetic Evaluation (NAV) and has been described in detail in Negussie et al. (2010). In brief, the model in matrix notation was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Tk} + \mathbf{F}_a\mathbf{a} + \mathbf{F}_p\mathbf{p} + \mathbf{e}$$

where **y** is the vector of observations for all nine traits; **b** is the vector of fixed effects; vector **k** contains random herd-year effects for CM, UA and UD and random herd-test-day effects for SCS; vector **a** has the animal additive genetic effects; vector **p** has the random animal non-additive genetic effects and **e** is the random residual. The $\mathbf{F}_a$ and $\mathbf{F}_p$ matrices have the trait-specific covariables from the covariance function. Covariance functions were used to model animal additive and non-additive genetic effects.

Scenarios

First, a standard ssGBLUP was implemented and the results were compared with those of weighted ssGBLUP. In a single-step evaluation, we need a relationship matrix that combines numerator relationship matrix (NRM) with genomic relation matrix (GRM) as follows:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

where **G** is the GRM and was calculated as $\mathbf{G} = \mathbf{ZZ'} + \mathbf{C}$, where **Z** is a centered and scaled marker matrix and $\mathbf{C} = w\mathbf{A}_{22}$ with w equal to the residual polygenic (RPG) proportion and $\mathbf{A}_{22}$ is the NRM of the genotyped individuals. The amount of RPG proportion was 0.10.

Second scenario was to apply a **Nonlinear** formula (VanRaden, 2008) to weigh the markers as follows:

$$\mathbf{G}_j = \frac{\mathbf{Z}_j\mathbf{W}_j\mathbf{Z}_j'}{\sum_{i=1}^{m} 2p_i(1-p_i)}$$

where m is the number of markers and p_i is allele frequency of marker i. W_j is a diagonal matrix containing the weights for eigenvalue trait j calculated by $1.25^{\frac{|\hat{u}_{ji}|}{sd(\hat{u}_j)}-2}$, where $|\hat{u}_{ji}|$ is the absolute value of the estimated SNP effect for marker i of the eigenvalue trait j and sd($\hat{\mathbf{u}}_j$) is the standard deviation of all estimated SNP effects for eigenvalue trait j.

In the third scenario, markers were weighted using the classical method (Falconer and Mackay, 1996), henceforth referred to as **2pqû²**.

In the last scenario, average weights of every 20 adjacent markers calculated by the classical method were applied (**20SNP_window**).

Validation

To create a reduced dataset for the validation of Legarra and Reverter (2018), the last four years of observations were excluded. Breeding values were predicted using both the reduced and full datasets for each of the scenarios. Combined genomic enhanced breeding values (GEBV) for both CM and SCS were calculated using lactation weights as applied by NAV.

Effective record contribution (ERC) for genotyped animals was calculated. Then, a bull could be a candidate if it had an ERC ≥ 2 using full data and that of zero using reduced data. Corresponding values were 0.9 and zero for cow candidates. All the analyses were implemented using the MiX99 program suite (Pitkänen et al., 2022).

Results & Discussion

Forward validation for CM

Regression of GEBVs using the full dataset on those using the reduced dataset showed slightly lower bias (b_0) for 2pqû² compared to the other scenarios (Table 1). The only exception was the 20SNP_window for RDC bull candidates. The standard ssGBLUP model yielded the lowest dispersion (b_1).

The reliability of predictions using standard ssGBLUP for RDC and JER bull candidates were 0.50 and 0.65, respectively. Corresponding values for RDC and JER cow candidates were 0.74 and 0.72, respectively. All marker weighting scenarios resulted in higher reliabilities (ranging from 0.5% to 13.8%) compared to the standard ssGBLUP, except for 20SNP_window in RDC and JER bulls. The highest prediction reliability was obtained by weighting the markers by the classical formula, i.e., 2pqû².

Table 1. Results of forward validation of bull and cow (within parentheses) candidates for combined clinical mastitis using standard single-step procedure as well as different SNP weighting scenarios for Nordic Red (RDC) and Jersey (JER) dairy cattle.

Breed	Group;n	Model	b_0	b_1	R^2	%gain*
RDC	Bull;86 (Cow;8,440)	standard ssGBLUP	0.002 (0.005)	0.75 (0.87)	0.50 (0.74)	
		Nonlinear	0.001 (0.005)	0.73 (0.85)	0.51 (0.74)	2.0 (1.1)
		2pqû²	0.001 (0.003)	0.68 (0.79)	0.57 (0.78)	13.8 (5.3)
		20SNP_window	0.0004 (0.005)	0.70 (0.85)	0.49 (0.75)	-1.6 (1.8)
JER	Bull;115 (Cow;8,224)	standard ssGBLUP	0.013 (0.010)	0.78 (0.89)	0.65 (0.72)	
		Nonlinear	0.015 (0.012)	0.77 (0.88)	0.66 (0.73)	0.5 (1.9)
		2pqû²	0.010 (0.008)	0.70 (0.79)	0.66 (0.76)	0.9 (5.3)
		20SNP_window	0.012 (0.011)	0.74 (0.87)	0.64 (0.74)	-2.4 (3.1)

* Percent of gain in prediction reliability relative to standard single-step evaluation.

Table 2. Results of forward validation of bull and cow (within parentheses) candidates for combined SCS using standard single-step procedure as well as different SNP weighting scenarios for Nordic Red (RDC) and Jersey (JER) dairy cattle.

Breed	Group;n	Model	b_0	b_1	R^2	%gain*
RDC	Bull;125 (Cow;18,112)	standard ssGBLUP	6.83 (6.11)	0.86 (0.97)	0.58 (0.77)	
		Nonlinear	7.40 (6.84)	0.83 (0.94)	0.60 (0.78)	2.6 (0.6)
		$2pq\hat{u}^2$	7.21 (5.82)	0.77 (0.87)	0.64 (0.79)	11.1 (2.3)
		20SNP_window	6.66 (6.63)	0.82 (0.94)	0.59 (0.78)	2.5 (1.0)
JER	Bull;119 (Cow;6,537)	standard ssGBLUP	8.17 (8.43)	0.81 (0.97)	0.61 (0.79)	
		Nonlinear	7.80 (8.43)	0.80 (0.96)	0.63 (0.80)	2.7 (0.9)
		$2pq\hat{u}^2$	4.06 (5.71)	0.70 (0.87)	0.65 (0.81)	5.4 (2.8)
		20SNP_window	7.55 (7.66)	0.80 (0.95)	0.64 (0.80)	4.0 (1.3)

* Percent of gain in prediction reliability relative to standard single-step evaluation.

The gain in prediction reliability by marker weighting differed by breed and was more advantageous for RDC. This might be due to the differences in the population structure.

Forward validation for SCS

Results of forward validation for SCS are shown in Table 2. Similar to CM, the lowest bias was obtained for the $2pq\hat{u}^2$ approach. Biases were higher for SCS (ranging from 4.06 to 8.43) than for CM.

The lowest and the highest dispersion were for the standard ssGBLUP and $2pq\hat{u}^2$, respectively, which is in line with the results for CM.

The reliability of predictions using standard ssGBLUP for RDC and JER bull candidates were 0.58 and 0.61, respectively. Corresponding values for RDC and JER cow candidates were 0.77 and 0.79, respectively. The amount of improvement in prediction reliability by applying marker weighting ranged from 0.6% to 11.1% for RDC and 0.9% to 5.4% in JER. Similarly, the $2pq\hat{u}^2$ approach resulted in the highest gain in prediction reliability in both breeds compared to the other scenarios. Prediction reliability was on average higher for SCS than for CM. This was expected as the heritability of SCS was higher than that of CM.

Conclusions

This study was conducted to compare predicted breeding values by the standard single-step genomic model with weighted approaches by using records of udder health traits in two Nordic dairy breed populations. Results indicated that marker weighting is beneficial as improvements in bias and prediction reliability were observed for clinical mastitis and somatic cell score. The classical formula to weigh the markers resulted in the highest gain in prediction reliability and the lowest bias. However, the highest dispersion was obtained by applying this approach. It seems that by marker weighting we accept slightly lower precision in exchange for higher accuracy.

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Technical options for all-breed Single-step GBLUP for US dairy cattle

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Abstract

The multi-step method for genomic prediction has worked remarkably well for US dairy cattle, but intense genomic selection makes recent genetic trends difficult to estimate in pedigree-only based BLUP evaluations. Thus, the introduction of routine single-step GBLUP (ssGBLUP) is under study. The large size of US dairy cattle data precludes naïve approaches for genomic prediction. Here we present the technical choices and needs of an all-breed (6 breeds and all existing crosses), ssGBLUP applied to different sets of traits within trait groups such as fertility, livability and health data. For each trait group, first, we prune pedigree to animals with records and their ancestors, reducing the size of pedigree and improving memory use and convergence. The model includes only genotypes of animals in this pruned pedigree, and we predict the other animals later either using Parent Average (if not genotyped) or sum of SNP effects (if genotyped). The set of markers is the usual CDCB set with 78,964 markers and included autosomes and sex chromosomes. The method for ssGBLUP was G-matrix with Algorithm for Proven and Young (APY) with metafounders (MF). APY largely reduces computational needs whereas MF provides smooth solutions for unknown origins and automatic compatibility of pedigree and genomic relationships within and across breeds. The gamma matrix was constructed based on base allele frequencies across breeds and increases of inbreeding within breeds. Core animals were chosen within breed, in a heuristic but complete and repeatable manner: genotyped sires with more than a certain number of daughters in records, and a deterministic subset of genotyped cows with records. This resulted in ~45K animals in the core and ~2M non-core animals for fertility evaluations. Still memory needs are large as G_APY inverse, stored in double precision, takes ~720 Gb. Thus, we used memory mapping (mmap) to assign memory to disk space. For the case of fertility (4 traits), computation of G-1_APY took 28h and 100 Gb of RAM using mmap. Solving MME took 22h, 120 Gb of RAM and 476 rounds of PCG. Genomic reliabilities took 120 Gb of RAM and 8h per trait. Backsolving for SNP solutions took negligible time and memory. Owing to the developments reported here, computations for ssGBLUP in this very large database can be done with reasonable time and memory.

Key words: metafounders, memory mapping, pedigree, genomic

Introduction

Genomic predictions in dairy cattle started with quite simple multi-step methods consisting in traditional pedigree-based evaluations followed by genomic predictions based on de-regressed proofs of the reference population – those animals with genotypes and some sort of information from traditional BLUP. However, multi-step methods do not use all available information and, probably more important, traditional evaluations produce biased genetic trends. Single-step methods (either in SNP-BLUP or GBLUP flavors) can instead use all

information to estimate unbiased trends and improve reliability.

Therefore, national dairy cattle evaluations are gradually shifting to single-step methods. Single-step methods are complex for two reasons. First, the elementary values handled are orders of magnitude larger than pedigree-based evaluations. For instance, a genetic evaluation with 1 million animals in pedigree uses a pedigree list of 3 million points. The same animals in a pure genomic evaluation would use 50 billion points: 50K (SNPs) times 1 million (cows). The second reason for the complexity is the easy algebra but complex operations in the single-step methods.

The US genetic evaluation system at Council on Dairy Cattle Breeding (CDCB) is very large, including roughly 60 million animals with records, 100 million animals in pedigree, more than 8 million animals genotyped and 50 traits grouped in different models. CDCB, AGIL (USDA) and University of Georgia are testing single-step methods using the blupf90 suite of programs. This led us to define technical options to avoid the use of very large resources (time, memory, disk space) or extensive reprogramming. We present these technical options here as they might be of interest for other practitioners.

Materials and Methods

Pruning pedigree and markers

The CDCB evaluates several trait groups (yield, somatic cell score, livability, productive life, fertility, gestation length, health, residual feed intake (RFI), heifer livability, calving ease and type traits) including a total of 50 traits – see https://uscdcb.com/individual-traits/ . Residual feed intake is a Holstein-only evaluation; type traits are separate purebred evaluations; the rest are all-breed evaluations. The number of animals with phenotypes varies enormously from ~8K for residual feed intake to 40M for yield traits. There are at this moment (June 2024) 9 million genotyped animals, all imputed to 79K SNPs. However not all this information is needed for the genomic evaluation itself. The CDCB receives pedigrees and genotypes for animals that are not directly related to the evaluations – because they are foreign animals or because they belong to herds that do not contribute information. They are related to records through pedigree, genotypes, or both.

Consider pedigree first. The set of animals in records for yield (the trait with largest database) and its ancestors constitute 60M animals. The set of animals in records for residual feed intake (the trait with smallest database) and its ancestors constitute roughly 60K animals. Although in theory one could include all 100M animals in the Mixed Model Equations (MME) for all traits, this is clearly an overkill. Preliminary analyses using the blupf90 family showed that solving of the Mixed Model Equations with all 100M animals in pedigree needed stricter convergence criteria (as some animals are very distantly related to phenotypes) than the trimmed 60M pedigree. Therefore we trim the pedigree, solve the MME, and then predict the trimmed animals by pedigree relationships (Henderson, 1977). This is done via Parent Average from oldest to youngest in the trimmed animals.

Then consider genotypes. One way of understanding single-step is that it improves pedigree relationships of non-genotyped animals via related genotyped animals. Thus, and contrary to pedigree BLUP, a young animal with no phenotype and no progeny with phenotype may contribute to improve the elements in **H** for its non-genotyped parent(s). However, it is commonly accepted that this improvement is very small. Thus, we decided to retain genotypes of animals directly related to phenotypes: animals with records and ancestors, reducing the number of genotypes from 9M to 2M for traits such as yield.

In other words, first we built a pedigree consisting of animals in records and all ancestors; then, we extracted the genotypes of animals included in this subset pedigree. The GEBVs of the remaining animals can be

predicted based on SNP effects and pedigree predictions (e.g., Vandenplas et al., 2023).

Metafounders

To model missing parentship and different breeds levels we fit metafounders defined by breed, year of birth, and selection path. Metafounders give smoother estimates (Legarra and VanRaden, 2023) and compatibility with genomic relationships (Legarra et al., 2015). Within trait group, we defined a joining strategy that first compacts the definitions "forward" until first phenotypes appear (e.g. for health traits) and then "backwards" to achieve a minimum "pseudo-count of records in progeny" per level of metafounders. These results in varying numbers of metafounders levels per trait group, up to approximately 300 at most. The relationship matrix across metafounders was obtained using base allele frequencies estimated from old genotypes in the database, plus a strategy using increase of inbreeding for more recent ones (Legarra et al., 2024). A "heatmap" of metafounders relationships is in Figure 1.

Using the algorithm for Proven and Young

For these tests we decided to use the Algorithm for Proven and Young, so called APY, which uses a sparse representation, $\mathbf{G}_{APY}^{-1}$, of the conditional covariances across individuals (Misztal et al., 2014; Misztal, 2016). It can also be seen as an approximate sparse inverse of the genomic relationship matrix. The APY algorithm has several advantages: it is fast and memory wise, easy to program. However, it requires the definition of a set of "core" animals representing the whole population. This was done using some ideas from Cesarani et al. (2022) and some new ones. We also wanted (1) to have both bulls and cows and (2) to avoid randomness, because it makes troubleshooting genetic evaluations more complex. The choice of core was done by breed as follows.

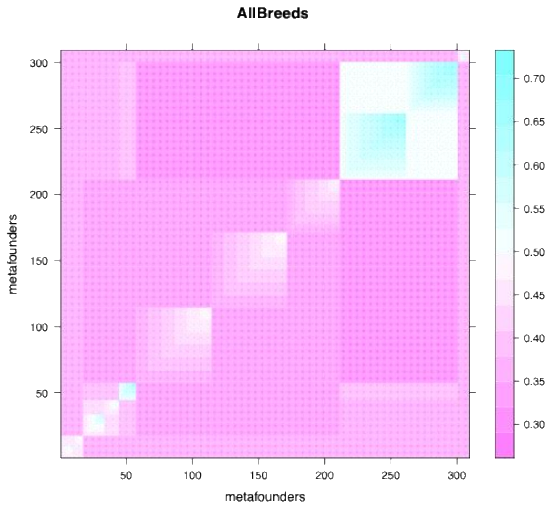

Figure 1. Gamma relationship for metafounders, sorted by breed and pedigree path

The genotypes that we used for large trait groups as fertility or yield consist in a very large number of Holsteins (almost 2M), a medium number of Jerseys (300K), a small number of crossbred animals (called "XX") (50K) and smaller number (<10K) for each of Ayrshire, Brown Swiss and Guernsey. At this stage it is unclear if XX genotypes will be included in the possible "routine" single-step, but we did that to test the most complex case. Very old genotyped animals (<1990) were *not* chosen as core as they are not truly representative of their respective periods. For AY and GU, all animals were included as core. For BS and XX 5K animals were needed as core, and 15K for JE and HO. These numbers have been found in previous studies – see Cesarani et al. (2022).

Then, within population: first, we first chose all genotyped sires with >100 daughters (Jersey, XX) or >500 (Holstein) with records. This left some spots to fill, that were filled with cows with records using a deterministic function module(anim_key,n) where anim_key is the unique integer used at CDCB for identification and n is a number to fill in the empty spots in the core. Table 1 gives an overview of the final numbers.

Table 1: number of animals and core genotypes for tests on livability

Breed	Genotypes	Core needed	Sires flagged	Cows flagged
Ayrshire	1,608	(all)	311	1175
Brown Swiss	9,560	5K	611	4313
Guernsey	3,561	(all)	219	3258
Holstein	1,669,795	15K	6890	8113
Jersey	300,976	15K	3186	11883
Crosses	56,528	5K	141	4616

Memory mapping

Even with APY, $\mathbf{G}_{APY}^{-1}$ stored in double precision requires ~700 GB for 45K animals in core and 2M animals in non-core. This is still less than the matrix of genotypes in double precision for the ~2M animals and 79K genotypes. This matrix is first formed by cross-products of blocks with program preGSf90, blended (5% or 10%) with a residual polygenic relationship matrix $\mathbf{A}_{(\Gamma)22}$ based in pedigree, then inverted. Allele frequencies are fixed to 0.5 as assumed by theory of metafounders. Then $\mathbf{G}_{APY}^{-1}$ is used by program blup90iod3 (solving the MME) and accGS2f90 (accuracies as in Bermann et al., 2022a).

The iterative method by Preconditioned Conjugate Gradients in blup90iod3 essentially consist in multiplications of the MME times a vector of solutions. This has a low cost for the pedigree + pedigree relationships part, which in addition can be easily solved by iteration on data algorithms. However, the contributions of $\mathbf{G}_{APY}^{-1}$ to the MME is more expensive if handled in memory. To alleviate memory needs, we used the programming technique called memory mapping (mmap) (https://en.wikipedia.org/wiki/Mmap) which allows mapping memory to disk space. Using this technique, RAM is reduced to 120Gb instead of 700Gb.

Backsolving for SNP solutions

The SNP effects estimates are needed for Indirect Predictions of animals not included explicitly in the MME, and also for new animals arriving to the database in between full runs. The SNP effect estimates can be obtained backsolving from GEBVs of core animals obtained in the full run (Bermann, 2022b). This has low computation cost as the core animals are a reduced number and $\mathbf{G}_{core,core}^{-1}$ is already available as part of $\mathbf{G}_{APY}^{-1}$.

Rough timings and memory

Here we give some crude numbers. We have made different tests across different servers, trait groups and options. The examples are for fertility traits: four traits, low heritability, with records dating back to 1960 – see Legarra and VanRaden (2023) for a more complete description.

There are roughly 100M lactation records belonging to 40M animals, with different patterns of missing values and different models across traits. The pedigree including animals in records and ancestors contains 60M animals. Of these, 2M animals are genotyped and their genotypes included in the prediction, and of these, 45K constitute the core, in a manner similar to Table 1.

We used 16 threads. Preparing $\mathbf{G}_{APY}^{-1}$ with preGSf90 took 16h and 720Gb of RAM or 28h and 120 Gb of RAM using mmap. For all the next operations we used mmap. Solving MME by blup90iod3 took 22h, 120 Gb of RAM and 476 rounds of PCG. Genomic reliabilities using an approximation to the inverse of the MME (Bermann et al., 2002a) took 120 Gb of RAM and 8h per trait. Backsolving for SNP solutions took negligible time and memory. These numbers are very similar to Cesarani et al., 2022.

Conclusions

Testing single-step forces to make explicit the choices and steps of the genetic evaluation systems and pipelines. The choices that we present here adapt easily to the diverse variety of information, traits and population at CDCB, while they should guarantee a fair, unbiased evaluation on time without using extensive computing resources.

Correct handling of missing pedigree and different breeds, e.g., using metafounder, is important for unbiased results. Choices of core and trimming pedigree are essential to save memory and computing time.

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Guidelines for Approximating Genomic Reliabilities of the Single-Step Genomic Model

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Abstract

A genomic reliability method developed by the Interbull Working Group on Genomic Reliability Calculation approximated reliabilities of estimated genomic breeding values for the multi-step genomic model as well as the single-step genomic model. Several modifications and improvements have been made thereafter, with a main optimization of making the genomic reliability method feasible for large-scale national genomic evaluations. The calculation of exact reliabilities of direct genomic values was proven to be computational demanding for large, genotyped populations. Therefore, this step of the original genomic reliability method, along with other steps, is no longer required in routine genomic evaluation but it is still needed when a genomic model or a major change in the national model is introduced. Consequently, two guidelines have been developed separately for the routine national single-step genomic evaluation and for deriving genomic effective daughter contribution gain via the Interbull GEBV Test. Detailed technical steps have been described in the new guidelines to assist the countries in applying the methods to the routine single-step evaluation and the derivation of the genomic effective daughter contribution gain parameter in a genomic validation. These guidelines should harmonize the calculation of genomic reliabilities and make the genomic reliabilities of marketed genomic bulls comparable across countries.

Key words: genomic reliability, single-step model, genomic validation, dairy cattle evaluation

Introduction

For conventional evaluations without genomic information, accurate reliability calculation methods were developed and have been routinely used in dairy cattle evaluations, e.g. a single-trait reliability method by VanRaden and Wiggans (1991) for a repeatability animal model, and multi-trait reliability methods (Liu et al. 2002; Tier and Meyer 2004) for a multi-trait animal model. For all types of genetic evaluation models, including a maternal-effect model for calving traits, fairly accurate and highly efficient reliability methods have been utilized for national dairy cattle evaluations.

Soon after the introduction of genomic selection in 2008, diverse genomic reliability methods (Liu et al. 2010; Wiggans and VanRaden 2010) were developed to consider a bull reference population, which covered multi-step genomic models as well as single-step genomic models (Misztal et al. 2013). To make national genomic reliabilities comparable across countries, an Interbull working group was set up in 2016 aiming to develop a standard genomic reliability (GREL) method for dairy cattle evaluations (Liu et al. 2017). The standardized GREL method by the working group was applicable for both multi-step and single-step models. However, at that time large-

scale female animal genotyping just started in few countries and thus the number of genotyped animals was still manageable.

The aims of this study were 1) to develop guidelines for routine genomic evaluations with millions of genotyped animals; 2) to address technical issues related to routine reliability calculation, and 3) to identify topics for future research and development projects.

Interbull genomic reliability method for the single-step model

Main Features of the Genomic Reliability Calculation Method

The Interbull standardized genomic reliability method (Liu et al. 2017) has the following features:

1) Keep using traditional reliability methods for the conventional part of the single-step model (SSM), including the calculation of effective daughter contribution (EDC) of bulls or cows according to the Interbull standardized methods,
2) Genotype data are treated as a new source of information contributing to the total reliability,
3) Calculate exact reliability values of direct genomic values (DGV) using all genotypic data of all genotyped animals, and
4) Adjust theoretical genomic reliability level via a genomic validation test.

Complex statistical models have been used for many trait groups when calculating the conventional part of reliability, e.g., a multi-lactation random regression model for test-day traits, a maternal-effect model for calving traits, or a multi-parity multi-trait animal model for fertility traits. Young animals and all genotyped animals must be included in the step of calculating the conventional part of reliability. Having completed the conventional reliability calculation, a model containing a general mean and additive genetic effect is assumed to compute the genomic contribution to the total reliability.

In contrast to approximating genomic reliabilities of candidates based on genomic or pedigree relationship to reference animals (Liu et al. 2010; Wiggans and VanRaden 2010), exact DGV reliability values are calculated using all genotypic data of all animals, including those with own phenotypic data and young candidates via the software *snp_blup_rel* (Ben Zaabza et al. 2020a). A single-trait SNP BLUP model without a residual polygenic effect (RPG) was assumed here for the computation of the exact reliability values of DGV, being equal to genomic breeding values (GEBV) under the assumption of no RPG effect at this step. An overestimation of genomic reliability by ignoring the RPG effect will be accounted for in a later step of adjusting GREL via Interbull GEBV Test (Mäntysaari et al. 2010).

Need for A Downward Adjustment of the Theoretical Genomic Reliabilities for Large Genotyped Population

The Interbull genomic reliability method was applied to the single-step evaluations of four test-day traits and 25 conformation traits in German Holstein (Liu et al. 2023). Phenotypic, genotypic and pedigree data stemmed from German Holstein official evaluation in April 2023. Genotype data of 1,318,780 genotyped Holstein animals were evaluated jointly with 264 million of test-day records or deregressed MACE proofs of 13,528,444 cows and bulls for each of the four test-day traits. For the 25 conformation traits, the number of national cows and MACE bulls with own phenotypic data was 3,144,366. The size of reference population was 524,187 for the test-day trait protein yield and 386,062 for the conformation trait stature, respectively (See Table 1 in Liu et al. 2023). According to the exact DGV reliabilities of the genotyped Holstein AI bulls (Figures 5 and 6 in Liu et al. 2023), it was clear that the exact, theoretical DGV reliability for 1-year-old genomic AI bulls born in 2022 was

way too high, with an average of 0.97 for milk yield and 0.83 for the conformation trait angularity which was recently introduced with a new trait definition and had much less data than all the other conformation traits. The extremely large reference population of the German Holstein led to the exceedingly high level of the exact, theoretical DGV reliability for the young genomic AI bulls of just 1 year old. With more animals genotyped, the level of theoretical DGV reliability will keep increasing. Therefore, a downward adjustment for the theoretical genomic reliabilities (VanRaden and O'Connell 2018) is, in general, needed for large, genotyped populations.

Ignoring the Individual Variability in DGV Reliabilities for Large Genotyped Population

In addition to the level of DGV reliabilities, variation in theoretical DGV reliabilities was investigated for the German Holstein animals (Liu et al. 2023). Standard deviations of the DGV reliabilities by birth year were plotted for all the genotyped German Holstein AI bulls (Figures 7 and 8 in Liu et al. 2023). Both graphs clearly showed that the standard deviation of DGV reliabilities was extremely small for the young genomic AI bulls without daughters, being as low as 0.005 for all the four test-day traits and about 0.01 for the conformation traits, indicating that the theoretical DGV reliabilities of the young animals had little variation among themselves, probably caused by the very large genotyped population and a fairly complete list of ancestor animals in the reference population for the young animals. These two graphs suggested a constant value of genomic EDC may give a satisfactory approximation of the exact, theoretical DGV reliabilities which usually required considerable computing time to calculate even with the highly efficient software *snp_blup_rel* (Ben Zaabza et al. 2020a). The simplification of the genomic reliability calculation makes it feasible for routine single-step evaluation of millions of genotyped animals.

Results & Discussion

As the number of genotyped animals increased over time e.g. by a large-scale female animal genotyping program and reached a high level for the German Holstein population, the variation in theoretical DGV reliabilities or total genomic reliabilities became smaller among the young, genotyped animals without own phenotypic data, also due to more complete ancestry in the genomic reference population for the young animals. The level of genomic reliabilities for the young animals was more important to ascertain than accounting for the individual variation in the DGV reliabilities. Therefore, a constant value of genomic EDC gain may be safely assumed for all the genotyped animals, which needs to be determined via a genomic validation study.

Since the calculation of the exact, theoretical DGV reliabilities of the original genomic reliability method (Liu et al. 2017) took a considerably long time for the very large genotyped population like German Holstein and the consideration of individual DGV reliabilities became less important for the large genotyped population, the step of calculating theoretical DGV reliabilities via *snp_blup_rel* was moved from the routine single-step evaluation pipeline to the genomic validation test conducted usually with much less time pressure than the routine genomic evaluation. Therefore, two Guidelines were developed separately for the routine single-step evaluation and for the genomic validation test deriving the genomic EDC gain parameter (see Appendices for the two Guidelines). Both Guidelines were approved by the Interbull Steering Committee in April 2024.

The standardized Interbull genomic reliability method was successfully implemented in all 10 trait groups of the German Holstein single-step evaluation according to the two Guidelines (see Appendices).

Technical Issues Related to Implementing the Guidelines on Genomic Reliabilities

A Multi-Breed Genomic Evaluation Model

Some countries or dairy populations may evaluate multiple dairy breeds jointly in a single-step evaluation, with some of the breeds having genotype data. For instance, Jersey and Holstein breeds would be evaluated together in a joint system, with both breeds having own genotypic data. Due to the vast difference in the size of reference populations of the two breeds, it is expected that young candidates of the Jersey breed would have lower genomic reliabilities than those of the Holstein breed. Regardless how the genotypic data are modelled for the two breeds, separate populations of genotyped animals and reference animals need to be defined according to the Guidelines (see Appendices). In addition, the adjustment step for genomic reliabilities must be conducted for each breed separately. Following all the steps of the two Guidelines, different levels of genomic reliabilities between the Jersey and Holstein candidates are ensured.

Applicability to Small Genotyped or Reference Populations

The step of adjusting genomic reliabilities of the Interbull GREL method plays a key role in determining a proper level of genomic reliabilities for young candidates. Applicability of the GREL method is limited to whether the required Interbull GEBV Test (Sullivan 2024) can be conducted for a small population with a limited number of genotyped animals or reference animals, such as a small breed with a small number of genotyped animals or a new trait with a small number of reference animals. If enough validation bulls can be defined for the GEBV Test, then the GREL adjustment and derivation of the genomic EDC gain parameter can be done properly.

For new traits like dry matter intake that have no reasonable number of validation bulls available, further research is required to investigate how to use validation cows with low reliability for the GREL adjustment. If a genomic validation via forward prediction cannot be performed due to a small number of reference cows for a new trait like dry matter intake, new research will be needed to extend the GREL method for a different validation procedure such as cross-validation.

