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Using genomic data to estimate genetic correlations between countries with different levels of connectedness

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Abstract

Genetic correlations (r_g) between countries are required for international evaluations. The estimation of those r_g is challenging or even unfeasible using only pedigree and phenotypes when poor connectedness between countries is structural in the data due to a limited number of bulls having recorded (grand-)offspring across countries. Genomic information could be used to estimate r_g between countries by capturing connectedness that is not traced by pedigree recordings. Indeed, populations that appear as (completely) disconnected through pedigree can, theoretically, be connected through genomic data. Thus, our study aimed to investigate if estimates of r_g between countries based on genomic information are more accurate compared to estimates based on pedigree data, considering different levels of genetic connectedness. A maternally affected trait mimicking weaning weight was simulated for two beef cattle populations of the same breed. Different levels of connectedness between populations were simulated by exchanging different proportions of top sires in the last five generations: 0% (completely disconnected), 2.5% (lowly connected), 5% (medium), and 20% (high). Genomic data in the form of individual SNP genotypes at medium density were stored in the last three generations and used only for the estimation process. r_g between populations were estimated using three different relationship matrices: i) a pedigree-based relationship matrix (**A**) including all phenotyped animals; ii) a genomic relationship matrix (**G**) including phenotyped and genotyped animals only from the last three generations; and iii) a combined pedigree and genomic relationship matrix (**H**) including all phenotyped and genotyped animals. With disconnected and lowly connected populations, estimates of direct and maternal r_g were, on average, close to the simulated values when using genomic data through **G** or **H**. With lowly connected populations, estimates of direct r_g were close to the simulated values when using **A**, but estimates of maternal r_g showed large variation. With more connected populations, estimates obtained with **A**, **G**, and **H** matrices were overall similar. For all scenarios, when using genomic data in the estimation process, estimates of r_g had smaller standard errors. Our results show that genomic data can help the estimation of r_g between countries and especially reduce their standard errors for populations that appear as completely disconnected or lowly connected through pedigree information, such as in beef and (small) dairy cattle populations.

Key words: genetic correlations between countries, international evaluations, genomic data, GREML, maternal traits, cattle.

Introduction

International genetic evaluations allow breeders to appropriately compare the genetic merits of domestic and foreign animals. Animals' estimated breeding values (EBVs) obtained from different national evaluations are not directly comparable due to differences in scales and genetic bases, trait and model definitions, and environmental differences between countries (Philipsson, 1987; Zwald et al., 2003; Jakobsen et al., 2009; Nilforooshan and Jorjani, 2022). International evaluations, such as those performed by Interbeef (2006) for

beef cattle and Interbull (1983) for dairy cattle, combine data between countries into a single evaluation that takes into account such differences and computes animals' international EBVs. Through these international EBVs, foreign animals (mainly sires) can be compared with domestic ones, helping breeders to make their selection decisions. To account for differences between countries, international evaluations use multi-trait models that treat the same trait recorded in different countries as different correlated traits (Schaeffer, 1994; Phocas et al., 2005). A genetic correlation (r_g) between countries below unity accounts for differences in trait and model definitions, scale and genetic bases, and for genotype-by-environment interactions (Falconer and Mackay, 1996; Mark, 2004; Nilforooshan and Jorjani, 2022). Moreover, the r_g between countries effectively models how much the information from one country contributes to the animals' international EBV in another country (Weigel et al., 2001). Thus, r_g between countries are crucial for international evaluations and directly impact the international EBVs.

Genetic connections are needed to estimate r_g between countries used in international evaluations. These genetic connections are usually provided by sires having recorded offspring in two or more countries, also called "common bulls" (CB). Moreover, for maternally affected traits, which are common in beef cattle, genetic connections established through common maternal grand-sires (CMGS) having recorded (grand-)offspring in two or more countries are needed to estimate maternal r_g between countries (Jorjani et al., 2005; Pabiou et al., 2014; Bonifazi et al., 2020). However, in beef cattle and small dairy cattle populations, there is often a low level of genetic connectedness, mostly due to the low usage of artificial insemination in the former (Berry et al., 2016) or the low past exchange of bulls' genetic material between countries in the latter (e.g., Jorjani, 2000; Mark et al., 2005a). The low genetic connectedness in beef and (small) dairy cattle populations makes the estimation of r_g between countries challenging with current pedigree-based methods. Such challenges results in long computational times, uncertainty around the estimated r_g (i.e., large standard errors), and even in inestimable r_g in the extreme case of two completely disconnected populations (Jorjani et al., 2005; Mark et al., 2005a; Venot et al., 2009; Pabiou et al., 2014).

Individual genomic information in the form of single-nucleotide-polymorphisms (SNP) markers is increasingly becoming available at the national level for beef and (small) dairy cattle breeds (e.g., Van Eenennaam et al., 2014; Lourenco et al., 2015; Berry et al., 2016; Venot et al., 2016; Johnston et al., 2018; Bonifazi et al., 2022a; Adekale et al., 2023; Council on Dairy Cattle Breeding, 2023). In beef cattle, Bonifazi et al. (2022a) showed the feasibility and advantages of pooling national phenotypes and genotypes into an international single-step evaluation. In such settings, genomic data could also be used to estimate r_g between countries and possibly aid the estimation process, especially for lowly connected populations. In theory, populations that may appear as completely disconnected according to the pedigree can be connected through genomic information (Wientjes et al., 2015; Wientjes et al., 2018). Therefore, our study aimed to investigate if genomic data help to estimate r_g between countries more accurately than pedigree data, considering different levels of genetic connectedness between populations.

Materials and Methods

Two beef cattle populations (POP1 and POP2) originating from the same breed were simulated, mimicking data from two different countries (Figure 1). Each population had data on a maternally affected trait simulating weaning weight as a representative trait in beef cattle international evaluations. Genetic parameters were simulated following those observed by Bonifazi et al. (2020) in real data. The trait heritability was 0.30 and 0.15 for

direct and maternal genetic effects, respectively, and the within-population direct-maternal r_g was -0.2. The r_g between populations was 0.8 and 0.7 for direct and maternal genetic effects, respectively, and the between-population direct-maternal r_g was 0. About 2,000 QTLs were simulated to be randomly distributed across 30 chromosomes of 1 Morgan length each, and marker effects were sampled from a Gaussian distribution. Each population was independently selected for 20 generations (G; Figure 1). Selection was first at random (from G0 to G9), followed by selection on the total EBV, defined as the sum of direct and maternal EBVs with equal weights. Pedigree and phenotypic information were assumed to be recorded from G7 and G10, respectively. Genomic information in the form of individual genotypes at medium density (~50,000 SNPs) were assumed to be recorded for animals from G18 to G20 but not used for selection, similar to what has been observed in real data in Bonifazi et al. (2022a).

To simulate different levels of connectedness between the two populations, top sires from each population were exchanged throughout the last five generations (G16 to G20), called hereafter common bulls (CB). Four scenarios were simulated based on the exchanged proportions of top sires being: 0% (scenario 'disconnected'), 2.5% ('low'), 5% ('medium'), and 20% ('high'), corresponding to exchanging 0, 1, 2, and 8 sires, respectively, out of the 40 selected in each population and generation (Table 1). Each scenario was replicated 10 times. Following observations from Bonifazi et al. (2020), preferential treatment was simulated such that daughters of CB were used as dams in the next generation, ensuring the presence of common maternal grand-sires (CMGS) and, therefore, genetic connections to estimate maternal r_g between populations. The names of the scenarios are based on the level of genetic similarity (GS) coefficient for CB (Rekaya et al., 1999; Rekaya et al., 2003; Bonifazi et al., 2020) and follow the definition used in Bonifazi et al. (2020): low (GS < 0.05), medium (GS between 0.05 and 0.10) and high (GS > 0.10). The GS coefficients for CB and CMGS in each scenario are in Table 1.

r_g between populations were estimated using a bi-variate model in which each population's trait is modelled as a different correlated trait with uncorrelated residuals. In each of the four simulated scenarios, r_g between populations were estimated using three different sources of information and relationship matrices:

- **A**: using phenotypes from G10 to G20, with a pedigree relationship matrix.
- **H**: using phenotypes from G10 to G20 and genotypes from G18 to G20, with a combined pedigree and genomic relationship matrix following Legarra et al. (2009).
- **G**: using phenotypes and genotypes from G18 to G20, with a genomic relationship matrix following VanRaden (2008) method 1.

The relationship matrices were built considering all 14 generations of pedigree information available (G7 to G20). Due to the presence of maternal genetic effects, one extra generation of pedigree information (i.e., G9 for **A** and **H**, and G17 for **G**, respectively) was included in the relationship matrix used for the estimation of r_g between populations to link the maternal genetic effect of the dam with the phenotype of the offspring in the first generation (i.e., G10 for **A** and **H**, and G18 for **G**, respectively). Therefore, the **G** matrix was effectively built as an **H** relationship matrix (Legarra et al., 2009).

The simulation was performed using the R-package MoBPS (Pook et al., 2020). The relationship matrices were built using calc_grm (Calus and Vandenplas, 2016). mtg2 (Lee and van der Werf, 2016) was used to estimate EBV and r_g between populations, employing a CORE GREML approach (Zhou et al., 2020) to account for maternal effects and using a convergence criterion of $1.0 \cdot 10^{-4}$. Starting values were provided for within-population (co)variances, while between-population (co)variances starting values were set to 0, mimicking the procedure used in international evaluations (Bonifazi et al., 2021).

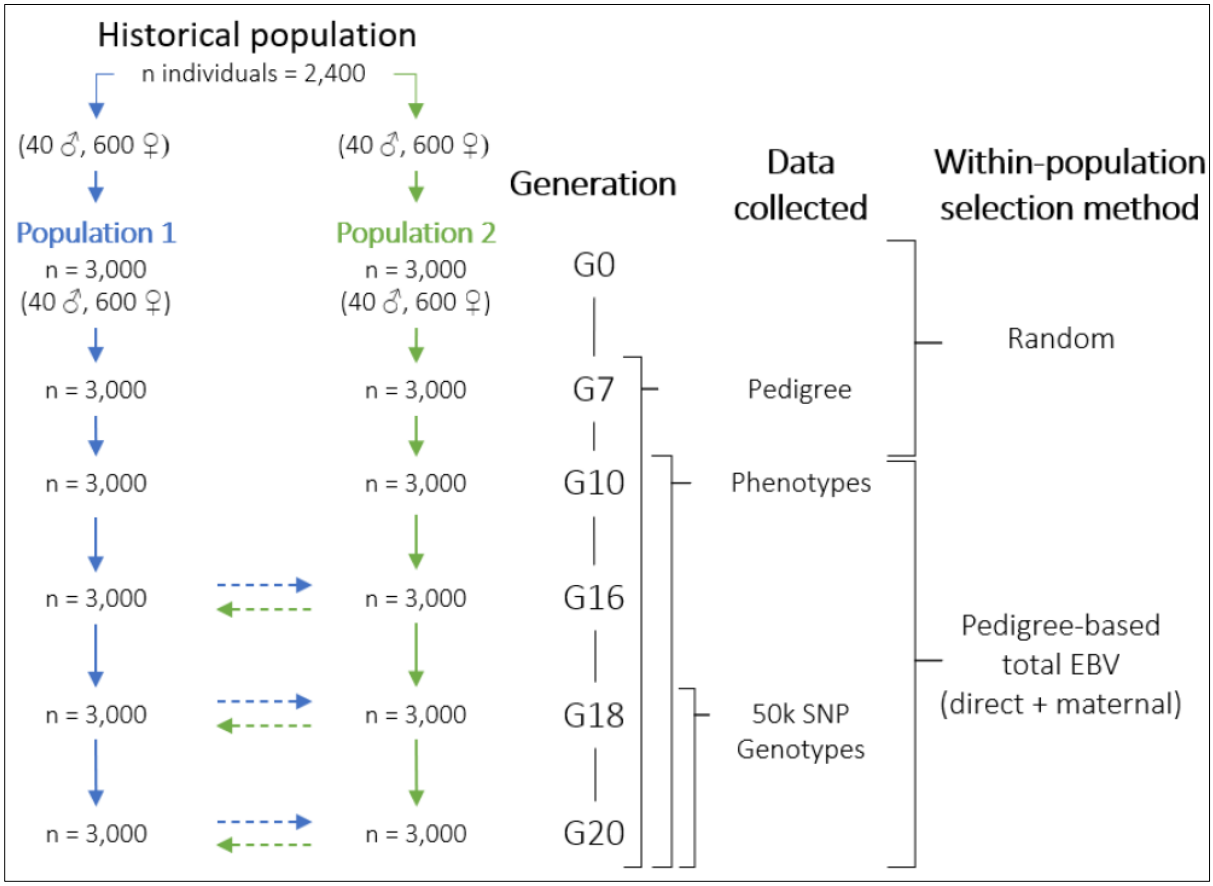

Figure 1. Schematic overview of the two simulated populations (POP1 and POP2), data collected, and selection method. n: number of individuals, G: generation, ♂: sires, ♀: dams. Horizontal arrows indicate the exchange of top sires between populations.

Table 1. Simulated scenarios and connectedness levels between populations [1, 2].

Scenario	n. of CB	n. off. from CB	GS_{CB} [3]	Average n. CMGS	Average n. grand-off. from CMGS	Average GS_{CMGS} [3]
Disconnected	0	0	0	0	0	0
Low	10	1,500	0.02	8	2,322	0.04
Medium	20	3,000	0.05	16	4,544	0.07
High	80	12,000	0.18	63	15,364	0.23

[1] Connectedness is computed from G10 to G20; results are averages of 10 replicates.
[2] n: number, CB: common bulls, GS: genetic similarity, CMGS: common maternal grand-sires.
[3] GS for CB (and CMGS) between two populations is defined as the proportion of recorded offspring (grand-offspring) born from CB (CMGS) over the total number of recorded offspring (grand-offspring) in the two populations.

Results & Discussion

Figure 2 shows the estimated r_g between populations using different relationship matrices. As expected, in the disconnected scenario, using conventional sources of information (i.e., pedigree and phenotypes) through the **A** matrix did not allow to estimate r_g: estimates did not move from the provided starting values. However, using genomic data through **G** or **H** matrices resulted in estimated direct and maternal r_g close to the simulated underlying true values. With lowly connected populations, using the **A** matrix resulted in estimated direct r_g close to the simulated values, while there was a large variation for the estimated maternal r_g (Figure 2). For medium and highly connected populations, there were no large benefits of using genomic data compared to using conventional sources of information: overall, estimated r_g using **A**, **G**, and **H** were similar for medium and high scenarios.

With increased connectedness between populations, the SE of direct and maternal r_g were smaller, regardless of the relationship matrix used (Figure 3). Furthermore, larger SE were observed for maternal r_g than for direct r_g. These results follow the findings of previous studies using real data where low levels of connectedness between populations were associated with large SE of the estimated r_g (e.g., Venot et al., 2009; Bonifazi et al., 2020). In all scenarios, the SE for direct and maternal r_g were smaller and showed less variation across replicates when using genomic information through **G** or **H** compared to **A**. Overall, using the **H** matrix resulted in the smallest SE of estimated r_g, while using the **G** matrix resulted in SE between those obtained with **A** and **H**. Thus, estimates of r_g between populations became more accurate, i.e., had smaller SE, when genomic information was included in the estimation process.

Computational requirements can partly be explained by mtg2 using dense relationship matrices for the estimation process instead of their inverses. As such, the estimation using **A** and **H** matrices showed similar computational resources (Table 2). The estimation using the **G** matrix required 12.5% of the memory of **A** and **H** matrices but required 2.43 times more computational time. Such computational requirements are likely due to the **G** matrix including only the last 4 generations of animals with 3 generations of phenotypes and genotypes, which, although resulted in a smaller matrix size, also led to an increased time to convergence (Table 2).

Overall, the more accurate estimation of r_g between populations with increasing numbers of CB and CMGS, agrees with Mark et al. (2005a). This relationship highlights the importance of establishing genetic links across countries by exchanging frozen semen to accurately estimate r_g between populations, especially when only conventional data is available. However, creating such genetic links is time-consuming since sires need recorded offspring in both populations.

The results of this study indicate that genomic data can be helpful to estimate r_g more accurately for disconnected and lowly connected populations and to reduce the associated SE compared to only using pedigree and phenotypic data. This was especially the case for maternal r_g between populations, which are reportedly challenging to estimate with low connectedness levels (Pabiou et al., 2014; Bonifazi et al., 2020; Bonifazi et al., 2021). In international evaluations, GS is usually reported to estimate connectedness between countries. The GS levels of the simulated scenarios are close to the values reported in beef and dairy cattle international evaluations. In particular, low to medium levels of connectedness are common in beef cattle. In Limousin, GS between countries was equal to 0.04 in Phocas et al. (2005) and ranged between 0.02 and 0.15 in Bonifazi et al. (2023; 2022b; 2020). Venot et al. (2009) reported values of GS between countries as low as 0.01 for both Limousin and Charolais. Therefore, using genomic data could

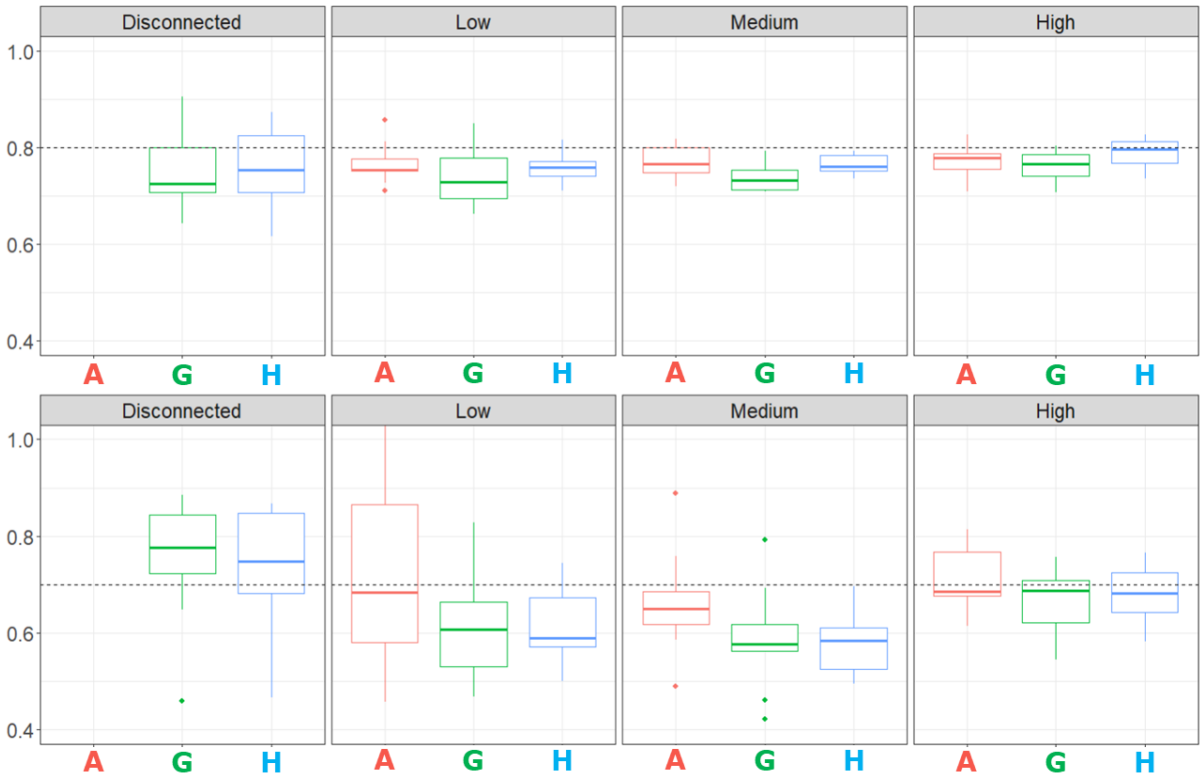

Figure 2. Boxplots of direct (top row) and maternal (bottom row) estimated r_g between populations across four connectedness scenarios (panels). **A**, **G**, and **H** indicate the different sources of information and relationship matrices used in the estimation process. Horizontal dotted lines indicate the simulated values of 0.8 for direct r_g and 0.7 for maternal r_g. Boxplots report estimated values of 10 replicates. One estimated maternal r_g in scenario "low" using **A** was out of parameter space (r_g >1).