Some countries or populations may have trait groups containing sub-traits with similar heritability values and data structure, assuming the same GREL adjustment factor for all the sub-traits of the trait group might simplify the genomic reliability calculation steps under this circumstance.

Frequency for Updating the Parameter of Genomic EDC Gain

As stated above, the core parameter of genomic EDC gain is used in the routine genomic reliability calculation (see Guidelines I) and determined via the Interbull GEBV Test (see Guidelines II). Because the derivation of the genomic EDC gain parameter is linked to Interbull GEBV Test, an update of this parameter value needs to be done whenever a new GEBV Test is requested. According to the current validation rules by Interbull, the update will be mandatory when a new national evaluation model is implemented, major changes are introduced to a national evaluation, or a routine validation of every 2 years is called. The same phenotypic, genotypic and pedigree data are used for the derivation of the genomic EDC gain parameter as for the Interbull GEBV Test.

Level of Genomic Reliabilities in Case of an Inflated Prediction

A country or population may pass the Interbull GEBV Test for a given trait, even when an inflation of prediction exits, with a regression slope being evidently but not yet statistically significantly less than 1. A legitimate concern was raised, if the adjusted genomic reliabilities would be too high for this situation, as GEBV variance of the validation bulls in the truncated validation data set appeared to be too high.

With dependent variable being GEBV of a later full single-step evaluation, a linear regression test (Legarra and Reverter 2018) can be applied according to the Interbull GEBV Test (Sullivan 2024):

$$\hat{u}_L = b_0 + b_1 \hat{u}_E + \epsilon \quad [1]$$

where $\hat{u}_L$ and $\hat{u}_E$ represent GEBV of a validation bull from the later full evaluation and the early truncated evaluation, respectively; b_0 and b_1 denote the intercept and slope of the regression line; and ϵ is a residual. Let *r* denote the correlation of two sets of GEBV for the validation bulls. For the simple linear regression model 1, the regression slope and correlation have the following relationship:

$$b_1 = r \sqrt{var(\hat{u}_L)} / \sqrt{var(\hat{u}_E)} \quad [2]$$

and

$$var(\hat{u}_L) = \frac{b_1^2}{r^2} var(\hat{u}_E) \quad [3]$$

According to the Interbull GREL method (Formular 11 in Liu et al. 2017), variance of the difference between the two sets of GEBV of the validation is needed for adjusting the theoretical genomic reliabilities. It can be shown that the variance of GEBV differences is:

$$var(\hat{u}_E - \hat{u}_L) = var(\hat{u}_L) - (2b_1 - 1)var(\hat{u}_E). \quad [4]$$

The Interbull GREL method with an adjustment for genomic reliabilities uses $var(\hat{u}_E - \hat{u}_L)$ but not $var(\hat{u}_L) - var(\hat{u}_E)$. The two variance terms are equal:

$$var(\hat{u}_E - \hat{u}_L) = var(\hat{u}_L) - var(\hat{u}_E) \quad [5]$$

only when $b_1 = 1$ for the case of no over- or underprediction.

In case of an inflated prediction, $b_1 < 1$, we can show that:

$$var(\hat{u}_E - \hat{u}_L) > var(\hat{u}_L) - var(\hat{u}_E) \quad [6]$$

which indicates that the expected average reliability of the early truncated evaluation (Formula 12 in Liu et al. 2017) is lower than the case of $b_1 = 1$.

For the third case of an underprediction $b_1 > 1$, we can see that

$$var(\hat{u}_E - \hat{u}_L) < var(\hat{u}_L) - var(\hat{u}_E) \quad [7]$$

indicating that the expected average reliability of the early truncated evaluation of the validation bulls be higher than the scenario of $b_1 = 1$.

We can draw a conclusion that the genomic reliability adjustment method of the Interbull GREL method does not result in too high genomic reliabilities in case of an inflated prediction.

Future Research Topics

Most countries or populations apply *multi-trait models* for routine conventional or single-step evaluations. However, a simple univariate model with only additive genetic effects is assumed to model the genomic information by the Interbull GREL method (see the two Guidelines in Appendices). Logically, applying the multi-trait model to the genomic part of the Interbull GREL method should be envisioned. By assuming the multi-trait model at all steps of genomic reliability calculation, genomic reliabilities would be more consistent with the multi-trait GEBV of the single-step model.

To ascertain the genomic EDC gain, a SNP BLUP model was assumed ignoring the RPG effect. Ben Zaabza et al. (2020b) extended the *SNP BLUP model with the RPG effect* added and developed a Monte Carlo sampling-based approach. New research will be needed to further improve the computational efficiency of the SNP BLUP model with the RPG effect.

As shown in the two Guidelines (see Appendices I and II), *many steps* are required to be conducted to calculate accurate genomic reliabilities for all animals and particularly for young marketed genomic bulls whose genomic reliabilities must be comparable across

countries. Some of the steps may need to be merged to reduce the complexity of the genomic reliability method. The structure of left-hand-side of mixed model equations of the single-step genomic model may be further explored to make the genomic reliabilities even more accurate.

Conclusions

The Interbull genomic reliability method was further optimized and modified to allow an efficient implementation for routine single-step evaluation with millions of genotyped animals. Several steps of the original genomic reliability method, which required considerable computing time for large, genotyped populations, were no longer required for the routine evaluation. Instead, those steps were taken out only for the purpose of deriving the parameter of genomic EDC gain via Interbull GEBV Test. Therefore, two separate guidelines were developed for the routine single-step evaluation and for the derivation of the core parameter of genomic reliability calculation. The step of adjusting genomic reliabilities via Interbull GEBV Test ensured a realistic level of genomic reliabilities, especially for young, genotyped animals. All countries or evaluation populations are encouraged to apply the Interbull standardized genomic reliability method according to the two Guidelines.

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Appendix I:

Guidelines for Approximating Genomic Reliabilities of the Single-Step Genomic Model

A genomic reliability method (Liu et al., 2017) developed by the Interbull Working Group approximates reliabilities of estimated genomic breeding values (GEBV) for a multi-step or a single-step genomic model. Several modifications and improvements have been made thereafter. This document describes technical details of the calculation of genomic reliabilities (GREL) of the single-step genomic model.

The Interbull GREL method assumes that Interbull member countries applies an accurate method to calculating pedigree-based conventional reliabilities, by ignoring genotype data, for either a single-trait repeatability model (VanRaden and Wiggans, 1991) or a multi-trait animal model (Liu et al. 2004; Tier and Meyer 2004) such as a random regression test-day model for milk production traits or a maternal-effect model for calving traits. Besides animals with own phenotypic records, genotyped animals without own phenotypic records must also be included in the calculation of the conventional reliabilities.

The required data for approximating genomic reliabilities using the Interbull GREL method are:

1) A pedigree file which is used for the single-step genomic evaluation of an evaluated trait or a linear index of evaluated traits. The pedigree file must be sorted from the oldest to the youngest animals (or in the opposite order) and should include both genotyped and ungenotyped animals,
2) An estimate of the heritability (h^2) of the evaluated trait or index of interest,
3) Pedigree-based conventional reliability values of all animals in the pedigree file, including genotyped animals without own phenotypic records, for the evaluated trait or index of the evaluated traits, and
4) Genomic effective daughter contribution (EDC) gain (φ_c) for the evaluated trait or index of the evaluated traits, which was derived by the countries following the Interbull GREL procedure (see Appendix for the *Guidelines for Deriving Genomic Effective Daughter Contribution Gain*).

The technical steps for calculating the final GREL for genotyped and ungenotyped animals are given below:

1. *Propagation of genomic information of the genotyped animals to their non-genotyped relatives*
   In the propagation process the trait-specific constant of the genomic EDC gain φ_c is treated as weight on genotypic data for each of the genotyped animals to approximate genomic reliabilities of their non-genotyped relatives. The propagation involves two steps (VanRaden and Wiggans, 1991; Liu et al. 2004): 1) accumulating progeny contribution by passing the genomic information φ_c of the genotyped animals to their non-genotyped ancestors through the pedigree from the youngest to oldest animals (while skipping genotyped ancestors), and 2) then collecting parental contribution by passing the genomic information from the oldest to youngest animals through the pedigree (while skipping genotyped progeny). Having completed these two steps of propagation through the pedigree, the *i*-th non-genotyped relative receives a reliability value, $\mathfrak{R}_i^{propg}$. According to the concept of genotype confidence (Eding, 2022), $\mathfrak{R}_i^{propg}$ is then multiplied with

$$\mathfrak{R}_c = \frac{\varphi_c}{\varphi_c + \lambda} \qquad [1]$$

   where the variance ratio λ of the animal model is $\lambda = \frac{1-h^2}{h^2}$. Genomic EDC for the *i*-th non-genotyped relative is then converted from its reliability $\mathfrak{R}_i^{propg}\mathfrak{R}_c$ as

$$\varphi_i^{propg} = \lambda \frac{\Re_i^{propg}\Re_c}{1-\Re_i^{propg}\Re_c}. \qquad [2]$$

2. *Combining the genomic reliability gain with the conventional reliability to obtain final genomic reliability value for all animals in the pedigree*
   For a *i*-th animal included in the single-step genomic evaluation, its conventional reliability value $\Re_i^{conv}$ is converted to EDC with:

$$\varphi_i^{conv} = \lambda \frac{\Re_i^{conv}}{1-\Re_i^{conv}} \qquad [3]$$

   If the animal is genotyped, then its total EDC contributed by both the conventional and genomic information is:

$$\varphi_i^{total} = \varphi_i^{conv} + \varphi_c \qquad [4]$$

   Otherwise, a total EDC for the animal without genotype data is:

$$\varphi_i^{total} = \varphi_i^{conv} + \varphi_i^{propg} \qquad [5]$$

   The genomic reliability of the *i*-th animal contributed by phenotypic, pedigree and genomic data is then:

$$\Re_i = \frac{\varphi_i^{total}}{\varphi_i^{total}+\lambda} \qquad [6]$$

It is worth noting that the approximated genomic reliabilities depend on the genomic EDC gain φ_c, which should be derived following the *Guidelines for Deriving Genomic Effective Daughter Contribution Gain* (see Appendix II) and be regularly updated, e.g., when an Interbull member country implements the single-step model or introduces major changes to its national single-step genomic evaluation.

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Appendix II:

Guidelines for Deriving Genomic Effective Daughter Contribution Gain

The Interbull genomic reliability method (Liu et al., 2017) has been optimised to make the genomic reliability calculation feasible for routine single-step genomic evaluations with millions of genotyped animals (see the *Guidelines for Approximating Genomic Reliabilities of the Single-Step Model*). A parameter, called hereafter genomic effective daughter contribution gain (φ_c) and required by the Interbull genomic reliability method, must be derived for every trait evaluated by the Interbull member countries.

Conventional reliability values are assumed to be reasonably accurate using an accurate reliability method for a single-trait model like VanRaden and Wiggans (1991) and a multi-trait model like Liu et al. (2004) or Tier and Meyer (2004).

Genomic breeding values (GEBV) of a single-step evaluation using the full phenotypic, genotypic and pedigree data as well as GEBV of an early single-step evaluation using a sub-set of the phenotypic data are needed. According to VanRaden and O'Connell (2018), following data are required for deriving the genomic EDC gain parameter φ_c:

1) A pedigree file (PED_{full}) that is used for a single-step evaluation using the full phenotypic and genotypic data. This pedigree file should also include genotyped animals without own phenotypic records;
2) An extracted pedigree file containing only genotyped animals and their ancestors (PED_{geno});
3) Heritability value (h^2) of the evaluated trait or a linear index of breeding values of evaluated traits and variance ratio of the animal model $\lambda = \frac{1-h^2}{h^2}$;
4) Conventional reliability values of all animals, including genotyped animals without own phenotypic records;
5) A file containing effective daughter contribution (EDC) of genotyped bulls and/or effective record contribution (ERC) of genotyped cows. When a genotyped cow with phenotypic records and her sire are both genotyped, her sire's EDC must be adjusted for her contribution to avoid a double counting of her own phenotype information. Interbull proposed an adjustment method for EDC of bulls and technical details of the EDC adjustment are given in Interbull (2018);
6) A list of genotyped animals for the single-step evaluation;
7) A file of allele frequencies for all SNP markers used in the genomic evaluation;
8) A SNP genotype file for all the genotyped animals containing ID of the animals and genotype string of all the SNP markers;
9) A list of validation bulls for *Interbull GEBV test* (Mäntysaari et al. 2010); and
10) GEBV of the validation bulls from the single-step evaluation with the full data set and from the early evaluation with the truncated subset of data.

The technical steps for deriving the genomic EDC gain constant φ_c are given below. Steps 1 to 5 must be run for both the full evaluation and the truncated, early evaluation.

1. *Computing reliabilities of direct genomic values (DGV) for all genotyped animals via software snp_blup_rel* (Ben Zaabza et al. 2020)
   A SNP-BLUP model without a residual polygenic effect is assumed for computing reliability values of DGV or genomic breeding value estimates (GEBV = DGV), denoted as $\mathfrak{R}^{DGV}$. The software *snp_blup_rel* reads heritability value of the trait, ERC values of the genotyped cows with own phenotypic data and adjusted EDC values of genotyped bulls with daughters, SNP genotypes of all the genotyped animals, and the corresponding

allele frequencies. Multiple single-traits can be evaluated jointly to reduce the total clock time. Reliabilities of DGV will be calculated for all the genotyped animals, including those without own phenotypic records. As an option, the inverse matrix of left-hand-side of the mixed model equation of the SNP BLUP model may be saved in a file for later use.

2. *Computing reliabilities of conventional EBV for all the genotyped animals*
Ignoring genotype data of the genotyped animals, reliabilities of conventional EBV, denoted as $\mathfrak{R}^{A22}$, need to be approximated using the EDC / ERC of the genotyped bulls / cows and pedigree file for all the genotyped animals. The same genotyped animals with the same EDC or ERC values must be considered as in Step 1 of calculating reliabilities of DGV. In addition, the smaller pedigree for the genotyped animals, PED_{geno}, are used here for faster speed.

3. *Calculating theoretical genomic EDC gain for every genotyped animal*
For a genotyped animal *i*, its theoretical gain in genomic EDC can be calculated by comparing the reliabilities of DGV and conventional EBV:

$$\varphi_i = \lambda\left(\frac{\mathfrak{R}_i^{DGV}}{1-\mathfrak{R}_i^{DGV}} - \frac{\mathfrak{R}_i^{A22}}{1-\mathfrak{R}_i^{A22}}\right). \qquad [1]$$

If $\varphi_i < 0$ for any reason, set $\varphi_i = 0$. For all the genotyped animals, average of their theoretical genomic EDC gain is denoted as $\bar{\varphi}$. [2]

4. *Propagating the genomic information from the genotyped animals to their non-genotyped relatives*
Using the theoretical genomic EDC gain (φ_i) as input data of the genotyped animals, genomic reliabilities of their non-genotyped relatives can be computed by processing the full pedigree file, PED_{full}, containing all animals with or without genotypic data. Firstly, progeny contribution to every animal is accumulated by processing the full pedigree from the youngest to oldest animals, and secondly parental contribution to the animal is collected by processing the full pedigree from the oldest to youngest animals. For a non-genotyped relative *i*, its genomic reliability, $\mathfrak{R}_i^{propg}$, contributed by its genotyped relatives after the two steps, is converted to EDC as:

$$\varphi_i^{propg} = \lambda\,\mathfrak{R}_i^{propg}\bar{\mathfrak{R}}/(1-\mathfrak{R}_i^{propg}\bar{\mathfrak{R}}) \qquad [3]$$

where $\bar{\mathfrak{R}} = \bar{\varphi}/(\bar{\varphi}+\lambda)$.

5. *Combining genomic with conventional reliabilities for all animals*
If animal *i* is genotyped, then its total theoretical EDC, $\varphi_i^{T\_total}$, contributed by conventional and genomic information is calculated:

$$\varphi_i^{T\_total} = \varphi_i^{conv} + \varphi_i \qquad [4]$$

where φ_i^{conv} represents the *i*-th animal's EDC converted from its total, conventional reliability $\mathfrak{R}_i^{conv}$:

$$\varphi_i^{conv} = \lambda\,\mathfrak{R}_i^{conv}/(1-\mathfrak{R}_i^{conv}). \qquad [5]$$

Similarly for a non-genotyped animal, its total theoretical EDC is:

$$\varphi_i^{T\_total} = \varphi_i^{conv} + \varphi_i^{propg}. \qquad [6]$$

A total theoretical genomic reliability is finally calculated by converting the total EDC:

$$\mathfrak{R}_i^{T_{total}} = \varphi_i^{T\_total}/(\varphi_i^{T_{total}} + \lambda) \qquad [7]$$

6. *Deriving an adjustment factor for EDC using validation animals from Interbull GEBV test*
   Based on the same validation bulls used in Interbull GEBV test (Mäntysaari et al. 2010; Sullivan 2024), expected change in genomic reliability is calculated:

$$E(\Delta\Re) = var(\hat{u}_L - \hat{u}_E)/\sigma_u^2 \qquad [8]$$

where $\hat{u}_L$ and $\hat{u}_E$ represent GEBV of the validation bulls from the later evaluation with full data set and the early evaluation with truncated data, respectively; and σ_u^2 is additive genetic variance of the evaluated trait or the linear index of interest. Sire variance estimates provided in routine MACE evaluation by Interbull may be used here as the genetic variance of own country.

Denote average genomic reliability values of the validation bulls from the later full evaluation $\overline{\Re}_L$, which is assumed to be reasonably accurately approximated due to daughter phenotypic information of the validation bulls. Average genomic reliability of the validation bulls in the early, truncated evaluation is expected to be:

$$E(\Re_E) = \overline{\Re}_L - E(\Delta\Re) \qquad [9]$$

The expected average genomic reliability is then converted to EDC:

$$E(\varphi_E) = \lambda\ E(\Re_E)/(1 - E(\Re_E)) \qquad [10]$$

Let $\Re_{i\_E}^{T\_total}$ represent the theoretical genomic reliability of validation bull *i* from the early, truncated evaluation using Equation [7], the average of the theoretical EDC for all the validation bulls is then:

$$\bar{\varphi}_E = \frac{1}{n}\lambda\sum_1^n\left(\frac{\Re_{i\_E}^{T\_total}}{1-\Re_{i\_E}^{T\_total}}\right) \qquad [11]$$

where *n* is the number of validation bulls.

A ratio of the expected and theoretical EDC values is defined as an adjustment factor:

$$f = E(\varphi_E)/\bar{\varphi}_E \qquad [12]$$

The EDC adjustment factor $f < 1$ or $f > 1$ indicates an overestimation or underestimation of genomic reliabilities from the early evaluation, respectively.

7. *Repeating Step 3 of calculating genomic EDC gain for all the genotyped animals*
   For the genotyped animal *i*, its *adjusted* gain in genomic EDC can be calculated using their DGV and EBV reliabilities and the adjustment factor *f* :

$$\varphi_i^{adj} = \lambda\left(\frac{\Re_i^{DGV}}{1-\Re_i^{DGV}} * f\ - \frac{\Re_i^{A22}}{1-\Re_i^{A22}}\right). \qquad [13]$$

Average of the *adjusted* genomic EDC gain for the validation bulls can be used for genomic reliability calculation in routine single-step evaluation (see *Guidelines for Approximating Genomic Reliabilities of the Single-Step Model*):

$$\varphi_c = \frac{1}{n}\sum_1^n \varphi_i^{adj}. \qquad [14]$$

The Interbull genomic reliability method is linked to the new Interbull GEBV test (Sullivan 2024), i.e. countries need to develop a new adjustment factor for genomic EDC using Formula [8]. Every time a country is required to conduct a GEBV test for a particular trait, this country is automatically also required to perform the genomic reliability validation by deriving a new genomic EDC gain parameter for this trait.

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Genomic-free EBVs computed from Single-Step evaluations as proofs for MACE in France

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Abstract

In France, all dairy breeds and all traits have been evaluated using a Single-Step approach since April 2022. Hence, polygenic evaluations are no longer used to select bulls in this country. Furthermore, polygenic evaluations are known to be biased due to genomic preselection. With the aim of providing to Interbull partners MACE proofs that are as unbiased and as close to the national EBVs used in France for the selection of bulls as possible, genomic-free Single-Step EBVs were computed and validated for their routine submission as proofs for MACE. Among the methods proposed by the Interbull working group for removing the genomic part from Single-Step EBVs, the most appropriate one for France was to run a BLUP evaluation using Single-Step YDs as phenotypes. Consequently, a novel pipeline was implemented to compute the genomic-free Single-Step EBVs for all traits (including milk production, fertility, longevity, calving, conformation, workability and udder health traits) and breeds (Brown Swiss, Simmental, Montbéliarde and Holstein). Then, genetic trends estimated using the EBVs resulting from a BLUP model were validated by means of the Interbull trend validation procedure based on the analysis of within-bull yearly daughter deviations. All the traits successfully passed validation tests. Genomic-free Single-Step EBVs revealed a genetic gain that was intermediate between polygenic EBVs and Single-Step EBVs. The correlations between MACE EBVs, based on polygenic or genomic-free Single-Step EBVs in Holstein ranged from 0.94 to 0.994 depending on the trait. In conclusion, France successfully transitioned from providing polygenic EBVs to genomic-free Single-Step EBVs as proofs for MACE for all traits and breeds.

Key words: genomic-free Single-Step EBVs, MACE, single-step

Introduction

The transition from polygenic and genomic evaluations to Single-Step evaluations has been a significant aera of focus for both research and evaluation centre teams in France since 2018. This was motivated by previous studies (Patry and Ducrocq, 2011), that had demonstrated biases in polygenic EBVs due to genomic preselection, whereas Single-Step evaluations are free from these biases (Legarra et al., 2014)

Thanks to the successful development of a new genetic evaluation software that enables a SNP-BLUP Single-Step evaluation and the validation of all breed-trait evaluations, the French national genomic evaluations for all traits in dairy cattle moved to a Single-Step approach in April 2022 (Croué et al. 2022). In addition, Interbull GEBV tests were successfully passed the same year to participate to GMACE.

Consequently, French EBVs provided to Interbull for Multiple Across Country Evaluation (MACE) are no longer employed to select bulls in French dairy cattle breeds (Croué et al. 2022), and sending polygenic EBVs to MACE is is not relevant anymore.

However, Single-Step EBVs cannot be used as input data for MACE, given that they include a genomic component whereas MACE is a strictly polygenic evaluation. This is why a solution had to be devised to send genomic-free EBVs based on the national Single-Step evaluations.

An Interbull working group proposed several methods to remove the genomic part from Single-Step EBVs and, hence, to obtain genomic-free Single-Step (GFSS) EBVs (Sullivan, 2021). One of the recommended methods is to run a pedigree based BLUP evaluation using pre-adjusted performances obtained from Single-Step evaluations as phenotypes. In our case, it was the most promising scientifically speaking and it represented a reasonable amount of work to implement and to maintain routinely.

The use of GFSS EBVs instead of polygenic EBVs for MACE was the last challenge of the transition from polygenic and genomic evaluations to Single-Step evaluations for the French national evaluations.

The objective of this paper is to present the first French experience of participation in MACE with GFSS EBVs. This was achieved by 1) developing a new pipeline to compute the GFSS EBVs, 2) validating estimated genetic trends using Interbull Trend Tests that are necessary to any participation to MACE, and 3) analyzing the impact of the transition from French national polygenic to GFSS EBVs in MACE on the MACE results.

Materials and Methods

Computation of genomic-free Single-Step EBVs

We performed the same work for all breed-trait combinations involved in MACE, *i.e.* milk production, female fertility, longevity, calving, conformation, workability and udder health traits in 4 breeds (Brown Swiss, Simmental, Montbéliarde and Holstein).

Among the methods proposed by the Interbull working group to compute GFSS EBVs, the most appropriate was to run a BLUP evaluation using Single-Step YDs as phenotypes.

The current Single-Step French evaluation incorporates foreign Holstein and Brown-Swiss bulls using MACE EBVs, with the objective of enhancing the reference evaluation. For this purpose, pseudo phenotypes of foreign bulls' progeny are assessed thanks to deregressed MACE EBVs after the removal of French information. Only pseudo phenotypes of bulls with few domestic daughters with performances (in Holstein: less than 600 daughters) are used in the French routine Single Step.

The Single-Step evaluation produces YDs for all animals. In order to avoid double-counting, Single-Step YDs for foreign daughters are excluded from the BLUP evaluation. We consider YDs for domestic daughters to include only a negligeable amount of foreign information.

Computation of genomic-free Single-Step EBVs for milk production, longevity, udder health, female fertility, conformation and workability traits

For all traits except calving traits, Single-Step YDs were calculated as such:

- performances were adjusted for all non-genetic effects estimated from the routine Single-Step evaluation. If a given animal has only one phenotype, its adjusted performance is its YD
- In the genetic evaluations with repeatable traits, the YD of each animal was the weighted average of adjusted performances

More details about the non-genetic effects included in Single-Step models for each trait can be found on GenEval's website (2024).

An adjusted weight for each Single-Step YD was also calculated, as the sum of the adjusted weights of the performances considered in the YD.

The pedigree based BLUP model used to estimate GFSS EBVs was:

$$y_i = by_i + a_i + \varepsilon_i \quad (1)$$

where y_i is the Single-Step YD for animal i, by_i is the fixed effect of the birth year of animal i, a_i is the additive genetic effect for animal and $\varepsilon_i \sim N(0, vare/w_i)$ is the residual, with w_i the weight of y_i.

Computation of genomic-free Single-Step EBVs for calving traits

Single-Step YDs for calving traits were computed correcting performances for all effects included in the Single-Step model, except for the genetic animal and maternal effects.

For these traits, the BLUP model used to estimate GFSS EBVs was as (1), with the addition of the maternal genetic effect of the dam j of animal i (m_j).

$$y_i = by_i + a_i + m_j + \varepsilon_i \quad (2)$$

Models (1) and (2) are univariate models, as are all Single-Step evaluation models in France for these traits.

Validation of genetic trends

Interbull trend validation procedures were used in order to validate GFSS EBVs so that they could then be provided as proofs for routine MACE runs.

In the present study, Trend Test method II was chosen as reference method for most of traits (Boichard et al., 1995), as it is the most stringent of the Trend Test methods. Few traits could not be validated using Trend Test method II. In this case, they were validated with Trend Test method III.

Investigation of the impact of transition from polygenic EBVs to genomic-free Single-Step EBVs on MACE EBVs of Holstein bulls

The aim of this study was to analyze the impact of the transition from French polygenic to GFSS EBVs on the MACE EBVs of bulls, on the Holstein Breed only, so that we could have an idea of the consequences of this change on the national evaluation.

For this, we compared the output of MACE evaluations, depending on whether polygenic information or GFSS was used. We also looked at them in comparison to the routine Single-Step evaluation and the GFSS in order to compare genetic gain between these four evaluations.

Overall, this work was based on 4 categories of EBVs of Holstein bulls:

- French Single-Step EBV (SSEBV) published by the French breed societies in August 2023;
- French genomic-free Single-Step EBVs (GFSSEBV) sent to Interbull for the September 2023 test-run;
- MACE EBVs (MACE EBV) based on polygenic French proofs and expressed in the French scale. These EBVs were performed by Interbull during the routine evaluation of August 2023;
- MACE EBVs expressed in the French scale (SSMACE EBV) and based on French genomic-free Single-Step EBVs. These EBVs were performed by Interbull during the test run of September 2023.

We considered six traits, in order to focus on a large panel of heritabilities: production traits (milk, protein and fat yield), somatic cell score, mastitis and heifer conception rate.

In some parts of this study, two sub-populations were considered: bulls with only domestic daughter information taken into account in the routine evaluation (FR) and bulls with foreign information included in the evaluation (FOR). As mentioned before, the FOR bulls have less than 600 domestic daughters (as only French performances are used for bulls with more domestic progeny).

Several statistical analyses were conducted: genetic trends estimated with the four categories of EBVs, correlations and regressions of SSMACE on MACE EBVs for FOR animals, and reranking between MACE

and SSMACE, comparing the top100 bulls between the two rankings.

The changes in genetic correlations between France and the other countries estimated by Interbull were also analyzed, but they are not reported in this article.

Table 1: number of bulls considered in the impact study.

Trait	FR	FOR
Milk yield	16495	2532
Protein yield	16883	2144
Fat yield	16883	2144
Somatic cell score	16140	2861
Mastitis	8677	2714
Heifer conception rate	13483	2127

Results & Discussion

Validation of genetic trends

All traits, except cow conception, cow interval and locomotion in Holstein and longevity in Brown Swiss, successfully passed the genetic trend validations using Trend Test method II. For the four aforementioned traits, genetic trend was successfully validated by means of Trend Test method III.

The validation of the estimated genetic trend in all populations and traits involved in the international evaluations represented the last step of an extensive validation process conducted in France for more than 5 years and involving research partners, GenEval and the users (AI industry and breed societies). This enabled the use of French GFSS EBVs in MACE since Decembre 2023. As a result, all French domestic proofs sent to Interbull for routine international evaluations are now derived from Single-Step evaluations, as domestic GEBVs calculated with the Single-Step methodology have been included in GMACE since 2022.

Impact of the transition from polygenic EBVs to genomic-free Single-Step EBVs on MACE results

Genetic gain estimated with bulls in FOR for milk yield was lower with MACE EBV than with the SSEBV. Genomic-free evaluations (SSMACE and GFSS) had an intermediate genetic trend. These results indicate that the correction for genomic preselection in the genomic-free Single-Step evaluation is only

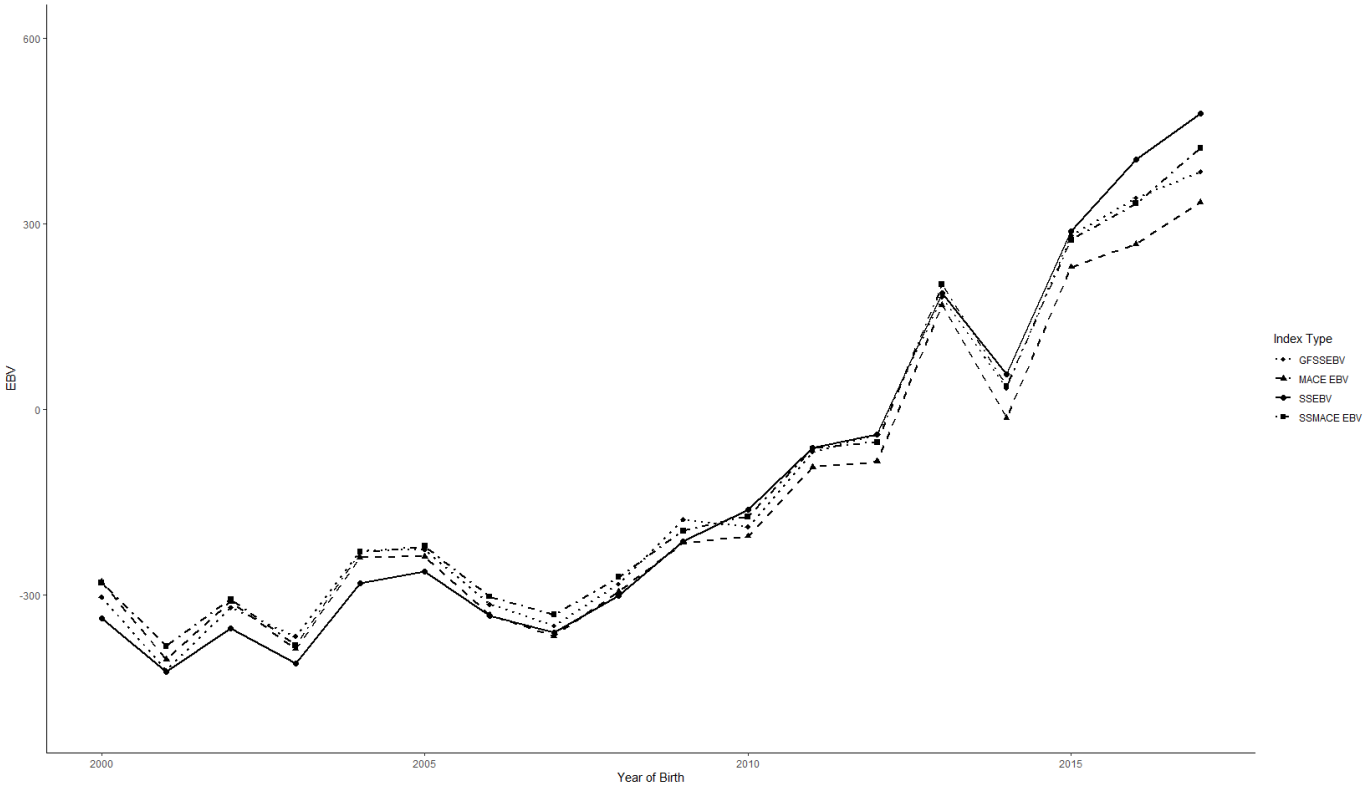

Figure 1. Genetic trend for milk production in the FOR population

Table 2. Correlation between SSMACE EBVs and MACE EBVs and slope of the regression of SSMACE EBVs on MACE EBVs, in the FOR population.

Trait	Correlation	Slope
Milk yield	0.994	0.963
Protein yield	0.996	0.951
Fat yield	0.996	0.979
Somatic cell score	0.994	1.025
Mastitis	0.996	0.997
Heifer conception rate	0.971	0.913

partial, which is consistent with the conclusions of the Interbull working group dedicated to this topic (Sullivan, 2021). The same pattern was observed for protein and fat yield. For heifer conception rate and mastitis, the genetic trends for all evaluations are much lower, and more stable between evaluations, with no type of evaluation showing a clearly higher or lower genetic trend.

The differences between MACE and SSMACE EBVs of bulls were limited (Table 2), with regression slopes between 0.951 and 1.025 and correlations above 0.994 for all traits except for heifer conception rate (above 0.97). This lower correlation might be due to the very low heritability of heifer conception rate (0.019) and the subsequent low reliability of EBVs. EBVs on this trait are more susceptible to vary due to a change in the evaluation approach and are the ones for which the removal of genomic information might be the most detrimental.

Reranking was mostly moderate: for all traits except heifer conception rate, more than 80% of the bulls in the top 100 bulls are common between ranking based on MACE or SSMACE EBVs (Table 3). Once again, we observed higher reranking for lower heritability traits, and especially for heifer conception rate.

All these results indicate that some variations are expected between former MACE EBVs and new SSMACE EBVs, but that the magnitude of these changes is limited.

Table 3. Reranking in the top100 bulls when ranking is based on MACE vs SSMACE EBVs.

Trait	Common[1]	In[2]	Out[3]
Milk yield	91	/	/
Protein yield	90	0	1
Fat yield	95	/	/
Somatic cell score	80	7	1
Mastitis	87	1	1
Heifer conception rate	77	8	2

[1]: Number of bulls common in the two top100. [2]: Number of French bulls getting in the top100 in SSMACE compared to MACE. [3]: Number of French bulls getting out of the top100 in SSMACE compared to MACE.

Therefore, the impact of the inclusion of SSMACE EBVs of foreign bulls included in the reference population used in the next French Single-Step evaluation is expected to be negligible.

Conclusion

For the past years, the transition from multi-step genomic evaluations to Single-Step genomic evaluations has been one of the main challenges for French research teams, evaluation center, breeding companies and breed societies.

In this paper, we presented a practical approach to estimate genomic-free Single-Step proofs, based on the recommendations of an Interbull working group (Sullivan, 2021). This approach is easy to implement at a national level and represents a reasonable amount of routine work to prepare the information needed for MACE evaluations. For all traits and breeds, the proofs produced by this approach passed the Interbull Trend Tests, most using the stringent method II. Consequently, the transition to Single-Step is now complete for dairy breeds, and, since December 2023, French proofs sent to MACE are based on Single-Step evaluations.

The estimated genetic trends based on genomic-free Single-Step EBVs were intermediate between those estimated with polygenic and Single-Step evaluations. This

confirms that there is a correction for the genomic preselection bias using genomic-free Single-Step EBVs, but that it is only partial. MACE and SSMACE EBVs are highly correlated, with regression slopes close to one and rankings are mostly preserved, hence French breeders can expect only minor differences in the subsequent national SSEBVs.

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Differential handling of missing parents in genetic evaluation of dairy cattle using single-step test-day SNP-BLUP model

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Abstract

Single-step genomic models use all available information on animals' phenotype, genotype and pedigree. Nowadays, many countries aim towards implementing single-step models and replacing the existing conventional models for routine evaluation. Even in the area of genomic evaluation, the pedigree data has still a significant impact on estimated genomic breeding values, and therefore it is very important to obtain the most informative structure of the pedigree. The crucial aspect of the pedigree editing is handling missing parents information. Missing data can arise either due to truly missing parentage information, or due to the fact that not all generations are utilized. We focused on three scenarios for handling missing parents: 1) raw pedigree, where missing parents IDs were set to missing; 2) genetic groups, where missing parents in the raw pedigree were replaced by genetic groups based on year of birth, country of origin, and sex; 3) metafounders, which are created based on genetic groups and genomic information. The genomic breeding values for fat yield were estimated using the single-step test-day SNP-BLUP model implemented by the MiXBLUP software. The analysed data corresponds to the population of Polish Holstein Friesian cattle used for routine genetic evaluation. We compared the results of the validation obtained by the three pedigree handling approaches and observed that the best results of validation were achieved by the scenario with metafounders (3), followed by scenario fitting pedigree with genetic groups (2), and finally by the raw pedigree (1). The metafounders scenario uses most of the information including genotype data, therefore, it provides the best classification of unknown animals into groups, which improves validation results.

Key words: single-step models, genetic groups, metafounders, validation

Introduction

The structure of pedigree data is important for the routine genetic and genomic evaluations of dairy cattle (Bradford et al., 2019). To reduce the amount of missing data and the corresponding bias in the pedigree file, genetic groups (phantom parents) are used to divide the missing ancestors into different categories (Westell et al., 1988, Legarra et al., 2007). Nowadays, single-step genomic models are the models of choice of many countries that are working on implementing routine breeding value evaluation. The single-step model incorporates all available sources of information, i.e., phenotype, genotype, and pedigree.

In this study, we focused on a single-step random regression SNP-BLUP test-day model for fat yield and investigated three approaches to handle missing parents in a pedigree: 1) *raw pedigree* with missing parents IDs set to missing; 2) *genetic groups* with missing parents replaced by unrelated genetic groups, which are defined based on year of birth, sex, and country of origin; 3) *metafounders* with missing parents replaced by metafounders, which are genetic groups with

relationships estimated from genomic information of descendants. The goal of this study was to compare results of genetic trend validation, number of iterations required to estimate all solutions, and computing times of the single-step evaluations with the three different pedigree handling scenarios. We also compared the results of the conventional pedigree-based BLUP (single-trait random regression test-day BLUP) with or without genetic groups with the single-step random regression test-day SNP-BLUP.

Materials and Methods

The data set (Table 1) corresponds to the Polish national evaluation for fat yield from April 2024 and contains 63,484,231 records of 3,701,610 cows in full data set, and 58,441,242 records of 3,224,577 cows in the truncated data set with the individuals born from 2019 removed. Genomic information from 46,118 SNPs was available for 182,143 animals. Pedigree information included 4,513,226 individuals and was extracted up to the third generation from animals with phenotypes or genotypes.

Table 1: Number of animals in the analysed data set.

Data	Sex	Number of animals	Number of records
Phenotype (fat yield)	Cows	3,701,610	63,484,231 Full data set 58,441,242 Truncated data set
Genotype	Cows Bulls	113,171 68,972	182,143
Pedigree	Cows Bulls	4,418,710 94,516	4,513,226

Genetic groups were defined according to the year of birth, sex, and country of origin of the animals with at least one missing parent (Table 2). All animals born before 1961 were removed from the pedigree. About 70% of the animals included in the pedigree had both parents known. Briefly, each genetic group was associated with at least 20 animals. The genetic group -31 that corresponds to the birth year 2010-2019, sex male, and country Poland was associated with most missing sires and assigned to 1,002,069 individuals. The largest number of missing dams was assigned the '-32' group (that is, birth year 2010-2019, sex female and country Poland) and contains 174,954 individuals.

Table 2: Genetic groups definition

Country	Year of birth	Male	Female
	<1960	-99	-99
POL	1960-1969	-1	-2
USA/CAN	1960-1969	-3	-4
OTHERS	1960-1969	-5	-6
POL	1970-1979	-7	-8
USA/CAN	1970-1979	-9	-10
OTHERS	1970-1979	-11	-12
POL	1980-1989	-13	-14
USA/CAN	1980-1989	-15	-16
OTHERS	1980-1989	-17	-18
POL	1990-1999	-19	-20
USA/CAN	1990-1999	-21	-22
OTHERS	1990-1999	-23	-24
POL	2000-2009	-25	-26
USA/CAN	2000-2009	-27	-28
OTHERS	2000-2009	-29	-30
POL	2010-2019	-31	-32
USA/CAN	2010-2019	-33	-34
OTHERS	2010-2019	-35	-36
POL	2020-present	-37	-38
USA/CAN	2020-present	-39	-40
OTHERS	2020-present	-41	-42

The following single-step random regression SNP-BLUP test-day model (Liu et al., 2004) was applied:

$$y = Xh + Wf + Vp + Vu + e,$$

where **y** contains cow's test day fat yield records from the first three lactation, **h** is a vector of fixed effects of herd-test-date-parity-milking frequency, **f** is a vector of fixed lactation curve coefficients which was modelled by the Wilmink function (Liu et al., 2004), **p** is a vector of permanent environmental effects expressed as random regression coefficient coefficients of the Legendre polynomial, **u** is a random additive genetic effects also described by the random regression coefficients of the Legendre polynomials.

GEBVtest method was chosen to perform the validation (Mäntysaari et al., 2010). It involves the preparation of two data sets, a full data set that includes all phenotypic data, and a

truncated data set that corresponds to the whole dataset with the latest 4 years of phenotypic data removed. Validation bulls were defined as bulls with daughters associated with records in the whole dataset but none in the truncated datasets.

The validation bulls were selected based on the full data set based on the following criteria: born between 2015-2019, have over 20 daughters with records.

Validation results was prepared for three lactation and total EBV, which includes:

$$Total\ EBV = 0.5 * 1st\ lactation\ EBV + 0.3 * 2nd\ lactation\ EBV + 0.2 * 3rd\ lactation\ EBV$$

Analyses were conducted using MiXBLUP 3.0 (Vandenplas et al., 2022)

Results & Discussion

For 815 validation bulls, we prepared validation results. For pedigree BLUP with and without genetic groups, validation resulted in b_1 of 1.03 (Table 3) and 1.01 (Table 4), respectively. Using the single-step random regression SNP-BLUP test-day model without genetic groups resulted in b_1 equal to 0.82 (Table 5). After defining the genetic groups, b_1 increased to 0.92 (Table 6). Finally, considering metafounders in ssSNP-BLUP improved the validation performance that achieved a $b_1$1.05.

Adding genotype information and using the single-step random regression SNP-BLUP test-day model resulted in a decreased b_1 of the validation. However, adding genetic groups and metafounders led to an increase of b_1 (Figure 1). Expressed by the R^2 value and correlation between GEBVs from the whole and truncated data sets, the same growing trend can be observed (Figure 2, Figure 3). For Pedigree BLUP without and with genetic groups and single-step random regression SNP-BLUP test-day model without genetic groups, the values of R^2 and correlation are similar, 0.43, 0.46, 0.45 respectively for R^2 and 0.66, 0.70, 0.67 for correlation. They changed when genetic groups and then metafounders were included in the pedigree. The best results were obtained for the scenario with metafounders, yielding R^2 of 0.73 and correlation of 0.86, while for the scenario with genetic groups the R^2 is 0.62 and correlation is 0.76.

Table 3: Results of validation for pedigree BLUP without genetic groups.

Bulls	b_0[1]	b_1[2]	R^2[3]	corr.[4]
1st lactation	-21.928	0.984	0.420	0.648
2nd lactation	-26.448	1.045	0.444	0.667
3rd lactation	-31.238	1.092	0.448	0.670
Total EBV	-8.471	1.030	0.435	0.660

Table 4: Results of validation for pedigree BLUP with genetic groups.

Bulls	b_0	b_1	R^2	corr.
1st lactation	-23.766	0.969	0.473	0.688
2nd lactation	-29.896	1.024	0.492	0.702
3rd lactation	-33.439	1.060	0.492	0.701
Total EBV	-9.304	1.009	0.485	0.696

Table 5: Results of validation for single-step random regression SNP-BLUP without genetic groups

Bulls	b_0	b_1	R^2	corr.
1st lactation	-30.391	0.818	0.441	0.664
2nd lactation	-30.428	0.809	0.457	0.676
3rd lactation	-27.911	0.823	0.467	0.684
Total EBV	-9.990	0.815	0.450	0.670

Table 6: Results of validation for single-step random regression SNP-BLUP with genetic groups

Bulls	b_0	b_1	R^2	corr.
1st lactation	-19.690	0.934	0.621	0.788
2nd lactation	-18.783	0.907	0.613	0.783
3rd lactation	16.873	0.907	0.614	0.784
Total EBV	-6.285	0.919	0.617	0.785

Table 7: Results of validation for single-step random regression SNP-BLUP with metafounders.

Bulls	b_0	b_1	R^2	corr.
1st lactation	-19.655	1.005	0.708	0.841
2nd lactation	-30.137	1.067	0.750	0.866
3rd lactation	-35.647	1.098	0.756	0.869
Total EBV	-8.774	1.046	0.733	0.856

[1] b_0 - intercept

[2] b_1 - slope

[3] R^2 - coefficient of determination

[4] corr. - correlation

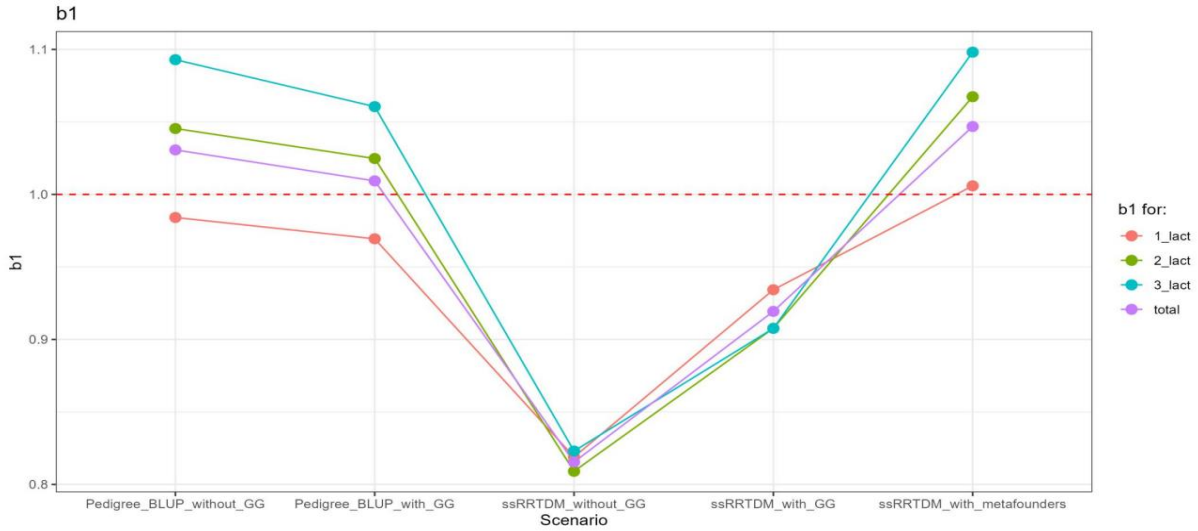

Figure 1. Validation regression coefficient (b_1) for different scenarios.

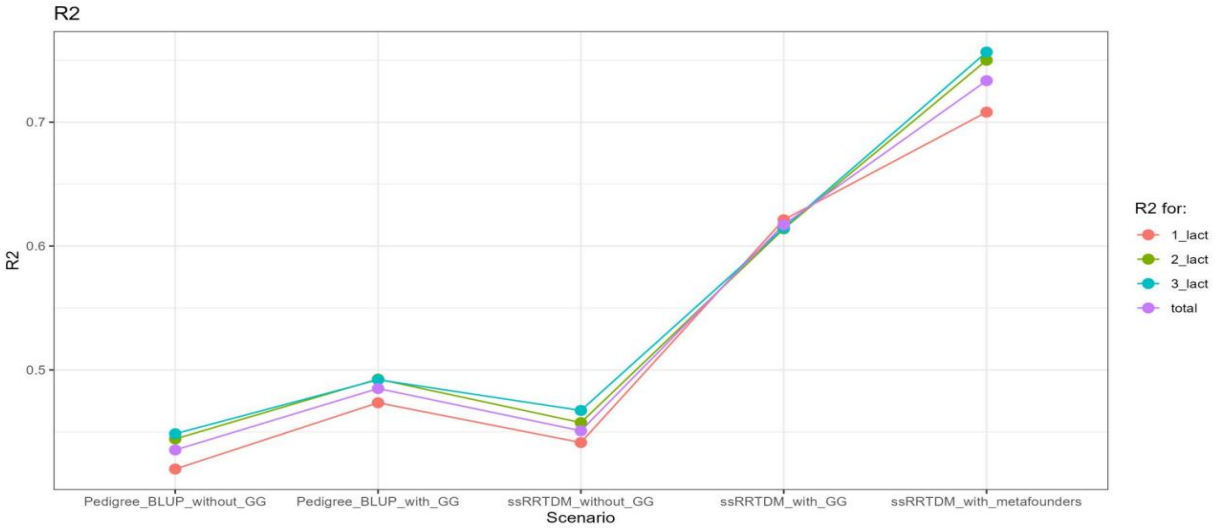

Figure 2. R^2 for different scenarios.

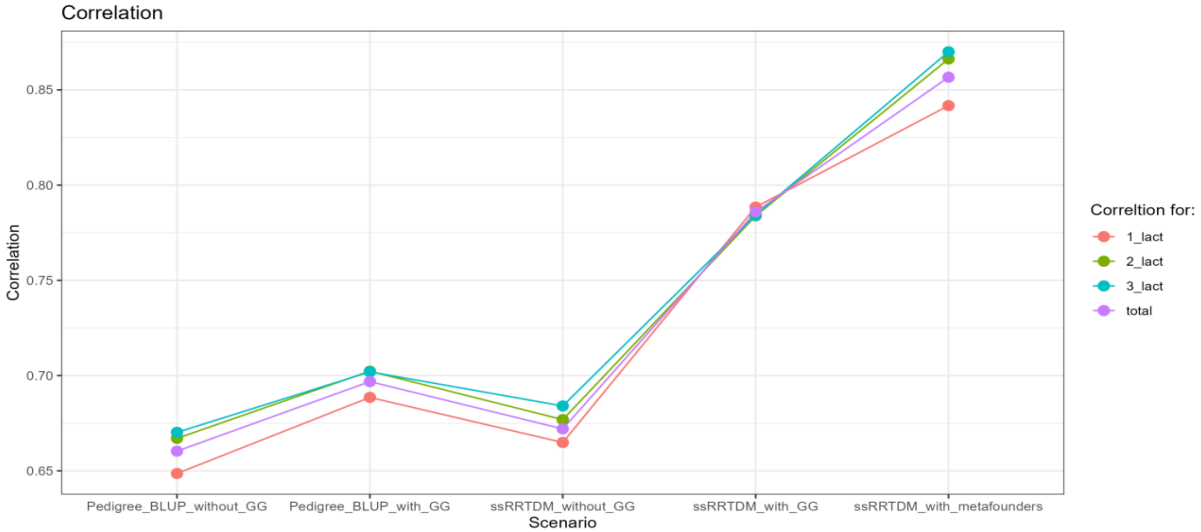

Figure 3. Pearson correlations between (genomic) estimated breeding value obtained from the whole and truncated datasets for different scenarios.

Results & Discussion

Models fitting a pedigree without genetic groups achieved much faster convergence with less iterations, and, obviously, the pedigree BLUP model converged faster than the SNP-BLUP model when fitting the same pedigree. The difference between both pedigree BLUP scenarios is large. The scenario with genetic groups needed 83 minutes more and 2,007 iterations more to get convergence. Similar situations were observed for the three single-step scenarios. The scenario with genetic groups needed 210 minutes more and 2,353 iterations more to get convergence than scenario without genetic groups, while the scenario with metafounders resulted in an intermediate number of iterations and thus the elapsed time (Table 8).

Table 8: Time and iteration per scenario

Scenario	Wall clock time (min)	Number of iterations
Pedigree BLUP without genetic groups	55	273
Pedigree BLUP with genetic groups	137	2280
ssRRTDM SNP-BLUP without genetic groups	154	949
ssRRTDM SNP-BLUP with genetic groups	372	3302
ssRRTDM SNP-BLUP with metafounders	283	2496

Conclusions

The use of alternatives to missing parents in the form of genetic groups or metafounders markedly improves the validation results. Particular improvements are seen in the single-step random regression SNP-BLUP test-day model, where the use of genetic groups first and then metafounders improved the b_1, yielded a model with the higher R2, and achieved higher correlation between GEBVS obtained from the whole and truncated datasets of validation bulls. The reason for this improvement may be the large amount of missing pedigree data for individuals born between 2010 and 2019, so the use of genetic groups and metafounders complements the missing information. The downside of using a more sophisticated pedigree architecture is the increased number of iterations and elapsed time until convergence.

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Single-step evaluation for milking cow survival in Poland

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Abstract

In Poland, the current genetic evaluation of dairy cow survival is performed using Survival Kit with sire model. Genomic evaluation is implemented using a two-step approach. The Centre for Genetics of Polish Federation of Cattle Breeders and Dairy Farmers together with National Research Institute of Animal Production in Balice are currently undertaking the review of the Polish genetic evaluation system for all traits to implement single-step genomic evaluations using BLUPF90 family of programs. The goal of this research was to develop single-step evaluation of cow survival. The following approaches to defining cow survival phenotype were considered: 1) length of productive life in months form first calving to culling (with and without dry period included, continuous); 2) survival from one calving to the next (binary) implemented as either four-trait model (survival from 1st to 2nd, 2nd to 3rd, 3rd to 4th and 4th to 5th calving) or as a repeated records model (up to ten parities included); 3) survival to a given day in milk during lactation (binary) with lactations divided into three parts based on when most culling for specific reasons occurs (1-74, 75-250, and >250 to next calving days in milk). This approach was implemented using a nine-trait model (first 3 lactations split into 3 periods each) or a three-trait repeated records model (each part of lactation as separate repeated records trait in up to ten parities); 4) random regression model with survival defined per month from first calving up to 72 months; 5) number of completed lactations (categorical treated as continuous); 6) number of days survived within each lactation (continuous). Variance components were estimated for all phenotypes, and alternative modeling approaches were tested, with a primary focus on assessing the feasibility of correcting for levels of milk production in the model. Next, the list of phenotypes of interest was narrowed down to options 1-3 listed above. For those phenotypes, both conventional pedigree-based evaluations and single-step evaluations were performed using BLUPF90 with the APY approach. Formal validation was carried out for all runs, including the Interbull trend test and Mendelian sampling test. This paper will present the results of the validation work which leads to the choice of the cow survival phenotype for single-step implementation.

Key words: dairy, genetic evaluation, genetic parameters, single-step evaluation, survival

Introduction

In Poland, the current genetic evaluation of dairy cow survival is performed using Survival Kit with sire model (Ducrocq, 2005; Morek-Kopec and Zarnecki, 2012). Genomic evaluation is implemented using a two-step approach. The Centre for Genetics of Polish Federation of Cattle Breeders and Dairy Farmers (PFHBiPM, CGEN) together with National Research Institute of Animal Production in Balice are currently undertaking the review of the Polish genetic evaluation system for all traits to implement single-step genomic evaluations using BLUPF90 family of programs (Aguilar, *et al.*, 2018). Although

survival hazard model might be statistically superior for genetic evaluation (GE) of cow survival, its implementation in single step methodology is problematic and not available in BLUPF90 software. The goal of this research was therefore to develop and implement a single step evaluation of milking cow survival for Polish Holstein-Friesian population using BLUPF90 software.

Materials and Methods

Phenotypic data

Phenotypic records were obtained from national database maintained by PFHBiPM. Data from 1995 were included for Holstein-Friesian and Holstein-Friesian Red cows. Many versions of possible survival phenotype definitions were tested. In this paper the focus is on the five most promising options used in final testing and validation runs.

Trait definitions

Option 1 – Length of productive life defined as time in days from first calving to culling. It was modeled as linear trait. Pros of this option are that it is simple single trait model, the phenotype would be the closest to the currently evaluated one, heritability is reasonable. Cons: phenotype is only available after cow's death. Abbreviation: *prodlife*.

Options 2-5 use binary phenotypes modeled on an observable scale.

Option 2 – Nine-trait model (MT-ML). Survival to a given DIM during lactation. Data from the first three lactations is used and each is split into periods of time representing culling for different reasons. Time periods were decided based on DIM at culling typical for main culling reasons (1-74, 75-249, 250-next calving). Dry period was included in the last period. This model was very similar to the one implemented in Germany (Taubert, *et al.*, 2017). Abbreviation: *surv9*.

Option 3 – Four-trait model. Phenotype is defined as survival from one parity to the next (one calving to next calving), parities 1-2, 2-3, 3-4, and 4-5 are considered. Abbreviation: *surv15*.

Option 4 – Repeated records variation of Option 2. Each parity is split to the same three periods as in Option 2, but each period is modeled as repeated records. It results in a three-trait repeated records model. Up to ten parities are included. Abbreviation: *prep*.

Option 5 – Repeated records variation of Option 3. Survival from one parity to the next is modeled as repeated records. It is a single trait model with up to ten parities included. Abbreviation: *rep*.

Models

In all the options phenotypes were modeled on the observable scale. Fixed effects included age at first or previous calving, contemporary group (herd-year-season of first/previous calving) and lactation number for repeated records model. Additional effects like the level of milk production were tested in earlier stages of the project but discarded.

Variance components data

For variance components estimation a subset of herds with larger size and data of higher and consistent quality was used. The dataset used included over 300,000 records for over 100,000 cows from 160 herds collected across 10 years. Pedigree included over 250,000 individuals. Variance components were estimated using ASReml software (Gilmour *et al*., 2015).

Genetic evaluation data

Genetic evaluation runs were performed using all available data from 1995. Contemporary groups with less than 5 observations and no variation in phenotypes were excluded from the analysis. For Option 1 there were 2.2M records with phenotypic average of 35 months of productive life. For Options 2&3 there were 2.4M of cows with records available. Phenotypic averages are presented in Table1.

For Options 4&5 there were 3.6M of cows with records. Phenotypic averages are presented in Table 2.

Table 1. Phenotypic averages for survival (to the next stage) phenotypes for Option 2&3.

Parity	Option 2			Option 3
	DIM1-74	DIM75-249	DIM250+	
1	0.92	0.90	0.82	0.76
2	0.93	0.87	0.76	0.68
3	0.89	0.83	0.71	0.61
4	---	---	---	0.55

Table 2. Phenotypic averages for survival (to the next parity) phenotypes for Option 4&5.

Parity	Option 4			Option 5
	DIM1-74	DIM75-249	DIM250+	
1	0.95	0.93	0.85	0.79
2	0.95	0.90	0.78	0.72
3	0.93	0.87	0.74	0.64
4	0.91	0.83	0.69	0.59
5	0.89	0.80	0.65	0.53
6	0.89	0.76	0.62	0.50
7	0.89	0.73	0.58	0.46
8	0.86	0.69	0.59	0.43
9	0.84	0.65	0.52	0.39
10	0.83	0.62	0.48	0.35

All genetic evaluation runs were performed using BLUPF90 family of programs (Aguilar, *et al.*, 2018). Pedigree based (conventional; PBLUP) evaluations were performed on all models as well as single step evaluations (SSBLUP) using APY approach (Misztal, *et al.*, 2014).

Combining EBVs

The EBVs from multiple trait models were combined into one EBV using the following weights: 1) Option 2 (nine-trait model) – 0.06, 0.09, 0.15, 0.05, 0.075, 0.125, 0.09, 0.135, 0.225; 2) Option 3 (four-trait model) – 0.3. 0.25, 0.2, 0.25; 3) Option 4 (three-trait repeatability model) – 0.2, 0.3, 0.5.