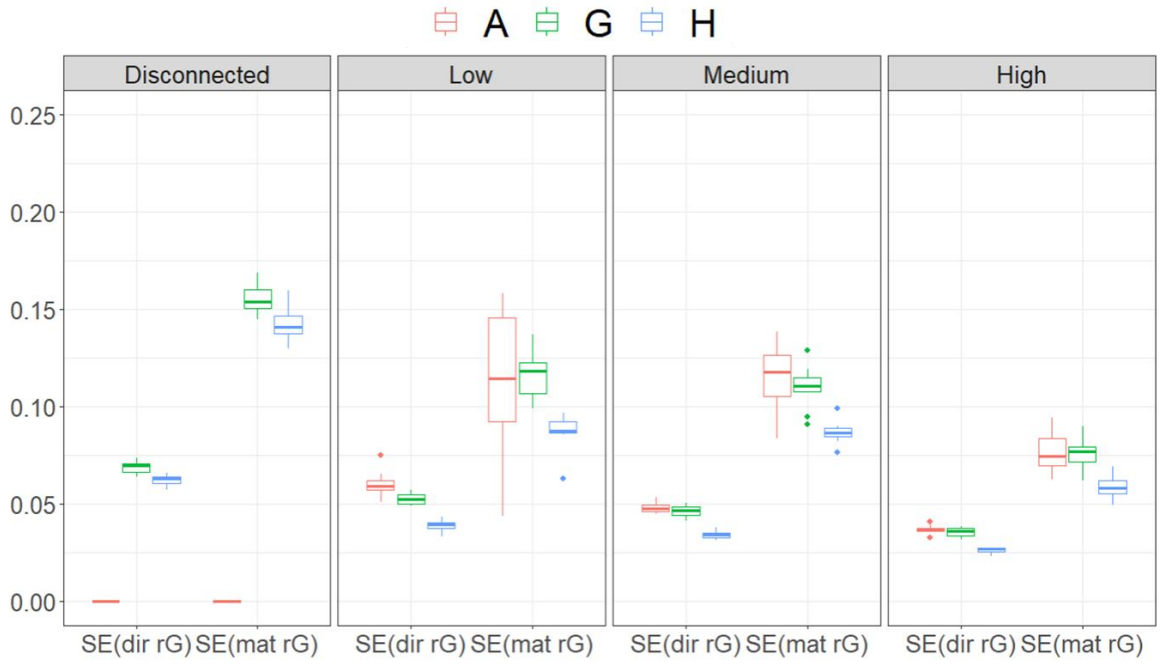

Figure 3. Boxplots of direct (left-side) and maternal (right-side) standard errors (SE) of estimated r_g between populations across four connectedness scenarios (panels). **A**, **G**, and **H** indicate the different sources of information and relationship matrices used in the estimation process.

help the estimation of r_g between beef cattle populations with a low exchange of bulls and low levels of GS and could reduce the uncertainty of the estimated r_g, i.e., the associated SE (Figure 2 and Figure 3). Similar to beef cattle, including genomic data in the estimation process could be beneficial for small and weakly linked dairy cattle populations such as Ayrshire, Guernsey, and Jersey (Jorjani, 1999; Jorjani, 2000; Mark et al., 2005b). On the other hand, for large dairy cattle international evaluations in which connectedness levels between populations are high, such as those of Holstein-Friesian (2000), it is unlikely that including genomic data would improve the estimation of r_g between countries (Figure 2).

Genomic information is increasingly becoming available at the national level for beef and small dairy cattle populations. Therefore, the proposed approach could be applied to estimate r_g between countries in (small-breed) international beef and dairy cattle evaluations. The **G** matrix used 3 generations of data and gave estimated r_g between populations similar to those obtained with **A** and **H** matrices, in which 10 generations of data were used. These results suggest that three complete generations of phenotypes and genotypes could be sufficient to estimate r_g between countries. However, in real data, additional challenges may be expected due to an unbalanced number of genotyped and phenotyped animals, missing records and incomplete pedigrees, and, depending on the population, a low number of offspring per dam. Finally, the genomic REML estimation approach used (Lee and van der Werf, 2016; Zhou et al., 2020) assumes that raw genomic data is available at the international level to calculate the relationship matrices. When sharing data is not possible due to privacy or political constraints, an approach based on summary statistics such as LDSC (linkage disequilibrium score regression analysis; Bulik-Sullivan et al., 2015; van Rheenen et al., 2019) could be investigated, albeit it is expected to require a larger amount of data and to be less accurate (Ni et al., 2018; van Rheenen et al., 2019).

Table 2. Computational requirements.

	A	G	H
Animals in matrix (number)	66,000	24,000	66,000
Elapsed time (hours)	3.1	7.3	2.9
RAM peak usage (GBytes)	106	13	102

Averages across scenarios and replicates.

Conclusions

Our simulation results showed that genomic data may help to obtain more accurate estimates of r_g between countries and especially reduce their associated standard errors compared to current methods using only pedigree and phenotypes. Larger advantages were observed for estimates of maternal r_g and for populations that appear completely disconnected or lowly connected through pedigree information, such as in beef and (small) dairy cattle populations.

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Effect of modelling unknown parent groups and metafounders on the historical genetic trend of fertility traits

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Abstract

Unknown parent groups (UPG) allow modelling unobserved selection in unknown parents. Because UPG are defined at least partly by year of birth, biased estimates can also bias estimates of environmental trends like management. Different modelling of UPG can reduce biases and standard errors. The 4 fertility traits daughter pregnancy rate (DPR), cow conception rate (CCR), heifer conception rate (HCR) and early first calving (EFC) in US dairy cattle make a good study case, because those have been affected by selection on correlated traits such as milk yield and have greatly differing recording patterns. Traits DPR and CCR are strongly correlated but DPR was recorded since ~1960 and CCR was recorded since ~2000. For missing traits, current traditional evaluation compress UPG definitions for missing years to avoid solving for UPGs with no direct information, and treat UPG as correlated across traits but uncorrelated across years (RandomUPGs). New models included: Fixed UPGs; Metafounders fitting average coancestry across UPGs based on year of birth (MFDeltaF); or including expected magnitude of change due to selection on a correlated trait (MFDeltaG). The data set consisted in 94 million records with potentially large numbers of missing values depending on trait and year, a pedigree including 94 million animals. Genetic evaluations were by BLUP and results are presented for Holstein. In all cases UPGs are treated as "a priori" correlated across traits. Genetic trends resulted in all cases in a fast decrease of DPR from 1960 until 2000. For DPR, this descent was most pronounced with RandomUPGs, closely followed by MFDeltaF and MFDeltaG, which yielded slightly less change because the inclusion of average coancestry results in smaller a priori changes. Similar trends but with larger differences across methods were observed for the correlated trait CCR, where the trend is inferred from correlations because of absence of records. Trends from 2000 to 2020 for both CCR and DPR were positive, with MFDeltaF showing slightly faster increases. Solutions of UPGs/MFs were most noisy with FixedUPGs, followed by RandomUPGs, followed by MFDeltaF which was the smoothest. Overall, for traits with years of missing records and with selection due to correlated traits not included in the data, modelling UPGs as random, and possibly correlated across years, is useful for correct genetic trends.

Key words: Unknown parent groups, fertility traits, genetic trends, metafounders

Introduction

Pedigrees are usually incomplete across all birth years in dairy cattle pedigrees and are classically modelled using Unknown Parent Groups (UPG). The theory of UPG (Masuda *et al.*, 2022) becomes more difficult in multiple trait situations with complex missing patterns. If fit as fixed, UPGs (**g**) are not *a priori* correlated to each other as shown in the pre-QP equations which include them as covariates of the form $(\mathbf{Q}'\mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z}\mathbf{Q})\,\hat{\mathbf{g}} = \mathbf{Q}'\mathbf{Z}'\mathbf{R}^{-1}\mathbf{y}$ (Quaas, 1988) – however "contributions" from descendants of the groups do account for the covariance across traits. If UPGs are fit as random, usually $Var(\mathbf{g}) = \mathbf{I}\otimes\boldsymbol{G}_0$, which implies *a priori* covariance across traits ($\boldsymbol{G}_0$) but not across levels. However, in a population with steady genetic trend the average genetic value of missing parents is expected to evolve smoothly from one generation or year to the next. The situation in which levels of UPGs are

correlated across levels, $Var(\mathbf{g}) = \mathbf{\Sigma} \otimes \boldsymbol{G}_0$ (Masuda *et al.*, 2022) with $\mathbf{\Sigma}$ a (not diagonal) covariance matrix, has not really been studied. Equivalently, the notion of metafounders (MF) generalizes the use of UPGs to include changes in inbreeding due to (missing) relationships among (missing) parents, and allows to "refer" relationships to genotypes. MF is also conceived to better model across-breed relationships.

Fertility traits in US dairy cattle make a good study case for multiple traits and UPGs or MFs. Fertility traits evaluations are difficult in a multiple trait model with UPGs because of (1) low heritability with different lactation weights and data pattern for each trait; (2) correlated, negative genetic trends caused by selection for yield, which is not included in the multiple-trait evaluation; (3) correlations might change (natural mating vs. AI; hormonal treatments; heat detection) and (4) the latest UPG is also unstable because heifer fertility arrives before cow fertility.

This work analyses results of different modelling of UPGs and MFs on the genetic trends of fertility traits and compares the results with expectations based on genetic progress.

Materials and Methods

Official data files from CDCB tri-annual all-breed BLUP evaluation of December, 2022 included 94 million records for four traits: daughter pregnancy rate (DPR) and early first calving (EFC), both recorded since 1960; cow conception rate (CCR) and heifer conception rate (HCR), both recorded since 2000. Our focus is on DPR and CCR with a high genetic correlation of 0.86. Missing records ranged from 4% for DPR to 87% for HCR.

We computed expected decrease in fertility from 1960 to 2000 for DPR and CCR due to selection on milk yield, based on negative correlation of -0.34, estimated ΔG of 4.2 genetic s.d. for milk yield, and genetic s.d. of 4.9 for DPR and similarly for CCR.

Pedigree included 94 million animals and 417 UPGs defined by breed, year of birth and pathway (sex of the animal with missing parent, sex of its ancestor, and foreign/local origin). The Holstein breed had 219 UPGs across 5 different pathways, where four pathways had 39 to 56 UPGs (roughly, but not always, every year) and unknown parents of foreign bulls had 12 UPGs. Smaller breeds had far fewer groups combined across years and pathways. The mininum number of offspring (not necessarily with record) to create an UPG was 5000.

Models included animal and permanent effects, contemporary groups (different per trait), heterosis and inbreeding. Multiple trait MME included ~800 million equations which were solved in ~8h using blup90iod3 from University of Georgia, with the PCG algorithm. The different models for UPGs and MFs are detailed next.

Models for UPGs and MFs

We first try a model with fixed UPGs. The second model was "RandomUPGs" with $Var(\mathbf{g}) = \mathbf{I} \otimes \boldsymbol{G}_0$. Then we run two models with metafounders. Thus the third model (MFDeltaF) with $Var(\mathbf{g}) = \mathbf{\Gamma} \otimes \boldsymbol{G}_0$ was inspired by (Sorensen and Kennedy, 1983) which describe that the means μ of each generation have the following covariance structure:

$$\boldsymbol{\Gamma} = \begin{pmatrix} \bar{A}_0 & \bar{A}_0 & \bar{A}_0 & \dots \\ \bar{A}_0 & \bar{A}_1 & \bar{A}_1 & \dots \\ \bar{A}_0 & \bar{A}_1 & \bar{A}_2 & \dots \\ \dots & \dots & \dots & \dots \end{pmatrix} \approx \begin{bmatrix} 0 & 0 & 0 & \dots \\ 0 & 2t_1\Delta F & 2t_1\Delta F & \dots \\ 0 & 2t_1\Delta F & 2t_2\Delta F & \dots \\ \dots & \dots & \dots & \dots \end{bmatrix}.$$

However, $\mathbf{\Gamma}$ obtained in this manner is not positive definite. A correct pseudo-inverse in this case is of the form, for invertible $\mathbf{B}$,

$$\begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{B} \end{bmatrix}^{-} = \begin{bmatrix} \mathbf{1}'\mathbf{B}^{-1}\mathbf{1} & -\mathbf{1}'\mathbf{B}^{-1} \\ -\mathbf{B}^{-1}\mathbf{1} & \mathbf{B}^{-1} \end{bmatrix}$$

But we did not attempt so. Instead, we used a version of **Γ** that is compatible with genomic relationships, i.e. (Wicki *et al.*, 2023)

$$\boldsymbol{\Gamma} = \begin{bmatrix} \Gamma_{1,1} & \Gamma_{1,1} & \Gamma_{1,1} & \dots \\ \Gamma_{1,1} & \Gamma_{1,1} + 2t_1\Delta F_\Gamma & \Gamma_{1,1} + 2t_1\Delta F_\Gamma & \dots \\ \Gamma_{1,1} & \Gamma_{1,1} + 2t_1\Delta F_\Gamma & \Gamma_{1,1} + 2t_2\Delta F_\Gamma & \dots \\ \dots & \dots & \dots & \dots \end{bmatrix}$$

with $\Gamma_{1,1} = \frac{2}{nsnp}\left(2\boldsymbol{p} - \frac{\mathbf{1}}{2}\right)\left(2\boldsymbol{p} - \frac{\mathbf{1}}{2}\right)'$, $\boldsymbol{p}$ a row vector of base allele frequencies and $\Delta F_\Gamma = \Delta F_\gamma = \Delta F\left(1 + \frac{\Gamma_{11}}{2}\right)$. Matrix **Γ** was constructed within breed and pathway. The inverse of **Γ** is a tri-diagonal matrix linking each MF to its immediate neighbors. The value of ΔF was estimated to be 0.0014 per year. Inspection shows that this is almost identical to inverting a structure of the form $\left(\begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{B} \end{bmatrix} + \mathbf{1}\mathbf{1}'k\right)$, with k a large constant that gets confounded with the mean.

For the fourth model, MFDeltaG, given that trend of fertility traits was initially due to selection on the correlated trait milk yield, we also tried a version of the above that would include putative change $\boldsymbol{\Delta G}$, as follows:

$$\boldsymbol{\Gamma} = \begin{pmatrix} \mathbf{0} & \mathbf{0} & \mathbf{0} & \dots \\ \mathbf{0} & (\boldsymbol{t_1\Delta G})^2 & (\boldsymbol{t_1\Delta G})^2 & \dots \\ \mathbf{0} & (\boldsymbol{t_1\Delta G})^2 & (\boldsymbol{t_2\Delta G})^2 & \dots \\ \dots & \dots & \dots & \dots \end{pmatrix}$$

In this case, we used the regular Moore-Penrose $\boldsymbol{\Gamma}^-$ (not the optimal choice). The value of ΔG was estimated to be 0.034 genetic standard deviations/year, per the correlation of DPR with, and observed genetic trends for, milk yield.

Results & Discussion

Estimated trends are presented in Table 1 and Figure 1. Genetic change in Table 1 was larger than expected, and all methods gave similar results for DPR (with actual 1960-2000 records) but not for CCR (no records in the period, inferred from genetic covariances). The expected genetic gain may have been underestimated because fertility also suffered from selection on "dairy form" (i.e. more angular) cows. In Figure 1 there is a genetic decrease in CCR followed by an increase. The trends differ at the beginning but as UPGs and MFs get more descendants and the database becomes larger, trends get closer to each other and are quite similar over the last 20 years when both traits have data. Note that for CCR the genetic trend 1960-2000 is entirely inferred from genetic covariances with DPR. The DPR phenotypic trend is partitioned into 53% genetic and 47% environmental trends (Figure 2), illustrating that deterioration of fertility is due to correlated response for selection for milk yield, but *also* to management changes (Lucy, 2001).