Validation methods

In order to validate the five options, records from the last four years were removed from the validation datasets (2018-2022), while keeping the pedigree unchanged. The results from the truncated runs were used for three types of validation. 1) Legarra and Reverter (LR) validation method as described by Legarra and Reverter (2018); 2) Quintile analysis, where validation cows are classified into quintiles (5 groups of equal size) based on EBVs from truncated runs. Validation phenotypes are then fit as dependent variables in model including quintile groups. Least square means solutions for those quintile groups are obtained. The differences between best and worse quintile groups are used to compare predictive abilities of the models with assumption that higher value of the difference means better prediction; 3) Interbull trend test III and Mendelian Sampling test.

There were three focal groups used in LR validation. 1) "Young Sires" – bulls with no daughters in truncated data and minimum 25 daughters in full data (N=679); 2) "Proven Sires" – bulls with 5-25 daughters in truncated data and minimum twice as many daughters in full data (N=196); 3) "Cows" – females with good quality phenotypes from large herds that calved first time in 2018 with phenotypes removed from truncated data (N=21,977). Only the "Cows" focal group was used for Quintile validation.

Correlations between EBVs from the current official survival evaluation and tested approaches were also assessed as well as genetic trends.

Results and Discussion

Variance components

The Heritability for Option 1 was estimated to be 0.12. Variance components obtained for Option 2 are presented in Table 3 (last page). Heritabilities for the nine-trait model were lower than for other options, but results in general align with those obtain in Germany for similar model (Taubert, *et al.*, 2017). Variance components obtained for Option 3 are presented in Table 4. Heritabilities for the four-trait model were substantially higher than for nine-trait model.

Heritabilities, genetic and residual correlations obtained for three-trait model (Option 4) are presented in Table 5. Permanent environmental variances were estimated to be very close to

Table 4. Heritabilities (diagonal) genetic correlations (above diagonal) and residual correlations (below diagonal) obtained for Option 3.

Trait	Surv12	Surv23	Surv34	Surv45
Surv12	0.034	0.74	0.69	0.64
Surv23	-0.09	0.046	0.78	0.71
Surv34	-0.07	-0.12	0.040	0.79
Surv45	-0.04	-0.09	-0.09	0.038

Table 5. Heritabilities (diagonal) genetic correlations (above diagonal) and residual correlations (below diagonal) obtained for Option 4.

Trait	DIM1-74	DIM75-249	DIM250+
DIM1-74	0.012	0.84	0.79
DIM75-249	0.07	0.013	0.74
DIM250+	0.05	0.06	0.026

zero, therefore permanent environmental effects were excluded from genetic evaluation models and repeatabilities are not presented for repeated records models (Options 4&5).

Also here, heritabilities for model where lactation is being split into parts are lower, especially for DIM 1-74 and DIM75-249. This might be due to the fact that most culling occurs in the last part of the lactation.

The heritability for single trait repeatability model (Option 5) was estimated to be 0.040.

Current vs new EBVs

The correlations between EBVs from 5 options tested and current official EBVs published for longevity for chosen groups of focal cows and bulls are presented in Table 6 (last page).

Single and three-trait repeatability models (Options 4&5) had the highest correlations with current official EBVs for longevity.

Genetic trends

Genetic trends for current official evaluation and for the five tested alternatives for bulls and cows are presented in Figure 1. As with correlations, single and three-trait repeatability models (Options 4&5) had the closest genetic trends to the current official EBVs for longevity.

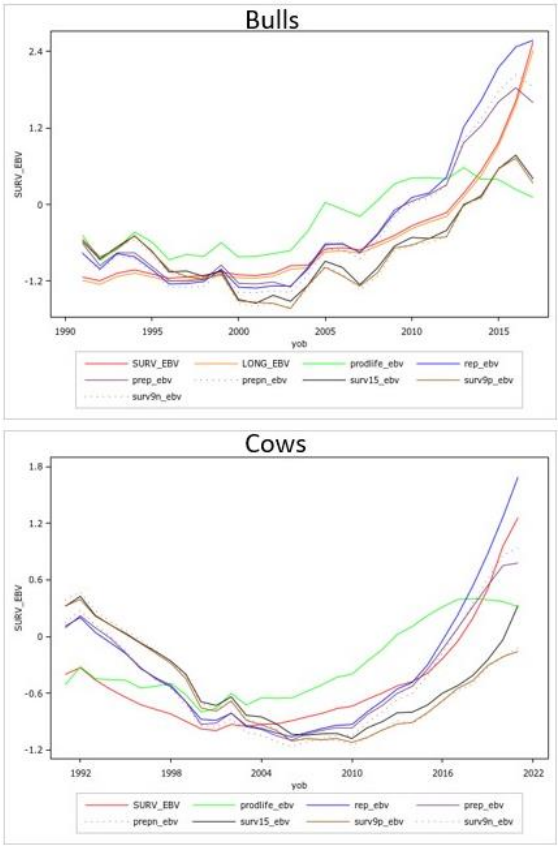

Figure 1. Genetic trends for current official EBVs (SURV_EBV & LONG_EBV) and for tested alternative Options 1-5 (prodlife_ebv, surv9_ebv, surv15_ebv, prep_ebv, rep_ebv) for bulls and cows.

LR validation

The results of the validation performed using the method of Legarra and Reverter are presented in Table 7. The values obtained for bias, slopes and accuracy are presented. All results are standardized to the same standard deviation of 1. Bias is the difference between mean EBVs obtained for full and truncated runs. The expectation of it is zero. A positive value means that animals with partial information are over evaluated. Slope is a linear regression of full on truncated EBVs. The expected value is one. Values lower than one mean that selected candidates are overestimated. Accuracy is calculated as correlation between EBVs from full and truncated runs. Values close to one indicate that truncated evaluation was as accurate as whole evaluation, but both evaluations could have low accuracy.

Table 7. Results for LR validation for pedigree based (PBLUP) and single step (SSBLUP) models for Option 1-5 for young sires (YoungS), proven sires (ProvenS) and cows.

	PBLUP			SSBLUP		
Group	bias	slope	acc	bias	slope	acc
surv9_ebv (Option 2)						
YoungS	-0.012	1.011	0.606	0.163	0.819	0.814
ProvenS	0.025	0.920	0.757	0.094	0.893	0.865
Cows	0.029	0.953	0.782	0.095	0.907	0.846
surv15_ebv (Option 3)						
YoungS	0.061	0.892	0.570	0.181	0.783	0.806
ProvenS	0.041	0.867	0.740	0.087	0.849	0.858
Cows	0.038	0.909	0.759	0.087	0.887	0.834
prep_ebv (Option 4)						
YoungS	-0.161	1.113	0.658	0.187	0.831	0.820
ProvenS	0.149	1.028	0.738	0.285	0.905	0.838
Cows	0.132	1.023	0.768	0.262	0.930	0.843
rep_ebv (Option 5)						
YoungS	0.271	1.017	0.631	0.004	0.852	0.818
ProvenS	0.554	0.933	0.727	0.105	0.860	0.829
Cows	0.535	0.930	0.751	0.078	0.896	0.831
prodlife_ebv (Option1)						
YoungS	0.263	0.397	0.468	0.518	0.449	0.828
ProvenS	0.056	0.671	0.789	0.218	0.594	0.847
Cows	0.069	0.719	0.768	0.233	0.643	0.820

For pedigree based (conventional) models, bias was the lowest in nine-trait model (Option 2), slopes were closest to one in three-trait repeatability model (Option 3), accuracy was the highest for Option 2 again.

For single step models, bias was the lowest in single trait repeatability model (Option 5), slopes were closest to one in three-trait repeatability model (Option 3), accuracy was the highest for Option 2.

The single trait model had slightly higher bias and slopes further from one than PBLUP models. However, all SSBLUP models resulted in higher accuracy than PBLUP models. With differences being largest for young sires, which is desired outcome.

It is worth noticing that differences between models were not big and all models from Options 2-4 performed very well in this validation.

Quintile validation

In quintile style validation many different phenotypes (N=35) were evaluated. The advantage of this approach is that any phenotype can be used as dependent variable, also one that is completely independent from the phenotypes used to derive EBVs. Here only one example is presented (Figure 2) for probability of surviving from first to fourth calving. This phenotype was not used to derive any of the EBVs validated.

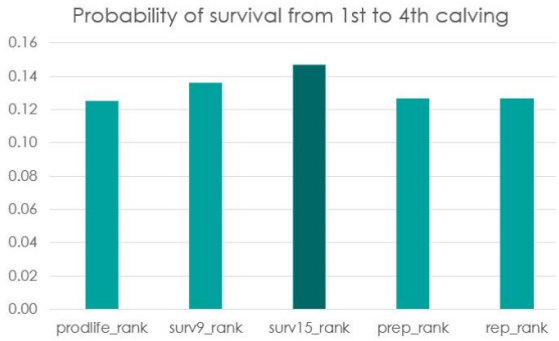

Figure 2. Example result from quintile validation for Options 1-5. Probability of surviving from first to fourth calving.

For this phenotype (as well as for many others, results not presented), the four-trait model (Option 3) showed the highest predictive ability. Validation shows substantial differences between the best and the worse EBV animals, for example difference between top and bottom 20% of cows based on surv15 (Option 3) combined EBV for probability of survival from first to forth calving is 14.7%.

In general, the differences between models were relatively small (for phenotype above, best EBV predictions differ by 14.7%, the worst differ by 12.5%) and satisfactory results were obtained for all tested options. Overall, the four-trait model (Option 3) performed best, followed by nine-trait models (Option 2) and three-trait repeatability model (Option 4).

Interbull test

All models except Option 4 (three-trait repeatability model) passed the Interbull trend test III. Number of bulls available for this test varied between traits from 142 to 215. All models except Option 5 (single trait repeatability model) failed Mendelian sampling test (Table 8). Options 2-4 failed for bulls only, while Option1 failed by a significantly higher margin for both sexes.

Table 8. The results of Interbull Mendelian sampling test.

Option	Bulls	Cows
1 (prodlife)	-13.4	-12.3
2 (surv9)	-5.9	1.0
3 (surv15)	-4.6	1.2
4 (prep)	-5.3	-1.2
5 (rep)	0.0	2.0

Run times

Consideration was also given to the time it takes to run each model. Single trait models (Options 1 & 5) took around 10 minutes for PBLUP and 30-40 minutes for SSBLUP. Three- and four-trait models needed less than one hour for PBLUP and almost 2 hours for SSBLUP. Nine-trait model took the longest, over five hours for PBLUP and almost nine hours for SSBLUP. While differences in run times between different options are substantial, for none of the models they would be considered problematic for implementation.

Conclusion

Based on the presented results no one model was a clear winner. The only clear "no" would be Option 1 – Length of productive life. Although this was the only phenotype with normal distribution and heritability >0.1, the phenotype itself has more disadvantages. It takes the longest to collect phenotypes, because as long as cow remains in a herd her phenotype would be missing. The resulting EBVs from this option has the lowest correlation with current official evaluation for longevity and genetic trends deviated the most from current ones. Additionally, this model resulted in the poorest results for Interbull Mendelian sampling test and LR validation (especially slopes).

For Options 2-5:

- Based on comparisons with current official proofs for longevity, both correlations and genetic trends, both repeatability models performed better than Options 2 &3.
- Based on LR validation a nine-trait model (Option 2) looked slightly better but followed closely by three-trait repeatability model (Option 4) and four-trait model (Option 3).
- Based on quintile validation, the four-trait model performed best across many validated phenotypes.
- Based on Interbull tests Option 4 (three-trait repeatability mode) should be discarded as it did not pass trend test III. Additionally, only the single trait repeatability model (Option 5) passed Mendelian sampling test for both sexes.

Based on the results presented in this paper no clear winner has been identified. However, based on further analysis and industry consultations, Option 3 – the four-trait model has been chosen as preferred for implementation for Polish dairy population.

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Table 3. Heritabilities (diagonal) genetic correlations (above diagonal) and residual correlations (below diagonal) obtained for Option 2.

	Lactation 1			Lactation 2			Lactation 3		
	DIM1-74	DIM75-294	DIM250+	DIM1-74	DIM75-294	DIM250+	DIM1-74	DIM75-294	DIM250+
Trait	1	2	3	4	5	6	7	8	9
1	0.014	0.91	0.58	0.68	0.67	0.58	0.68	0.61	0.52
2	0.05	0.010	0.75	0.75	0.75	0.63	0.65	0.65	0.68
3	0.03	0.05	0.029	0.66	0.65	0.72	0.50	0.65	0.64
4	-0.01	-0.01	-0.13	0.016	0.88	0.59	0.67	0.67	0.58
5	-0.17	-0.13	-0.08	0.00	0.009	0.80	0.75	0.75	0.63
6	-0.02	-0.04	-0.19	-0.04	0.02	0.026	0.65	0.64	0.72
7	0.00	-0.02	-0.10	-0.10	-0.06	-0.10	0.010	0.88	0.69
8	-0.14	-0.11	0.01	-0.10	-0.09	-0.04	-0.05	0.006	0.80
9	0.02	-0.02	-0.14	-0.03	-0.05	-0.13	0.03	0.07	0.022

Table 6. Correlations between current official EBVs and the five tested Options.

	All bulls		Available (young) bulls		Cows
Evaluation type	Domestic	MACE	Domestic	MACE	Domestic
Number of animals	19,568	36,099	621	777	2,490,297
Average reliability	0.60	0.45	0.45	0.50	0.30
prodlife (Option 1)	0.46	0.34	-0.15	-0.14	0.54
surv9 (Option 2)	0.58	0.47	0.34	0.44	0.59
surv15 (Option 3)	0.58	0.48	0.32	0.42	0.59
prep (Option 4)	0.72	0.60	0.43	0.52	0.77
rep (Option 5)	0.73	0.64	0.49	0.57	0.78

Genetic correlations: a parameter or a latent phenotype in genetic evaluations?

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Abstract

Genetic correlations are relevant parameters in genetic evaluations, particularly when a breeding program aims to achieve genetic progress for multiple traits altogether. These correlations are usually estimated from a base population as one of the many parameters that define the distribution used to predict breeding values for the selection candidates. In such a fashion, genetic correlations are assumed to be identical for all selection candidates. However, with a preliminary study on the output predicted breeding values of sires with more than 500 daughters from the French Montbéliarde population, we observed that the genetic correlation among daughters from different sires may differ substantially, *i.e.*, different sires expressed different genetic correlations between traits through their daughters. Thus, if genetic correlations are specific values inherent to each individual, they could be considered as a phenotype; in other words, genetic correlations may be the observable consequence of a concealed regulatory trait guiding the relationship between observable traits. For antagonistic traits (*e.g.* production and fertility in dairy cattle), it is reasonable to believe that individuals on the extremes of the trade-off distribution are likely to present a low breeding value for this concealed regulatory trait. However, due to our inability to directly measure this potential regulatory trait, it can be considered a latent phenotype. Although a method to consider such hypothesis that genetic correlations may be a latent phenotype is yet undefined, there is no doubt that such hypothesis has an impact on the medium to long-term perspectives of a breeding program, given its breeding goals. Hypothesizing that genetic correlations are latent phenotypes, simulations can then be used to assess the genetic progress for multiple traits of interest in a breeding program over many generations, as well as to assess the trajectory of genetic correlations between traits and the genetic progress of the latent regulatory phenotype driving such correlations. Such comprehension of the genetic progress for the latent phenotype is of particular relevance, since a regulatory trait is likely to impact more than only two antagonistic traits, but many of the traits selected for in a breeding program.

Key words: correlated traits; multi-trait evaluation; physiological trait regulation; non-linear genetic correlation; genetic progress

Introduction

Genetic correlations (GC) are relevant parameters in genetic evaluations, particularly when a breeding program aims to achieve genetic progress for multiple traits altogether. These correlations are typically estimated from a base population as parameters from the joint distribution of the breeding values of the traits of interest, a distribution that is then used to predict the breeding values (BV) for the selection candidates (Patterson and Thompson, 1971; Henderson et al., 1959; Henderson, 1975). In such a fashion, GC are assumed to be identical for all evaluated individuals. In terms of statistical modelling, the assumption that

GC are population parameters, thus identical to all selection candidates, enables the implementation of the best linear unbiased prediction (BLUP) (Henderson, 1975) and Bayesian methods (Meuwissen et al., 2001; Gianola and van Kaam, 2008), widely used to predict BV in genetic evaluations.

While the assumption that GC is a population parameter is of great value to describe the underlying genetic architecture that drive the relationship between traits, such assumption ignores potential physiological genetic effects in the regulation of multiple traits (Berry et al., 2016). A preliminary study on the output predicted BV of sires with more than 500 daughters from a dairy cattle population, showed that the GC among daughters from different sires may differ substantially, *i.e.*, different sires may express different GC between traits through their daughters.

Physiological traits may impact both positive and negatively correlated traits. Our study focused on the latter case, particularly on the classic antagonism between production and fertility traits in dairy cattle (Boichard and Manfredi, 1994; Hoekstra et al., 1994; Veerkamp et al., 2001), traits of great commercial interest for this production system. Our hypothesis is that, rather than a modelling parameter, GC may be the observable consequence of an underlying physiological trait, responsible to regulate the trade-off between production and fertility, and such regulatory trait is not directly measurable.

Under this hypothesis that GC is a consequence of an underlying physiological trait, it is reasonable to believe that individuals on the extremes of a trade-off distribution (*i.e.* individuals who present a very high breeding value for production and a very low breeding value for fertility, or *vice-versa*) are likely to present a low breeding value for this concealed regulatory trait. Conversely, individuals on the center of a trade-off distribution, with average breeding values for both commercial traits, are likely to present a good regulatory capacity. Therefore, GC would be a measure inherent to each individual, representing their genetic capacity to regulate a trade-off. However, due to our inability to directly measure this potential regulatory trait, it can be considered as a latent phenotype, making it difficult to be evaluated and included in the unified index for the selection candidates.

Rather than aiming on how to include the hypothesis that GC are latent phenotypes in a genetic evaluation, the objective of the present work was to compare the genetic progress of production and fertility traits in a simulated breeding program, with data simulated under the assumptions that GC was either a parameter or a latent phenotype. Simulations were performed for different scenarios of selection, and the consequences of these scenarios on the regulatory trait and on the observed GC between the measurable traits was also studied.

Although the objective of our study was the discussion of the hypothesis that GC are latent phenotypes, without neither developing novel methods to evaluate antagonistic traits, nor proposing a manner to consider the possibility that GC are latent phenotypes in the unified index for the selection candidates, the discussion of this hypothesis is still relevant, since it sheds a light on the medium to long-term consequences of breeding decisions on the genetic progress of traits of interest.

Materials and Methods

Preliminary study on real data

The data set analyzed consisted of records from production (PROD) and fertility (FERT) traits from the French Montbéliarde population. PROD consisted of milk yield on first lactation corrected for 305 days, and FERT consisted of the cow conception rate at the first insemination after the beginning of the first lactation. Records on both traits were available for 806,159 cows, for which pedigree data with ~ 4 million animals were available. The phenotypes analyzed were recorded for

cows that began their first lactation between the years of 2002 and 2021.

The model used for both the variance component estimation and the genetic evaluation was a two-trait model (PROD and FERT), with an overall mean, age, and herd-year-season included as fixed effects for both traits; lactation length was included as a fixed effect only for PROD, and calving-insemination interval, sexed semen, artificial insemination (AI) operator, and day of the week as fixed effects for FERT only; for both traits, the random additive genetic effect was included assuming a normal distribution with mean zero and variances $\boldsymbol{A}\sigma^2_{PROD}$ and $\boldsymbol{A}\sigma^2_{FERT}$ for PROD and FERT respectively, and a covariance $\boldsymbol{A}\sigma_{P,F}$ between the two traits, such that $\boldsymbol{A}$ was the nominal relationship matrix, σ^2_{PROD} and σ^2_{FERT} were the total additive genetic variances of PROD and FERT respectively, and $\sigma_{P,F}$ was the additive genetic covariance between the two traits evaluated; the random effect of the AI bull was included into the model for FERT only, assuming independence between the bulls and a normal distribution with mean zero and variance $\sigma^2_{bull\times year}$; finally, for both traits the random residuals were considered to be normally distributed with mean zero and a heterogeneous variance per herd-year group.

Variance components were estimated using the residual maximum likelihood (REML), and the genetic evaluation was performed using the BLUP, to obtain the estimated breeding values (EBV) for all animals in the pedigree. After the evaluation model was performed, from the pedigree we subset 247 sires with more than 500 daughters evaluated among those 806,159 cows with records on both traits. For each of these sires, we calculated the mean EBV of their daughters for both PROD and FERT per year of their first lactation, and the GC between their daughters' EBVs for PROD and FERT, over all the years, and separated by year of the daughters' first lactation. This descriptive study per sire was performed to confirm the genetic progress for both traits (thus, selection for both PROD and FERT), and to verify whether different sires expressed different GC between the traits of interest, through their daughters.

Simulation study

Datasets were simulated to contemplate the two hypotheses we intended to discuss with this present study: (1) GC are statistical parameters modulating the genetic relationship between two traits; (2) GC are observable consequences from a latent physiological trait (RGLT) that regulates the genetic relationship between two traits. Under both hypotheses, PROD and FERT were simulated with heritabilities $h^2_{PROD} = 0.3$ and $h^2_{FERT} = 0.04$ respectively, and with a GC $\rho_{P,F} = -0.2$ between them. The total phenotypic variances were 50 and 75 for PROD and FERT respectively.

For the simulated datasets under each of the two hypotheses, a base population with 2,000 individuals was simulated, with 50k SNPs allocated in 29 chromosomes, such that the number of SNPs per chromosome and the linkage disequilibrium (LD) pattern were adjusted to resemble the cattle genome.

Genomic data simulations were performed in R language (R core team, 2018), using routines from the GenEval package (https://github.com/bcuyabano/GenEval), and correlated traits were simulated using self-coded routines. All evaluations were also performed in R language.

Selection scenarios with different weights for the two traits in the breeding goals were defined to evolve the population over many generations. For every simulated scenario, selection was performed on sires only, by selecting the top 20% bulls in agreement with the scenario's breeding goal. The choice of selection of sires only was made so that the simulation resembled a dairy cattle breeding program.

Genetic correlation as parameter

When GC was simulated as a parameter, its origin was purely quantitative. This means that part of the correlation was due to pleiotropic quantitative trait loci (QTL) for both PROD and FERT, and part of this correlation was due to different QTL for each trait, but that were in close proximity, such that these QTL were in a sufficient level of LD for GC to arise. For each trait, 3,000 out of the 50k simulated SNPs were assigned as QTL; 1,000 of these QTL were shared by both traits (pleiotropic); 1,000 QTL were trait-specific, but in close proximity to those trait-specific from the other trait so that these QTL were in LD; 1,000 QTL were trait-specific and far enough from any QTL from the other trait, so that their between-trait effects were completely independent. Figure 1 illustrates the described scheme of the QTL display on the simulated genome.

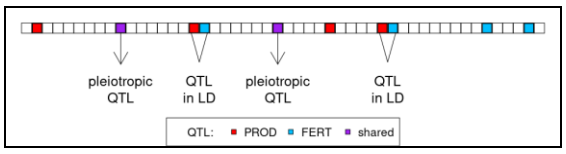

Figure 1. Scheme of the QTL display on the simulated genome, indicating the QTL responsible for creating genetic correlation between PROD and FERT (pleiotropic QTL, and QTL in LD), and the QTL that created independent effects on each trait.

For the simulation study under the hypothesis that GC was a parameter, the population was evolved over 40 generations under five different scenarios, one scenario in completely random mating, and four scenarios with selection of the top 20% bulls, with different weights for %PROD-%FERT in the breeding goal: (1) 100-0; (2) 90-10; (3) 80-20; and (4) 50-50. Each scenario was replicated 100 times.

Genetic correlation as a latent phenotype

When GC was simulated as a latent phenotype, we initially simulated RGLT was with heritability $h^2_{RGLT} = 0.1$, and then both PROD and FERT were simulated to have a concave parabolic relationship with RGLT, following the simulation method in Shokor et al. 2024. Figures 2 and 3 illustrate the relationship between the simulated BV for the three traits.

For the simulation study under the hypothesis that GC was a latent phenotype, the population was evolved over 50 generations under three selection scenarios of the top 20% bulls, with different weights for %PROD-%FERT-%RGLT in the breeding goal: (1) 100-0-0; (2) 80-20-0; and (3) 80-10-10. Each scenario was replicated 1,000 times.

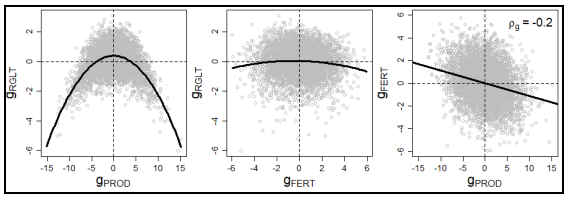

Figure 2. Scatterplots of the BV, denoted as g, simulated for the three traits when the genetic correlation between PROD and FERT was the consequence of a latent phenotype. The full black line in all the panels indicate the mean relationship between the pairs of traits.

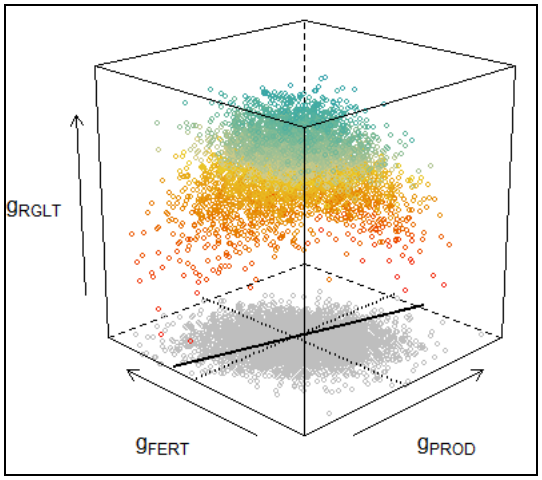

Figure 3. 3D scatterplot of the BV (colored dots, with the gradient red-yellow-blue representing negative-zero-positive values for RGLT), denoted as g, simulated for the three traits when the genetic correlation between PROD and FERT was the consequence of a latent phenotype. The gray dots are the projection of the simulated BV for PROD and FERT only, which are the observable traits. The full black line indicates the mean relationship between PROD and FERT, perceived as a linear correlation.

Results & Discussion

Descriptive results on real data

On the group of 247 subset sires, we could observe a clear pattern of genetic progress from 2002 for PROD, and from 2009 for FERT, as shown in Figure 4. Although FERT has been included in the breeding goals for the French Montbéliarde population in 2001, at this moment this breeding goal was defined mostly for the AI sires. Therefore, the genetic progress is not immediately perceived. Given the low heritability of FERT and considering the generation interval needed for a change in breeding goals to take effect, it not surprising that the clear pattern of genetic progress arises from 2009. Moreover, to further explain the trajectory of the genetic progress for FERT, it is only from 2006 that females began to be more systematically selected for fertility traits.

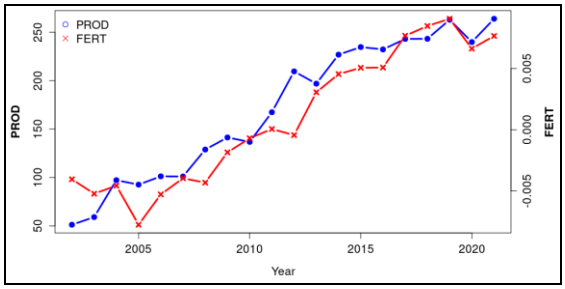

Figure 4. Yearly mean breeding values of PROD and FERT from 2002 until 2021, for the daughters of 247 sires with more than 500 daughters evaluated, all with both traits recorded.

With respect to the sire-specific GC between PROD and FERT, from Figure 5 we can observe that their values range from -0.3 to 0.3, a great dispersion around the GC of 0.051 estimated by REML. This dispersion is observed both on the sire-specific GC disregarding their daughters' year of birth and taking the year into account. When observing the distributions of the sire-specific GC per their daughters' year of birth, we observed that this distribution changes most visibly from 2009. From the year 2002 until 2008, sire-specific GC were on average negative, with a mean of -0.055. From 2009 on, these mean shifts to approach zero, and finally become mildly positive, with a mean of 0.063.

Based on our knowledge of the historical breeding goals for the French Montbéliarde population in the period from 2002 to 2021, and the observed response in genetic progress for PROD and FERT, it is then of no surprise that visible changes in the distribution of the sire-specific GC arise from 2009, as shown in Figure 5.

The great dispersion of the sire-specific GC between PROD and FERT suggests that considering these correlations as a static parameter may not reflect the true nature of what drives the relationship between PROD and FERT.

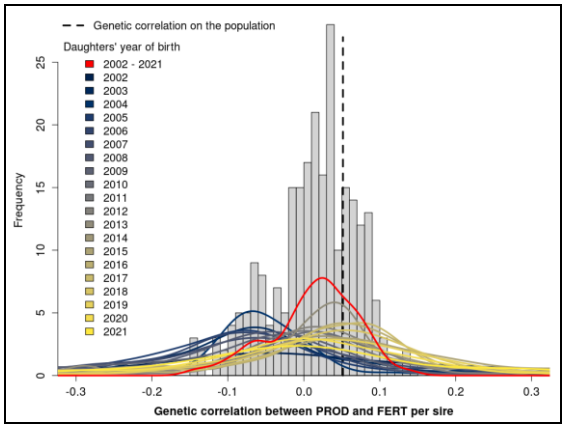

Figure 5. Histogram of the sire-specific genetic correlations (GC) between PROD and FERT, for 247 sires with more than 500 daughters evaluated, all with both traits recorded.

Genetic progress and genetic correlation on simulated data

The different assumptions of what causes GC between the antagonistic traits have a remarkably different impact on the trajectory of GC over generations for populations under selection.

When GC was simulated as a parameter, different selection scenarios generally presented significantly different outcomes. Under this hypothesis, a single-trait selection resulted in an attenuation of GC, *i.e.*, the negative GC evolved towards zero as generations progressed, as shown in Figure 6. Although we solely present the trajectory of

the GC for single-trait selection on PROD, the exact same trajectory was observed when selection was performed for FERT only.