Table 1. Genetic change 1960-2000 for DPR and CCR

	DPR	CCR
Phenotypic	-16.00	-
Expected genetic	-6.86	-5.17
FixedUPGs	-9.23	-9.03
RandomUPGs	-8.56	-8.33
MetafoundersDeltaF	-8.21	-7.93
MetafoundersDeltaG	-8.38	-6.49

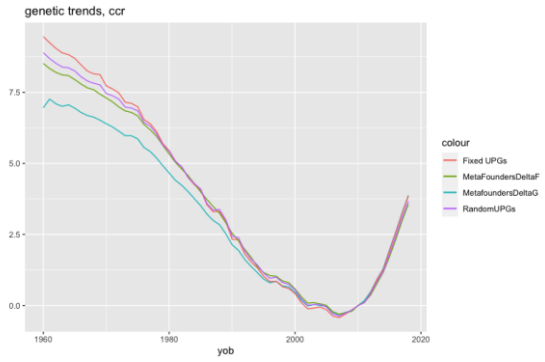

Figure 1. Estimated genetic trends for CCR under different models

Finally, Figure 3 shows estimates of UPGs/MFs for CCR and different models in pathway "21" (unknown sires of foreign dams) in Holstein. The estimates align well with those for DPR, even if CCR recording started in 2000. All models capture correctly the overall trend,

but Fixed UPG is very noisy, Random UPG is smoother and MFDeltaF gives a smooth, continuous line that provides the same long-term genetic trend

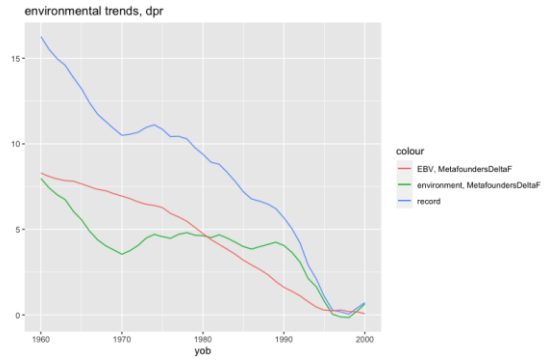

Figure 2. Decomposition of phenotypic into genetic and environmental trend, DPR.

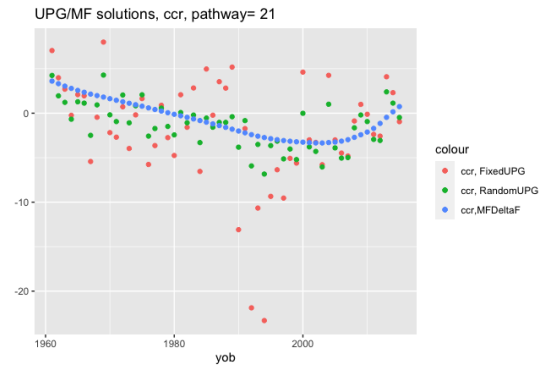

Figure 3. Different solutions of UPGs/MFs for the unknown sires of foreign dams pathway along time, CCR.

Conclusions

Modelling differently UPGs and MFs does result in different genetic trends, although the effect was small in this large data set. More research is needed to ascertain the effect of this modelling in smaller data sets with unequal recording across traits. Fixed or Random UPGs or MFDeltaF provided meaningful results and are computationally easy. MFDeltaG gives less noisy solutions when there is not enough data. MFDeltaG is not recommended as the value for ΔG is trait dependent. MFDeltaG values for ΔG could in theory be estimated from each trait's covariance with the index but is less practical for most uses.

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Software project 'miraculix': Efficient computations with large genomic datasets

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Abstract

We present mathematical approaches for CPU accelerations to calculate matrix multiplications between a Single Nucleotide Polymorphism (SNP) matrix and another SNP matrix or a real-valued matrix. These accelerations are important in crucial time-relevant calculations of single-step evaluations and other methods in genetics. The presented algorithms are much faster than previous algorithms. The C-code is released as part of the software project 'miraculix', which has been integrated into existing software such as MoBPS and MiXBLUP. We also discuss precision problems and missing SNP genotypes.

Key words: CPU, fast calculation, matrix multiplication, SIMD, SSE

Introduction

Many free and commercial software packages offer a broad range of methods in quantitative genetics, such as PLINK (Chang et al., 2015) and GCTA (Yang et al., 2011) to name a few. Others deal only with specific aspects, e.g., MiXBLUP (Vandenplas et al., 2022) with breeding value estimation or MoBPS (Pook, 2020) with breeding program simulation. In many of these applications, the most time-consuming steps are related to the Single Nucleotide Polymorphism SNP-matrix $Z \in \{0,1,2\}^{n\times s}$, which is multiplied to its transposed or a real-valued matrix. Here, n is the number of individuals and s the number of SNPs per individual. Many packages uncompress the 2-bit-packed SNP-matrix in some way before further calculations. Here, some approaches for CPUs are presented that avoid this unpacking partially or fully.

We will deal with matrix products of the form $Z^\top Z$ and $ZZ^\top$, which is the so-called unweighted genomic relationship matrix (GRM), up to a factor (Fragomeni et al., 2017). Afterwards, we will deal with products of the form $Z^\top V$ where $V \in R^{n\times p}$. As matrix products boil down mathematically to a collection of scalar products, we consider here scalar products, only. We first assume that missing values are absent. Afterwards, SNP matrices with missing values are considered together with certain precision considerations and centring of SNP matrices, since all three problems have similar mathematical foundations. We refer to Freudenberg et al. (2023a, 2023b) for benchmarks, including GPU solutions, and to Schlather (2020) for related and former methods.

Materials and Methods

For simplicity and clarity, we will primarily refer to commands of the Intel SSE instruction set family (128 bits). We comment on AVX2 and AVX512 explicitly when extensions of SSE are not obvious or when SSE is not enough for the given instructions. Note that most SSE commands can be easily transferred to the NEON instruction set through the header file sse2neon.h, for instance, in contrast to AVX commands.

Notations

In the subsequent pseudo-codes, &, |, and >> denote bitwise and, bitwise or, and shift to the

right, respectively. The signs '+' and '-' denote addition and subtraction in the decimal system. They can be interpreted as parallel operations on k-bit pieces if it is guaranteed that no k-bit overflow or underflow appears. We will use this fact several times, for $k = 2,4,6,8$ bits.

In case a register is filled by a repeated sequence s of bits we write (s)*. For instance, (01)* means that zeros and ones are alternated. The variable 'sum' refers to some register that accumulates summands; in case partial sums must be calculated first, sum is further added up in a variable called `total'.

Variables in the code pieces refer to Single Instruction Multiple Data (SIMD) registers, if not indicated differently; a and b indicate SNP values with a certain compressed coding. Finally, indexing assumes little endian.

Mini Lookup Tables

The SSE command _mm_shuffle_epi8 offers a lookup table with 16 entries of 1 Byte. AVX implementations realize only more parallel lookups, while the size of the lookup table does not change. The lower 4 bits of each byte in the SIMD register are used to realize 16 lookups at once at a cheap prize of at most 1 clock cycle.

Such mini lookup tables have a broad field of applications. For instance, they can be used for data transformation, adding-up neighboured 2-bit values, and to implement population counts (i.e., the number of bits in a register that equal 1) on systems without genuine popcnt command (Mula et al., 2016). We define

shuffle(x) :=

_mm_shuffle_epi8(x & (00001111)*, table) + _mm_shuffle_epi8((x>>4) & (00001111)*, table)

where the values of the table depend on the context and can always be obtained by simple calculations. For instance, the popcnt table is {0,1,1,2, 1,2,2,3, 1,2,2,3, 2,3,3,4}. Since 'sum' may not exceed the value 255, regular clearance of 'sum' is necessary. In case of popcnt this must happen after 31 iterations, the latest.

Large Lookup Tables

A lookup table with more than 16 entries can still be accessed in a reasonable time if the table fits well into the L1 cache. Hence, lookup tables for AVX registers should be addressed by at most 8-bit, and ALU registers by at most 14 bits.

Strassen algorithm

An important algorithm for calculating a matrix product between large matrices is the Strassen algorithm (Strassen, 1969). For a quadratic matrix $Z \in R^{n \times n}$ the standard costs for the product $ZZ^\top$ are of order n^3, whereas the costs of the Strassen algorithm are of order $n^{2.807}$. Indeed, in a standard set-up of a double-precision matrix Z, the Strassen algorithm is faster than the standard algorithm if n is larger than about 10^3. Numerical experiments suggest that the Strassen algorithm will be beaten in a SNP-SNP matrix multiplication by the best algorithms presented below up to $n \approx 10^6$. Note that the Strassen algorithm performs best in case of quadratic matrices. Otherwise, the smallest edge length is decisive for its performance. Hence the Strassen algorithm will never be an option for calculating $Z^\top V$ in a single step framework, where V is a vector or a small matrix. A further disadvantage of the Strassen algorithm is that its numerical errors are larger than those of the approaches presented here. Since the fast multiplication of matrices is still an active area of research, the limit $n \approx 10^6$ may change in future.

SNP-SNP scalar products by integer product

An immediate way of calculating the scalar product from a compressed 2-bit representation is to extract the first two bits of each of the two vectors a and b, and to continue with integer arithmetic. Then, the next two bits are extracted using shifting, and so on. Clearly, this procedure can be vectorized. Of advantage here is the SSE command _mm_madd_epi16, which multiplies and adds two consecutive 16-bit integers so that only 7 shifts are necessary. This method is based on the 2-bit standard binary coding of $\{0,1,2\}$; in case of PLINK 1 binary coding, a preceding transformation is necessary to the standard 2-bit binary coding.

The speed can be improved by the following consideration. Let $a_1, a_2, b_1, b_2 \in \{0,1,2\}$ be 4 SNP numbers. The two products $a_1 b_1$ and $a_2 b_2$ can be calculated in a single multiplication through

$$(a_1 + 2^c a_2)(b_1 + 2^c b_2) =$$
$$a_1 b_1 + 2^c (a_2 b_1 + a_1 b_2) + 2^{2c} a_2 b_2$$

provided the result is identifiable, i.e., the three summands on the right-hand side occupy different bits in the binary representation of the above value. This is the case when $c > 3$. Hence, convenient choices for c are $c = 4,6$ or 8. For instance, choosing $c = 8$ reduces the number of calls of _mm_madd_epi16 to 4 and the number of shifts to 3 by the following code:

```
for (i=0; i<8; i+=2)
  sum += _mm_madd_epi16((a >> i) &
        (00000011)*, (b >> i) & (00000011)*)
```

Clearance of the variable 'sum' is necessary after 7 iterations,

```
total += ((char *) sum)[0] + ((char *) sum)[2]
```

The analogue AVX512 command is _mm512_dpbusd_epi32, which sums up 4 products of adjacent 8-bit integers into a 32-bit integer. Hence, $c = 4$ and $c = 8$ are not possible and $c = 6$ leads to 3 calls of _mm512_dpbusd_epi32.

SNP-SNP scalar products by lookup tables

The following algorithm relies on data with PLINK 1 binary format, where the coding $00_p = 0_d$, $10_p = 1_d$ and $11_p = 2_d$ is used. Here, the index p and d denote PLINK 1 binary coding and decimal coding, respectively,

```
c:= a xor b
d:= ~(c >> 1) & c & (01)*
sum += shuffle( (a & b) - d )
```

Note that $d = 01_b$, if the decimal result is 2, and $d = 00_b$ otherwise.

SNP-SNP scalar product for chromosome data

If data are available per chromosome, we have two matrices $Z_{11}, Z_{12} \in \{0,1\}^{n \times s}$ where the value 1 indicates a deviation from the reference allele and $Z_{11} + Z_{12}$ equals the SNP matrix Z. Then, the non-centred relationship matrix is given by

$$(Z_{11} + Z_{12})(Z_{11}^\top + Z_{12}^\top) =$$
$$Z_{11} Z_{11}^\top + Z_{11} Z_{12} + Z_{12} Z_{11}^\top + Z_{12} Z_{12}^\top$$

Note that all scalar products on the right-hand side are between binary data, so that the multiplication step can be realized by the bitwise & and the adding-up by popcnt. Obviously, this algorithm can be used also for genomic data after a preprocessing step, where the genome data are artificially split into data per chromosome.

SNP-SNP scalar product based on the Hamming Distance

An interesting algorithm has been introduced in PLINK (Purcell et al., 2007; Chang et al., 2015) and has been based on the idea that a value can be represented by the number of bits that equal 1 in a 4-bit representation. The values of the vectors a and b must be coded asymmetrically by two mappings f and g, say, as a coding by a single mapping is not possible. Then, the bitwise &-operator is applied before popcnt is applied. Table 1 gives a possible realisation.

Table 1. Values for the Hamming distance method.

$f(\cdot) \wedge g(\cdot)$	$g(0)=0000_b$	$g(1)=0011_b$	$g(2)=1111_b$
$f(0)=0000_b$	000_b	0000_b	0000_b
$f(1)=0110_b$	0000_b	0010_b	0110_b
$f(2)=1111_b$	0000_b	0011_b	1111_b

Overview over SNP-SNP algorithms

Tables 2-6 give an overview over some properties of the divers approaches.

Table 2. Amount of additional cache/memory.

Method	Cache/memory needs
Integer product	Space for partial sums
Mini lookup table	Space for partial sums
Per chromosome	No extra needs for AVX512
Hamming distance	Each SNP needs 8 bits instead of 2

Table 3. Rough speed of the algorithm; the speed depends on the hardware and the specific coding.

Method	Speed
Integer product	Highly hardware dependent; fast on AVX512 & GPU
Mini lookup table	Intermediate
Per chromosome	High on AVX512
Hamming distance	High on AVX512

Table 4. Generality of the algorithm with respect to the hardware. Note that AVX512 has a lot more commands available and that the available set of commands differs between CPU and GPU.

Method	Hardware generality
Integer product	Any; currently, hardware is being developed in favour of this algorithm
Mini lookup table	All SIMD variants
Per chromosome	Well adapted to GPU & AVX512; modifications work for all SIMD variants
Hamming distance	All SIMD variants

Table 5. Number of registers needed for the calculations.

Method	Register need
Integer product	Several
Mini lookup table	Many
Per chromosome	Few
Hamming distance	Few

Table 6. Generality of the algorithm with respect to the coding of a SNP. If the algorithm is not general, much more memory is needed as a preceding re-coding is necessary.

Method	SNP coding generality
Integer product	Standard binary coding needed; re-coding on the fly possible
Mini lookup table	Principle suits any 2-bit coding; adaptions necessary
Per chromosome	Inherent coding; ideal for information per chromosome
Hamming distance	Inherent coding

SNP-double scalar products

In contrast to the bunch of algorithms for SNP-SNP scalar products, the spectrum of possible approaches to perform SNP-double scalar products is narrower and the algorithms simpler.

SNP-double scalar products can be performed by preceding conversion to double, essentially in the same way as for the integer product, except that the obtained, intermediate integer value is transformed into a double-precision value before being multiplied.

Since a SNP can take only the three values 0, 1 and 2, the implementation by addition is another, ensnaring approach. There are at least two variants of this idea. First, GPUs and AVX512 allow a conditional addition by indirect or direct masking, e.g., _mm512_mask_add_pd in AVX512, without loss of speed in comparison to a simple add command. Second, the if-condition is a moderately expensive command provided it does not lead to a far jump. Hence, the multiplication can be implemented by two nested if-conditions.

The last approach given here is more intriguing and mathematically more complex. It is called 5codes (Freudenberg et al., 2023b). For convenience, we repeat the algorithm here. Let $Y = Z^\top V$ and start with the well-known fact, that for fixed, real-valued values $V_j \in R$, the product $Z_{i,j}^\top V_j$ takes only 3 different values for arbitrary $Z_{i,j}^\top \in \{0,1,2\}$. Hence, a partial scalar product $Z_{i,j}^\top V_j + \cdots + Z_{i,j+k-1}^\top V_{j+k-1}$ can take at most 3^k different values. So, by creating a lookup table $H_{j,k}$, we can replace

```
for (j=0; j<nrow(Z); j+=k)
  Y[i] += Z[ j,i]*V[j]+…+Z[ j+k-1,i]*V[j+k-1]
```

by

```
for (j=0; j<nrow(Z); j+=k)
  Y[i] += H_{j,k}(Z[ j,i], … Z[ j + k - 1,i]) .
```

Since $3^5 = 243$, we can use $k = 5$ SNP values to index H by a single byte. Hence, a lookup table of doubles has less than 2000 Bytes. Now, m tables may fit into the L1 cache, so that the final pseudo-code reads

```
for (j=0; j<nrow(Z); j += m * k)
  Y[i] += H_{j,k}(Z[ j,i], …, Z[ j + k - 1,i]) + … +
    H_{j+(m-1)k,k}(Z[j + (m-1) k, i], …, Z[j + m k-1, I])
```

Overview over SNP-double algorithms

Tables 7-11 give a comparative overview of the properties of the different approaches.

Table 7. Amount of additional cache/memory.

Method	Cache/mem need
Conversion to double	Space for converted values
Conditional adding (mask)	None
Conditional adding (if)	None
5-codes	Lookup table in L1

Table 8. Rough speed of the algorithm; the speed depends on the hardware and the specific coding.

Method	Speed
Conversion to double	Intermediate
Conditional adding (mask)	Very high
Conditional adding (if)	Very dependent on the implementation
5-code	High

Table 9. Generality of the algorithm with respect to the hardware. Note that AVX512 has a lot more commands available and that the available set of commands differs between CPU and GPU.

Method	Hardware generality
Conversion to double	Any
Conditional adding (mask)	AVX512 & GPU
Conditional adding (if)	Any
5-codes	Any

Table 10. Number of registers needed for the calculation.

Method	Register need
Conversion to double	Few extra registers
Conditional adding (mask)	Few extra registers
Conditional adding (if)	Extra ALU registers
5-codes	Extra ALU registers

Table 11. Generality of the algorithm with respect to the coding of a SNP. If the algorithm is not general, much more memory is needed as a preceding re-coding is necessary.

Method	SNP coding generality
Conversion to double	General; adaptions necessary
Cond. adding (mask)	Adaptions necessary
Conditional adding (if)	General; adaptions necessary
5-codes	Inherent coding

Centring, missing values and precision

The above sections have considered the scalar product for the non-centred GRM, only. There, it has also been assumed that no missing values are present. In this section, we extend the above results to centred GRM and allow for missing values. We assume, however, that the portion of missing values is small.

A typical situation in genetics is that the phenotype V is non-negative. Hence, all products in $Z^\top V$ are non-negative, so that the calculation of the scalar product cannot profit from cancellations. A simple measure for an increased precision is to centre V and/or Z before calculation. Of course, further action to increase precision can be taken, e.g., using higher precision formats such as long double.

Below, we choose an approach that includes considerations for calculating both GRM and LD, in a rather general set-up.

Centred GRM

Schlather (2020) has shown that centred and normalized GRM (VanRaden, 2008; Wals and Lynch, 2018) can be calculated without loss of performance. Indeed, first the non-centred GRM can be calculated as above. Afterwards, the result can be corrected at low costs. To this end, let I_k be the vector of length k whose components are all equal to 1. The centred and normalized GRM G is defined as

$$G = (Z - Q)\,(Z - Q)^\top/\sigma^2$$

where

$$Q = 2I_n p_s^\top$$

$$\sigma^2 = 2\sum_{i=1}^{s} p_{s,i}(1 - p_{s,i})$$

and the $p_{s,i}$ are the allele frequencies. Then,

$$\sigma^2 G = ZZ^\top - I_n(2Zp_s)^\top - (2Zp_s)I_n^\top + 4I_n({p_s}^T p_s){I_n}^\top.$$

Obviously, the matrix $\sigma^2 G$ can be calculated from $ZZ^\top$ at low computational costs of order $n[s + n]$. As the calculation of σ^2 has costs of order s, the total computational costs for retroactive centring are some magnitudes smaller than the costs for calculating the cross-product $ZZ^\top$.

If there are no missing values and p_s equals the empirical allele frequency $n^{-1}Z^\top I_n/2$, the value $2n^2\sigma^2$ and the matrix $n^2\sigma^2 G$ are integer-valued and hence can be calculated exactly, so that the numerical errors in G can be reduced to a minimum. The costs for calculating $2n^2\sigma^2$ and

$n^2\sigma^2 G$ from $ZZ^\top$ are also of order $n[s+n]$, see Schlather (2020) for details. Note that the components of $ZZ^\top$ are unsigned 32-bit integers in standard applications, whereas $2n^2\sigma^2$ and $n^2\sigma^2 G$ need a 64-bit integer representation.

Allele frequencies in presence of missing values

Let $N \in R^{s\times s}$ and $S \in R^{n\times n}$ be the diagonal matrices whose diagonal elements equal to n (respectively s) minus the number of missing values in the respective row (column) of $Z^\top$. Then, the vector of empirical allele frequencies might be defined as

$$f_s := \frac{1}{2} N^{-1} Z^\top I_n$$

Let

$$g_n := \frac{1}{2} S^{-1} Z I_s$$

be the analogue mean taken in the direction of the SNPs, which appears in LD calculations.

Numerical centring

While the centring of GRM should always be performed retroactively, a preceding centring of Z and/or V in $Z^\top V_n$ or ZV_s increases the precision of the result. A retroactive correction of this numerical centring is of low cost. Let

$$B = Z - 2cI_n p_s^\top,$$

where $p_s \in R^s$ is any arbitrary vector. It is close to f_s in standard practical applications. For an advantageous centring of V_n, we aim to minimize

$$\| B(V_n - \mu_n e_n) \| = \min_{\mu_n}!, V_n \in R^n$$

for some fixed vectors $e_n \in R^n$, which may depend on Z. The minimization problem has the solution

$$\mu_n = \frac{e_n^\top B^\top B}{e_n^\top B^\top B e_n} V_n,$$

where

$$e_n^\top B^\top B =$$

$$e_n^\top ZZ^\top - 2m_n c q_n^\top \left[\left[c - \frac{e_n^\top Z I_s}{m_n}\right] I_{n\times n} - \frac{S}{s}\right]$$

with $I_{n\times n}$ the identity matrix and $m_n = 2se_n^\top q_n$.

If there are only a few missing values, i.e. $S/s \approx I_{n\times n}$, we have

$$e_n^\top B^\top B \approx e_n^\top ZZ^\top - 2m_n c(c-2) g_n^\top.$$

If we further choose $e_n = I_n$, then

$$I_n^\top B^\top B \approx 2n f_s^\top Z^\top + 2m_n c(c-2) g_n^\top$$

and

$$I_n^\top B^\top B I_n \approx I_n^\top ZZ^\top I_n + c(c-2)\frac{m^2}{s}$$

where $m = I_n^\top Z I_s$. Analogous formulae hold for an advantageous centring of V_s.

Genetic centring

In genetics, the centred matrices

$$Z - 2I_n p_s^\top \text{ and } Z^\top - 2p_s I_n^\top, z \in \{0,1\}.$$

are of interest, where p_s is the or any given allele frequency. Here, we combine these centred matrices with the numerical centring above in a rather general way. To this end, let $z \in \{0,1\}$ denote whether genetically motivated centring is of interest, i.e., we consider

$$Z - 2zI_n p_s^\top \text{ or } Z^\top - 2zp_s I_n^\top, z \in \{0,1\}.$$

Let $c, \nu \in \{0,1\}$ denote whether numerical centring of Z and V, respectively should be performed. Then we get for arbitrary $\mu_n, \mu_s \in R$, $p_s \in R^s$, and $q_n \in R^n$, that

$$(Z^\top - 2zp_s I_n^\top)V_n =$$
$$(Z^T - 2cI_s q_n^\top)(V_n - \nu I_n \mu_n)$$
$$- 2z(I_n^\top V_n)p_s + 2c(q_n^\top V_n)I_s + \nu\mu_n Z^\top I_n$$
$$- c\nu\mu_n(q_n^\top I_n)I_s$$

and

$$(Z - 2zI_n p_s^\top)V_s =$$
$$(Z - 2cI_n p_s^\top)(V_s - \nu I_s \mu_s) + \nu\mu_s Z I_s +$$
$$2(c-z)(p_s^\top V_s)I_n - c\nu\mu_s(p_s^\top I_s)I_n$$

so that the first term on each right side is critical concerning computational costs, and the remaining summands can be considered as correction terms. Note that $Z^\top I_n$ needs to be calculated only once in for every genotype matrix

Z. More generally, let $\zeta \in \{0,1\}$ indicate the centring in SNP direction. Then formulae for

$$(Z^\top - 2zp_s I_n^\top - 2\zeta I_s q_n^\top)V_n$$

and

$$(Z - 2zI_n p_s^\top - 2\zeta q_n I_s^\top)V_s$$

can easily be derived from the above equations. Note that all the above formulae hold independent of the values of p_s and q_n. We have only assumed that the numerical centring of the matrices Z and $Z^\top$ uses the same vectors.

Missing values

Assume we aim to calculate

$$x = (Z^\top - 2zp_s I_n^\top - 2\zeta I_s q_n^\top)V_n$$

or

$$y = (Z - 2zI_n p_s^\top - 2\zeta q_n I_s^\top)V_s$$

for arbitrary vectors $p_s \in R^s$ and $q_n \in R^n$ with a missing value in the position (j_k, i_k) of the matrix Z for $k = 1, \dots, \ell$. We define $Z_{j_k,i_k} := 0$ for all k and let I be the set of coordinates of all ℓ positions. Then, we have for $z, c, \nu \in \{0,1\}$ and arbitrary $\mu_n, \mu_s \in R$, $p_s \in R^s$, and $q_n \in R^n$, that

$$x_\alpha = \sum_{\beta,(\alpha,\beta)\notin I} (Z^\top - 2zp_s I_n^\top - 2\zeta I_s q_n^\top)_{\alpha\beta}\,(V_n)_\beta$$

$$= ((Z^\top - 2zp_s I_n^\top - 2\zeta I_s q_n^\top)V_n)_\alpha + 2\sum_{\beta,(\alpha,\beta)\in I} (z(p_s)_\alpha + \zeta(q_n)_\beta)(V_n)_\beta$$

$$y_\alpha = ((Z - 2zI_n p_s^\top - 2\zeta q_n I_s^\top)V_s)_\alpha + 2\sum_{\beta,(\beta,\alpha)\in I} (\zeta(q_n)_\alpha + z(p_s)_\beta)(V_s)_\beta.$$

This shows that matrix multiplications can be corrected for missing values retroactively even in very general set-ups. The corrections terms, i.e., the second summands in the above two equations, cause total computational costs proportional to the number of missing values in Z. The proportionality constant is large, however, because of cache misses, the outage of SIMD commands and the outage of tiling, at least in simple implementations.

Implementation of the numerical centring

While the centring of V_n and V_s is simple, the centring of Z and $Z^\top$ comes with extra computational costs for the conditional adding algorithms. Both the conversion to doubles and the 5-code algorithm do not lose speed and the implementation of the numerical centring is simple.

Conclusion

Algorithms for compressed SNP data can differ largely from simple approaches, such as decompression. Fast algorithms are hardware dependent and so change over time. Centring and missing values do not need to be considered in fast algorithms provided the number of missing values is small. Some increase in precision is possible without loss of speed, but with additional programming effort and use of special coding, e.g., 5-codes.

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Accelerating Single-Step Evaluations Through GPU Offloading

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Abstract

Single Nucleotide Polymorphism (SNP) genotype datasets used in empirical research are steadily growing in size which has introduced challenges in the calculation of population statistics that are based on large parts of the genome. In other fields, similar computational challenges have been tackled with the help of Graphics Processing Units (GPUs). We have developed a range of algorithms for the calculation of SNP genotype matrix operations widely used in empirical studies, which take advantage of modern NVIDIA GPUs. We provide an implementation in the C library miraculix and exemplary interfaces in Julia and Fortran. To ease adaptation, we also supply functions to calculate a number of derivatives, such as the genomic relationship matrix (GRM), linkage disequilibrium (LD) statistics, the genomic BLUP, and principal components analysis. Source code is released under the Apache 2.0 license and is freely available at GitHub. The library is developed in C, C++ and CUDA.

Key words: Genomic prediction, single-step models, high-performance computing, GPU

Introduction

Due to the emergence of high-throughput sequencing technology, the recent decades have seen the collection of massive genomic datasets, furthering the research in various fields in genetics such as human medicine or animal breeding and plant breeding. The consideration of large amounts of data helps to increase the accuracy of predictive models (Canela-Xandri et al., 2016; Zhao et al.,2021; Singh and Prasad, 2021) and some authors show that big data can contribute towards the closing of the missing heritability gap (Kim et al., 2017; Pallares, 2019). For breeding purposes, the use of genomic information leads to more accurate breeding values at earlier life stages, thus allowing for earlier selection to both reduce housing cost and increase genetic gain (Schaeffer, 2006). However, the computational analysis of these datasets places a significant burden on researchers and practitioners.

A genomic relationship matrix (GRM) describes the proportion of the genome that is shared between individuals in a population (Mrode, 2014) and is used in various selection methods such as genomic BLUP (VanRaden, 2008), single-step genomic BLUP (Misztal et al., 2009), extended genomic BLUP for modeling epistatic effects (Jiang and Reif, 2015) or (selective) epistatic random regression BLUP (Vojgani et al., 2021). Similarly, linkage disequilibrium (LD) measures the statistical similarity of pairs of SNPs in a population. For instance, LD quantities are used in human genetic studies to infer information on disease causes or population history (Pritchard and Przeworski, 2001; Gazal et al., 2017). Due to the large dimensions of modern genomic data sets, a naive calculation of the GRM, LD and their derivatives would inflict extraordinarily high computational demands, both in terms of memory requirements and calculation times. Since the SNP genotype of an individual is coded as 0 for one homozygous genotype, 1 for the heterozygous genotype, or 2 for the alternate homozygous genotype, each SNP value can be stored in 2 bits of memory. An example of this compressed storage format is the PLINK 1 binary format (Chang et al., 2015). While many statistical quantities in genomics can be calculated using highly optimized BLAS libraries, similar utilities are not available for these compressed storage formats. There exist two main approaches to mitigate this problem. The first one decompresses SNP genotype data

before further processing. For instance, the R packages AGHmatrix (Amadeu et al., 2016), qgg (Rohde et al., 2019), rrBLUP (Endelman, 2011) and snpReady (Granato et al., 2018) use custom floating-point matrix operations for the calculation of the GRM or rely on BLAS libraries. The R package SNPRelate (Zheng et al., 2012) benefits from explicit SIMD instructions in the calculation of LD and the GRM. Standalone solutions for the calculation of LD include HaploView and LDkit (Barrett et al., 2004; Yao, 2020). The calculation of the GRM and LD statistics is also implemented in the software packages PLINK and GCTA (Yang et al., 2011; Chang et al., 2015) which have popularized the second approach for processing compressed genotype data. They both utilize bit-compressed algorithms for an efficient calculation of the dot product of SNP vectors. Motivated by the remarkable speed improvements of these implementations, a number of tailored algorithms for the dot product have been developed for different instruction set architectures which are up to 48 times faster than a naïve BLAS-based implementation (Schlather, 2023).

Additionally, some software solutions have studied the benefit of offloading genotype matrix operations to the GPU. PLINK 2.0 provides a BLAS-based calculation of the GRM on GPUs. However, according to the documentation, this functionality is just provided as a proof-of-concept. The Julia package SnpArrays.jl (Zhou et al., 2020) offers a pure-Julia solution for accelerating the multiplication of SNP matrices by a floating-point vector on GPUs.

Over the past few years, there has been a rising interest in low-precision arithmetics in the field of deep learning (Hubara et al., 2017), which has led to hardware improvements. For example, recent NVIDIA® architectures have introduced a number of new assembler instructions for this purpose. In deep learning, the method of quantization reduces the cardinality of possible values of a parameter by using low-precision integers and has been used in neural networks to increase the number of parameters (Gholami et al., 2022; Dettmers et al., 2022; Kim et al., 2022). This progress has opened new paths to explore for acceleration in genomic calculations.

We present the library miraculix which implements functions for the GPU-based multiplication of compressed SNP matrices by itself or floating-point matrices, which helps to accelerate the calculation of the GRM, LD and other essential quantities in genomics. In contrast to some of the aforementioned software packages such as PLINK, the package miraculix offers only a narrow, highly fine-tuned functionality and is designed to allow a neat integration into genomic analysis pipelines. Furthermore, it differentiates itself from other GPU software solutions by leveraging low-precision instructions available on NVIDIA® GPUs to operate on compressed SNP data. This technique reduces device memory requirements and is substantially faster than solutions in floating-point format. We provide interfaces which can be used by existing libraries for genomic analysis or in higher-level programming languages such as Julia (Bezanson et al., 2017).

Materials and Methods

For a diploid species, the SNP genotype matrix $\mathbf{Z}$ describes the genomic information of a set of genetic markers in the population. That is, $\mathbf{Z} \in \{0,1,2\}^{n \times k}$, where n is the number of individuals in the population and k is the number of SNPs. Due to the dramatic decrease in sequencing costs over the last decades, it is now possible to genotype millions of SNPs in vast populations or, alternatively, impute incompletely genotyped individuals. Therefore, researchers regularly deal with extraordinarily large data sets. For instance, the UK Biobank comprises broad genetic data of hundreds of thousands of human individuals (Bycroft et al., 2018). The SNP genotype matrix is used for a wide range of genomic analyses. For instance, the SNP genotype matrix is used for computing the VanRaden 1 GRM $\mathbf{G}$, which is defined by

$$\mathbf{G} = \frac{\mathbf{PZ}(\mathbf{PZ})'}{2\mathbf{p}' \cdot (1_k - \mathbf{p})}$$

with $1_k = (1, \ldots, 1)'$ denoting a vector of length k consisting only of 1s, $\mathbf{p}$ denoting the vector of allele frequencies and the matrix $\mathbf{P} = \mathbf{I} - 2 \cdot 1_n \mathbf{p}'$ for the identity matrix **I**. Here, the matrix **P** scales **Z** to have zero-centered allele counts (VanRaden, 2008). In genome-wide analysis studies (GWAS), the SNP genotype matrix **Z** is used to calculate regression coefficients of traits on one or multiple SNPs (Jiang et al., 2019). In the analysis of LD, the SNP genotype matrix is used to approximate the correlation statistic r^2 through the computation of the correlation between allele counts in **Z**. Due to the intrinsic properties of a SNP matrix, the efficient computation of $\mathbf{ZZ}'$ or $\mathbf{Z}'\mathbf{Z}$ is in fact the problem of a {0,1,2}-matrix multiplication (Chang et al., 2015; Schlather, 2023). Memory-efficient storage formats for **Z**, such as the PLINK 1 binary format, only use 2 bits per entry and the conceptual arrangement of these bits yields different multiplication approaches. A number of highly efficient SIMD-based algorithms for Central Processing Units (CPUs) have been suggested (Schlather, 2023; Chang et al., 2015). Here, we rely on an allele-count encoding for our GPU implementation MMAGPU, which stores counts in unsigned 2-bit integer format. This allows us to target the 4-bit matrix multiplication assembler instructions on modern NVIDIA® GPUs of compute capability 7.5 and higher. Through bit-masking and shift operations, we obtain a straightforward matrix multiplication microkernel. For fast data movement from global memory to shared memory to the cores and back, our library extends the CUTLASS library (NVIDIA, 2023) with 2-bit specializations, utilizing the available fast tile iterators. Since the resulting multiplication function is mainly bound by data transfers between the GPU and main memory, we divide the multiplication into blocks of rows and parallelize the multiplication of these rows into different threads and streams respectively.

Deviating from the above computations **ZZ'** and $\mathbf{Z}'\mathbf{Z}$, an efficient multiplication of the SNP genotype matrix by a floating-point matrix is required for other essential operations in genomics, e.g., in GWAS. Recently, we have presented functionality in miraculix for offloading this type of computation to GPUs and how this functionality can be used for accelerating single-step evaluations (Freudenberg et al., 2023).

To our knowledge, miraculix is the first software library which offers a GPU-based implementation of optimized matrix multiplications on compressed genotype data. The R packages MoBPS (Pook et al., 2020) and EpiGP (Vojgani et al., 2023), as well as the proprietary software MiXBLUP (ten Napel et al., 2021), have integrated miraculix.

Results & Discussion

Since multiplications of the SNP genotype matrix are an essential operation in a number of computational tasks in genomics, miraculix can be used as the backend for various calculations. In this section, we describe four possible applications of our high-performance GPU implementation and demonstrate how it enables the processing and analysis of datasets in previously unattainable computing times.