Still under the hypothesis that GC is a parameter, with the exception of the scenario 90%PROD-10%FERT, for which GC remained stable around its original value, selection scenarios for both PROD and FERT inevitably lead to an intensification of GC, *i.e.*, the negative GC evolved to a farther more negative value as generations progressed, as shown in Figure 6. Moreover, the greater the equilibrium in the breeding goal between the two antagonistic traits, the faster this intensification of GC was observed.

Although the observed results in Figure 6 were initially surprising, these trends were statistically supported when we performed the calculus on the expected GC for the truncated bivariate normal distribution to select progressively increasing values for both means (calculus not shown), and can be explained in terms of loss of genetic diversity, assuming that the hypothesis that GC is a parameter is true, *i.e.*, that GC arises uniquely due to QTL effects. Nonetheless, questions remained about whether the observed trends in the simulated data were biologically sound, specially when compared to the results observed with the real data, as presented in Figures 4 and 5. This, combined with research in bovine physiology (Berry et al., 2016) lead us to hypothesize that genetic correlations may be a latent phenotype, or in other words, the observable consequence of a concealed physiological trait, responsible to regulate the trade-off between the antagonist traits.

When GC was simulated as the consequence of a latent phenotype, as shown in Figure 7, we observed that the different selection scenarios did not present the great differences as previously observed in Figure 6. In fact, in the short to medium term (up to approximately generation 15), the trajectory of GC was statistically the same for all selection scenarios. During the first 15 generations

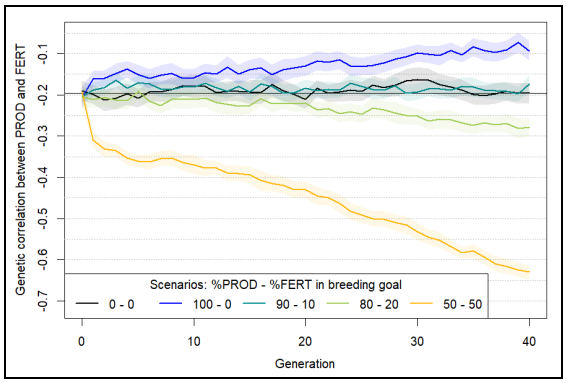

Figure 6. Trajectory of GC between PROD and FERT over 40 generations of populations under selection according to the five simulated scenarios of breeding goals (%PROD-%FERT). The full lines in the plot represent the mean GC observed with 100 replicates of each scenario, and the shaded area around the mean GC represent their 95% confidence interval.

under selection, the negative GC tended to be attenuated.

From generation 15 on, the overall trend of GC under selection for a single trait differs from that of selection for both traits. While under all simulated selection scenarios the GC reached a peak of attenuation, and then presented a trend of slow re-intensification, this trend seemed to be temporary when selection was performed for more than one trait, with GC reaching an apparent stabilization in its trend, from generation 30. When selection was performed for a single trait (in our simulations, PROD), the trend of re-intensification seemed constant throughout all generations after generation 15. These results presented in Figure 7 suggest that, although initially any breeding goal for a breeding program will lead to the attenuation of GC, in the long-term, single-trait selection will inevitably lead to stronger negative GC, compared to multi-trait selection.

To conclude the discussion with respect to the trends observed for the GC over generations in the simulated populations under different selection scenarios, the contrasting results from the two hypotheses considered to

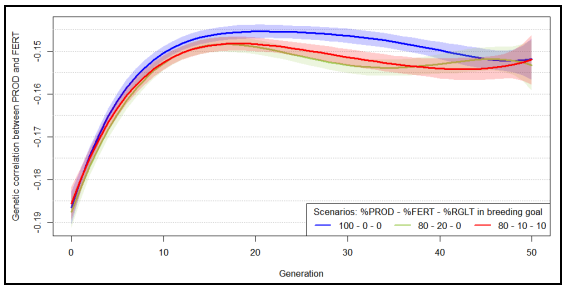

Figure 7. Trajectory of GC between PROD and FERT over 50 generations of populations under selection according to the three simulated scenarios of breeding goals (%PROD-%FERT-%RGLT). The full lines in the plot represent the mean GC observed with 1,000 replicates of each scenario, and the shaded area around the mean GC represent their 95% confidence interval.

simulate correlated traits presented in Figures 6 and 7, when compared to the results observed in real data, gives us information to support the hypothesis that GC is a consequence of a latent phenotype.

Finally, we evaluated the genetic progress achieved with the different selection scenarios, when GC was simulated under the hypothesis that they are a consequence of a latent phenotype. The results presented in Figure 8 show that, as expected, after 50 generations the average BV for production was mildly lower when multi-trait selection was in place. Since breeding goals for multi-trait selection kept a weight of 80% for production, although significant, the difference in PROD between the simulated scenarios was small. Therefore, the inclusion of FERT and RGLT did not largely decrease PROD. On the other hand, the inclusion of FERT alone, or FERT+RGLT to the breeding goal resulted in great changes to the average BV for these two traits, compared to the scenario in which selection was performed uniquely for PROD.

It was interesting to observe that, in the scenario for which selection was performed for PROD and FERT (without RGLT), the dual selection did impact positively the genetic progress of RGLT. Although not surprising, this result is reassuring that, if GC are the observed consequence of a latent physiological trait, selection for the observable traits is indirectly selecting for the latent trait.

To conclude the discussion with respect to the genetic progress, a final remark has to be done with respect to the selection including the latent physiological trait (RGLT) responsible to regulate the trade-off between PROD and FERT. If RGLT can be measured either directly or indirectly, and the included in the breeding goals, the genetic progress of this trait is relevant, counterbalancing the mild loss in genetic progress for the other traits of commercial interest (in the simulation PROD and FERT). The great genetic progress in RGLT due to its inclusion in the breeding goal has an importance, because such trait is very likely to have an influence in many other traits of commercial interest, beyond PROD and FERT. Thus, including RGLT in the breeding goals is expected to improve many of the traits considered in a real breeding program, which are far more traits than PROD and FERT.

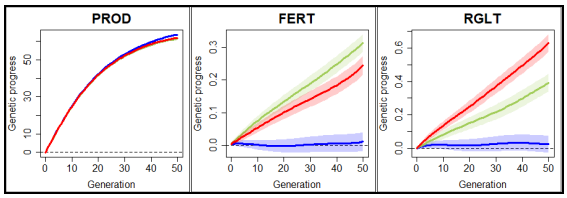

Figure 8. Genetic progress per generation, for PROD, FERT, and RGLT over 50 generations of populations under selection according to the three simulated scenarios of breeding goals (color-coded as in Figure 7, *i.e.* blue: 100%PROD-0%FERT-0%RGLT; green 80%PROD-20%FERT-0%RGLT; red 80%PROD-10%FERT-10%RGLT). The full lines in the plot represent the mean breeding values observed with 1,000 replicates of each scenario, and the shaded area around the mean GC represent their 95% confidence interval.

Conclusions

This work had the objective open a discussion about the nature of genetic correlations between traits. We evoked two hypothesis, one that assumes genetic correlations as a parameter driving the genetic architecture of correlated traits, and another that assumes that genetic correlations are the observable

consequence of a latent physiological trait responsible to balance the expression of measurable traits. Using simulations of breeding schemes considering different breeding goals under these two hypotheses, and comparing our simulated medium to long-term results with observations in real data, we believe that our study provides information to support the hypothesis that GC are a consequence of a latent phenotype. This hypothesis is relevant to define breeding objectives, since a regulatory trait may impact not two, but many traits altogether. Last but not least, although our study focused on the antagonism between production and fertility traits, the concept that genetic correlations may be the consequence of a latent phenotype can be extended to many other traits.

Acknowledgments

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Incorporation of external GEBV in the Dutch-Flemish dairy genetic evaluation

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Abstract

Incorporation of external breeding values in an evaluation is a convenient way to increase the information underlying the breeding values from a national evaluation. This provides improved estimates of breeding values of animals with mostly or wholly foreign pedigrees. In genomic analyses external breeding values can be used to increase the reference population. In this paper we present the approach to incorporating foreign or external breeding values taken for the Dutch-Flemish genetic evaluation. It consists of 1) deregression of external breeding values, removing national information to arrive at the deregressed proof containing foreign information only and 2) transformation of the deregressed proof to pseudo-observation records that can be used as 'own observations' in the routine evaluation. Solutions are presented for linear single animal effects models, correlated animal effects models and random regression models. Results are shown of a validating procedure in a random regression test day model for milk production.

Key words: External breeding values, deregression, genomic evaluation

Introduction

Incorporation of external breeding values in an evaluation is a convenient way to increase the information underlying the breeding values from a national evaluation. This provides improved estimates of breeding values of animals with mostly or wholly foreign pedigrees. In genomic analyses external breeding values can be used to increase the reference population. In this paper we present the approach to incorporate foreign or external breeding values taken for the Dutch-Flemish genetic evaluation.

Materials and Methods

The method to incorporate foreign or external BV in routine evaluations consists of 1) deregression of external breeding values, removing national information to arrive at the deregressed proof containing foreign information only and 2) transformation of the deregressed proof to pseudo-observation records that can be used as 'own observations' in the routine evaluation.

Deregression

The method of deregression is based on the work by Pitkänen et al. (2019). It requires the following components:

1. A VanRaden (2009) deregressed proof of the external breeding value (DRP_X) with corresponding expected record contribution (ERC_X)
2. A VanRaden deregressed proof of the national breeding value DRP_N with corresponding ERC_N

Note that for a trait the *expected daughter contributions* EDC and ERC are proportional, such that ERC = k*EDC and $k = (1 - h^2)/(4 - h^2)$. The relative weight of components does not change by using either ERC or EDC to deregress.

The target DRP to include in genetic evaluations is then obtained through:

$$ERC = (ERC_X - ERC_N)$$
$$DRP = [\ DRP_X * ERC_X - DRP_N * ERC_N] / ERC$$

The reliability of the DRP can be obtained by back transforming ERC. Foreign information of a bull is included in the evaluation if the reliability of the DRP is at least 0.10.

Transformation

To derive the correct transformation function it is necessary to distinguish between 1) *m* input traits, 2) *k* analyzed traits and 3) *n* target traits, where 1) input traits are the traits for which a BV or DRP are available, 2) analyzed traits are the traits actually in the evaluation and 3) target traits are the traits for which a pseudo-observation is desired. Usually two or all three trait categories are identical, but particularly in the case of random regression models this may not be the case. For example, in a milk production test day model, the input traits are cumulative 305 day BV, the analyzed traits are the polynomial variables shaping the production curve and the target traits are observations of milk production on a particular day in lactation.

General form

The general form of the transformation function is:

$$\mathbf{o} = \mathbf{Tb}$$

Where **o** is a vector with *n* desired pseudo-observations or target traits, **b** is a *m* size vector with input DRP/BV and **T** is the $n \times m$ transformation matrix derived from the genetic covariance matrix used in the evaluation.

The transformation matrix **T** is obtained through:

$$\mathbf{T} = \mathbf{DCV}^{-1}$$

Where **V** is a $m \times m$ genetic (co)variance matrix of *m* input traits and **C** is a $k \times m$ matrix with covariance between *m* input traits and *k* analyzed traits. The $n \times k$ matrix **D** links analyzed traits with target traits.

To obtain **C** and **V** a $m \times k$ matrix **F** is constructed linking input traits to analyzed traits. Both **C** and **V** are obtained using **F** through:

$$\mathbf{C} = \mathbf{GF'}$$
$$\mathbf{V} = \mathbf{FGF'}$$

Where **G** is the genetic (co)variance matrix for analyzed traits. This is usually the matrix used as parameter in genetic evaluations.

The explicit form of the complete transformation matrix is:

$$\mathbf{T} = (\mathbf{DGF'}) \cdot (\mathbf{FGF'})^{-1}$$

Linear trait breeding value

The most trivial of cases is when input, analyzed and target traits are identical. In that case **F** = **I** and **D** = **I**. If **F** = **I**, then **T** necessarily also is equal to **I**, reducing the transformation function to:

$$\mathbf{o} = \mathbf{Tb} = \mathbf{Ib} = \mathbf{b}$$

If the input trait is an index of underlying traits, for which pseudo-observations are required (with analyzed and target traits identical), we construct a *1 x n* matrix **F** = **w**, where **w** is a vector with index weights of the analyzed traits. For instance, if the input trait is an index of three underlying traits with index weights, such that:

$$\mathbf{C} = \mathbf{GF'} = \mathbf{Gw} = \mathbf{c}$$
$$\mathbf{V} = \mathbf{FGF'} = \mathbf{wGw'} = v$$

Since analyzed and target traits are identical **D** = **I** and can be omitted, reducing the transformation matrix **T** to a vector:

$$\mathbf{o} = \mathbf{Tb} = (\mathbf{CV}^{-1})b = b\mathbf{c}/v$$

The above can be readily extended to include multiple traits by extending **F** with lines for every index trait to be included.

Random regression breeding values

The proper construction of transformation matrix **T** in random regression model is illustrated using a milk production test day model as an example.

Assume a milk production RR model with 3 lactations and 5 Legendre polynomials for 15 analyzed traits in total. The input DRP are based on cumulative 305 day BV. Target traits are (expected) milk productions on day 60 of lactation for each lactation. Assume furthermore that **G** is ordered traits within polynomial (e.g. $poly_1$(lac1…lac5), $poly_2$(lac1…lac5), etc.). Additionally, we assume the presence of a c x l matrix **L** with l Legendre coefficients for each day in a lactation curve of c days in length. In this example $\mathbf{L} = \mathbf{L}_{5:420}$.

The transformation matrices **F** and **D** are of the following form:

$$\mathbf{F} = \mathbf{s} \otimes \mathbf{I}_3$$
$$\mathbf{D} = \mathbf{t} \otimes \mathbf{I}_3$$

Where **s** is a vector with cumulative Legendre coefficients $\sum \mathbf{L}_{5:305}$ and **t** is a vector with Legendre coefficients for DIM = 60, $\mathbf{L}_{60}$ and $\otimes$ denotes the Kronecker product of **s**/**t** and identity matrix **I** of size equal to the number of lactations.

These **F** and **D** are then used to construct **T** to provide pseudo-observations at DIM=60 corresponding to the DRP based on 305 day BV.

Weight or ERC of pseudo-observations

To accurately account for the reliability of the input DRP, weights must be calculated for the observations on the target traits. These can be obtained by transformation of the single trait ERC into corresponding multi trait ERC.

This cannot be done analytically, but a simple iterative procedure to obtain MT-ERC from ST-ERC is the following:

Reliability function

A vector with reliabilities **b** is a function of $\Re(\mathbf{Y}, \mathbf{G}, \mathbf{F})$, which calculates reliabilities according to the MT-ERC matrix **Y** (Liu et al. 2001), using genetic covariance matrix **G** and matrix **F**, where **F** is as before. The function is as follows:

1) $\mathbf{G_t} = \mathbf{G}[\mathbf{I} - (¼\mathbf{YG} + \mathbf{I})^{-1}]$
2) $\mathbf{b} = diag(\mathbf{F'G_tF})/(diag(\mathbf{F'GF})$, where **b** is now a vector with reliabilities for each trait, corresponding to observations enumerated in **Y**, on the diagonal. In this instance the operator / denotes element-by-element division.

Deriving multi-trait ERC from single trait ERC

Let **O** be a diagonal matrix with ERC. With function $\Re$ in place we can iterate on **O** until some convergence conditions are met. Let **b** be the matrix with DRP reliabilities and $\mathbf{b}_i$ the reliabilities from the ERC iterated on ($\mathbf{O}_i$). Since **O** corresponds to number of repeated observation records we assume values on the diagonal of $\mathbf{O}_i$ are integer values.

1) Calculate $\mathbf{Y}_i = 4(\mathbf{O}_i\mathbf{D})'\mathbf{R}^{-1}\mathbf{D}$
2) Calculate $\mathbf{b}_i = \Re(\mathbf{Y}_i, \mathbf{G}, \mathbf{F})$
3) Calculate $\mathbf{t} = (\mathbf{b}_i - \mathbf{b})$
4) Calculate convergence $c = (\mathbf{t't})/(\mathbf{b'b})$
5) Compare $\mathbf{b}_i$ with **b**
6) If $b_i(x) < b(x) - r$: $\mathbf{O}_{i+1}(x,x) = \mathbf{O}_i(x,x) + 1$
7) If $b_i(x) > b(x) + r$: $\mathbf{O}_{i+1}(x,x) = \mathbf{O}_i(x,x) - 1$ (with a certain minimum value, e.g. 1)
8) Repeat until c reaches a threshold value e or **O** stops changing: $\mathbf{O}_{i+1} - \mathbf{O}_i = \mathbf{0}$.

A theshold value can be $e = n*r^2/(\mathbf{b'b})$, where n is the number of elements in **b** and r is the maximum allowed error on $\mathbf{b}_i$ described above.

Validation of the transformation procedure

We applied the procedure described above to the MACE breeding values of a set of 54,194 Eurogenomic bulls for milk, fat and protein and incorporated the resulting pseudo observations in a full conventional milk production evaluation. Input MACE BV were from the Aug. 2023 Interbull evaluation. The full conventional evaluation was based on data for the Dutch/Flemish Dec. 2023 genetic evaluation. Pseudo-observations were fitted as own observations with a separate herd-testday-class. Apart from a random animal effect no other fixed or random effects were fitted.

From the evaluation we selected bulls without national information present in the data, only data from DRP, and compared the input Interbull BV to the BV estimated in the full conventional evaluation.

Results & Discussion

The results are presented in Table 1. Correlations between input BV and output BV were high (~0,99) with comparable standard deviations.

Table 1. Number of selected bulls, standard deviations and correlation *r* of input (MACE) and output EBV for milk production traits.

	N	*std MACE*	*std EBV*	*r*
Milk	37,429	851.6	865.8	0.988
Fat	37,398	31.6	33.2	0.985
Protein	37,413	27.6	26.7	0.990

Bulls were selected which did not have daughter data present in the evaluation, only DRP based on MACE BV.

The procedure outlined above provides a generalized and relatively convenient method of transforming deregressed proofs into pseudo-observations that can be used in a genetic evaluation directly. It precludes the need for the definition of correlated pseudo-traits and the redefinition of the statistical modelling of the evaluation.

The method relies on two matrices, **F** and possibly **D**, which must be constructed explicitly to carry out the transformation. If these are correctly defined in relation to input traits, analyzed traits and target traits, the construction of **T** is straightforward and the transformation of large numbers of DRP or EBV is a relatively simple and fast process.

Conclusions

A generalized approach to derive pseudo-observations from deregressed proofs was presented. The pseudo-observations thus obtained can be used in a genetic evaluation directly, as observations on existing traits, without the need for the definition of additional correlated pseudo-traits. The method is applicable to a variety of models and types of DRP.

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Modeling unknown parent groups or metafounders in single step genomic BLUP – results of a simulation study

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Abstract

The concepts considering for unknown parents are crucial in improving genetic evaluations in animal breeding by accounting for genetic differences within base populations. This study builds on a previous simulation study for the German-Austrian-Czech Fleckvieh population, presenting results that compare metafounders (MF) and unknown parent groups (UPG) for single-step genomic best linear unbiased prediction, and includes detailed analyses for scaling variance components when using MF. The results show that in both settings with complete and incomplete pedigree, evaluations using MF show the best bias and dispersion results, with minimal impact from incomplete pedigree information. In contrast, evaluations without UPG or MF and evaluations where UPG were incorporated via Quaas-Pollak-transformation in the pedigree-based and genomic relationship matrix (UPG_fullQP) exhibit substantial overestimation and overdispersion, emphasizing the importance of accurate relationship modeling in genetic evaluations. This study found that estimating variance components using MF and scaling variance components lead to the same heritability. However, using adapted variance components results in moderate overestimation and slight overdispersion of GEBV. The validation method based on the linear regression method could not detect the significant overestimation and overdispersion in UPG_fullQP. This means that commonly used validation methods tend to underestimate the advantages of MF in populations with numerous unknown pedigrees, highlighting challenges in model optimization for handling unknown parents.

Key words: ssGBLUP, unknown parents, metafounder, simulation, dairy cattle

Introduction

Thompson (1979) and Quaas (1988) published the concept of unknown parent groups (UPG) to account for genetic differences within subgroups of base populations, incorporating animals with missing parents and diverse genetic backgrounds into genetic evaluations. UPGs can have non-zero means but are assumed to be non-inbred and unrelated, similar to the base population. For single-step genomic best linear unbiased prediction (ssGBLUP) Legarra et al. (2015) extended this concept and introduced metafounder (MF), which can model relationships within and across subpopulations.

ssGBLUP uses an integrated relationship matrix (H), combining the pedigree-based (A) and genomic (G) relationship matrices. Ideally, both matrices should refer to the same base population (Christensen, 2012), though this is often not the case in cattle populations without adjustments. Methods to align G with A include those by VanRaden (2008), Vitezica et al. (2011), and Christensen (2012). MF is addressing this alignment by adapting A to match G.

In the German-Austrian-Czech Fleckvieh population, the first ssGBLUP genomic evaluation was published in April 2021 (Himmelbauer et al., 2021), using 15 UPGs for most fitness traits. MF is considered the gold

standard for ssGBLUP implementations (Meyer et al., 2018). Therefore, it is likely to be one of the next development steps in the national genomic evaluation system.

A small preliminary study for the case without unknown parents has already been published in Himmelbauer et al. (2023a). The detailed results based on a simulated cattle population based on two base populations, several scenarios and different pedigree settings were published in Himmelbauer et al. (2024). The results presented in this paper are in part a small selection from Himmelbauer et al. (2024) supplemented by more detailed analyses for scaling the variance components.

Materials and Methods

Simulating metafounders

The fundamental methodology employed for simulating the population is analogous to that described in Himmelbauer et al. (2024). The procedure begins by dividing the founder population into two subpopulations, with each subsequently selected independently. The populations are then reunited, forming the basis of the pedigree. From this point onwards, the pedigree is recorded, while the heritability (h^2) for the trait under selection is set to 0.3. Subsequently, a period of 30 years was simulated with selection based on PBLUP, followed by an 8-year period of selection based on ssGBLUP. Figure 1 provides a schematic representation of the simulation process.

Dataset

The entire simulation documented all pedigree information, phenotypes, genotypes, and TBV for all animals across all years. This data was used to create the study's dataset, described in detail by Himmelbauer et al. (2024) as "low pedigree completeness." In the simulation, all females with offspring had phenotypes. To mimic routine datasets, 90% of the phenotypes from the first 15 generations were randomly deleted. Additionally, 75% of males and 30% of females born in the last eight years were randomly genotyped. The final dataset includes approximately 154,500 phenotypes, 143,400 genotypes, and a total of around 1,105,500 animals in the pedigree.

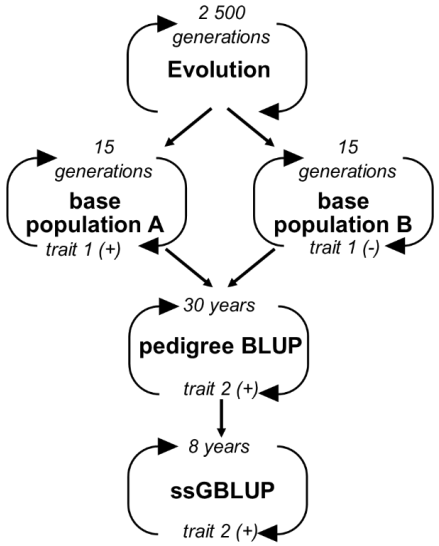

Figure 1. Schematic overview of the simulation process.

A reduced dataset was created for validation, using the same animals and genotypes but excluding the phenotypes from the last three years. Specifically, the phenotypes of all females born in years 32, 33, and 34 were excluded, resulting in 133,500 phenotypes in the reduced dataset.

For most analyses, some animals are assumed to have unknown sires and/or dams. The proportions of missing parents are 7.5% for sires and 10% for dams and are consistent across all birth years. Animals with unknown parents are randomly selected, but the potential for genomic parentage verification was considered, such that parents that can be identified with certainty (genotyped sires and dams of genotyped animals) or with a high probability (e.g., genotyped dam's sires of genotyped animals) are not deleted. This approach reflects practical scenarios and prevents double counting in genetic evaluations (Pimentel et al., 2022).

Pedigree settings

Two pedigree settings were tested, resulting in different classifications of unknown parents as UPG or MF.

Full pedigree

This setting uses the complete pedigree with no missing parents, except for animals born in year 0, forming the pedigree base. Base animals are assigned to their true subpopulations (purebred A or B), forming two UPG or MF.

True missing pedigree

This setting simulates unknown parents according to the previously described procedure. UPG or MF classification is based on subpopulation (purebred A, B, or crossbred AB), sex (missing sire or dam), and year of birth (grouped in five-year intervals). Since the full pedigree is known, true subpopulation and year of birth for missing parents are used.

Genetic evaluations

In order to test different methods of accounting for unknown parents, a series of genetic evaluations were conducted for the two pedigree settings. To calculate the estimated linear regression validation statistics (LR) (Legarra and Reverter, 2018), all evaluations were also computed for the truncated datasets. Except for the evaluation with scaled variances, we used the simulated genetic variance ($\sigma^2_{unrelated} = 0.3$) and for all evaluations, true base allele frequencies were used to construct the genomic relationship matrix.

All evaluations were conducted using MiX99 (MiX99 Development Team, 2019). The G matrix for ssGBLUP was prepared as in Himmelbauer et al. (2023b) using the HGINV program (Strandén and Mäntysaari, 2020), based on VanRaden's method 1 (VanRaden, 2008) and the approach for Proven and Young (Misztal et al., 2014a). For evaluations using the MF approach, base allele frequencies were set to 0.5, as outlined by Legarra et al. (2015).

1) ssGBLUP without UPG (no_UPG):

An ssGBLUP was used to estimate GEBV without UPGs. All unknown parents were set to 0, assigning them to a single base population.

2) ssGBLUP with UPG in A (UPG_alteredQP):

This ssGBLUP used UPGs in the pedigree, modeled as random. UPGs were included in the inverse pedigree relationship matrix (A^{-1}) and the inverse pedigree relationship matrix for genotyped animals (A_{22}^{-1}), but not in the inverse genomic relationship matrix (G^{-1}). This approach follows Masuda et al. (2018, 2022) and Strandén et al. (2022).

3) ssGBLUP with UPG in H (UPG_fullQP):

This method also used UPG and QP transformation was applied to A^{-1}, A_{22}^{-1} and G^{-1} as described in (Misztal et al., 2013).

4) ssGBLUP with MF and true Γ (MF_true):

In this ssGBLUP, unknown parents were represented by MF, with relationships defined by the true Γ. The variance-covariance matrix for breeding values was

$$var(u) = H_\Gamma \cdot \sigma^2_{unrelated},$$

where $\sigma^2_{unrelated}$ is 0.3 and H_Γ is the combined relationship matrix as described in Legarra et al. (2015).

5) ssGBLUP with MF, true Γ and scaled variances (MF_sc):

This evaluation is similar to MF_true, but with scaled variance components according to Legarra et al. (2015). The additive genetic variance was scaled using:

$$\sigma^2_{related} \approx \frac{\sigma^2_{unrelated}}{1 + \frac{\overline{diag(\boldsymbol{\Gamma})}}{2} - \overline{\boldsymbol{\Gamma}}}$$

(Legarra et al., 2015). The variance-covariance matrix for breeding values was then

$$var(u) = H_\Gamma \cdot \sigma^2_{related}.$$

Estimation of variance components

The variance components were estimated using AIREML (Misztal et al., 2014b). The data used

correspond to those used for the "full pedigree" pedigree setting, i.e., a complete pedigree with two base populations and all phenotypes that were also used for all test runs analyzed. Genotypes were not used in the variance component estimation.

Two different approaches were tested. On the one hand, the variance components were estimated in the case that the relationships between the two base populations (Γ) were not taken into account, and on the other hand with consideration of (Γ) in the creation of A. In addition, the results from the first approach were scaled using the scaling method of Legarra et al. (2015) and compared with those results from the second approach.

Analyzing results

All comparisons are based on 10 repetitions of the previously described simulation.

True validation statistics:

Two measures, bias and dispersion, are used to compare the different evaluations. These are calculated using the youngest animals with genotypes born in the last year of the simulation, totaling 14,672 animals.

Bias, the mean difference between (G)EBV and TBV, is calculated as

$$b = \overline{EBV} - \overline{TBV}$$

Positive bias values indicate overestimation. Given that the genetic standard deviation for the trait is 1, the bias can be interpreted as genetic standard deviations.

Dispersion is measured by the regression coefficient b_1 from the regression:

$$TBV = b_0 + b_1 \cdot EBV + e$$

where b_0 is the intercept, b_1 the regression coefficient and e the residuals.

Estimated validation statistics using linear regression (LR) method:

To obtain validation statistics, (G)EBV for certain validation animals based on a full dataset are compared with those from a reduced dataset. Two validation groups were defined: a male group (around 530 genotyped bulls born between years 30-32) and a female group (around 12,400 genotyped females born between years 32-34). Bulls in the male group have no daughters with records in the reduced dataset but at least 20 daughters in the full dataset. Cows in the female group have no phenotypes in the reduced dataset but have records in the full dataset.

Based on Himmelbauer et al. (2023b), the LR method accurately estimates bias, dispersion, and validation reliability (Legarra and Reverter, 2018; Macedo et al., 2020). Bias, the mean difference of GEBV between reduced and full datasets, is calculated as:

$$b = \overline{GEBV_r} - \overline{GEBV_f}$$

A bias of 0 indicates unbiased (G)EBV. Positive values indicate overestimation, and negative values indicate underestimation.

Dispersion is calculated as:

$$b_1 = \frac{\mathrm{cov}(GEBV_f, GEBV_r)}{\mathrm{var}(GEBV_r)}.$$

If $b_1 = 1$, there is no over- or underdispersion, $b_1 < 1$ indicates overdispersion, and $b_1 > 1$ indicates underdispersion.

Reliability is calculated as:

$$r^2 = \frac{\mathrm{cov}(GEBV_f, GEBV_r)}{\sigma_g^2},$$

where σ_g^2 is the true genetic variance in the validation group.

These statistics were calculated for both male and female animals across all pedigree settings, and genetic evaluations, and are based on 10 replicates.