Genomic Relationship Matrix

For large dimensions of **Z**, a straightforward calculation of **G** becomes computationally prohibitive and a careful treatment of the involved operations is required. The decomposition

$$c\mathbf{G} = \mathbf{M} - 1_n \mathbf{p}'\mathbf{M} - \mathbf{M}1_n\mathbf{p} + \mathbf{M}1_n\mathbf{p}'\mathbf{p}1_n\mathbf{M}$$

with $\mathbf{M} = \mathbf{ZZ}'$ and $c = 2\mathbf{p}'(1_k - \mathbf{p})$, reveals that the matrix **G** can be obtained from **M** at relatively low computational costs of order $n^2 + nk$, whereas **M** requires $O(kn^2)$ (Schlather, 2023). In Figure 1, we compare the computation time of our GPU implementation with the CPU-targeted solution in PLINK. As these evaluations are performed on different

hardware, we also benchmark a naive GPU implementation, which involves unpacking compressed genotype data into unsigned integers and uses the NVIDIA® cuBLAS library for multiplication. This approach resembles the proof-of-concept GPU solution implemented in PLINK, though we opted to store the input data in integers of 8 bits to save memory, while PLINK uses single-precision floating-point values. We simulated three different sets of genotype markers for a population of 22 000 individuals with the simulation utility in PLINK: A low-density array with 50 241 markers ("Low"), a medium-density array with 250 000 markers ("Medium") and a high-density one with 1 000 000 markers ("High"). For reference, the UK Biobank currently comprises about 850 000 directly measured variants. We tested the GPU functions on an NVIDIA GPU A100 with 80GB of device memory, while running PLINK on a dual-socket AMD® EPYC 7513 (2.6 GHz) with 32 dedicated cores each using the PLINK options *--make-rel square cov*. The results displayed are the median of 5 evaluations. Though direct conclusions on the efficiency of each solution are hard to draw due to the different hardware involved in the benchmarks, it can be observed that wall clock times in miraculix are smaller by a factor of at least 18 across the three test sets compared to PLINK. On the large dataset, the computation time was reduced from approximately 20 minutes to 56 seconds. Juxtaposing our solution to the simple cuBLAS-based solution, we see that significant speed gains can still be achieved by using our stack of microkernels for sub-byte integers and efficient memory management.

Yet, the significantly higher price tag of the A100 GPU has to be considered when evaluating these results: It is available at about 15 000 USD with a thermal power design (TDP) of 300W, while each of the two AMD® EPYC 7513 (2.6 GHz) CPUs has a recommended price of 2 840 USD with a TDP of 200W. Considering the power consumption of the evaluated methods, it is reasonable to assume that the GPU approaches are significantly more efficient than PLINK. Making a rough estimate based on the involved TDPs and computing times, a reduction in the magnitude of 20 in terms of power consumption can be presumed.

The gBLUP model

The genomic BLUP (gBLUP) model is widely used in population analysis to capture additive genetic effects (Misztal and Legarra, 2017) and is the basis for various extensions such as the extended gBLUP model, the single-step gBLUP model or the epistatic random regression BLUP model (Misztal et al., 2009; Jiang and Reif, 2015; Vojgani et al., 2021). In the gBLUP model, a quantitative trait $\mathbf{y}$ is assumed to be in a linear relationship with the genetic markers and environmental influences, captured in a matrix $\mathbf{X} \in \mathbb{R}^{n \times p}$. The effects of SNPs are traditionally assumed to be random, resulting in the model

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{PZu} + \boldsymbol{e},$$

where $\mathbf{b}$ is a vector of fixed effects, $\mathbf{u} \sim N(0, \sigma_u^2 \mathbf{I})$ is a vector of random effects and $\mathbf{e} \sim N(0, \sigma_e^2 \mathbf{I})$ is an error term independent of $\mathbf{u}$. Alternatively, the term $\mathbf{g} = \mathbf{PZu}$ can be used to denote the breeding values. Then, $\mathbf{g}$ is normally distributed with mean 0 and covariance matrix $\sigma_g^2 \mathbf{G}$ for $\sigma_g^2 > 0$. Furthermore, denoting $\mathbf{V} = \frac{\sigma_e^2}{\sigma_g^2}\mathbf{I} + \mathbf{G}$, the best linear unbiased estimator (BLUE) for $\mathbf{b}$ is given by

$$\widehat{\boldsymbol{b}} = \left(\boldsymbol{X'V^{-1}X}\right)^{-1}\boldsymbol{X'V^{-1}y},$$

and the best linear unbiased predictor (BLUP) for $\mathbf{g}$ is given by

$$\widehat{\boldsymbol{g}} = \boldsymbol{GV^{-1}}\left(\boldsymbol{y} - \boldsymbol{X}\,\widehat{\boldsymbol{b}}\right).$$

In practice, the variance components σ_g^2 and σ_e^2 are either derived from previous estimates on the heritability of the trait $\mathbf{y}$ (e.g., by comparing offspring phenotypes with parental phenotypes) or estimated through Restricted Maximum Likelihood (REML), for instance, using the software package ASReml (Butler et al., 2017). Considering the above identities, the quantities $\hat{\mathbf{g}}$ and $\hat{\mathbf{b}}$ can be derived from the GRM $\mathbf{G}$ and estimates for σ_g^2 and σ_e^2 through a Cholesky

decomposition. To this end, we utilized the cuSOLVER library to offload this computation to the GPU. In a recent empirical study, the full gBLUP calculation with miraculix showed an acceleration of up to 100 times compared to traditional software in the case where the heritability is known (Pook et al., 2021).

Additionally, two recent studies investigating the effects of epistasis utilized the efficiency of optimized CPU functions in miraculix (Vojgani et al., 2021, 2023). However, it should be noted that the memory requirements for setting up the GRM increase quadratically with the number of individuals which puts a limit to potential problem sizes.

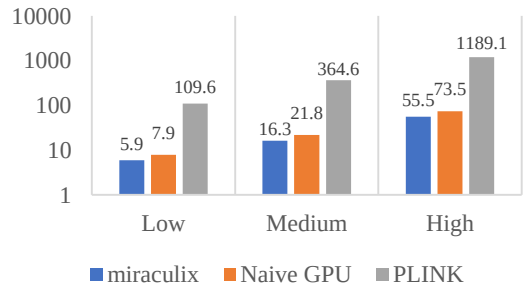

Figure 1. Wall clock times for the calculation of the GRM on three simulated sets of SNP genotypes.

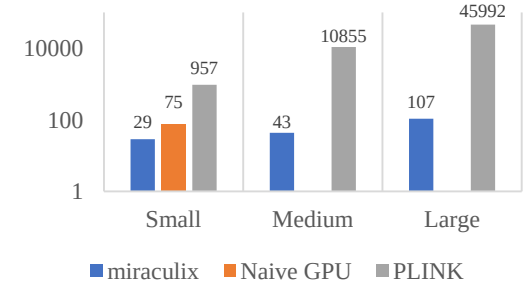

Figure 2. Wall clock times for the calculation of the LD on three simulated sets of SNP genotypes.

Linkage Disequilibrium

LD is a way of describing the dependence structure between pairs of alleles in a set of markers and there exist different statistics to capture this information in a population (Pritchard and Przeworski, 2001). The software PLINK implements the LD statistics r^2, D and D', which can be thought of as correlation measures between alleles. Though the true linkage value is based on haplotypes, it is sometimes approximated by the allele count correlations (e.g., in PLINK). That is, the squared correlation between the columns *i* and *j* of $\mathbf{Z}$ is used as value for r^2. Since the correlation matrix $\mathbf{R}$ can be written as

$$\boldsymbol{R} = \boldsymbol{D}^{-1/2}\,\widetilde{\boldsymbol{M}}\boldsymbol{D}^{-1/2}$$

for $\widetilde{\mathbf{M}} = \mathbf{Z}'\mathbf{Z} - 4n \cdot \boldsymbol{pp}'$ and $\mathbf{D} = \mathrm{diag}(\widetilde{\mathbf{M}})$, the matrix of pairwise r^2 values can be computed from $\widetilde{\mathbf{M}}$ at low cost. While the computation of $\mathbf{R}$ for a small block of SNPs with a limited number of individuals is straight-forward, a simple algorithm for the detection of LD between distant SNPs (so-called long-range LD) or the calculation of the average LD decay in a large part of the chromosome becomes cumbersome.

In our experiments, we calculated the matrix $\mathbf{R}$ of 50 241 markers across three simulated populations: A small population of 102 000 individuals (“Small”), a medium-sized one comprising 751 000 individuals (“Medium”) and a large population of 3 101 000 individuals (“Large”). As inflating the large population to single-precision floating-point values would require approximately 580GB of memory, this approach is impractical for LD calculation. Since miraculix processes SNP data in compressed format and subdivides the computation of the SNP matrix multiplication into blocks, only about 6 GB of device memory was required. Using the same hardware set-up as above, we compare our solution with the implementation in PLINK on 64 cores and a simple GPU solution in cuBLAS. However, due to its inherently higher device memory requirements, the latter could only be evaluated on the small dataset. Results are displayed in Figure 2 and are the median of 5 evaluations for the GPU functions. PLINK calculations were only performed once as wall clock times on these test sets made further evaluations unreasonable. For LD calculation, the PLINK options *--r square* were used. We observe that compute times in PLINK were more than 400 times higher on the large dataset.

Principal component analysis

For a column-wise standardized matrix $\mathbf{X} \in \mathbb{R}^{n \times p}$ the first m principal components (PCs) are defined by $\mathbf{X}\mathbf{v}_1, \ldots, \mathbf{X}\mathbf{v}_m$, where $\mathbf{v}_1, \ldots, \mathbf{v}_m$ solve the maximization problems

$$\max_{\mathbf{v}_1 \in \mathbb{R}^p, \|\mathbf{v}_1\| = 1} \|\mathbf{X}\mathbf{v}_1\|$$

and

$$\max_{\mathbf{v}_i \in \mathbb{R}^p, \|\mathbf{v}_i\| = 1, \mathbf{v}_1' \mathbf{v}_i = 0, \ldots, \mathbf{v}_{i-1}' \mathbf{v}_i = 0} \|\mathbf{X}\mathbf{v}_i\|$$

for $i = 2, \ldots, m$. Even though the transfer of the principal component analysis (PCA) to non-continuous data is not straightforward and a topic of ongoing research (see, e.g., Schlather and Reinbott (2021) for an approach to non-Euclidean data), PCA is still regularly used as an auxiliary tool in statistical genomics. Since PCA is a dimension-reducing method aimed at capturing large parts of variation in the dataset, PCs of the GRM are used in empirical studies in genetics to investigate population structure (e.g., by Steyn et al. (2022)) or as an auxiliary tool in the REML-based estimation of variance components (Thompson and Shaw, 1990; Lee and van der Werf, 2016). Principal components of the SNPs are regularly used to control for population stratification in GWAS studies or in breeding value estimation to reduce computational costs (Price et al., 2006). Popular software solutions include Eigensoft and PLINK (Price et al., 2006; Chang et al., 2015). Since PCA requires the multiplication of an orthonormal matrix of eigenvectors of $\mathbf{X}'\mathbf{X}$ by $\mathbf{X}$, miraculix can help to accelerate the PCs of a population by a fast computation of the GRM. Similarly, the PCs of SNPs can be derived from the $\mathbf{Z}'\mathbf{Z}$ matrix. However, if the dataset contains a lot of markers, constructing this matrix is challenging. The functionality of miraculix to multiply a SNP matrix by a floating-point matrix helps to alleviate this burden since there exists randomized algorithms for the singular value decomposition that do not require an explicit construction and calculate the first m eigenvalues and their corresponding eigenvectors with high accuracy (Halko et al., 2011). We provide an exemplary implementation in Julia.

gBLUP computing times

To evaluate the performance of miraculix in a practical setting, we simulated a population of $50\,000$ animals with $727\,605$ SNP variants based on the Illumina BovineHD BeadChip (Cunningham et al., 2021). Our supplementary Julia functions are linked to the interface of the library and perform low-cost post-processing operations on its return values. Emulating typical computational tasks in practice, we first load and process our data, which is stored in PLINK binary format on the disk, then calculate the GRM of the population and the SNP-wide PCs for later usage in inferring the parameters of the gBLUP model. PCs were modeled as fixed effects. Data processing operations were performed on an AMD® EPYC 7513 (2.6 GHz), while SNP matrix operations were offloaded to an NVIDIA® GPU A100. Since the CPU was mainly used for data preprocessing, we only used 8 dedicated cores. Due to the memory efficiency of our implementation, we were able to use the version of the A100 GPU with only 40GB of device memory. Computation of the GRM involved calculating the SNP matrix cross-product of dimensions $50\,000$ times $50\,000$ while retrieving the first 10 principal components required the multiplication of the SNP matrix by a floating-point matrix to obtain the approximate eigenvectors of SNP-wide covariance matrix. To estimate the vectors $\hat{\mathbf{b}}$ and $\hat{\mathbf{g}}$ of the gBLUP model, the Cholesky decomposition of the stretched GRM needed to be computed to solve the involved equation systems. Since heritability was assumed to be known, the ratio of variance components did not need to be estimated on the data.

Results are displayed in Table 1. We note that data processing now constitutes a significant portion of the total compute resource requirements both in terms of memory and computing times, as it requires a 2-bit format

conversion and reordering of the bit-level values. The construction of the GRM was performed in just approx. 30 seconds, whereas the PCA calculation and the Cholesky decomposition needed 18 seconds and 13 seconds respectively. In total, approx. 36 gigabytes of main memory and 19 gigabytes of device memory were used.

Table 1. Computing times for various steps in a gBLUP calculation.

Calculation	Wall clock time (s)	Main memory usage (GB)	Device memory usage (GB)
Data set-up	20.20	26.24	-
GRM	30.48	18.67	5.57
PCA	17.63	5.01	17.23
Cholesky	12.73	0.13	18.67
Total	95	36.08	18.67

Computations were performed on a single NVIDIA® GPU A100-40GB using the Julia interface of miraculix. Total wall clock time includes additional system start-up time.

Conclusions

We have presented the capability of miraculix to offload essential operations on genomic data to the GPU. We illustrated its benefits in four applications. The approach outperforms existing CPU-based software solutions significantly and thereby enables much faster processing of genomic datasets of substantial size. Furthermore, it works on compressed data and therefore allows the processing of huge datasets. Overall, our experiments showed that a full gBLUP on a population of 50 000 individuals could be performed in little more than 1.5 minutes. Considering that a similar task was assumed to be computationally infeasible by VanRaden (2008) at the time, we find the performance improvements to be promising and encourage the use of GPUs to accelerate the processing of large datasets in genomics. While there is a suite of established methods to deal with extraordinary dimensions, e.g., Algorithm for Proven and Young (APY) (Misztal et al., 2014) or the use of iterative solvers (Strand´en and Lidauer, 1999), these approaches can similarly benefit from the techniques introduced in this article. To allow miraculix to handle further increases in dataset sizes, future software versions might include distributed calculations for high-performance clusters via a Message Passing Interface (MPI), which would extend its applicability to datasets that still cannot be fully stored in device memory. Furthermore, since the variance component estimation through REML is another computational bottleneck in genomic analyses, it would be interesting to offload this procedure to the GPU as well. Extensions of miraculix to AMD® or Intel® GPUs would be useful to allow researchers to take full advantage of existing computer hardware.

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Methods to Estimate Erosion Factors of Genomic Breeding Values of Candidates due to Long-Distance Linkage Disequilibrium

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Abstract

Most validation studies of genomic evaluation observe inflation, *i.e.* regression coefficients of the later phenotypes on early predictions smaller than one. This pattern does not reflect a bias in the evaluation model, it rather reflects long distance associations between markers and quantitative trait loci (QTLs). Due to linkage disequilibrium (LD), SNP effects estimated from a reference data capture non-zero contributions from distant QTLs located not only in the same, but also in the other chromosomes, and we show that some across-chromosome LD does exist in different French dairy cattle breeds. This LD results from limited effective population size and, more importantly, from the relationship within the reference population. Long distance associations are partly broken and rebuilt at random at each generation. Therefore, corresponding SNP effects are partly lost in the next generations and we shall refer to this effect loss as erosion. This erosion can be predicted by different methods based on the following equations applied to simulated QTLs. If the breeding values are **Pq** with **P** the QTL genotypes and **q** their effects, the expected contribution of QTL j to the estimated SNP effect i is $\mathbf{c}_i \mathbf{M'} \mathbf{P}_j q_j$, where **M** is the matrix of SNP genotypes and $\mathbf{c}_i$ is line i (corresponding to SNP i) of $\mathbf{C} = (\mathbf{M'M} + \lambda \mathbf{I})^{-1}$. Two methods based on simulations are proposed to estimate the erosion factor ρ. In Method 1, the direct genomic value (DGV) of the progeny based on SNP effects estimated in this new simulated generation are regressed on the DGV of the same progeny based on SNP effects estimated in the reference population. In Method 2 all the QTL contributions to SNP effects are regressed based on SNP-QTL recombination rates and summed to predict the breeding value at the next generation. The regression coefficient of the DGV based on eroded contributions on the raw DGV is also an estimate of erosion. An illustration is given with the French Normande female reference population in 2021. Method 1 is simpler to implement on a routine basis, and yields good estimates of erosion over one generation. Erosion is also dependent on the distance between the young candidates and their reference population and formulae are proposed to apply erosion. We recommend accounting for erosion in genetic evaluations to provide unbiased predictions for the young candidates. Accordingly, erosion has been accounted for in the French Single Step bovine evaluation since March 2022.

Key words: Genomic evaluation, inflation, erosion of genomic values, validation methods

Introduction

In genomic evaluation, single nucleotide polymorphism (SNP) effects are estimated in a reference population and applied to selection candidates. This method is extensively used to select candidates at an early stage of their life or not yet with phenotypic information. The standard interpretation is that SNPs are in close LD with causal mutations (or QTL) and therefore, good proxies for these QTL. Implicitly, this assumes that estimated SNP effects reflect those of the neighbouring causal mutations. Under this assumption, SNP effects observed in the reference sample should be very similar in the next generation as short-distance LD erodes slowly due to recombination. It is, however, well known that genomic evaluation

efficiency is highly dependent on the close relationship of the candidates to the reference sample (Habier et al, 2007, 2013; Legarra et al, 2008; Pszczola et al, 2012). Many studies have shown the limited gain in accuracy in multi-breed evaluation (Erbe et al, 2012; Hozé et al, 2014), illustrating that distant reference data are not informative. Other studies have shown a decrease in accuracy over generations when the reference population is not updated (Soneson et al, 2009; Solberg et al, 2009). Moreover, it has been observed that the absence of parents in the reference population directly influences the prediction accuracy of the selection candidates. All these results suggest that SNP effects erode as the distance between candidates and reference sample increases.

Validation studies of genomic evaluations are generally based on the regression of later performances on the early predictions. These studies frequently observe an inflation pattern, *i.e.*, the regression coefficient is systematically lower than 1, meaning that later performances of the best candidates are below those initially predicted (and later performances of the worst candidates, if any, are above those initially predicted).