Results & Discussion

Bias and dispersion

Figure 2 presents bias and dispersion results for the two pedigree settings and different evaluations. Regarding bias in the full pedigree setting, no_UPG, UPG_alteredQP, and UPG_fullQP show a slight overestimation of around 0.04 genetic standard deviations, with UPG_fullQP slightly higher at 0.07. MF with

true Γ underestimates by approximately 0.02 genetic standard deviations, while scaled variance components overestimate by 0.03 genetic standard deviations. In the true missing pedigree setting, no_UPG and UPG_fullQP exhibit substantial overestimation of 0.24 and 0.40 genetic standard deviations, respectively. MF evaluations show slight underestimation, with minimal impact from incomplete pedigree information.

Similar trends apply to dispersion (Figure 2, second row). In the full pedigree setting evaluations no_UPG, UPG_alteredQP, and UPG_fullQP show similar results regarding dispersion of around 0.96. MF_true and MF_est perform best with a regression coefficient of 1.00. However, scaling variance components with MF slightly worsens dispersion. In the true missing pedigree setting UPG_alteredQP maintains a similar dispersion level of 0.96, while no_UPG decreases to 0.93 due to incomplete pedigree data. UPG_fullQP shows the most significant impact, decreasing dispersion coefficients from 0.96 (full pedigree) to 0.74 (true missing pedigree). MF evaluations show consistent dispersion values, with MF_true at 1.00 and MF_sc at 0.97 for the true missing pedigree setting.

The evaluation using MF and true Γ shows the best bias and dispersion results in both pedigree settings, aligning with Bradford et al. (2019). Clear differences are observed in evaluations with and without UPG. The upward bias in no_UPG, along with overdispersion, arises because relationships in A, which only considers known relationships, do not match those in G, where all genomic relationships are fully considered.

UPG_fullQP exhibits significant bias and overdispersion due to double-counting when UPG is considered in G, despite G's complete genomic relationships. Similar issues have been identified in other studies (Bradford et al., 2019; Masuda et al., 2021; Meyer, 2021). UPG_alteredQP yields results similar to those with a complete pedigree because G accurately accounts for relationships and is unaffected by incomplete pedigrees.

All results presented here are also part of the study published by Himmelbauer et al. (2024). Beside a more detailed discussion of the presented results, in Himmelbauer et al. (2024) a comparison of these results with the results of a second scenario with less unknown parents, two additional pedigree settings and two additional genetic evaluations are presented.

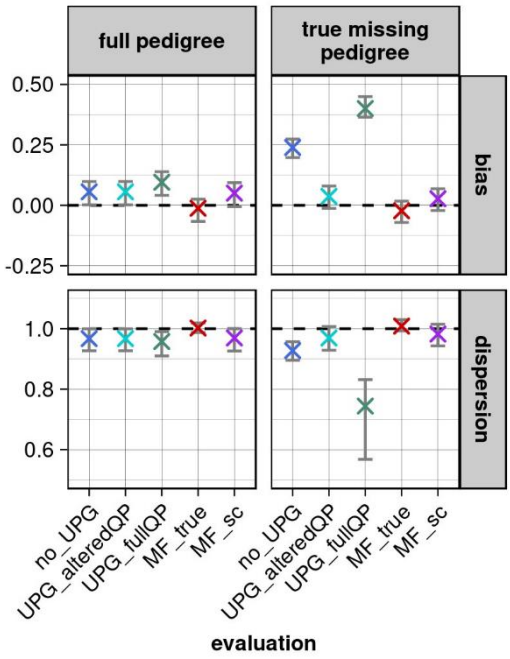

Figure 2. Comparison of true validation statistics (bias, dispersion) for 2 pedigree settings and 5 evaluation methods. The error bars in the plot show the range from minimum to maximum and the "x" show the means over 10 repetitions.

Scaling or estimating variance components

This study demonstrated that scaled variance components, compared to non-scaled ones, tend to result in a moderate overestimation rather than slight underestimation of GEBV. In terms of dispersion, scaled variance components have a negative effect, causing slight overdispersion. Similar effects were observed in a scenario with a complete pedigree and only two MF (Himmelbauer et al., 2023a). The effects of scaled variance components in this study are comparable, but to a lesser extent, with those reported by Himmelbauer et al. (2023b) in a

scenario with excessively too high heritability. This suggests that scaling may lead to a slightly too high heritability estimate.

Variance component estimation was performed to analyze this aspect in detail. Using A (pedigree relatedness without considering metafounder) it was possible to accurately estimate the simulated heritability (h^2) (Table 1 without MF/Γ). However, using A_Γ (pedigree relationship matrix considering MF relationships) resulted in an average h^2 of 0.3887, significantly higher than the simulated h^2. Yet, this h^2 corresponds closely to the scaled value calculated using the formula from Legarra et al. (2015), shown in Table 1 (scaled) as 0.3854. These analyses confirm that the scaling method of Legarra et al. (2015) works well and yields nearly identical results as estimating variance components with MF relationships. However, why the scaled h^2 provides slightly poorer validation results compared to unscaled h^2 remains unresolved.

Additional analyses in Himmelbauer et al., (2024) indicated that scaled variance components appear to influence the GEBV of the animals themselves but not the estimation of the MF effects.

Table 1: Results of variance component estimation with and without using MF and scaling variances.

	without MF/Γ		
	genetic variance	residual variance	h^2
mean	1.0663	2.3891	0.3088
min	0.9625	2.1878	0.2708
max	1.1142	2.6268	0.3224
	with MF/Γ		
	genetic variance	residual variance	h^2
mean	1.5212	2.3941	0.3887
min	1.3761	2.1918	0.3435
max	1.5941	2.6303	0.4045
	scaled		
	genetic variance	residual variance	h^2
mean	1.4970	2.3891	0.3854
min	1.3565	2.1878	0.3405
max	1.5644	2.6268	0.4006

Estimated validation statistics using LR method

Figure 3 displays the results of validation using the LR method for both pedigree settings and all genetic evaluations. Reliability shows minor variations among genetic evaluations, with those using MF performing slightly less effectively compared to other ssGBLUP evaluations. Notably, the validation does neither detect the full extent of the significant overestimation of no_UPG and UPG_fullQP, nor the extent of the pronounced overdispersion of UPG_fullQP in settings with incomplete pedigree. Validation statistics reveal no substantial differences between full and missing pedigree settings. In terms of bias, MF_true

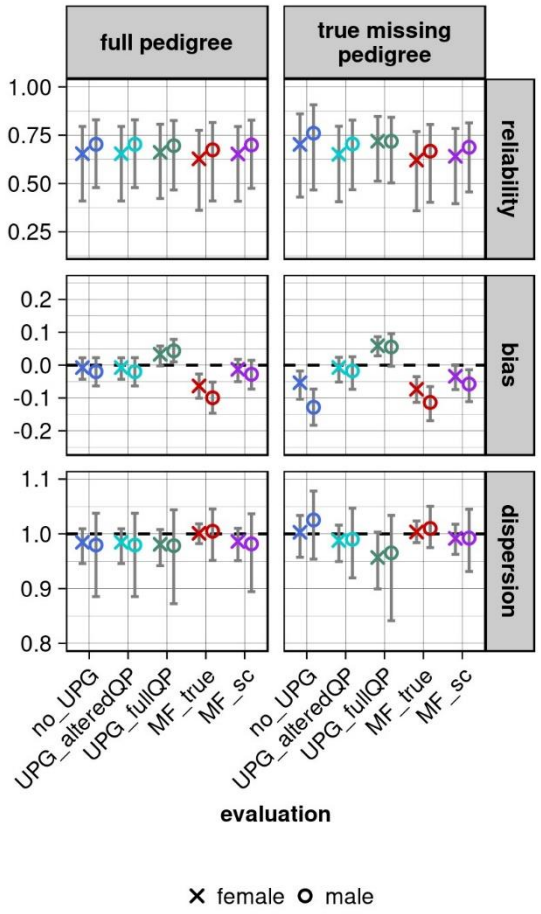

Figure 3. Comparison of estimated validation statistics (reliability, bias, dispersion) based on the LR method for 2 pedigree settings and 5 evaluation methods. The error bars in the plot show the range from minimum to maximum and the "x" and "o" show the means over 10 repetitions.

exhibits slight downward bias compared to other evaluations. However, regarding dispersion, both MF_true and MF_sc show a regression coefficient close to 1.00 across both pedigree settings, while no_UPG and UPG_alteredQP demonstrate slightly worse but still quite good results based on this validation.

The main conclusion is that also with this validation method evaluations using MF generally perform very well or at least better compared to other evaluation methods. However, differences in validation statistics are notably smaller than with true validation statistics. It is important to note that the significant bias and dispersion observed with UPG_fullQP is not detected by LR validation statistics. Such methods can only detect bias and dispersion if these issues are corrected in evaluations using complete datasets (Himmelbauer et al., 2023b). This is not the case here, as GEBV from UPG_fullQP appear nearly unbiased.

In summary, in populations with numerous unknown pedigrees, commonly used validation methods tend to underestimate the advantages of MF compared to other evaluations. This emphasizes the challenge of identifying an optimal model for dealing with unknown parents in practice.

Conclusions

In conclusion, the findings of this study indicate that MF has a positive effect on reducing bias and dispersion. The study highlights the potential of significant bias and dispersion when UPG is considered in an incorrect manner. Furthermore, the scaling of variance components was found to have a small detrimental effect on true validation statistics, rather than an enhancing one. The study also shows that the method of scaling variance components proposed by Legarra et al. (2015) leads to similar results as those obtained by estimating variance components using metafounder. Finally, the study identified limitations in the use of the LR method for assessing the effectiveness of MF in this context. These findings emphasize some of the challenges and outcomes associated with implementing MF in dairy cattle populations.

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Genomic validation software: USA update including truncated MACE

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Abstract

With the need to establish a standardized method to validate genomic estimated breeding values (**GEBV**) to meet the requirements for marketing the semen of young bulls in Europe, the Interbull Centre has routinely added new features to the GEBVtest software. In 2023, the United States (**US**) conducted a GEBV validation and reported that large population breeds and traits with high heritability were more stable, whereas smaller populations and complex traits often failed due to various reasons. In addition, the use of Truncated MACE (**TMACE**)-based genomic evaluations was recommended to verify if this model would outperform 4-year-old official results. A new version of the GEBVtest software will become the standard for GEBV validation in 2024. The new version includes bootstrapping to improve and expand significance, with better tests for slopes, validation accuracy, and bias and does not allow bulls with GEBV foreign proof to be included as candidates. In this study, GEBV validation was performed using the newest version of the GEBVtest software while validating truncated domestic plus TMACE instead of using official US predictions from 4 years ago and applied to US dairy cattle populations. Nine traits were tested and GEBV from August 2023 were used as the full dataset, whereas TMACE-based GEBV were used as the truncated dataset. A TMACE-based model can accommodate model or data changes over time as well as a validation on traits that were not even implemented four years ago such as mastitis for more breeds implemented in 2020 and 2022 in the US. In general, the inclusion of TMACE improved results for all breeds. For Holstein, all traits passed validation except for one trait that failed with a slope ($\mathbf{b_1}$) of 1.31 (>1.2). The b_1 standard error was 0.02, which confirms an underestimation of this trait. In smaller breeds, a few other traits failed validation due to a $b_1 < 0.8$, but showed clear improvement of the b_1 by including TMACE. Finally, the smallest breeds showed several inconclusive passes and fewer failures compared to a previous study. The results may be due to the complexity of traits and the small number of candidate bulls. The use of TMACE-based genomic evaluations improves the validation test and is a tool to be considered as standard when performing GEBV validation, especially for smaller breeds.

Key words: bootstrapping, foreign information, model change, reference population

Introduction

With the need to establish a standardized method to validate genomic estimated breeding values (**GEBV**), the Interbull Centre in Uppsala, Sweden, https://interbull.org/index, has developed and routinely improved the GEBVtest software (Interbull, 2021; Mäntysaari et al., 2011). Recognizing that standardized software may be the easiest and most practical way to validate GEBV, the United States of America (**US**) conducted a genomic validation in 2023 using the software proposed by the Interbull working group. The software incorporated new features such as different validation targets, base adjustments, and a larger birth year window for candidate bulls (Mota et al., 2023a).

It was reported by Mota et al. (2023a) that large population breeds and traits with high

heritability were more stable, whereas smaller populations and complex traits often failed due to various reasons, such as the small number of candidate bulls, the slope (**b_1**) being more or less than expected from the standard error (**S.E.**), the b_1 upper biological limit being higher than 1.20, and the prediction accuracy (**R^2**) of parent average (**PA**) exceeding the GEBV with small sample sizes.

In addition, the use of extra regressions to assist other tests (VanRaden, 2021) and Truncated MACE (**TMACE**)-based genomic evaluations was recommended to verify if this model would outperform validation results using 4-year-old official breeding values. TMACE is a relatively new voluntary base service introduced by Interbull Centre, scheduled annually in October. This service aims to supply validation inputs for countries that include foreign bulls without domestic daughters in their reference population. It follows the same logic as conventional MACE but requires countries to use the current conventional model with the most recent four years of data truncated. The truncated EBV are then submitted to Interbull for a TMACE evaluation (Jorjani and Dürr, 2011). The use of a TMACE-based model in countries that blend their domestic with international evaluations, allows for accommodating model or data changes over time, in any and all countries participating in MACE and TMACE, as well as validating traits that were not implemented four years ago where neither domestic estimated breeding values (**EBV**) nor GEBV were available. In the US, this is the case for traits such as clinical mastitis in the Jersey and Brown Swiss breeds, which were implemented in 2020 and 2022, respectively (Norman et al., 2020; Mota et al., 2021; CDCB Connection, 2022; CDCB Connection, 2023; Mota et al., 2023b).

A year later, Interbull developed a new version of the GEBVtest software (**gebvtest.py**) that will become the official validation standard in December 2024. This new version adds bootstrapping to improve and expand significance testing, with better tests for slopes, validation accuracy, and bias. In addition, the software automatically excludes bulls from the validation group if they have type of proof = 24 in the truncated data, which denotes GEBV that included performance records of foreign daughters. This new edit prevents countries from using foreign bulls that are already progeny-proven in the truncated data, with no domestic daughters within the country but having daughters worldwide. If the genomic evaluation includes MACE data, as was the case of this study, the GEBV will include all daughters, both foreign and domestic. Moreover, if a wider birth year window is used to add more validation bulls with smaller data sets, it could become a more significant issue that the bulls with foreign daughters only from four years ago should be excluded from the validation test group, as will be the case with this new edit.

Therefore, a genomic validation was performed using the newest version of the GEBVtest software, validating truncated domestic plus TMACE instead of using official US predictions from 4 years ago, and applied to US dairy cattle populations.

Materials and Methods

To provide updated results on the US dairy cattle populations, a genomic validation was conducted using the gebvtest.py software version *gebvtest_2023C2.py*. A new version *gebvtest_2024A.py* is under testing and will likely become the official version.

The genomic prediction datasets in US dairy cattle populations were GEBV extracted from the August 2023 genomic evaluation (full dataset), which included MACE input, and truncated GEBV to the year of 2019 plus TMACE input (truncated dataset).

In this study, five breeds were evaluated: Holstein (**HOL**), Jersey (**JER**), Brown Swiss (**BSW**), Red Dairy Cattle (**RDC**), and Guernsey (**GUE**). Nine traits were tested: milk yield (**MIL**), fat yield (**FAT**), protein yield (**PRO**), longevity (**DLO**), somatic cell score (**SCS**), clinical mastitis (**MAS**), heifer conception rate (**HCO**), cow conception (**CC1**), and calving

interval (**INT**). All breeds were evaluated for all nine traits with one exception: MAS was only tested in HOL, JER, and BSW since the US had no evaluations for this trait for the RDC and GUE breeds.

In addition to using bootstrapping and the exclusion of bulls with GEBV foreign proof, the following parameters were applied as in Mota et al. (2023a): (1) Predicted deregressed GEBV (**dGEBV**) were used rather than the conventional deregressed EBV (**dEBV**) or daughter yield deviations (**DYD**). Validating using later GEBV is easier for the public to understand and allows national evaluations that have adopted single-step methods to apply the validation straightforwardly (VanRaden, 2021). This was done by using the option "*--target DGEBV*" from the software; (2) Base adjustments were applied to the GEBV and not EBV as conventionally done by using the option "*--baseadj GEBV*". The minimum birth year used was 2015, which reflects the current year of data (2023) minus eight years, as recommended by Interbull; (3) Foreign bulls were included as candidate bulls to increase the validation group size for the small breeds only: BSW, GUE, and RDC.

The criteria for candidate bulls are reported in Interbull (2021) and Mota et al. (2023a). The number of candidates bulls ranged from 9 to 3,277 depending upon the trait and breed evaluated (Table 1).

Results & Discussion

In general, the inclusion of TMACE improved results for all breeds (Table 1) compared to the results reported by Mota et al. (2023a) when official GEBV from August 2018 were used as truncated data. One of the main reasons for better US genomic validation results in the present study is that both the US and Canada participated in TMACE simultaneously.

For the HOL breed, a PASS was observed for all traits except HCO. This trait failed due to a b_1 of 1.31, higher than the upper biological limit (1.20). The b_1 standard error was 0.02, which confirms an underestimation of this trait. An important point to highlight is the trait MAS. As seen in Table 1, with the use of the TMACE methodology, a PASS was observed, whereas Mota et al. (2023a) reported a FAIL using official GEBV from 4 years prior due to the b_1 being higher than the biological limit. This is because model and data ingestion differences (Gaddis et al., 2020) between full and truncated GEBV used as input by Mota et al. (2023a) were overcome by the use of the TMACE methodology. In addition, the use of TMACE provided a less biased PA prediction (10% vs. 17%) and a b_1 within the biological interval of 0.80 and 1.20 (1.08 vs. 1.30). Another example of the benefits of TMACE is if the same data is used in this study as full but replaced the truncated data with official GEBV from 4 years ago (i.e., 2019), a FAIL for MAS continues to be observed (Table 2). After the implementation of MAS, the model was changed, and there was a significant effort to include much more data for this trait, which clearly impacted GEBV predictions over time. Therefore, current GEBV and those from four years ago are not directly comparable (Mota et al., 2023a).

The JER breed passed for most traits except the fertility traits of CC1 and INT (Table 1). There was a clear improvement in b_1 with the inclusion of TMACE compared to results reported by Mota et al. (2023a), as shown in Table 2. However, it was still not enough to pass the validation test due to high standard error. Fertility traits are under significant work to improve their predictions. As with HOL, MAS is again a noteworthy trait. This is because MAS evaluations for JER were implemented after 2018 (Norman et al., 2020; Mota et al., 2021), the year of the GEBV predictions used by Mota et al. (2023a) as truncated data input. Therefore, while Mota et al. (2023a) were not able to genetically validate this trait, the use of TMACE methodology allowed us to do so for this breed and trait combination, accounting for the current model in use and all data and model changes over time.

Table 1. GEBV Validation results for the five breeds evaluated in this study: Holstein, Jersey, Brown Swiss, Red Dairy Cattle and Guernsey

Holstein

Trait	*Bulls*	b_1 ±*S.E.*	R^2 *GEBV*	R^2 *EBV*	Pass
MIL	2,767	1.08±0.01	68	36	Yes
FAT	2,767	1.07±0.01	74	48	Yes
PRO	2,767	1.03±0.01	70	44	Yes
DLO	2,509	1.18±0.02	65	43	Yes
SCS	2,731	1.09±0.01	75	36	Yes
MAS	1,738	1.08±0.03	50	10	Yes
HCO	3,277	1.32±0.02	53	20	No
CC1	3,277	1.10±0.02	68	31	Yes
INT	3,277	1.03±0.01	65	27	Yes

Jersey

Trait	*Bulls*	b_1 ±*S.E.*	R^2 *GEBV*	R^2 *EBV*	Pass
MIL	486	1.07±0.03	79	51	Yes
FAT	486	1.05±0.03	75	44	Yes
PRO	486	1.02±0.03	76	51	Yes
DLO	435	0.86±0.05	41	36	Yes
SCS	481	1.09±0.04	63	37	Yes
MAS	222	0.81±0.15	13	12	Yes
HCO	516	0.98±0.08	27	10	Yes
CC1	445	0.83±0.05	40	28	No
INT	480	0.81±0.04	45	32	No

Brown Swiss

Trait	*Bulls*	b_1 ±*S.E.*	R^2 *GEBV*	R^2 *EBV*	Pass
MIL	71	0.86±0.07	66	46	Yes
FAT	71	0.77±0.08	54	31	No
PRO	71	0.82±0.08	60	45	Yes
DLO	63	0.73±0.15	33	18	hSE
SCS	69	0.74±0.10	39	32	No
MAS	NA				
HCO	75	1.04±0.20	22	6	Yes
CC1	63	0.93±0.13	35	27	Yes
INT	71	0.84±0.11	43	31	Yes

Red Dairy Cattle

Trait	*Bulls*	b_1 ±*S.E.*	R^2 *GEBV*	R^2 *EBV*	Pass
MIL	18	0.68±0.15	43	43	hSE
FAT	18	0.83±0.22	55	57	Yes
PRO	18	0.75±0.16	52	53	hSE
DLO	9	0.59±1.19	5	13	hSE
SCS	18	0.90±0.51	16	30	Yes
MAS	NA				
HCO	16	2.18±0.84	30	5	hSE
CC1	16	-0.21±0.8	1	4	hSE
INT	17	-0.04±0.5	4	0.1	No

Guernsey

Trait	*Bulls*	b_1 ±*S.E.*	R^2 *GEBV*	R^2 *EBV*	Pass
MIL	16	0.87±0.25	35	8	Yes
FAT	16	0.31±0.33	5	4	hSE
PRO	16	0.08±0.54	0.3	0.1	hSE
DLO	NA				
SCS	16	1.79±0.59	41	22	hSE
MAS	NA				
HCO	NA				
CC1	12	2.19±0.58	68	77	No
INT	16	1.87±0.31	70	52	No

MIL: milk yield; FAT: fat yield; PRO: protein yield; DLO: longevity; SCS: somatic cell score; MAS: clinical mastitis; HCO: heifer conception rate; CCR: cow recycling; INT: calving interval; hSE: high standard error; EBV/GEBV R^2 are expressed in %.

Table 2. Comparison of three genomic validation scenarios (S) using different truncated data as input

S	*Bulls*	b_1 ±*S.E.*	R^2 *GEBV*	R^2 *EBV*	Pass
Brown Swiss - INT					
S1	88	0.64±0.16	16	27	No
S2	77	1.30±0.19	40	40	hSE
S3	71	0.84±0.11	43	31	Yes
Jersey - INT					
S1	588	0.79±0.03	47	31	No
S2	500	0.71±0.04	32	32	No
S3	480	0.81±0.04	45	32	No
Holstein - MAS					
S1	2,379	1.30±0.03	40	17	No
S2	1,548	0.60±0.05	9	10	No
S3	1,738	1.08±0.03	50	10	Yes
Jersey - MAS					
S1		NA			
S2		NA			
S3	222	0.81±0.15	13	12	Yes

S1: 2022-2018 official GEBV; S2: 2023-2019 official GEBV; S3: 2023 official GEBV and 2019 truncated MACE; INT: calving interval; MAS: clinical mastitis; hSE: high standard error.

The BSW breed had a FAIL for FAT and SCS due to a $b_1 < 0.8$, an inconclusive PASS test for PRO, but also with a $b_1 < 0.8$, and a PASS was observed for the other traits (Table 1). The S.E. of b_1 were much larger than those observed for the aforementioned larger breeds HOL and JER. This is likely linked to the much smaller number of candidate bulls and the fact that more than 50% of the BSW reference population in the US is composed of foreign bulls, primarily from Switzerland and France (Mota et al., 2023b). These foreign bulls likely have daughters outside the US earlier than within the country. So, even if the effective daughter contribution (**EDC**) in the US is equal to zero when truncating the data, this is not true worldwide, making these bulls ineligible as candidate bulls.

As seen in Table 1, there are no results for MAS for the BSW breed. This is because there were no bulls to validate MAS at this time. However, if there were candidate bulls, this trait would have been validated using the TMACE methodology, even if it is a trait recently implemented by the Council on Dairy Cattle Breeding (**CDCB**), i.e., in 2022 (CDCB Connection, 2022; CDCB Connection, 2023; Mota et al., 2023b).

Finally, the smaller breeds RDC and GUE showed several inconclusive passes and far fewer failures compared to the results reported by Mota et al. (2023a), as shown in Table 1. These results may be due to the complexity of traits and the small number of candidate bulls.

For RDC, a PASS was observed for FAT and SCS, even though the R-squared for the GEBV is smaller than for PA. This indicates a high S.E. for the R^2 test and it cannot be concluded that the R^2 is significantly lower with the GEBV ($P > .05$). Numerical differences are often due to sampling bias, making the R^2 too high for the PA, and the (lower) R^2 for GEBV is actually more reasonable. A FAIL in this case is likely an unreliable decision because the small sample inflation of R^2 for PA are ignored and combined with a reasonable alignment of model R^2 with truncated GEBV and the corresponding genomic reliabilities. With the new software, the R^2 test is still applied as additional information, but it causes an overall FAIL in the GEBV test if the R^2 is significantly lower ($P < .05$) for the GEBV than it is for the PA.

The GUE results here were very similar to RDC with several inconclusive passes, mostly due to the small number of candidate bulls (Table 1). DLO and HCO had no bulls to apply a genomic validation for GUE, whereas MAS is not implemented for either of these breeds, RDC and GUE.

Significance testing of the validation slope parameter was theoretically improved with the implementation of bootstrapping in the updated validation test software. In practice, the

bootstrap tests were very similar to t-test results applied in earlier versions of the software (e.g. Mantysaari et al, 2011), as verified by both Canada (Table 3) and similarly by USA in the present study. Several new tests were also added which make use of the full posterior probability distributions now available from bootstrap samples. For example, the software now includes new significance tests for bias in top young genomic bulls specifically, for the average bias across all young genomic bulls, and additionally the new tests described earlier for significance of R^2 improvements due to genomics.

In summary, the use of TMACE-based genomic evaluations improves the validation test and is a tool to be considered as standard when performing genomic validation, especially for smaller breed populations.

Table 3. Estimated t-values based on bootstrap samples (Boot.) versus the standard t-test, for *n* traits by breed in Canadian research data described by Sullivan (2023)

Breed	*Value*	*Mean*	*SD*	*Min*	*Max*
RDC	Boot.	-0.47	1.43	-3.7	2.4
n=32	t-test	-0.56	1.54	-4.1	2.7
JER	Boot.	-0.58	1.89	-5.6	3.4
n=30	t-test	-0.63	1.93	-5.3	3.3
HOL	Boot.	-1.31	6.55	-18.0	9.1
n=36	t-test	-1.58	7.05	-18.1	9.5

Conclusions

Countries must ensure they use candidate bulls with no daughters, domestic or foreign, from four years ago. The tests continued to fail for smaller breeds and less heritable traits due to b1 underestimation, the biological interval being between 0.80 and 1.20, and/or not enough bulls to validate. As in CAN, bootstrapping provided trivial differences to the results. TMACE resulted in a fairer test, and countries are strongly encouraged to use Truncated MACE service when performing GEBV validation, especially those with small populations. Finally, for maximum benefit, it is recommended that groups of countries share their genotypes to all participate in TMACE simultaneously.

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The mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by CDCB. The CDCB is an equal opportunity provider and employer.

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PREP database: Extension to Genomic Form

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Abstract

The Performance Recording, Evaluation and Publication (PREP) database is an online platform developed by Interbull Centre under the umbrella of the Centre's activities as the European Union Reference Centre (EURC) for Zootechnics, specifically designed for breed associations and National Genetic Evaluation Centres in order to submit and share descriptive information for a broad range of breeds and traits for both dairy and beef cattle. The main purposes of PREP include transparency, comparison and harmonization of information collected and used at the national level. To date, there are different electronic forms available to collect descriptive information for both dairy and beef cattle regarding *conventional* genetic evaluation for several trait groups. Next steps would be to expand PREP, so to be able to also collect descriptive information for *genomic* information. To do this, different approaches, and their level of pros and cons, were considered leading to identify a slightly different form's structure, compared to the one currently applied for conventional evaluations, as the most convenient for recording genomic information. The new form's structure will have all breeds and trait groups in one form, rather than having different forms per trait groups, as specific general traits' information regarding, for example, trait definition, method of recording, heritability etc. will be already available in the dedicated conventional forms. Moreover, the selected approach will allow to copy/paste information across different breeds/trait groups, which often do appear to be very similar (if not identical) when dealing with genomic evaluations. In summary, the new electronic genomic form will be considered as the general form for users to provide descriptive information related to their national genomic evaluations.'

Key words: PREP, harmonization, genomic form, national genomic evaluation, EURC

Introduction

The Performance Recording, Evaluation and Publication database (PREP) was developed, and is hosted, at the Interbull Centre within the context of the Centre's function as the European Union Reference Centre (EURC) for Zootechnics (Bovine breeding) with the aim of promoting transparency of methodologies applied, and easier comparisons of best practices (via a standardized set of options available for each question), all in all leading to harmonization and/or improvement of the methods of performance testing and/or genetic evaluation applied.

Regarding traits' harmonization, trait correlations play an important role in the quality of the estimations for international evaluations. On the other hand, harmonization of traits helps to improve correlations in order to achieve more accurate international and national evaluations. In this regard, extracting and reviewing information for each individual trait from PREP can lead the given correlated traits to International Committee for Animal Recording (ICAR)-Interbull guidelines in order to improve the across-country compatibility of traits. To date, the ICAR-Interbull guideline for trait harmonization has been published for Calving traits in 2022 (https://interbull.org/ib/eu_detailed_technical_reports), and Fertility traits harmonization has been approved in 2024 and will be included in the ICAR-Interbull guideline in the near future.

PREP is an online platform available for all breed societies and National Genetic Evaluation Centres (NGECs) beyond their direct involvement with any of the international evaluations currently offered by Interbull Centre.

Benefits of PREP

PREP has several benefits over document-based records:

a) Provides easier harmonization and standardization of information by comparing evaluation methods, trait definitions etc. across countries, breeds and traits in an easier and more efficient way

b) Represents a common database accessible to all cattle breeding organisations and third parties (both inside and outside Europe) including NGECs, researchers and competent authorities regardless of their direct involvement in any of the Interbull Centre's International evaluations for dairy (Interbull) and/or beef (Interbeef)

c) Collected information is freely available to the world wide web, either by directly accessing of specific country-breed-trait forms or through ad-hoc query options that can be easily defined by the user under "*Submission*" and "*Data queries*" tabs.