One plausible interpretation is the existence of long-range LD, even across different chromosomes. Consequently, many markers may capture partial effects of supposedly unlinked QTL. Although long-distance LD is notably lower than short-distance LD, the number of long-distant variants is considerably higher and their combined effects can account for a substantial proportion of the genetic variance in a genomic prediction.

In the first part of this study, we demonstrate that markers do capture part of the effects of distant QTL due to the long-distance LD. Because this long-range LD gradually decays over generations, it is imperative to account for the erosion of marker effects to predict the genomic values for the candidates. In the second part, we propose two methods to estimate the specific erosion factor of a reference population and suggest how to use it in practice to adjust DGV.

Materials & Methods

Evidence for linkage disequilibrium across chromosomes

LD across chromosomes was assessed using data from the 2021 female reference populations of six French dairy cattle breeds (Holstein, Montbéliarde, Normande, Abondance, Tarentaise, Vosgienne). The reference populations exhibited varying sizes, ranging from 2617 to 362,363 animals. It is worth noting that Vosgienne, Tarentaise, and Abondance are local mountain breeds, whereas Montbéliarde and Normande are national populations, comprising 18% and 7% of the French dairy herd, respectively. On the other hand, the international Holstein breed accounts for 70% of the French dairy cattle population. In our analysis, we selected one every 20 SNP of the Illumina EuroGMD BeadChip on the 29 autosomes, resulting in a sample of ~3 million r^2 values for each breed (vs ~1.2 billion in total for all SNPs).

Table 1 presents various LD statistics across chromosomes in the female reference populations of six French dairy cattle breeds. While the average r^2 values appear to be small, suggesting limited across-chromosome disequilibrium, it is important to note that the focus here is on the parameter r, as the impact of a QTL on a SNP effect is directly proportional to the correlation between them. These correlation values decreased when the size of the breed (reference population, number of females in the breed, or effective size) increased. The proportion of SNP pairs with |r| exceeding 5% fluctuated notably, ranging from 1.5% to 33%, depending on the breed. Notably, as the reference population size increased, this proportion also tended to decrease. Nevertheless, it is worth highlighting that even with a small percentage, we still observe non-null correlations between 600 to several thousand SNP with a QTL located on a different chromosome (assuming that these r distributions between SNP and QTL are the same as between SNP).

Table 1. Statistics of |r| and r^2 values across the 29 chromosomes in female reference populations of six French dairy cattle breeds (selection of one every 20 SNP within chromosome).

Breeds	# cows	Mean (|r|) *	% |r| > 0.05	Mean(r^2)
Vosgienne	2617	0.0420	33	0.0029
Tarentaise	3788	0.0225	18	0.0015
Abon-dance	7115	0.0268	15	0.0012
Normande	69,220	0.0206	7	0.00073
Montbe-liarde	185,053	0.0173	4	0.00053
Holstein	362,363	0.0148	1.5	0.00038

*statistics based on 2,812,741 to 3,231,800 SNP pairs per breed

Impact of long distance LD on genomic predictions

To investigate the impact of long-distance LD on SNP effects, we focused on the Normande population, which consisted of 69,220 cows (N) with genotypes and phenotypes. In this study, we considered the first five chromosomes comprising 13,608 SNP. Two hundred additive causal mutations (nq=200) were randomly sampled among SNPs with a minor allele frequency (MAF) higher than 0.02. The additive effects of these mutations were independently drawn from a normal distribution, assuming a heritability of 0.3. Two scenarios were tested: (1) the SNP-BLUP model accounted for the ns=13,408 SNPs excluding the QTL; (2) in addition to these SNPs, the SNP-BLUP model also accounted for an additional residual polygenic effect explaining 20% of the genetic variance. Note that in a previous study (Boichard et al., 2022), we have shown with a similar approach that erosion was minimal when the causal variants were included in the analysis, and this scenario is not replicated here. This also agrees with de los Campos et al (2015) who showed that missing heritability does not exist when causal variants are in the model.

The strategy used to compute erosion relied on the determination of the contribution of each QTL to each SNP, as follows. Omitting fixed effects, the SNP-BLUP equations can be written as

$$[\, \boldsymbol{M'M} + \lambda \boldsymbol{I} \,]\, \hat{\boldsymbol{s}} = \boldsymbol{M'y}$$

with **M** the (*N* x *ns*) matrix of cantered and scaled genotypes, **s** the vector of SNP effects, **y** the vector of phenotypes adjusted for the fixed effects, and $\lambda = \sigma_e^2 / \sigma_s^2$ with σ_e^2 and σ_s^2 the residual and the SNP variances, respectively. According to the simulation, the phenotype can be written as **y = Pq + e**, *i.e.*, the sum of *nq* QTL effects and an error term, with **P** the (*N* x *nq*) matrix of genotypes at the QTL level and **q** the vector of true QTL effects. Therefore, the equations can be rewritten as

$$\hat{\boldsymbol{s}} = [\, \boldsymbol{M'M} + \lambda \boldsymbol{I} \,]^{-1} \quad \boldsymbol{M'(Pq + e)} \qquad [1]$$

Let us denote $\mathbf{C} = [\, \mathbf{M'M} + \lambda \mathbf{I} \,]^{-1}$ the inverse of the coefficients matrix. If $\mathbf{c_i}$ is line *i* (corresponding to SNP *i*) of **C,** the contribution of QTL *j* to SNP effect *i* is

$$f_{ij} = \boldsymbol{c_i M' P_j} q_j. \qquad [2]$$

There were *nq* x *ns* = 200 x 13,408 = 2,681,600 such contributions, distributed in the 4 following categories based on the distance (*d*) between the QTL and the SNP: (1) $d < 5$ Mb; (2) $5 < d < 20$ Mb; (3) $d > 20$ Mb with both the QTL and the SNP located on the same chromosome; (4) the QTL and the SNP are located on different chromosomes. Within each of these categories, we computed a partial DGV for each cow within the reference population. Summary statistics were then calculated over 30 replicates quantifying their relative contributions to the total DGV and their correlations.

The same strategy can also be applied in a model including a residual polygenic effect, denoted as **u**. The equations corresponding to **s** and **u** are as follows:

$$\begin{bmatrix} \boldsymbol{Z'Z} + \kappa \boldsymbol{A^{-1}} & \boldsymbol{Z'M} \\ \boldsymbol{M'Z} & \boldsymbol{M'M} + \lambda \boldsymbol{I} \end{bmatrix} \begin{bmatrix} \hat{\boldsymbol{u}} \\ \hat{\boldsymbol{s}} \end{bmatrix} = \begin{bmatrix} \boldsymbol{Z'y} \\ \boldsymbol{M'y} \end{bmatrix} \qquad [3]$$

with **Z** being the incidence matrix linking the records of **y** to **u** and $\kappa = \sigma_e^2 / \sigma_u^2$ the corresponding variance ratio.

The **u** equations can be absorbed into **s** equations, resulting in the following formula:

$$\left[\boldsymbol{M}'\left(\boldsymbol{I} - \boldsymbol{Z}\left(\boldsymbol{Z}'\boldsymbol{Z} + \kappa\boldsymbol{A}^{-1}\right)^{-1}\boldsymbol{Z}'\right)\boldsymbol{M} + \lambda\,\boldsymbol{I}\right]\hat{\boldsymbol{s}} = \mathbf{M}'\left(\mathbf{I} - \mathbf{Z}\left(\mathbf{Z}'\mathbf{Z} + \kappa\mathbf{A}^{-1}\right)^{-1}\mathbf{Z}'\right)(\mathbf{Pq} + \mathbf{e})$$

Let us denote **C*** the inverse of the coefficient matrix after absorption

$$\mathbf{C}^{*} = \left[\mathbf{M}'\left(\mathbf{I} - \mathbf{Z}\left(\mathbf{Z}'\mathbf{Z} + \kappa\mathbf{A}^{-1}\right)^{-1}\mathbf{Z}'\right)\mathbf{M} + \lambda\,\mathbf{I}\right]^{-1}$$

and **M*** the adjusted genotype matrix after absorption

$$\boldsymbol{M}^{*\prime} = \boldsymbol{M}'\left(\boldsymbol{I} - \boldsymbol{Z}\left(\boldsymbol{Z}'\boldsymbol{Z} + \kappa\boldsymbol{A}^{-1}\right)^{-1}\boldsymbol{Z}'\right)$$

Then the contribution of QTL *j* to each SNP effect *i* is:

$$f_{ij} = \mathbf{c}^{*}_{i}\ \boldsymbol{M}^{*\prime}\ \boldsymbol{P}_j\ q_j \qquad [4]$$

where $\mathbf{c}^{*}_{i}$ is line i of **C***

Table 2 presents the relative contribution of the 4 categories based on QTL-SNP distance to the total DGV variance.

Results and Discussion

In the model without a polygenic effect (scenario 1), the partial DGV derived from contributions of the QTL close to markers explained approximatively three-quarters of the DGV variance. Notably, more distant markers (d>20 Mb) and markers located on other chromosomes together accounted for ~13% of the total DGV variance. Markers located on other chromosomes explained more variance than markers at more than 20 Mb on the same chromosome. This result can be attributed to the larger number of marker-QTL pairs when markers are located on different chromosomes. The likely underlying reason of this pattern lies in the strong shrinkage of the effects of markers situated close to the QTL due to the influence of the prior information. Indeed, all markers receive the same prior variance, and the parameter λ had a relatively high value compared to the diagonal elements of the matrix **M'M**. Consequently, the estimated effects of markers in proximity to the QTL experience substantial shrinkage and are much smaller, even altogether, than the true QTL effect. As a result, the unexplained part of the true QTL effect becomes available for distant markers, potentially leading to their contribution to total DGV. This effect would be probably maximized when the number of true QTL is much lower than the number of SNPs ($\sigma_q^2 >> \sigma_s^2$), and when the reference population is relatively small (diagonal (**M'M**) does not dominate λ). It is important to note that when the size of the reference population is very large, the influence of prior information decreases, and this observed pattern is likely to gradually decrease.

Table 2. – Relative contributions (%) of each of the 4 classes of QTL-SNP pairs defined according to their distance (d). The contribution of a class is the percentage of DGV variance explained by each partial DGV in the reference population, over 30 replicates.

Classes of partial DGV defined according to QTL-SNP distance (*d*)	Scenario 1 Model without polygenic effect	Scenario 2 Model with polygenic effect
1: *d* < 5 Mb	73.5	68.9
2: 5 Mb < *d* < 20 Mb	13.7	14.3
3: *d* > 20 Mb	4.9	5.2
4: QTL and SNP located on different chromosomes	8.0	11.5

The inclusion of a polygenic effect in the model (scenario 2) resulted in a higher proportion of variance being explained by distant markers and by markers located on different chromosomes. At first glance this result may seem counterintuitive as one could

expect that the polygenic effect would help account for these long-distance effects since it captures the genetic relationships between individuals. Distant markers and markers on another chromosome altogether explained around 17% of the DGV variance whereas the share due to close markers (d<5Mb) decreased to 69%. As in scenario 1, a possible interpretation is the shrinkage of estimated SNP effects. Indeed, in the presence of a polygenic effect with variance σ_u^2, the total variance due to SNPs is reduced to σ_g^2 - σ_u^2 and results in an increased variance ratio λ (σ_e^2 / σ_s^2). It can then be concluded that inclusion of a polygenic effect into the model should primarily be motivated by the need to account for the genetic variance not captured by the SNPs, rather than as a means to reduce inflation.

Table 3a. Average correlations between partial DGV in Scenario 1, without polygenic effect. Results over 30 replicates

Partial DGV class	<5 Mb	5-20 Mb	>20 Mb
5-20 Mb	0.30		
>20 Mb	0.17	0.15	
Other Chromosomes	0.11	0.05	0.12

Table 3b. Average correlations between partial DGV in Scenario 2, with polygenic effect. Results over 30 replicates

Partial DGV class	<5 Mb	5-20 Mb	>20 Mb
5-20 Mb	0.54		
>20 Mb	0.26	0.29	
Other Chromosomes	0.25	0.22	0.27

Tables 3a and 3b present the correlations between the partial DGVs derived from different categories of QTL-SNP distances in scenarios 1 and 2, respectively. Both scenarios presented low to moderate positive correlations, illustrating that distant QTL contribute to the effects of many markers. Inclusion of a polygenic effect in the model (scenario 2, table 3b) increases these correlations showing that long distance effects are reinforced.

Methods to estimate erosion factor of SNP effects

The concept of erosion of the genomic breeding values has two distinct components: (a) a component that is characteristic of the reference population itself, and (b) a component that is specific to each candidate and its genetic distance from the reference population.

The extent of long-distance LD in the reference population is influenced by the effective population size (Ne). Notably, when Ne is small, a non-zero LD baseline persists. More importantly, the level of long-distance LD is also strongly dependent on the genetic relatedness within the reference population, which can be different from the relatedness in the overall population. A higher average relationship between individuals within the reference population results in more long-distance LD. It can be argued that, on average, the long-distance LD appears relatively stable across generations, but this stability does not hold for individual pair of markers. At a given generation, existing LD is halved in the subsequent generation due to recombination, but new LD can emerge from different marker pairs as a consequence of the random processes associated with the sampling of parents and genetic drift, making average LD stable.

The theoretical derivation of the erosion factor ρ requires additional investigation. Nevertheless, practical and efficient solutions can be obtained through simulation. In this paper, we present two simulation-based approaches which offer practical and effective means to address the erosion phenomenon and to estimate ρ.

Method 1: by simulating a new generation

The real reference population G_r of the breed for a given trait is considered with its SNP genotypes **M**. As previously, *nq* QTL are simulated in this reference population by sampling SNP which are thereafter excluded from the analysis. Expectations of SNP effects are estimated by $\widehat{\mathbf{s_r}} = (\mathbf{M'M} + \lambda\mathbf{I})^{-1}\,\mathbf{M'}\,\mathbf{P}\,\mathbf{q}$, assuming the same previous notations.

A new generation, G_n, is then simulated, by sampling parents (at random or following a predefined design) in the reference population and performing matings. The expected DGV of this new generation is obtained from the genotypes M_n and from the SNP effects estimated in the reference population: $\mathbf{DGV_r} = \mathbf{M_n}\,\widehat{\mathbf{s_r}}$

Assuming phenotypes are known in this new generation, new SNP effect estimates can be obtained from generation *n* only $\widehat{\mathbf{s_n}} = (\mathbf{M_n'}\,\mathbf{M_n} + \lambda\mathbf{I})^{-1}\,\mathbf{M_n'}\mathbf{P_n}\mathbf{q}$, and a new set of DGV is obtained from these new SNP estimates $\mathbf{DGV_n} = \mathbf{M_n}\,\widehat{\mathbf{s_n}}$

These new SNP effects are different from the previous ones if the covariances between markers and QTL $\mathbf{M'P}$ and $\mathbf{M_n'}\,\mathbf{P_n}$ differ. A large change in the covariances between markers $\mathbf{M'M}$ and $\mathbf{M_n'}\,\mathbf{M_n}$ (i.e., in LD between SNP) may also affect the results but probably to a lesser extent.

From these two sets of DGV, an estimate of the erosion ρ between the two generations is obtained through a regression analysis, where:

$$\boldsymbol{DGV_n = 1\mu + \rho\, DGV_r + e} \qquad [5]$$

Method 2: by regressing contributions of QTL to marker effects.

As above, the real reference population G_r of the breed for a given trait is considered with its genotypes **M** and *nq* QTL are simulated in this reference population. All contributions f_{ij} of the QTL *j* to the effect of SNP *i* are computed as shown in equation [2]:

$$f_{ij} = \boldsymbol{c_i\, M'\, p_j\, q_j}.$$

DGV_r in the reference population is the sum of all contributions:

$$\boldsymbol{DGV_r = M\, f\, 1_q}$$

with $\mathbf{1_q}$ being a vector of 1 of size q.

Note that the DGV can also be obtained as $\mathbf{DGV_r} = \mathbf{M}\,\widehat{\mathbf{s_r}}$, as in Method 1.

Then, all f_{ij} are regressed according to the genetic map, with coefficients $(1\text{-}r_{ij})$ varying from 1 to 0.5, r_{ij} being the recombination rate between the loci *i* and *j*:

$$h_{ij} = r_{ij}\, f_{ij}. \qquad [6]$$

New eroded DGV ($\mathbf{DGV_e}$) are the sum of all regressed contributions

$$\boldsymbol{DGV_e = M\, h\, 1_q}$$

An estimate of $\sqrt{\rho}$ is obtained through a regression analysis, where:

$$\boldsymbol{DGV_e = 1\mu + \sqrt{\rho}\;\; DGV_r + e} \qquad [7]$$

Comparison of both methods

Both methods are based on QTL simulation, and their results are influenced by assumptions, particularly regarding the number of QTLs. However, as far as the number of QTLs is smaller than the number of markers and long-distance LD is present, we can anticipate that erosion exists.

Method 1 is relatively straightforward to implement, as it involves simulating one additional generation and estimating expected SNP effects in both the reference population and in the new generation using standard software. In contrast, Method 2 requires a specific program to compute all the contributions and erode them. Nevertheless, it provides an explicit biological basis to understand and interpret erosion across generations.

Method 1 generates progeny from pairs of parents, leading to erosion on both sire-progeny and dam-progeny pathways. Method 2, on the

other hand, simulates erosion through recombination at only one meiosis, resulting in the erosion factor estimated by method 2 being the square root of that estimated by method 1. This scale difference should be considered when interpreting results.

Additionally, method 2 considers the whole reference population whereas method 1 generates a new generation based on assumptions about the number and the choice of parents sampled. Therefore, results between the two methods can exhibit slight variations.

Numerical example

In our numerical example, we applied both method 1 and method 2 to the same 2021 Normande female reference population. As before, we focused on only 5 chromosomes and simulated 200 QTLs. In method 1, a new generation was created by sampling 1,000 sires and 50,000 dams. Each sire had 50 progeny, while each dam had one progeny, resulting in a new generation of 50,000 animals. The results obtained with both methods are presented in Table 4. Recombination rates were based on ARS-UCD1.2 bovine genome assembly, assuming 1 cM for 1 Mb.