Current and upcoming forms on PREP

Several conventional genetic forms are at the moment available within PREP for different trait groups, both for dairy and beef cattle (http://prep.interbull.org). Available forms cover trait groups such as production, calving, female fertility and conformation traits for dairy cattle and adjusted weaning weight, calving and carcass traits for beef cattle. Throughout the course of this year, PREP will be expanded with new forms for udder health, longevity and workability traits for dairy cattle.

In addition to the above-mentioned forms, PREP also includes an "Other traits" form, aimed at collecting information regarding a wide range of traits and breeds recorded at the national level but not yet evaluated internationally. The relevance of such information is twofold: 1) Provide an overview of novel traits recorded in different countries and the status of their evaluation: implemented or still in a research phase; evaluated via a conventional or a genomic model (single step or two-step approach). 2) Explore the opportunity to identify new traits suitable for international evaluations, and the related challenges (i.e. new phenotype recording) for such traits. Examples of traits currently collected through the "Other traits" form are claw health, metabolic diseases (such as milk fever, clinical and sub-clinical ketosis), gestation length, and feed efficiency.

Overview of the structure of the current forms

Each of the conventional electronic forms currently available in PREP covers a specific trait group. Each form is made up by four different sections, with the first (and main) section allowing to select the different breeds/traits (within the specific trait group) the form will deal with, as shown in Figure 1.

The second section collects all general information regarding each trait selected. This section includes information referring to, for example, trait definition, method of recording, trait heritability, genetic variance, and data edits, as shown in Figure 2. Each set of questions is equipped with a list of standardized options that can be chosen from.

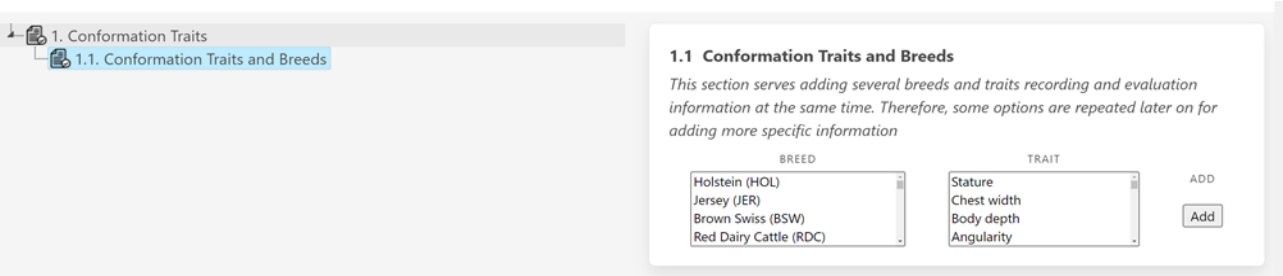

Figure 1: Section related to breed trait combinations of the current conventional form on PREP

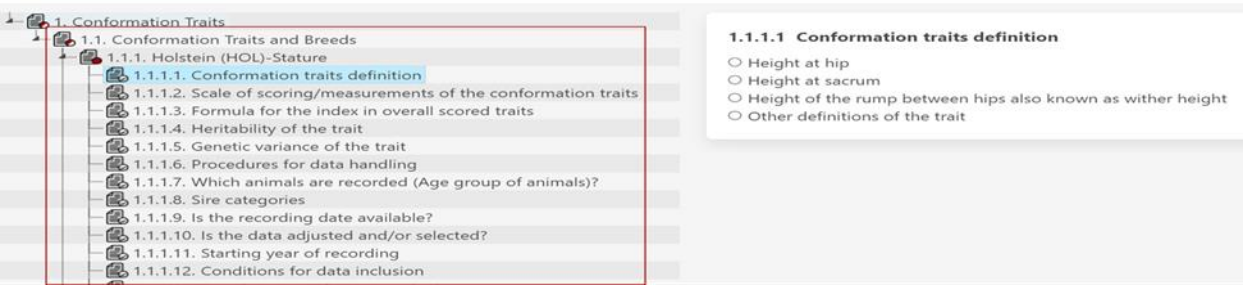

Figure 2: Section related to the general information for each individual trait of the current conventional form on PREP

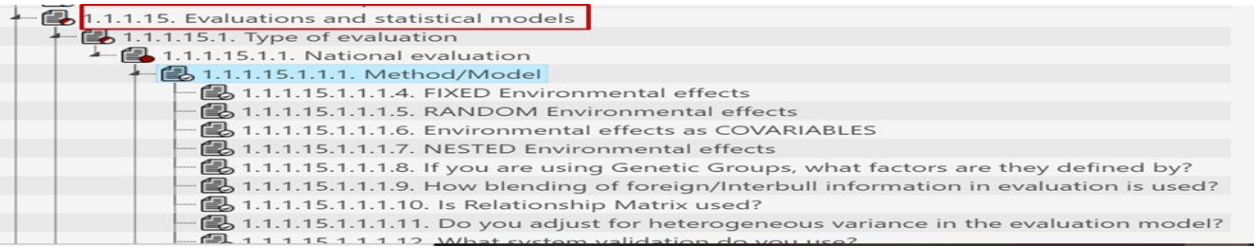

Figure 3: Section related to the evaluations and statistical models of the current conventional forms on PREP

The third section collects information regarding the evaluation methods and statistical models applied. After choosing the type of evaluation (national or international), questions regarding this section will focus for example on the model applied (i.e. Multi-breed (MB) or Multi-trait (MT), BLUP or Animal model), fixed and random effects used and publication criteria. An example of this section is shown in Figure 3. Database's tools will allow the user to copy and paste (and modify) the answers provided to one type of evaluation/breed to the others available within the same form.

The fourth and last section collects information regarding the scientific base, such as the scientific references that have been used for reliability or validation methodologies, as shown in Figure 4.

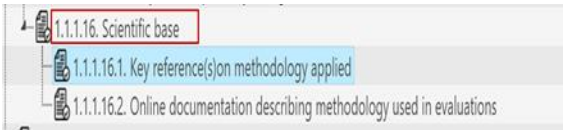

Figure 4: Section related to the scientific references of the current conventional form on PREP

Collecting genomic information via PREP

To complete the collection of descriptive information, specific forms need to be created in order to also collect information regarding genomic evaluations. Several approaches have been considered by the Interbull Centre. The approach described in this paper was the one found as the most efficient for collecting general genomic information in PREP.

Structure of the general genomic form

The structure of the genomic form remains similar to the current conventional form, but with questions and type of answers being more genomic-oriented. The new genomic form will consist of three main sections:

a) *Genomic information*: such as SNP-chip used, method for imputation and reference population.

b) *Genomic methods and models*: for example single-step genomic evaluation (ssGBLUP), bayesian or polygenic models.

c) *Genomic reliability and system validation*: including publication criteria, genomic reference base, scientific base etc. will also be part of the genomic form.

The new genomic form will therefore have one section less compared to the conventional form: the section reporting general information regarding the trait definition, method of performance recording, data edits, heritability etc., in fact, will not be part of the new genomic form as such information will already have been provided within the related conventional (genetic) form and will therefore already be available in PREP.

Another main difference will be within section 1: While in the conventional form section 1 will allow users to select breeds and individual traits, within the trait group the form was referring to (i.e. production, conformation, calving), section 1 of the genomic form will allow selecting *breed-trait group* combinations. This means there will be only one form for all available breed-trait group combinations (Figure 5).

Advantages of the general genomic form

The chosen approach has several advantages: It would be more efficient and easier for users to fill in only one form; as the trait-specific information has previously been provided in the PREP conventional forms, there is no need for repeating the same information for each trait or trait group. All information provided for one breed-trait group can be easily and rapidly copied across the remaining breed-trait groups selected. For example, information provided for production traits could be copied to calving or female fertility traits, or from Holstein to Jersey breed (https://interbull.org/ib/prep_user_manual), as shown in Figure 6. Should the copied information need some adjustments to make them fit properly the specific breed-trait groups they have been pasted into, such information can be easily modified. For example, if the definition of the reference population differs between the Holstein and Jersey breed, the information can be edited as shown in Figure 7.

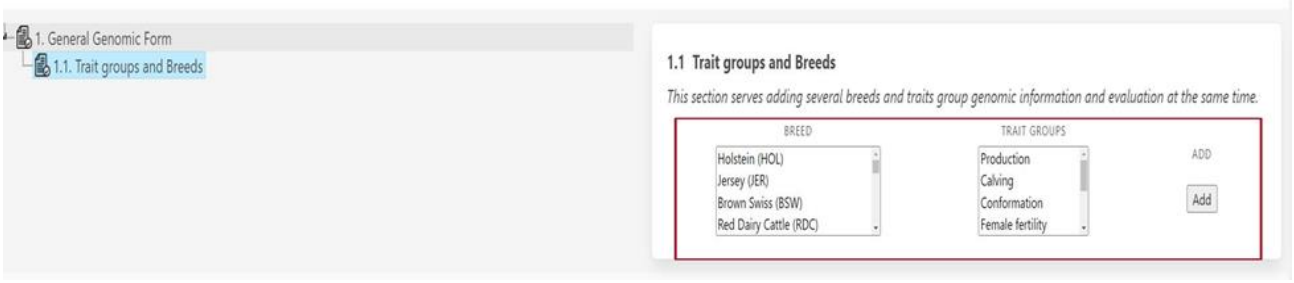

Figure 5: Structure of the proposed genomic form on PREP

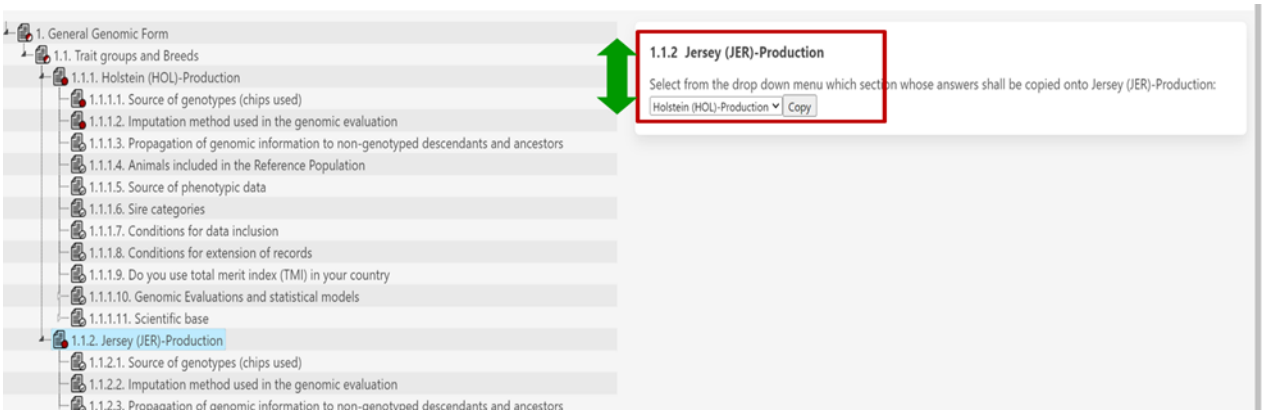

Figure 6: Feature for copying information among different breed-trait-groups in the proposed general genomic form.

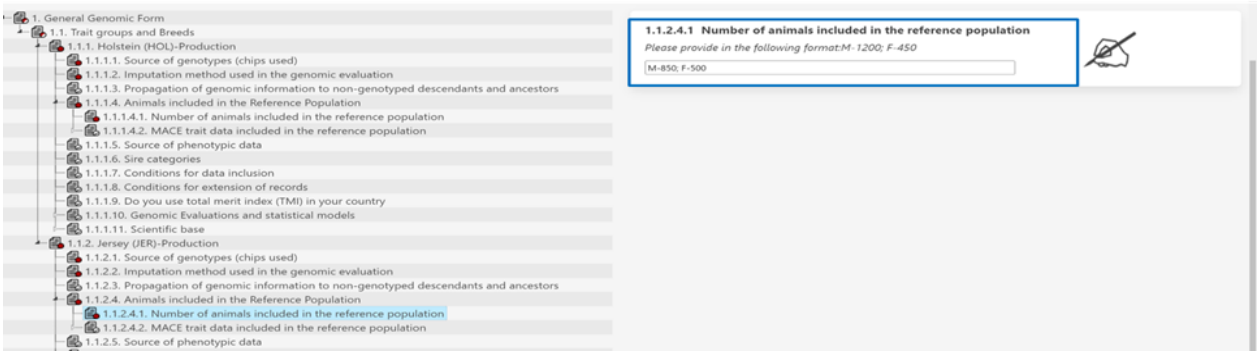

Figure 7: Feature for editing information for different breed-trait-groups in the proposed general genomic form.

Conclusion

PREP is an online platform with many benefits not only for Interbull users but also for breed organizations, NGECs, universities, students and other third parties, regardless of their involvement in international evaluations. Free access to the submitted data via *Submission* and *Data query* tabs makes it efficient and user-friendly to browse and look up information for different traits and breeds for both dairy and beef cattle. The development of a genomic information form is the next milestone in the development of the PREP database. A *general genomic form* with all trait groups in one form will make it easier for users and organizations to provide genomic information in one go, without the need to repeat the information already provided for each trait within the genetic form. The feature for copying and editing the information across different breeds and trait groups makes the genomic form more efficient. In order to fully benefit from all advantages of the new genomic form, users would still require to fill in all trait-specific information available within the conventional forms.

Acknowledgements

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https://interbull.org/ib/eu_detailed_technical_reports

Designing a validation application for genetic and genomic evaluation systems in the New Zealand dairy industry

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Abstract

New Zealand Animal Evaluation Limited (NZAEL) is considering a transition from their pedigree-based genetic evaluation system to a single-step genomic evaluation system, both of which use BOLT and Helical software. Central to the successful implementation of this system is a robust validation process that ensures the reliability of genomic breeding values (GEBVs) compared to current traditional estimated breeding values (EBVs). To accomplish this task, NZAEL and AbacusBio began a collaborative project to design an automated validation pipeline and accompanying R Shiny application. The objective was to create a tool that efficiently assesses the performance of the new genomic evaluation system across more than 30 traits, focusing on flexibility, minimal user intervention, and applicability to various stakeholder needs. The design process began with a facilitated workshop aimed at defining the project's scope. Key outcomes included the identification of critical validation analyses and metrics, criteria for evaluating (G)EBV performance, and the selection of relevant focal groups for the initial validation. This approach prioritized the needs of preliminary stakeholders, while also considering the broader interests of the New Zealand dairy sector. A significant aspect of the project was differentiating between 'routine' validation analyses, which would be directly integrated into the application, and 'exploratory' analyses, which required additional resources. This distinction allowed for a more focused development effort and a clearer understanding of the project's deliverables. The result of this collaboration was a validation application that streamlines the identification of problems and communication with stakeholders. Our experience underscores the importance of a user-centric design process in developing scientific tools, highlighting the need for clear communication, stakeholder engagement, and flexibility in project management.

Key words: dairy, model validation, genomic evaluation, New Zealand

Introduction

NZAEL is considering transitioning from their pedigree-based genetic evaluation system to a single-step genomic evaluation system, both of which use BOLT and Helical software (Garrick et al., 2018). This includes the introduction of a strict filtering process to ensure that only high-quality data enters the genetic evaluation. Central to the successful implementation of this system is a robust validation process that ensures the reliability of GEBVs compared to current traditional EBVs. To accomplish this task, AbacusBio was asked to develop a system for validating the models.

While the scientific literature extensively covers various validation methodologies such as bootstrapping (Weller et al., 2003), linear regression models (Legarra and Reverter, 2018), and bias estimation (Hickey et al., 2008), less attention has been paid to the practical implementation of these methods. In our experience, validation serves dual purposes: development and communication. From a development perspective, the aim is to refine a genetic evaluation model to ensure it produces the best predictions of genetic merit available

within the constraints of available data and resources. Validation results are used to fine-tune model parameters and the pre-processing of data extracts. On the other hand, communication through validation seeks to gain the approval of key decision-makers, facilitating adoption of the new system and building trust among stakeholders.

In scenarios where a considerable financial commitment has been made and the evaluations are likely to face scrutiny, it becomes especially beneficial to engage an independent third party for the validation. Engaging an independent validator not only supports efficient development, by ensuring comprehensive and unbiased evaluations, but also facilitates high quality communication among stakeholders. Independent expertise is also useful for building in-house capability with fresh perspectives, for validation systems that are complex and extensive, and when an objective confirmation of model performance is crucial for improving stakeholder confidence.

Materials and Methods

Planning workshop

A planning workshop was organized to build a consensus around the intended design. A key objective was to narrow the scope of the project by distinguishing 'routine' validation tests – i.e., those essential for initial screenings of the model – from 'exploratory' analyses, which delve deeper into specific issues as they arise. The primary focus was on the project team's own needs as a key stakeholder, ensuring clarity and relevance in the validation process without being prematurely influenced by broader stakeholder requirements.

Nominal Group Technique is a group process used to explore problems, generate solutions, and assist with decision-making (Delbecq and Van de Ven, 1971). Applying this framework in the validation workshop allowed us to systematically explore diverse opinions and leverage the scientific expertise within the project team. Participants were asked to individually consider key questions before the meeting (e.g., 'How will we know the new EBVs are better than the old EBVs?'), submit their answers anonymously, and then engage in a structured review of all responses during the workshop. This facilitated consensus-building by allowing participants to see both commonalities and outlier opinions. This approach encouraged convergence towards a group norm but also allowed space for discussing and integrating divergent views effectively.

Once the group had agreed on the EBV characteristics which would be targeted for assessment, a similar approach was taken to explore the metrics and analyses which could be investigated. Participants were asked to individually list their preferred analyses, before collating the responses as a group and categorizing the results. This allowed common themes to emerge from the data rather than to be defined *a priori*, reducing the risk of being unduly influenced by the facilitator's personal biases. The group then discussed the 'trigger points' for exploratory analysis, establishing the criteria by which each test result would be considered a failure. This must be done before conducting the validation to avoid the temptation to 'change the goalposts' to suit the results. A similar process was undertaken to explore the population subsets of interest (i.e., focal groups such as young genomic bulls or validation heifers).

Finally, a group prioritization exercise invited participants to cast votes for their preferred metrics and analyses. This was essential due to the scope of the validation; it would not have been possible to implement every desired test or analysis with the limited resources available to the project team, and within the project deadline.

This resulted in a detailed plan outlining the key EBV characteristics, metrics and analyses, and focal groups required to assess the new NZAEL 3.5 models. This was an essential step towards determining whether the new genomic

EBVs would represent an improvement over the current pedigree evaluations.

Validation pipeline development

Once the plan was drafted, we needed a validation pipeline that could undertake the specified analyses. The development of the pipeline centered on the need for robust, automated processes that could handle the high volume of data inherent in a national genomic evaluation including pedigree information for 34.5 million cattle. The pipeline was developed in R and designed to seamlessly integrate with the output files produced by Helical, using the *aws.s3* and *data.table* packages. This ensured that data could be directly fed into the validation processes without manual intervention. The data files required for the validation pipeline are shown in Table 1.

Table 1: Data used in the validation pipeline

Data	Description
Animal information	Pedigree file including animal ID, dam and sire IDs, sex, birth year and breed
Phenotypes	Files containing phenotype and fixed effect data for each trait
(Daughter) yield deviations	Files containing the (D)YD data produced for each trait from models run on all available data
Full EBVs	EBVs and reliabilities for each animal, produced from models run on all available data
Truncated EBVs	EBVs and reliabilities for each animal, produced from models run on training datasets excluding the most recent 4 years of data.

Although the pipeline can ingest high throughput data for analysis, it produces memory-efficient outputs such as plots, tables, and summary statistics. The modular nature of the pipeline ensures that plot generation is separate from the components for analysis, allowing changes to be made to the display of data without needing the entire pipeline to be re-run.

R Shiny application

Comparing three models across five key characteristics, each with approximately five metrics, over eight breed categories and for more than 30 traits would require the project team to assess over 18,000 plots, figures, and tables. This represented a significant mental overhead for the project team, who were split across multiple geographic locations and had differing levels of familiarity with the R programming language.

To facilitate the process, we developed an R Shiny application with a strong focus on user experience, aiming to provide a clear, intuitive interface for users to interact with the validation data. A list of key features is shown in Table 2.

Table 2: Key features of the R Shiny validation app

Feature	Description
Validation-specific tabs	Each key EBV characteristic has a dedicated tab, aligning with the validation design.
Overview summary	Dynamic heatmaps use color gradients (red to blue) to summarize the validation results for each trait, highlighting the best model for each breed and metric.
Dropdown selection	Users can select different traits and focal groups to compare via dropdown boxes.
Version control	A changelog button provides updates on the app's version and recent changes, ensuring that users are informed of modifications.
Security	User credentials are required for login. The app also has two display modes, with a cleaner version for external stakeholders.
Accessibility	The app is globally accessible through a secure server, administrated by AbacusBio.
Performance	The app contains minimal on-the-fly analyses, relying on summarized outputs. This makes it highly responsive, allowing reviewers to make quick comparisons between traits, metrics, and focal groups.

Results and Discussion

Overview

At this stage, the validation pipeline and R Shiny application have been successfully used to compare pedigree and genomic evaluation models across 30 traits. The initial EBV characteristics identified by the project team as essential for assessment are shown in Table 3. The metrics and analyses used to investigate these are also shown.

A detailed explanation of all metrics and analyses is outside the scope of this paper. However, in general, a forward prediction approach was used, where (G)EBVs from data truncated by four years were used to predict daughter performances (Mäntysaari et al., 2010) or (G)EBVs produced from the full dataset (Legarra & Reverter, 2018).

Table 3: Key EBV characteristics assessed, along with the analyses

EBV characteristic	Metrics and analyses
Sense-making	Genetic trends (means and standard deviations)[1] Breed differences (violin plots)[1] Table of summary statistics (count, mean, median, standard deviation)[1]
Predictive ability	Regression of adjusted phenotypes (YDs and DYDs)[2] on truncated EBVs (intercept, slope, and correlation/accuracy) Quintile analysis (difference between the adjusted phenotypic performance of the top and bottom 20% of animals, ranked on their parent average EBVs)
Stability	Regression of full EBVs on truncated EBVs (slope and correlation/accuracy)
Bias	Difference between whole EBVs and truncated EBVs (mean bias)
Interbull suitability	Interbull trend tests 2-4 (DYD trend, EBV trend accounting for new daughters, Mendelian Sampling variance trend)

[1]For each model, separated by breed and sex
[2]Yield deviations (YDs) and daughter yield deviations (DYDs)

This validation process confirmed the superior performance of the NZAEL 3.5 genomic models for most traits, while for others, it identified areas of improvement. In these cases, the process was then used to confirm that adjustments to the model and data processing had the intended positive outcomes.

The robust validation design and wide range of analyses performed helped improve the project team's confidence in the performance of the NZAEL 3.5 genomic models. This increased confidence informed communications when seeking internal funding approval and presenting the project to external stakeholders.

The R Shiny application was also shared with international reviewers, providing an additional layer of objective and scientific expertise to the validation. Positive feedback on the application was provided by the reviewers, who commented on its ease of use, the inclusion of heatmap summaries, and the convenience of switching between different traits and focal groups. Where appropriate, we incorporated several reviewer suggestions directly into the application design, further improving the validation process.

Key learnings and challenges

It was essential to start the validation project with a plan. However, as with any complex task, the team made early decisions which were then reassessed after improving our understanding of the process. By reporting these decisions here, we hope to assist other readers in their own validations.

For example, the validation initially focused upon the use of yield deviations (YDs) and daughter yield deviations (DYDs) rather than raw phenotypes to assess the predictive ability of the EBVs. This was a practical decision to avoid the need to incorporate trait-specific fixed effects into the validation pipeline and worked well for most traits. However, due to the differences in data pre-processing between the models, and the fact that YDs are products of the models that we were assessing, it was

difficult to know which set of YDs was to be used for validating three different models. For this reason, it became necessary to undertake exploratory analysis to validate the YDs for some traits, which may have been avoided by focusing on the raw phenotypes from the start of the project.

We also needed a highly disciplined approach to development, to avoid incorporating unnecessary features into the application or pipeline. It was essential to keep referring to the plan and to remind the project team of the distinction between 'routine' and 'exploratory' analysis, to avoid a continually expanding codebase and overly complicated user interface. In some cases, findings from the exploratory analysis needed to be incorporated into the core pipeline; these two concepts lie upon a continuum, and it can be difficult to know where one ends and the other begins. However, gentle resistance to design suggestions originating from stakeholders who are not part of the target audience is almost always a useful general guideline.

Finally, careful specification of file names, missing values, and column names was also needed to ensure that model results would be compatible with the pipeline. This required clear communication between the modelling and validation groups. A good understanding of data pre-processing and model specifications was essential, both to focus our attention on trait-specific areas of interest, and to interpret anomalies in the results.

Conclusions

This project demonstrates the utility of a comprehensive, independent validation application developed by NZAEL and AbacusBio, aimed at enhancing the credibility and acceptance of genomic breeding values in the New Zealand dairy industry. By integrating an automated validation pipeline with an R Shiny application, the project exemplifies a more structured and transparent approach to evaluating genomic predictions.

The project also highlights the importance of clear communication and collaborative planning in validating genomic models. By distinguishing between routine and exploratory analyses and defining the target audience for the validation, our approach concentrates resources on areas of critical importance to the project.

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Integration of estimates of SNP effects into a single-step genomic evaluation

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Abstract

The aim of this research was to develop and validate a method that integrates estimates of single nucleotide polymorphism (SNP) effects and the associated prediction error (co)variance (PECs) matrix from a genomic evaluation into a single-step SNP Best Linear Unbiased Prediction (ssSNPBLUP) evaluation. As the PEC matrix is a dense matrix, the developed method was also tested with two different chromosome-wise matrices (that is, ignoring off-diagonal elements among chromosomes), and with a prediction error variance matrix (that is, ignoring all off-diagonal elements of the PEC matrix). Using simulated data from two dairy cattle populations with a genetic correlation between their traits of 0.80, we compared the genomic enhanced breeding values (GEBVs) predicted by the different integration methods to those of a joint ssSNPBLUP evaluation of both populations. The developed method, using the whole PEC matrix, resulted in GEBVs for selection candidates highly correlated and consistent with those from the joint ssSNPBLUP evaluation. Ignoring off-diagonal elements among chromosomes resulted in similar accurate results, but ignoring all PECs resulted in biased GEBVs in comparison to those of the joint evaluation. Therefore, an accurate integration of estimates of SNP effects and the associated PEC matrix into a single-step genomic evaluation is feasible and accurate when PEC of SNP effects within chromosomes are at least considered. The developed method can be readily implemented in existing software that support ssSNPBLUP models and can be adapted for single-step genomic BLUP models, though further research is needed to address potential computational challenges with these models.

Key words: ssSNPBLUP, SNP effects, integration, Prediction Error Covariance

Introduction

For genomic evaluation in dairy cattle, single-step genomic models have emerged as the models of choice. A major advantage of these genomic prediction approaches is that they simultaneously analyze phenotypic and pedigree information of genotyped and non-genotyped animals with Single Nucleotide Polymorphism (SNP) genomic information of genotyped animals (Legarra *et al.*, 2014). Although the prediction of genomic enhanced breeding values (GEBVs) is the principal goal of the different equivalent single-step genomic evaluations, estimates of SNP effects can also be obtained for all of them, either simultaneously with the GEBV prediction (e.g., Fernando *et al.*, 2014; Liu *et al.*, 2014) or indirectly by back-solving GEBVs (e.g., Lourenco *et al.*, 2015). Models that directly predict GEBVs and SNP effects as random effects will hereafter be referred to as single-step SNP Best Linear Unbiased Prediction (ssSNPBLUP), while models that predict only GEBVs will hereafter be referred to as single-step GBLUP (ssGBLUP).

The exchange of genetic material among populations necessitates the comparison and combination of genetic and genomic evaluations across populations for animals of

interest. In dairy cattle, these needs have been addressed through meta-analysis approaches. These include, among others, the Multiple Across-Country Evaluation (MACE; Schaeffer, 1994), which combines individual-based pseudo-data of sires obtained from national genetic evaluations, the Genomic MACE (GMACE; VanRaden and Sullivan, 2010), which combines individual-based pseudo-data of sires derived from national genomic evaluations, and, more recently, the SNPMACE approaches (e.g., Jighly *et al.*, 2022; Kärkkäinen et al., 2024; Vandenplas *et al*., 2018), which combine SNP-based pseudo-data obtained from genomic evaluations. These meta-analyses facilitate the combination of genetic and genomic evaluations across multiple populations and an optimal use of all available across-country information in each population.

The increasing adoption of single-step genomic models for routine evaluations, along with the implementation of meta-analyses such as SNPMACE, may result in an increased exchange of estimates of SNP effects and their associated measures of precision, potentially replacing the exchange of individual-level pseudo-data (e.g., computed from (G)EBVs). This potential increased exchange of estimates of SNP effects and their associated measures of precision creates a need for methods that can accurately integrate them into national single-step genomic evaluations.

The objective of this research was to develop and validate a method that integrates external estimates of SNP effects and their associated measures of precision into a ssSNPBLUP evaluation. Our method was validated using simulated data from two dairy cattle populations. Results demonstrate that the developed method enables accurate integration of estimates of SNP effects into a single-step genomic evaluation.

Materials and Methods

To develop a method that integrates estimates of SNP effects and their associated measures of precision into a ssSNPBLUP evaluation, we consider two populations, respectively *A* and *B*, both associated with animals phenotyped and/or genotyped at identical SNP loci. We first describe in this section a population-specific ssSNPBLUP evaluation based on mixed model equations (MME) proposed by Liu *et al.* (2014). Second, we describe a joint ssSNPBLUP evaluation that simultaneously analyzes phenotypes and genotypes from both populations. Third, we describe a method to integrate estimates of SNP effects of population *B* into a ssSNPBLUP evaluation of population *A*, assuming an exact prediction error covariance (PEC) matrix is available for population *B*. Finally, we outline four approximations of the PEC matrix. In the second part of this section, we present the simulations used to validate these different methods.