Table 4. Estimation of erosion factors by Method 1 and Method 2 (30 replicates)

	$\hat{\rho}$	SD($\hat{\rho}$)
Method 1	0.87	0.015
Method 2	0.84	0.010

The number of replicates was set to 30. Variability across replicates was small (at least with such a large reference population) and this number of replicates was sufficient to obtain reliable estimates of ρ.

Discussion on how to apply erosion in practice

Here, we consider that, by definition, individuals in the reference population are assumed to possess non-eroded SNP effects. It is worth noting that this assumption may warrant discussion due to potential heterogeneity within the reference population. The estimated SNP effect represents an expectation and may not align with individual situations. However, this point goes beyond the scope of this initial study.

Erosion primarily concerns selection candidates, *i.e.* genotyped individuals without phenotype data and, therefore, out of the reference population. When their parents, referred to as *s* and *d*, are part of the reference population, we assume that the parent's average (PA) DGV, denoted as

$$PA = 0.5\,(DGV_s + DGV_d),$$

remains unaffected. Indeed, their DGVs are based on performances; and this is especially the case for sires with progeny evaluation, and therefore with very reliable DGVs. Erosion influences the deviation from PA, *i.e.*, the predicted Mendelian sampling term. We propose applying the following formula:

$$DGV_{eroded} = PA + \rho\,(DGV\text{-}PA) \qquad [8]$$

When the parents of a candidate are not in the reference population, erosion applies at each generation between the reference population and the candidate. Following a similar approach as described by Dekkers et al (2021), the number of generations between the candidate and its closest relatives within the reference population is determined on both the sire and dam pathways and k is their sum. Erosion is applied following equation [9]:

$$DGV_{eroded} = PA + \rho^{k/2}\,(DGV\text{-}PA) \qquad [9]$$

When the parents themselves are candidates, *i.e.,* genotyped and not in the reference population, erosion also applies to them. This erosion affects the PA of their progeny in the following way:

$$DGV_{eroded} = PA_{eroded} + \rho^{k/2}\,(DGV\text{-}PA)$$

This formula should be applied recursively, processing parents before progeny. This recursive approach highlights that the DGV of candidates born from very young parents experience significant erosion, which aligns

well with practical observations. Therefore, breeding schemes with accelerated generations without updating reference data tend to accumulate more erosion than initially anticipated. These schemes may be less appealing due to the rapid erosion effect on genomic values.

Furthermore, as shown by Dekkers et al (2021), erosion also affects reliability, but with a coefficient equal to ρ^2 instead of ρ. The loss in genomic accuracy is therefore very fast. Theoretical accuracies calculated based on the inverse of the coefficient matrix tend to overestimate the reliabilities for candidates and must be adjusted accordingly. In the French evaluation system, reliabilities are computed by combining effective record contributions (ERC) associated to polygenic information and genomic information, the latter (and the latter only) being eroded in candidates.

Conclusions

The practical implications of erosion in genetic evaluations and breeding programs are important. When considering the overall prediction of selection candidates, contributions from short-distance LD tend to remain relatively stable because they are only mildly eroded by recombination (Dekkers et al, 2021). However, contributions from long-distance LD are halved at each generation. The extent of erosion varies with the relative weight of short and long-distance LD, but it should never be disregarded.

Further investigations are needed to theoretically determine the erosion factor ρ. Nonetheless it is clear this factor depends on baseline LD in the population, *i.e.,* effective population size (Ne) and genome length (L), as well as on the structure of the reference population. Additionally, it is likely influenced by the genetic architecture of the traits (such as the number of QTL and magnitude of their QTL effects) and the model used (SNP-BLUP/GBLUP vs Bayesian models, the latter being likely less affected by erosion).

It is also relevant to explore the impact of the structure of the reference population, such as its heterogeneity in terms of time span, selection, and relationship. For instance, the impact on erosion of old generation data in the reference population would be worth investigating. While the proposed erosion methods consider the smallest distance between the candidate and the reference population, alternative approaches using the barycentre of the reference population warrant investigation.

However, one can anticipate that:

(1) Inflation factors, frequently observed between 0.8 and 0.9, give the magnitude of the erosion phenomenon;

(2) Models including causal variants tend to be more persistent and less subject to erosion, as demonstrated by Boichard et al (2022);

(3) Models that incorporate a residual polygenic component may appear to have less inflated predictions for candidates because they combine two estimates of the MS term: the genomic estimate, which is inflated, and a polygenic estimate, which is equal to zero (i.e., 100% deflated). However, the polygenic effect does not capture long-distance LD effects and, therefore, does not improve predictions in terms of persistence. We believe that accounting for erosion is a more rigorous and accurate approach, even if it requires post-processing.

This methodology has been implemented in the French Single Step bovine evaluation since March 2022.

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The Value of Increased Heterozygosity in Dairy Cattle

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Abstract

Today mating decisions are mostly based on pedigree information. However, genomic information could be used to minimize inbreeding or increase heterozygosity in mating decisions, because it contributes with more information on the expected heterozygosity (He) than pedigree information. The underlying hypothesis is that the more heterozygous the offspring of a mating is, the larger the dominance effect and less inbreeding depression in purebred offspring. The purpose of this study was to estimate the size of He using single nucleotide polymorphism (SNP) marker information and further the effect of increased heterozygosity on milk, fat and protein yield in Holstein (HOL), Red Dairy Cattle (RDC) and Jersey (JER) cows. He was calculated SNP by SNP for all couples of genotyped parents. Genome-wide He was calculated as the mean heterozygosity over all the SNPs. Data from 5,423 HOL, 2,245 RDC and 5,975 JER genotyped cows born between 2015 and 2017, which all had parents with GEBV were analyzed. The mean He levels were 0.328 for HOL, 0.336 for RDC and 0.308 for JER with standard deviations between 0.007 and 0.008. Results showed a significant effect of He on milk, fat and protein for all breeds. For HOL, a 1%-point increase in He corresponds to an increase in 305 days yield of 122 kg milk, 3.7 kg fat and 3.7 kg protein. For RDC the effect was 99 kg milk, 2.9 kg fat and 2.9 kg protein per heterozygosity percentage point. For JER the effect was 42 kg milk, 3.2 kg fat and 1.8 kg protein per heterozygosity percentage point. This indicates that it could be beneficial to include He in the mating plan decisions.

Key words: Dairy Cattle, mating decisions, inbreeding, heterozygosity, production traits

Introduction

Today mating decisions are primarily based on pedigree information. However, genomic information could be used to minimize inbreeding or increase heterozygosity in mating decision, because it contributes with more information on the expected heterozygosity (He) than pedigree information. Inbreeding depression has been shown to affect many traits affecting the profitability of dairy cows such as milk, fat and protein yield (Rokouei et al. 2010, Cassell 2009), but also mastitis (Sørensen et al. 2006) and some fertility traits (Rokouei et al., 2010). Accounting for genomic information increases the estimation accuracy of inbreeding coefficients because it captures realized autozygosity. As a result, it also allows for more accurate estimation of inbreeding depression effects than pedigree information. For example, Pryce et al. (2014) found that a 1% increase in pedigree inbreeding results in a decrease in milk yield of 21 L for Holstein dairy cows whereas the same 1% increase in genomic inbreeding leads to a decrease in milk yield of 27.8L and the effect of increased homozygosity was -63 L. Bjelland et al. (2013) also found a decrease in yield traits with an increase of genomic inbreeding.

The expected genome-wide heterozygosity for a progeny from a prospective mating can be computed from the parents' genotypes. The expected heterozygosity is assumed to reflect part of inbreeding depression effect that would be expected for an "average" progeny of a specific mating. When the effect of increased expected heterozygosity on traits is known, it

is possible to implement it in the mating plan. This will make it possible for the farmer to select the sire for the specific cows, that will give the highest profit considering both breeding values and heterozygosity.

In this study we calculated the expected heterozygosity of purebred animals, using SNP information and further the effect of increased heterozygosity on milk, fat and protein yield in Holstein (HOL), Red Dairy Cattle (RDC) and Jersey (JER) cows. The underlying hypothesis is that the more heterozygous the offspring of a mating is, the larger the dominance effect and less inbreeding depression in purebred offspring.

Material and Methods

Data

Data from 5423 Holstein (4833 dams and 517 sires), 2245 RDC (1978 dams and 212 sires) and 5975 Jersey cows (5148 dams and 214 sires) born in Denmark in 2015, 2016 and 2017 were included in the analyse. All cows were genotyped and had deregressed proofs. The parents of the cows were also genotyped and had GEBVs. For HOL 46,342 SNPs were used to calculate He, 41,897 SNPs were used to calculate He for RDC and 46,914 SNPs were used to calculate He for JER.

Methods

Expected heterozygosity was calculated SNP by SNP for the genotyped parents. If both parents were opposite homozygotes for the SNP, the He was set to 1. If both parents were homozygous for the same SNP, the expected heterozygosity was set to 0. If one or both parents were heterozygous for the SNP, He was set to 0.5. The genome-wide He was then calculated as the sum of all He over all the SNPs divided by the total number of SNPs.

Effect of He on each trait was estimated using a linear regression model with software SAS (version 9.4; SAS Institute Inc.).

$$\mathrm{DRP} = \mu 1 + \alpha \mathrm{He} + \beta \frac{\mathrm{GEBV_s} + \mathrm{GEBV_d}}{2} + e$$

where:

μ1 is the intercept, He is the expected heterozygosity fitted as covariate, and $\frac{\mathrm{GEBV_s} + \mathrm{GEBV_d}}{2}$ is the mean parental GEBV for the traits milk yield, fat yield and protein yield. DRP is the deregressed proof (Stránden and Mäntysaari, 2010) expressed in index units.

The phenotypic effect of He for each trait in trait units was calculated by scaling He according to the value of 1 index unit (see Table 1).

Table 1. Value of + 1 index unit (NAV 2023)

	HOL	RDC	JER
Milk (305-d, kg)	66.0	72.5	57.3
Fat (305-d, kg)	2.4	2.5	2.1
Protein (305-d, kg)	2.0	2.0	1.7

Results & Discussion

The mean He at SNP markers for HOL was 0.328 with a standard deviation of 0.007. RDC had, as expected the highest He of 0.336 with a standard deviation of 0.008, and JER the lowest He with a mean on 0.308 with a standard deviation of 0.008.

The effect of expected heterozygosity on milk, fat and protein yield is shown in Table 2 for HOL, in Table 3 for RDC and in Table 4 for JER. The estimates for regression coefficients represent the increased value, expressed in index units, when going from an He of 0 to 1 or 100%. All regression coefficients were significant, irrespective of the breeds and traits. They were consistently higher for HOL. The effect of an 1%-point increase in He was 1.85 index units for milk, equivalent to 122 kg milk. For fat yield the effect of 1%-point increase in He increases fat yield by 3.7 kg corresponding to 1.54 index units of fat. The effect of increased He is the same for protein yield as for fat yield, where 1%-point increase of He resulted in 3.7 kg (1.87 index units).

Table 2. Regression coefficient estimated for deregressed proofs on expected heterozyosity for Holstein

	Estimate	Standard error
He milk	185	52
He fat	154	54
He protein	187	47

He = estimated heterozygosity

For milk regression coefficients were lower for RDC and JER than for HOL: the effect of 1%-point increase in He was 1.37 index units in RDC (equivalent to 99 kg of milk) and 0.74 index units for JER (equivalent to 42 kg of milk). The effect of heterozygosity on fat yield of JER (1.51 index units per 1%-increase in He, i.e. 2.9 kg fat yield) was as high as the HOL estimate and higher than the RDC estimate.

Table 3. Regression coefficient estimates for deregressed proofs on expected heterozygosity for RDC

	Estimate	Standard error
He milk	137	50
He fat	115	49
He protein	187	47

He = estimated heterozygosity

In contrast, the regression coefficient of protein yield DRP on He was lower in JER (1.03 index units per 1% increase in He, i.e. 1.8kg protein yield) than in HOL and RDC.

Table 4. Regression coefficient estimated for deregressed proofs on expected heterozygosity for Jersey

	Estimate	Standard error
He milk	74	33
He fat	151	33
He protein	103	30

He = estimated heterozygosity

Pryce et al. (2014) and Bjelland et al. (2013) focused on increase in homozygosity instead of heterozygosity but because 1% increase in heterozygosity is the same as 1% decrease in heterozygosity these results are comparable, even though there are difference in the models used, where Pryce et al. (2014) used phenotypic data instead of deregressed proof. Pryce et al. (2014) found a decrease in milk yield of 63 L for Holstein and 71 L for Jersey, with a mean of 7286 L milk for Holstein and 5197 L milk for Jersey in the population, corresponding to a decrease of 0.9% and 1.4% in milk. Bjelland et al. (2013) found a decrease in milk yield of 53 kg with one percentage increase in homozygosity, this is for 205 days yield with a mean of 8453 kg which corresponds to a decrease on 0.6%. The mean milk, protein and fat yield (305 days) for Danish Holstein, RDC and Jersey cows are shown in Table 5. Converted to percentage of total milk yield the effect of 1%-point increase in estimated heterozygosity are between 0.6% increase in milk yield (Jersey) and 1.1% increase in milk yield (Holstein), which are similar to the results found by Pryce et al. (2014) and Bjelland et al. (2013). Bjelland et al. (2013) did not find a significant effect of increased homozygosity on fat- and protein percentages. Pryce et al. (2014) found a decrease in fat yield of 3 kg for Holstein and 3.9 kg for Jersey corresponding to 1.1% and 1.5% which is higher than our estimates, that, converted to increase in percentage of fat yield is between 0.7% (RDC and Jersey) and 0.8% (Holstein). The increase in fat yield with increased heterozygosity was between 0.6% (Jersey) and 0.9% (Holstein) which for Holstein is comparable to results by Pryce et al. (2013) with a decrease in protein yield of 0.8% for Holstein with increase in homozygosity, but lower for Jersey where Pryce et al. (2014) found a decrease in protein yield of 1.4%.

Table 5. Mean 305 days yield in Denmark 2021/2022 (Viking Danmark 2022)

	Milk	Fat	Protein
Holstein	11,271	462	396
RDC	9,735	428	360
Jersey	7,598	453	326

Conclusions

There is a significant effect of He on milk, protein and fat yield across all three breeds and traits. Including this information in the mating plan is straightforward. It is expected to increase the average genome-wide heterozygosity of offspring compared to random mating and thereby their phenotypic performance. Before being implemented in the mating plan with full added value, there is a need to estimate the effect of He for all other traits in the breeding goal and for the total merit.

Acknowledgments

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Udder Classification Based On AMS Data

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Abstract

Since April 2023, CRV uses AMS (Automatic Milking System) data to estimate breeding values for udder conformation traits in the Netherlands and Flanders in addition to data from herd classification. AMS data is used to derive traits for udder depth, distance between the front teats, distance between the rear teats and udder balance. Three teat coordinates are determined and stored by the AMS each milking, all given in millimetres: x (measure of the width), y (measure of the length), and z (measure of the depth). Based on these three teat coordinates, the four udder traits can be derived. Traits are derived for first three parities. The heritability for respectively udder depth, distance between the front teats, distance between the rear teats and udder balance in parity 1 is 0.56, 0.60, 0.45 and 0.45. Based on herd classification, heritabilities for udder depth, front teat placement and rear teat placement are respectively 0.39, 0.31 and 0.29. The genetic correlations between these three traits and corresponding heifer traits based on AMS data are respectively 0.98, 0.98 and 0.99. Traits for later parities show comparable heritabilities and genetic correlations. The repeated records lead to an increase of the heritability and reliability compared to udder depth, front teat placement and rear teat placement based on herd classification. Furthermore, the AMS gives more objective results compared to scores given by herd classifiers. The udder conformation traits based on herd classification are still the traits that are published, while udder balance is introduced as new trait. Due to the usage of AMS data, an increase of reliability for bulls breeding values was found ranging between 0.4 and 3.0%. Adding AMS data to the breeding value estimation of udder conformation traits leads to better estimates of the breeding values for existing udder conformation traits by using more information as well as having a breeding value for the new trait udder balance.

Key words: udder conformation, herd classification, AMS data, udder balance

Introduction

In April 2023, 4 805 dairy farms in the Netherlands were milking their cows with an AMS. This is 33% of the total number of dairy farms in the Netherlands. These farms had 9 825 AMS boxes in total, resulting in on average 2 boxes per farm (Stichting KOM, s.d.).

To attach the milkcup to the teats, the AMS should know the position of the teats. The teat positions make it possible to derive udder conformation traits based on information from data stored by the AMS.

By using AMS data, more information on the conformation of the animal is available in addition to herd classification data. As there are also animals in the AMS data without information from herd classification, more animals can be included in the breeding value estimation for udder conformation.

The data collection is automatic and therefore less time costly compared to herd classification. Dairy farmers can easily indicate via JoinData (JoinData u.a., Wageningen, the Netherlands) if they are willing to share their AMS data with CRV for the breeding value estimation. With their permission, CRV can upload all milkings on a daily basis. The automatic data collection makes the data also available faster compared to classification scores.

Materials and Methods

Data for breeding value estimation

AMS data from more than 1 400 Dutch dairy farms are available for the genetic evaluation. Those farms are all milking their cows with an AMS from Lely (Lely group, Maassluis, the Netherlands).

Each day, almost 400 000 milkings from the previous day are uploaded and added to the database. There is more data from recent years compared to the first year with data, 2014, because the number of farms milking with an AMS keeps growing.

From each milking, the x, y, and z-coordinates are known. The three teat coordinates are all given in millimetres and are illustrated in Figure 1. Based on those coordinates, four udder traits can be derived: udder depth, distance between the front teats, distance between the rear teats and udder balance. Udder depth is the average z-coordinate of the four teats. Distance between the front teats is the difference in x-coordinates of the front teats. Distance between the rear teats is the difference in x-coordinates between the rear teats. Udder balance is the difference in the average z-coordinates of the rear teats and the average z-coordinates of the front teats.

A positive udder balance means a higher rear udder compared to front udder. The opposite is the case if we speak about a negative udder balance.

Higher breeding values indicate a more positive udder balance. A too positive udder balance is not desired, because this can give problems for the AMS when attaching the milkcup to the rear teats. This makes udder balance an optimum trait.

For each cow, the first and every twentieth milking is used for the breeding value estimation to reduce the data size. The first milking is taken to ensure that each cow in the data is also in the breeding value estimation. 33% of the animals in the AMS data has no information on udder conformation traits from herd classification.

Data from parity 1 to 3, is used. In the April 2023 breeding value estimation, the number of milking was 5 499 248.