Population-specific ssSNPBLUP

A standard univariate mixed model for a single-step genomic evaluation for population i ($i = A, B$) can be written as:

$$\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i^* + \mathbf{W}_i\mathbf{u}_i^* + \mathbf{e}_i^*, \qquad (1)$$

where $\mathbf{y}_i$ is the vector of records for population *i*, $\mathbf{b}_i^*$ is the vector of fixed effects, $\mathbf{u}_i^* = \left[\mathbf{u}_{n,i}^{*\prime} \quad \mathbf{u}_{g,i}^{*\prime}\right]'$ is the vector of additive genetic effects for non-genotyped (n) and genotyped (g) animals, respectively, and $\mathbf{e}_i^*$ is the vector of residuals. The matrices $\mathbf{X}_i$ and $\mathbf{W}_i$ are incidence matrices relating records to the corresponding effects.

Additive genetic effects of the genotyped animals, $\mathbf{u}_{g,i}^*$, for population *i* can be decomposed as $\mathbf{u}_{g,i}^* = \mathbf{a}_{g,i}^* + \mathbf{Z}_i\mathbf{g}_i^*$, where $\mathbf{a}_{g,i}^*$ is the vector of the residual polygenic (RPG) effects, $\mathbf{g}_i^*$ is the vector of SNP effects, and $\mathbf{Z}_i$ is the centered matrix of SNP genotypes (coded as 0 for one homozygous genotype, 1 for the heterozygous genotype, or 2 for the alternate homozygous genotype). We assume a

multivariate normal (MVN) distribution for the additive genetic effects $\mathbf{u}_i^*$ and the SNP effects $\mathbf{g}_i^*$, with a mean equal to zero and a covariance matrix $\mathbf{H}_i^*\sigma_{u,i}^2$ with $\mathbf{H}_i^*$ being the covariance structure matrix and $\sigma_{u,i}^2$ being the additive genetic variance for population *i*. Finally, we assume that $var(\mathbf{e}_i) = \mathbf{I}\sigma_{e,i}^2$ where $\mathbf{I}$ is an identity matrix, and $\sigma_{e,i}^2$ is the residual variance for population *i*.

The inverse of $\mathbf{H}_i^*$ for population *i*, $\mathbf{H}_i^{*-1}$, is equal to (Liu *et al.*, 2014):

$$\mathbf{H}_i^{*-1} = \begin{bmatrix} \mathbf{A}_i^{nn} & \mathbf{A}_i^{ng} & \mathbf{0} \\ \mathbf{A}_i^{gn} & \mathbf{A}_i^{gg} + \frac{1-w}{w}\mathbf{A}_{gg,i}^{-1} & -\frac{1}{w}\mathbf{A}_{gg,i}^{-1}\mathbf{Z}_i \\ \mathbf{0} & -\frac{1}{w}\mathbf{Z}_i'\mathbf{A}_{gg,i}^{-1} & \mathbf{K}_i^* \end{bmatrix}$$

where $\mathbf{A}_i^{-1} = \begin{bmatrix} \mathbf{A}_i^{nn} & \mathbf{A}_i^{ng} \\ \mathbf{A}_i^{gn} & \mathbf{A}_i^{gg} \end{bmatrix}$ is the inverse of the pedigree relationship matrix partitioned between non-genotyped and genotyped animals, $\mathbf{A}_{gg,i}$ is the pedigree relationship matrix among genotyped animals, w is the proportion of variance (due to additive genetic effects) considered as RPG effects, and $\mathbf{K}_i^* = \frac{1}{w}\mathbf{Z}_i'\mathbf{A}_{gg,i}^{-1}\mathbf{Z}_i + \frac{1}{1-w}\mathbf{B}^{-1}$ with $\mathbf{B}^{-1} = \mathbf{I}2\sum p_j(1-p_j)$ and p_j being the allele frequency of the *j*-th SNP.

It is worth noting that these assumptions lead to the following MVN distribution for the SNP effects for population *i*, $\mathbf{g}_i$:

$$\mathbf{g}_i \sim MVN\left(\mathbf{0}, \mathbf{B}\sigma_{g,i}^2\right)$$

with $\sigma_{g,i}^2 = (1-w)\sigma_{u,i}^2$.

Joint single-step genomic evaluation

A standard bivariate mixed model for the joint analysis of the phenotypic, genomic, and pedigree datasets of both populations *A* and *B* can be written as:

$$\begin{bmatrix} \mathbf{y}_A \\ \mathbf{y}_B \end{bmatrix} = \begin{bmatrix} \mathbf{X}_A & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_B \end{bmatrix}\begin{bmatrix} \mathbf{b}_A \\ \mathbf{b}_B \end{bmatrix} + \begin{bmatrix} \mathbf{W}_A & \mathbf{0} \\ \mathbf{0} & \mathbf{W}_B \end{bmatrix}\begin{bmatrix} \mathbf{u}_A \\ \mathbf{u}_B \end{bmatrix} + \begin{bmatrix} \mathbf{e}_A \\ \mathbf{e}_B \end{bmatrix} \quad (2)$$

where $\mathbf{b}_i$ $(i = A, B)$ are the vectors of population-specific fixed effects, $\mathbf{u}_i = \begin{bmatrix} \mathbf{u}_{n,i}' & \mathbf{u}_{g,i}' \end{bmatrix}'$ are the vectors of population-specific additive genetic effects for non-genotyped and genotyped animals, and $\mathbf{e}_i$ are the vectors of population-specific residuals.

Similarly to the population-specific model (1), we assume a MNV distribution for the additive genetic effects with mean zero and a covariance matrix equal to $\mathbf{H}_J \otimes \mathbf{G}_J$, where the additive genetic covariance matrix $\mathbf{G}_J$ is equal to $\mathbf{G}_J = \begin{bmatrix} \sigma_{u,A}^2 & \sigma_{u,AB} \\ \sigma_{u,AB} & \sigma_{u,B}^2 \end{bmatrix}$, with $\sigma_{u,AB}$ being the additive genetic covariance between populations *A* and *B*. The inverse of $\mathbf{H}_J$ is computed as for the population-specific $\mathbf{H}_i^{*-1}$ using pedigree and genotype datasets of both populations. Similarly, we also assume a MVN distribution for the residuals, that is $\begin{bmatrix} \mathbf{e}_A \\ \mathbf{e}_B \end{bmatrix} \sim MVN\left(\begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{I}\sigma_{e,A}^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_{e,B}^2 \end{bmatrix}\right)$.

Integration of estimates of SNP effects

To develop a method that integrates estimates of SNP effects into ssSNPBLUP, we assume that the estimates of SNP effects and the associated PEC matrix of population *B* are known without approximation and are expressed on the same scale as the trait of population *A*. Furthermore, we assume that all SNP genotype matrices, i.e., $\mathbf{Z}_A$, $\mathbf{Z}_B$, and $\mathbf{Z}_J$, were centered with the same allele frequencies.

The integration of estimates of SNP effects from population *B* into the single-step genomic evaluation of population *A* can be achieved through a method analogous to that proposed by Gianola and Fernando (1986) for integrating external estimated breeding values (EBVs) and the associated PEC into internal genetic evaluations. Therefore, our method relies on the alteration of the mean and covariance matrix of the MVN distribution for SNP effects of population *A*, using the estimates of SNP effects $\hat{\mathbf{g}}_{B,A}^*$ and the associated PEC matrix, $\mathbf{\Delta}_{B,A}^*\sigma_{g,A}^2$,

obtained from a genomic evaluation of population *B* and expressed on the scale of the trait of population *A*, that is $\left[\mathbf{g}_A \middle| \hat{\mathbf{g}}^*_{B,A}, \boldsymbol{\Delta}^*_{B,A}\sigma^2_{g,A}\right] \sim MVN\left(\hat{\mathbf{g}}^*_{B,A}, \boldsymbol{\Delta}^*_{B,A}\sigma^2_{g,A}\right)$.

After some algebra, and ignoring fixed effects for readability, the ssSNPBLUP MME with an integration of estimates of SNP effects of population *B* can be written as follows:

$$\begin{bmatrix} \mathbf{W}'_{n,A}\mathbf{W}_{n,A}\sigma^{-2}_{e,A} + \mathbf{H}^{11}\sigma^{-2}_{u,A} & \mathbf{H}^{12}\sigma^{-2}_{u,A} & \mathbf{0} \\ \mathbf{H}^{21}\sigma^{-2}_{u,A} & \mathbf{W}'_{g,A}\mathbf{W}_{g,A}\sigma^{-2}_{e,A} + \mathbf{H}^{22}\sigma^{-2}_{u,A} & \mathbf{H}^{23}\sigma^{-2}_{u,A} \\ \mathbf{0} & \mathbf{H}^{32}\sigma^{-2}_{u,A} & \mathbf{H}^{33}\sigma^{-2}_{u,A} \end{bmatrix} \begin{bmatrix} \mathbf{u}_{n,A} \\ \mathbf{u}_{g,A} \\ \mathbf{g}_A \end{bmatrix} = \begin{bmatrix} \mathbf{W}'_{n,A}\mathbf{y}_{n,A} \\ \mathbf{W}'_{g,A}\mathbf{y}_{g,A} \\ \mathbf{0} \end{bmatrix} \sigma^{-2}_{e,A} +$$

$$\mathbf{H}^{*-1}_{A,B}\sigma^{-2}_{u,A} \begin{bmatrix} -(\mathbf{A}^{nn}_A)^{-1}\mathbf{A}^{ng}_A\mathbf{Z}'_A \\ \mathbf{Z}'_A \\ \mathbf{I} \end{bmatrix} \hat{\mathbf{g}}^*_{B,A},$$

$$\text{with } \mathbf{H}^{*-1}_{A,B} = \begin{bmatrix} \mathbf{H}^{11} & \mathbf{H}^{12} & \mathbf{H}^{13} \\ \mathbf{H}^{21} & \mathbf{H}^{22} & \mathbf{H}^{23} \\ \mathbf{H}^{31} & \mathbf{H}^{32} & \mathbf{H}^{33} \end{bmatrix} = \begin{bmatrix} \mathbf{A}^{nn}_A & \mathbf{A}^{ng}_A & \mathbf{0} \\ \mathbf{A}^{gn}_A & \mathbf{A}^{gg}_A + \frac{1-w}{w}\mathbf{A}^{-1}_{gg,A} & -\frac{1}{w}\mathbf{A}^{-1}_{gg,A}\mathbf{Z}_A \\ \mathbf{0} & -\frac{1}{w}\mathbf{Z}'_A\mathbf{A}^{-1}_{gg,A} & \mathbf{K}^*_{A,B} \end{bmatrix},$$

and with $\mathbf{K}^*_{A,B} = \frac{1}{w}\mathbf{Z}'_A\mathbf{A}^{-1}_{gg,A}\mathbf{Z}_A + \frac{1}{1-w}\boldsymbol{\Delta}^{*-1}_{B,A}$.

It is worth noting that the only difference between $\mathbf{K}^*_A$ in the MME without integration and $\mathbf{K}^*_{A,B}$ in the MME with integration is the replacement of the diagonal matrix $\mathbf{B}^{-1}$ by the dense matrix $\boldsymbol{\Delta}^{*-1}_{B,A}$.

Approximation of the PEC matrix

In practice, computing the PEC matrix of $\hat{\mathbf{g}}^*_{B,A}$, $\boldsymbol{\Delta}^*_{B,A}\sigma^2_{g,A}$, can be computationally challenging as it requires the inversion of the coefficient matrix of the MME. Therefore, the PEC matrix must be approximated. In this study, without loss of generality, we assume that the genomic evaluation of population *B* is a single-step genomic evaluation, and we approximate the PEC matrix $\boldsymbol{\Delta}^*_{B,A}\sigma^2_{g,A}$ by applying steps 1-3 of Gao *et al.* (2023) to a bivariate single-step genomic evaluation for both populations *A* and *B*, while considering only the phenotypic and SNP genotype datasets of population *B*. Considering the parameters of population *A* in this bivariate approach allow us to approximate the PEC matrix of population *B* expressed on the scale of population *A*.

Briefly, as a first step and in the context of this study, the approach consists of computing reliabilities for a bivariate pedigree-based BLUP for all animals in population *B* for the traits of both populations *A* and *B*. Second, deregressed equivalent record contributions (ERCs) for the genotyped animals in population *B* and for trait *A* are computed by reversing the method of Tier and Meyer (2004). Third, a coefficient matrix of a univariate SNPBLUP is constructed using all genotypes of population *B*, the residual and additive genetic variances of population *A*, and the deregressed ERCs of the genotyped animals in population B. Finally, an approximation of the PEC matrix $\boldsymbol{\Delta}^*_{B,A}\sigma^2_{g,A}$ associated with SNP effects in population *B* for the trait *A*, denoted by $\widetilde{\boldsymbol{\Delta}}^*_{B,A}\sigma^2_{g,A}$, is obtained by inverting the SNPBLUP coefficient matrix. This approach for approximating the PEC matrix is computationally feasible, even for large-scale genomic evaluations, as shown by Gao *et al.* (2023).

Additional approximations could be needed as the properties of the PEC matrix could lead to additional computational challenges when solving the single-step genomic evaluations. Indeed, it is a dense square matrix of size equal the number of SNPs multiplied by the number of traits, and these characteristics can make handling of the inverse of the PEC matrix in an iterative solver prohibitively demanding. To address these issues, we propose below three

approximations, assuming that $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}\sigma^{2}_{g,A}$ is available.

The first approximation of $\boldsymbol{\Delta}^{*-1}_{B,A}\sigma^{-2}_{g,A}$ involves ignoring off-diagonal elements among chromosomes of $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}$, which corresponds to inverting each block of $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}$ associated with a chromosome separately. This approximation results in $\boldsymbol{\Delta}^{*-1}_{B,A}\sigma^{-2}_{g,A} \approx \left(block\_diag\left(\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}\right)\right)^{-1}\sigma^{-2}_{g,A}$ with $block\_diag(.)$ denoting a chromosome-wise block diagonal matrix.

The second approximation of $\boldsymbol{\Delta}^{*-1}_{B,A}\sigma^{-2}_{g,A}$ involves ignoring off-diagonal elements among chromosomes after the inversion of $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}$, which results in $\boldsymbol{\Delta}^{*-1}_{B,A}\sigma^{-2}_{g,A} \approx block\_diag\left(\widetilde{\boldsymbol{\Delta}}^{*-1}_{B,A}\right)\sigma^{-2}_{g,A}$. This block matrix $block\_diag\left(\widetilde{\boldsymbol{\Delta}}^{*-1}_{B,A}\right)\sigma^{-2}_{g,A}$ corresponds to the SNPBLUP coefficient matrix used to compute $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}$ after absorbing the fixed effects and ignoring its off-diagonal elements.

The third approximation of $\boldsymbol{\Delta}^{*-1}_{B,A}\sigma^{-2}_{g,A}$ involves ignoring all off-diagonal elements of $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}$, which corresponds to inverting only the prediction error variances (PEV) of $\hat{\mathbf{g}}^{*}_{B,A}$, and results in $\boldsymbol{\Delta}^{*-1}_{B,A}\sigma^{-2}_{g,A} \approx \left(diag\left(\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}\right)\right)^{-1}\sigma^{-2}_{g,A}$.

Simulations

Two dairy cattle populations originating from the same breed were simulated following the procedure of Bonifazi *et al.* (2023a). Each population had simulated data on one trait with a heritability assumed to be equal to 0.30 in both populations. The genetic correlation between populations was assumed to be equal to 0.80. Briefly, about 2,000 QTLs were simulated to be randomly distributed across 30 chromosomes of 1 Morgan length each, and QTL effects were sampled from a Gaussian distribution. Each population was independently selected for 20 generations. In each population, 15,000 individuals were simulated per generation. Within each population and generation, 40 sires and 3,000 dams were selected to produce offspring for the next generation. Selection was first at random from generation 1 to generation 9, followed by a truncated selection based on within-population pedigree-based genetic evaluation. Pedigree and phenotypic information were assumed to be recorded from generation 7 and generation 10, respectively. The SNP genotypes included about 45,000 SNPs after quality control, and were assumed to be available for animals from generation 15 to generation 20 for both populations. Connectedness between the two populations was simulated by exchanging each generation the eight sires with the highest EBVs in each population throughout the last five generations.

Each scenario was replicated 10 times. The simulation was performed using the R-package MoBPS (Pook *et al.*, 2021), and pedigree-based genetic evaluations were performed with the software MiXBLUP (Vandenplas *et al.*, 2022).

Analysis

Using the simulated datasets, the aim was to validate the integration of estimates of SNP effects in population *B* into a ssSNPBLUP evaluation in population *A*, and to test the different approximations of the PEC matrix $\boldsymbol{\Delta}^{*}_{B,A}\sigma^{2}_{g,A}$.

For each replicate, datasets analyzed with ssSNPBLUP were built as follows. For population *A*, 60,000 phenotypes were randomly sampled for animals from generation 10 to 19. In addition, SNP genotypes for 7,000 animals were randomly sampled from generation 17 to 20. All the genotyped animals of generation 20 in population *A* were considered as selection candidates. For population B, all the 165,000 animals from generation 10 to 20 were associated with a phenotype, and all the 75,000 animals from generation 16 to 20 were associated also with a SNP genotype. Finally, the SNP genotypes of the exchanged sires were added to the genotype dataset of each population.

Using the datasets of both populations *A* and *B*, the following analyses were performed:

a) a joint ssSNPBLUP evaluation based on model (2) and using all datasets of both populations *A* and *B*;
b) a joint ssSNPBLUP evaluation based on model (2) but using genotype and phenotypic datasets of population *B* only. This evaluation is equivalent to a population *B* ssSNPBLUP evaluation based on the model (1), except that it provides also estimates of SNP effects of population *B* expressed on the scale of the trait of population *A*, $\hat{\mathbf{g}}^*_{B,A}$, and the associated approximated PEC matrix $\widetilde{\mathbf{\Delta}}^*_{B,A}\sigma^2_{g,A}$ computed as detailed in the section "Approximation of the PEC matrix", which are used in analyses d) to g) below;
c) a population *A* ssSNPBLUP evaluation based on the model (1) and using genotypes and phenotypes of population *A* only;
d) same as in c), but also integrating the population *B* information summarized by $\hat{\mathbf{g}}^*_{B,A}$, and $\widetilde{\mathbf{\Delta}}^*_{B,A}\sigma^2_{g,A}$;
e) the same as d) but by using $\left(block\_diag\left(\widetilde{\mathbf{\Delta}}^*_{B,A}\right)\right)^{-1}$ instead of $\widetilde{\mathbf{\Delta}}^*_{B,A}$;
f) the same as d) but by using $block\_diag\left(\widetilde{\mathbf{\Delta}}^{*-1}_{B,A}\right)$ instead of $\widetilde{\mathbf{\Delta}}^*_{B,A}$;
g) the same as d) but by using $\left(diag\left(\widetilde{\mathbf{\Delta}}^*_{B,A}\right)\right)^{-1}$ instead of $\widetilde{\mathbf{\Delta}}^*_{B,A}$.

All evaluations were performed with the software MiXBLUP (Vandenplas *et al.*, 2022). Without loss of generality, the pedigree of both populations was used in all evaluations. Furthermore, we assumed that the variance components were known and equal to the simulated variance components, and that the proportion w for RPG effects was assumed to be equal to 0.30. Finally, all genotypes in both populations were centered with the same allele frequencies. Therefore, a regression effect (often called J-factor; e.g., Strandén *et al.*, 2022) that makes the GEBVs independent of the allele frequencies used for centering was fitted for each evaluation.

To evaluate the accuracy of the integration of estimates of SNP effects in ssSNPBLUP, we compared the GEBVs of all population *A* selection candidates obtained with the different population *A* ssSNPBLUP evaluations (i.e., analyses c) to g) above). The joint ssSNPBLUP evaluation was used as reference, because it analyses simultaneously all data from both populations *A* and *B*.

The metrics computed for comparing the joint evaluation with the population *A* evaluations were: (i) Pearson correlations (r) between joint GEBVs and GEBVs without or with integration, (ii) regression coefficients (b_1) of joint GEBVs on GEBVs without or with integration, and (iii) root mean square errors (RMSE) of GEBVs without or with integration, defined as the square root of the mean of the squared differences between joint GEBVs and GEBVs without or with integration, and expressed in genetic standard deviation (SD) units. An accurate and consistent integration will result in Pearson correlation and regression coefficient equal to 1 and in RMSE equal to 0.

Results & Discussion

Integration with the complete PEC matrix

Based on our results, the developed method enables integration of estimates of SNP effects and the associated PEC matrix from a genomic evaluation into a single-step SNPBLUP. Table 1 compares joint GEBVs to GEBVs without or with integration for selection candidates in population *A*. The integration of estimates SNP effects with the approximated PEC matrix $\widetilde{\mathbf{\Delta}}^*_{B,A}\sigma^2_{g,A}$ resulted to almost the same GEBVs for the selection candidates as with the joint ssSNPBLUP, as shown by average correlations and regression coefficients close to 1 (that is, 0.98 and 0.97, respectively), and RMSE close to 0 (that is, 0.10 genetic SDs). For comparison, the average Pearson correlation between joint

GEBVs and GEBVs without integration was 0.74, the average regression coefficient was 0.78, and the average RMSE was 0.40 genetic SDs (Table 1).

Table 1. Comparison of joint GEBVs to GEBVs without or with integration for selection candidates in population *A*. Results are averaged across the 10 replicates (SE between brackets)[1].

Evaluation	R	b_1	RMSE
Pop. *A*	0.739	0.781	0.404
	(0.019)	(0.034)	(0.017)
PEC	0.982	0.973	0.104
	(0.001)	(0.004)	(0. 004)
Chromosome-wise PEC (v1)[2]	0.989	0.951	0.086
	(0.001)	(0.005)	(0.004)
Chromosome-wise PEC (v2)[3]	0.989	0.977	0.080
	(0.001)	(0.004)	(0. 004)
PEV	0.981	0.904	0.123
	(0.002)	(0.005)	(0.004)

[1] r = Pearson correlation between joint GEBVs and GEBVs without or with integration; b_1 = regression coefficient of joint GEBVs on GEBVs without or with integration; RMSE = root mean squared error of GEBVs without or with integration (in genetic standard deviation units).
[2] Off-diagonal elements among chromosomes ignored before inversion.
[3] Off-diagonal elements among chromosomes ignored after inversion.

Non-unity Pearson correlations and regression coefficients, as well as non-zero RMSE, for the integration of SNP effects using the approximated PEC matrix $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}\sigma^{2}_{g,A}$ could be explained by two approximations. First, differences between joint GEBVs and GEBVs with integration can be explained by the fact that the PEC matrices were approximated. Although our results show that our approach based on Gao *et al.* (2023) still results in an accurate integration of SNP effects, other approaches have been proposed in the literature (e.g., Jighly *et al.*, 2022; Vandenplas *et al.*, 2018), and should be also investigated in the context of single-step evaluations. Second, differences between joint GEBVs and GEBVs with integration can be explained by the fact that the contributions of the RPG effects to the additive genetic effects in the genomic evaluation of the population *B* are not integrated in the ssSNPBLUP evaluation of the population *A*. Future research is needed to explore the impact of ignoring the RPG effects in the developed procedure and to extend it for integrating RPG effects if needed.

Integration with chromosome-wise PEC and PEV matrices

Integrations based on chromosome-wise PEC matrices (that is, $\left(block\_diag\left(\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}\right)\right)^{-1}\sigma^{-2}_{g,A}$ and $block\_diag\left(\widetilde{\boldsymbol{\Delta}}^{*-1}_{B,A}\right)\sigma^{-2}_{g,A}$) resulted in accurate and consistent GEBVs, similarly to the integration based on the approximated PEC matrix $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}\sigma^{2}_{g,A}$, as shown in Table 1. Both versions of chromosome-wise PEC matrices resulted in metrics similar to those using $\widetilde{\boldsymbol{\Delta}}^{*}_{B,A}$ (that is, average Pearson correlations of 0.99, average regression coefficients higher than 0.95 and RMSE between 0.8 and 0.9 genetic SDs). These results suggest that the integration of estimates of SNP effects into a ssSNPBLUP evaluation can be performed without the whole PEC matrix. This is an appealing result because considering the whole PEC matrix in a multi-trait context could be challenging as it is a dense square matrix, and ignoring off-diagonal elements among chromosomes results in a relatively sparse block-diagonal matrix that can be easily handled with current computers.

Finally, the integration based on PEV only resulted in highly accurate, but biased, GEBVs, as shown by an average Pearson correlation of 0.98 and an average regression coefficient of 0.90. These results agree with those obtained by Vandenplas *et al.* (2018) in the context of SNPBLUP evaluations.

Implementation of the developed method

Implementing our developed method in existing software should be straightforward for those that already support a ssSNPBLUP model. First, the inverse of the (co)variance matrix of SNP effects must be replaced by the inverse of the (chromosome-wise) PEC matrix in the

coefficient matrix of the ssSNPBLUP MME. Second, the right-hand-side of the ssSNPBLUP MME requires the addition of a vector equal to the multiplication of $\mathbf{H}_{A,B}^{*-1}$ with a vector that includes imputed DGVs for non-genotyped animals ($-(\mathbf{A}_A^{nn})^{-1}\mathbf{A}_A^{ng}\mathbf{Z}_A'\hat{\mathbf{g}}_{B,A}^{*}$), direct genomic values (DGVs) for genotyped animals ($\mathbf{Z}_A'\hat{\mathbf{g}}_{B,A}^{*}$), and the estimates of SNP effects of population *B* ($\hat{\mathbf{g}}_{B,A}^{*}$). By implementing these changes, existing software can efficiently

$$\begin{bmatrix} \mathbf{W}_{n,A}'\mathbf{W}_{n,A}\sigma_{e,A}^{-2} + \mathbf{H}_g^{11}\sigma_{u,A}^{-2} & \mathbf{H}_g^{12}\sigma_{u,A}^{-2} \\ \mathbf{H}_g^{21}\sigma_{u,A}^{-2} & \mathbf{W}_{g,A}'\mathbf{W}_{g,A}\sigma_{e,A}^{-2} + \mathbf{H}_g^{22}\sigma_{u,A}^{-2} \end{bmatrix} \begin{bmatrix} \mathbf{u}_{n,A} \\ \mathbf{u}_{g,A} \end{bmatrix} = \begin{bmatrix} \mathbf{W}_{n,A}'\mathbf{y}_{n,A} \\ \mathbf{W}_{g,A}'\mathbf{y}_{g,A} \end{bmatrix}\sigma_{e,A}^{-2} +$$

$$\mathbf{H}_g^{*-1}\sigma_{u,A}^{-2}\begin{bmatrix} -(\mathbf{A}_A^{nn})^{-1}\mathbf{A}_A^{ng}\mathbf{Z}_A' \\ \mathbf{Z}_A' \end{bmatrix}\hat{\mathbf{g}}_{B,A}^{*},$$

with $\mathbf{H}_g^{*-1} = \begin{bmatrix} \mathbf{H}_g^{11} & \mathbf{H}_g^{12} \\ \mathbf{H}_g^{21} & \mathbf{H}_g^{22} \end{bmatrix} = \begin{bmatrix} \mathbf{A}_A^{nn} & \mathbf{A}_A^{ng} \\ \mathbf{A}_A^{gn} & \mathbf{A}_A^{gg} - \mathbf{A}_{gg,A}^{-1} + \mathbf{G}_{A,B}^{*-1} \end{bmatrix}$

where the inverse of the genomic relationship matrix $\mathbf{G}_{A,B}^{*}$ is equal to $\mathbf{G}_{A,B}^{*-1} = \left((1-w)\mathbf{Z}_A\boldsymbol{\Delta}_{B,A}^{*}\mathbf{Z}_A' + w\mathbf{A}_{gg,A}\right)^{-1}$.

It is worth noting that the form of $\mathbf{G}_{A,B}^{*}$ has the same form as the genomic relationship matrix of ssGBLUP with residual polygenic effects (Christensen and Lund, 2010), except that the diagonal matrix $\mathbf{B}$ is replaced by $\boldsymbol{\Delta}_{B,A}^{*}$. However, replacing $\mathbf{B}$ by $\boldsymbol{\Delta}_{B,A}^{*}$ for computing $\mathbf{G}_{A,B}^{*-1}$ might lead to computational challenges as $\boldsymbol{\Delta}_{B,A}^{*}$ is a dense square matrix of size equal to the number of SNPs multiplied by the number of traits in multi-trait evaluations. Further research to efficiently implement our method in ssGBLUP is therefore needed.

Potential uses of the developed method

Our analyses demonstrate that the integration of estimates of SNP effects and the associated PEC into a single-step genomic evaluation can be performed accurately. Because our developed method does not depend on the form of the genomic evaluation that provides the estimates of SNP effects, it is expected that similar results will be obtained with estimates of SNP effects integrate estimates of SNP effects obtained from a foreign genomic evaluation.

Our developed method can be also extended to ssGBLUP. As explained by Vandenplas *et al.* (2023), the absorption of the equations of SNP effects of the ssSNPBLUP MME result in the ssGBLUP MME based on the Woodbury matrix identity applied to the inverse of the genomic relationship matrix. Applying the same strategy to MME (3) results in the following MME:

computed, e.g., with a SNPMACE approach (Kärkkäinen et al., 2024; Liu and Goddard, 2018). Therefore, our developed method can be used by national organizations to integrate estimates of SNP effects computed by an international genomic evaluation into their national single-step genomic evaluation. As such, our method is an alternative to procedures that integrate pseudo-data computed from (G)EBVs into genetic evaluations (e.g., VanRaden *et al.*, 2014; Bonifazi *et al.*, 2023b)

Our developed method was tested under simple assumptions, such as datasets from only two populations, genotypes at the same SNP loci, same allele frequencies in all evaluations, and PEC matrices of population *B* available on the scale the trait of population *A*. These assumptions can be easily ignored by using or extending procedures developed in the context of SNPMACE (e.g., Jighly *et al.*, 2022; Kärkkäinen et al., 2024; Vandenplas *et al.*, 2018).

Conclusions

In this study, we developed a method that accurately integrates estimates of SNP effects and the associated PEC matrix into a single-step genomic evaluation. Our results demonstrates that the developed method yields GEBVs highly consistent with those of a joint single-step genomic evaluations when the whole PEC

matrix was used. Using chromosome-wise PEC matrices provided similarly accurate results, allowing for computationally efficient implementations in large-scale multi-trait single-step genomic evaluations.

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