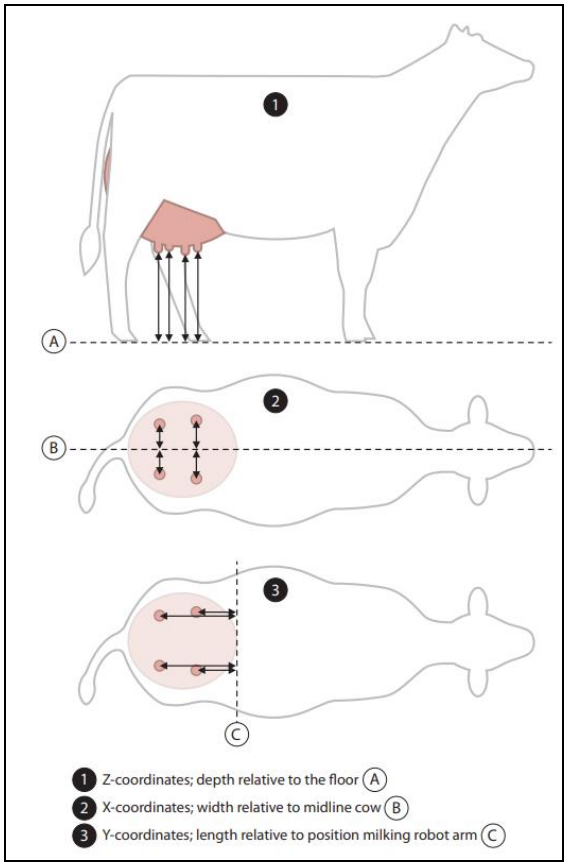

Figure 1. Illustration of teat coordinates.

Parameters

Parameter estimation was based on 868 396 milkings from 89 456 cows with 311 661 milkings belonging to parity 1, 279 191 milking to parity 2 and 277 544 milking to parity 3. All cows were at least 87,5% Holstein. Parameters were estimated using an animal model.

Model

The statistical model used for udder conformation based on AMS data is:

$$Y1_{ijklmnopqrs} = HYS_i + DIL_j + AFC_k + HY_l + HET_m + REC_n + INB_o + HGT_p + TLE_t + A_q + PME_r + Rest_s$$

In which:

Y observation on udder conformation on heifers (parity 1), young cows (parity 2) and cows (parity 3);
HYS herd x year x season x box of milking;
DIL lactation stadium;
AFC age of first calving;
HY herd x year of first calving;
HET heterosis effect;
REC recombination effect;
INB inbreeding effect;
HGT EBV stature;
TLE EBV teat length;
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, INB, HGT and TLE are covariables, the other effects are fixed. Effect AFC is only added to the model for parity 1 and parity 2.

The covariables HGT and TLE are only added to the model for udder depth. Udder depth is based on the z-coordinate, which is measured as the distance from the teat tip to the floor. Stature and teat length of the cow influence this distance, so correction in the model is needed to make udder depth independent form stature and teat length.

Results & Discussion

Udder balance

Udder balance is the average difference in udder depth between rear udder and front udder. The genetic correlations of udder balance with udder conformation traits based on herd type classification are shown in table 1 and are all moderate and positive. This explained the positive trend in phenotype for udder balance over the period 2014 – 2023 as shown in Figure 2. Over this period, udder balance has increased with 5 millimetres for all three parities.

Table 1. Genetic correlation of udder balance parity 1 with udder conformation traits based on herd classification.

trait	genetic correlation
front udder attachment	0.25
front teat placement	0.29
teat length	0.25
udder depth	0.24
rear udder height	0.53
udder support	0.34
rear teat placement	0.36

Table 1 shows that udder balance has the highest genetic correlation (0.54) with rear udder. Because all correlations are moderate, the udder traits based on herd classification cannot give a good prediction of the udder balance of a cow.

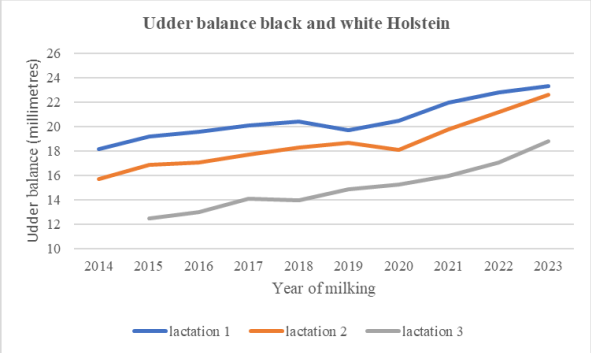

Figure 2. Phenotypic trend in udder balance for black and white Holstein cows in parity 1, parity 2 and parity 3 from 2014 to 2023.

Next to the upward phenotypic trend in udder balance, figure 2 shows that udder balance is most positive in parity 1 and declines over the parities. The decline from parity 1 to parity 2 is about 2 millimetres, from parity 2 to parity 3 the decline is about 3 millimetres.

Genetic parameters

Table 2 shows the heritabilities of the udder conformation traits based on AMS data. Table 3 shows the genetic correlations between similar traits based on herd classification and AMS data for the three different parities. Udder depth from herd classification is

compared to udder depth based on AMS data, and front- and rear teat placement from herd classification are compared to distance between the front- and rear teats based on AMS data. Table 4 shows the genetic correlations between the different parities for the traits based on AMS data.

Table 2. Heritabilities of the traits in parity 1 (p.1), parity 2 (p.2) and parity 3 (p.3).

	heritability		
trait	p.1	p.2	p.3
udder depth	0.56	0.56	0.52
distance front teats	0.60	0.53	0.45
distance rear teats	0.45	0.38	0.33
udder balance	0.45	0.42	0.43

The heritabilities in table 2 are higher compared to the heritabilities for similar traits based on herd classification. Udder depth has a heritability of 0.39, front teat placement has a heritability of 0.31 and rear teat placement has a heritability of 0.29. The increase in heritability by the traits based on AMS data is caused by repeated records of high-quality data.

Table 3. Genetic correlations between similar traits based on herd classification and AMS data for parity 1 (p.1), parity 2 (p.2) and parity 3 (p.3).

	genetic correlations		
trait	p.1	p.2	p.3
udder depth	0.98	0.97	0.97
distance front teats	0.98	0.98	0.97
distance rear teats	0.99	0.99	0.96

The genetic correlations between the traits in table 3 are, independent from parity, close to 1.0, ranging from 0.96 to 0.99. This means that the herd classifiers and AMS measure actually the same trait.

The heritabilities of the udder conformation traits based on AMS data and the genetic correlations with the linear traits from herd classification are comparable with results found in Scandinavia (Rius-Vilarrasa *et al.*, 2016).

Table 4. Genetic correlations for udder traits based on AMS data between parity 1 and 2 (p.1-2), parity 2 and 3 (p.2-3) and parity 1 and 3 (p.1-3).

	genetic correlations		
trait	p.1-2	p.2-3	p.1-3
udder depth	0.97	0.99	0.93
distance front teats	0.99	0.99	0.94
distance rear teats	0.97	0.98	0.90
udder balance	0.96	0.98	0.85

The genetic correlations in table 4 between the different parities are all above 0.90, only udder balance has a lower genetic correlation of 0.85 between parity 1 and 3 which is still considered a high correlation. The different parities give all the same information, therefore parity 1 is used as published trait for udder balance since parity 1 contains the most cows.

Reliabilities

Udder balance in parity 1 is the published trait, the other udder conformation traits based on AMS data are indicator traits. The udder conformation traits based on herd classification profit from the indicator traits using the genetic correlations between the traits because information of extra cows is added, resulting in more reliable breeding values. The increase in reliabilities is shown in table 5.

Table 5. Reliability for udder conformation traits without (old rel.) and with (new rel.) using udder conformation traits based on AMS data as indicator traits and the correlation (corr.) of the bull breeding values between both systems for Holstein bulls born since 2010.

trait	old rel.	new rel.	corr.
front udder attachment	78.1	81.7	0.98
front teat placement	78.7	82.7	0.97
teat length	81.3	81.7	0.99
udder depth	81.4	83.9	0.98
rear udder height	77.1	78.6	0.99
udder support	75.3	79.2	0.97
rear teat placement	77.8	81.8	0.97

The increase in reliability ranges from 0.4 to 4.0%, depending on the covariance structure with the indicator traits. Front udder placement, udder depth and rear teat placement have a relative large increase of respectively 4.0, 2.5 and 4.0%. As shown in table 3, the genetic correlations with the corresponding traits based on AMS data are high, so a relatively large increase in reliability compared to the other traits was also expected.

Conclusions

Teat coordinates from AMS data can be used to derive udder conformation traits. In the Netherlands and Flanders, four udder conformation traits are derived based on AMS data: udder depth, distance between the front teats, distance between the rear teats and udder balance.

Udder balance is a new trait, because it is not scored during the herd classification. Udder balance has a heritability of respectively 0.45, 0.42 and 0.43 for parity 1, parity 2 and parity 3. The genetic correlations with the other udder conformation traits are all moderate positive what causes the upwards trend of 5 millimetres in udder balance over the past ten years.

Repeated records are used for the breeding value estimation, which lead to an increase in heritability and reliability. The heritabilities in parity 1 are 0.56, 0.60 and 0.45 for respectively udder depth, distance between the front teats and distance between the rear teats based on AMS data, while based on herd classification the heritabilities are 0.39, 0.31 and 0.29. Genetic correlations between the traits based on herd classification and AMS data are close to 1.0, ranging from 0.96 to 0.99. Genetic correlations between the different parities for the traits based on AMS data are also close to 1.0, ranging from 0.85 to 0.99.

The use of AMS data in the breeding value estimation for udder conformation lead to an increase of the reliability of the published udder conformation traits. The increase in reliabilities ranges from 0.4 to 4.0%.

Using AMS data leads to better estimates of the breeding values for udder conformation traits by using more information.

Acknowledgments

AMS data is shared by dairy farmers via JoinData, a Dutch non-profit organization. JoinData is a data platform where farmers are in charge of their own data. Organizations who are willing to use data can only use data from farmers who agree with the use of their data.

The existence of JoinData made is possible for CRV u.a. to make use of AMS data from Dutch dairy herds.

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Quality and Value of Imputing Gene Tests for All Animals

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Abstract

Genomic selection is driven by genotyping arrays designed for uniform coverage of the genome because most quantitative trait loci (QTLs) underlying the heritability of the trait are unknown. Laboratories have improved the arrays since 2014 with custom content by adding selected QTLs discovered from whole-genome sequencing (WGS) and high-effect markers from higher-density arrays. Breed differences, missing data rates, and error rates were investigated for eight QTL gene tests currently imputed for all genotyped animals of 5 breeds plus crossbreds. Gene content for each gene test was predicted for non-genotyped relatives using mixed model methods like those used in single-step genomic evaluations, allowing potential direct selection across all animals. For the 8 QTL studied, Mendel error rates were low except for polled in Jerseys and *DGAT1* in most breeds. Allele effects for *DGAT1* were smaller than two nearby flanking single nucleotide polymorphism (SNPs) because *DGAT1* was genotype quality was poor on several arrays. For yield traits, 79K predictions including selected markers and QTLs had 1-2% higher reliability than 45K or 35K predictions excluding those SNPs.

Key words: Gene tests, imputation, marker selection, dairy cattle

Introduction

Genotyping laboratories began adding QTL gene tests in 2014 following the US Supreme Court decision that natural genetic variants should not be patented. Accuracy of imputing QTL genotypes for other animals can be affected by which arrays include the QTLs. Each year, new QTLs may be discovered and included. The SNP list used in US evaluations was updated frequently to include selected markers and QTLs from more breeds and higher density chips or from sequence (Al-Khudhair et al., 2021; Olson et al., 2012; VanRaden et al., 2009, 2017; Wiggans et al., 2016), with gains in reliability across traits expected to total about 3% (Table 1).

Some QTLs have effects larger than markers on traits we select or should select for. Goals of the project were to examine the most important QTLs currently used, summarize quality and breed differences of raw and imputed genotypes, estimate gene content for non-genotyped animals, and estimate gains in reliability of prediction from including or excluding the selected markers and gene tests.

Materials and Methods

Genotypes were examined from December 2022 official evaluations of the Council on Dairy Cattle Breeding (CDCB) for 5,669,157 Holstein, 663,366 Jersey, 65,172 Brown Swiss, 15,110 Ayrshire, and 7,620 Guernsey to summarize allele frequencies by breed (Table 2), Mendelian conflicts (Table 3) for eight important QTLs, and missing rates before and after imputation with *DGAT1* as an example (Table 4). Gene content was estimated for all non-genotyped relatives by predicting their genotypes from relatives using Gengler (2007) method. To potentially include such QTLs in a selection index, non-genotyped candidates for selection also need estimates of their unknown QTLs.

For the QTLs studied (Table 5), some have economic merit not yet included in national selection indexes such as 1) polled mutations near 1:2578598 (chromosome: position on ARS-UCD1 map) that suppress horn growth, improve animal welfare, and reduce farm labor, 2) β-casein allele (a2) at 6:84451299 in a milk protein gene that may improve

digestibility, and 3) two κ-casein alleles near 6:84451299 that affect cheese yield. The three casein QTLs are in a 200kb gene duplication region. Other QTLs mainly affect traits already in selection such as 4) *diacylglycerol O-acyltransferase 1 (DGAT1)* at 14:611019 affecting fatty acid metabolism, percentages, and yields of fat and protein, 5) *Bovine growth hormone receptor (BGHR)* at 20:31888449 affecting protein percentage, 6) *β-lactoglobulin (BLG)* at 11:103259232 with large effects on yield especially in Brown Swiss, and 7) *ATP binding cassette subfamily G member 2 (ABCG2)* at 6:36599640 with the largest effect for milk, fat %, protein %, and net merit in Holsteins, but the favorable allele is now nearly fixed at 2.5%, while fixed in other breeds (Table 2). Many other QTLs have recessive lethal effects and carrier status is reported, but those were not part of this study.

Genomic predictions using three SNP densities from 2019 yield trait data for 6,899 young Holstein bulls now proven allowed estimating the value of including selected markers and QTLs. The current 79K official list was compared to the 35K subset of only markers from the original 50K array and two 45K chips constructed by augmenting the 35K chip with independent sets of ¼ of the high density (HD) SNPs, respectively.

Results & Discussion

A true QTL is expected to have a better genetic signal (effect size or genetic SD) compared to nearby markers on the chip and that was true for most QTLs. For Holsteins, the *ABCG2* gene test had the best signal and the top ranked locus for milk, fat %, protein % and net merit. The *BGHR* gene test had the best signal and the second ranked locus for protein %. But the *DGAT1* gene test had a smaller effect than two nearby markers, and so attention was focused on *DGAT1*.

A locus from the 50K chip (ARS-BFGL-NGS-4939) on chromosome 14 at 609,870 bp had the largest genetic standard deviation (SD) genome-wide for the five Holstein yield traits: milk, fat, protein, fat % and protein %. That locus is 1,149 bp away from *DGAT1*, and another locus from the high-density chip (BovineHD1400000216) also had larger effects than *DGAT1*. Poor imputation quality was ruled out by comparing SNP regressions using only cows with direct calls for *DGAT1* and the 50K SNP. Genotypes from nine of the 52 chips and 1,377,604 Holsteins had both loci, 46,051 (6%) had discordant calls (gene test vs. marker), of which 6,830 had phenotypes. Six GeneSeek chips accounted for most of the data and had varying discordant rates (Table 6). The GeneSeek Genomic Profiler (GGP) 9K had the most genotyped animals (452,687), highest discordant rate (8.27%), and 92% (6281) of the phenotyped animals. GGP 9K regression effect sizes were greater and p-values smaller for the 50K SNP (Table 7). Genotype quality of GGP 9K was then assessed using SNP heritability (Gengler 2007) for 25,000 animals with discordant calls on that chip. The 50K SNP had heritability 0.98 and *DGAT1* only 0.16, indicating poor genotype quality as the likely source. Discordant calls for *DGAT1* on other chips also had low heritability although sample size was much smaller.

Because some valuable gene tests are sold by laboratories rather than delivered with array genotypes, freely imputed QTLs could benefit breeders and progress. Decreasing costs of whole genome sequence data will increase power of QTL discovery, and more QTL genotypes should increase imputation accuracy, prediction accuracy, and economic gain. Regressions averaged 1.07 and were nearly equal across the 3 densities. Reliabilities of yield traits for 79K averaged 1.2% higher than 45K and 2.0% higher than 35K, worth potentially > $10 million every year nationally. Eventually, more QTLs should be included to further improve predictions.

Conclusions

Gene tests were already imputed for all genotyped animals of all five breeds. Mendelian error rates were low for QTLs except for Polled in Jerseys and *DGAT1* in most breeds. Imputed *DGAT1* tests were statistically less significant for all yield traits compared to two nearby chip SNPs (one HD and one 50K), direct *DGAT1* gene tests also had smaller effects than the best markers, and SNP heritability indicated that *DGAT1* genotyping quality was the cause of later imputation errors, though the GGP 7K and linkage disequilibrium (LD) V4 had low discordance rates. Further investigation of problematic chips is warranted. Gene content was imputed for all non-genotyped animals by extracting QTLs from the imputed genotypes and using those as data to predict related animals. Accumulated gains in reliability for yield from adding selected markers and QTLs were 1-2%, a little less than previous studies indicated. Most gains were from larger reference populations.

Acknowledgments

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Table 1. History of US SNP list revisions and reported gains in reliability of Holstein predictions

Year	Reference	Breeds	Added information	Markers (1000s)		HOL Reliability	
				Added	Total	Gain (%)	Total
<2008		All	Parent average		0		27
2009	VanRaden	HO	Chip genotypes (50K)	38	38	23	50
2012	Olson	3	More breeds (JE, BS)	5	43	0	50
2013	Wiggans	HO	Add HD markers (GHD)	18	61	0.5	67
2016	Wiggans	HO	Add HD markers (GH2)	16	77	1.5	68
2019	VanRaden	HO	Add sequence SNPs	2	79	1.2	69
2020	Al-Khudhair	5	Add HD, other breeds	+5, -5	79	0	69

Table 2. Final allele frequencies for the eight QTLs including gene content for all animals of each breed

Breed	Polled	ABCG2	β-casein	κ-casein1	κ-casein2	β-Lact	*DGAT1*	BGHR
RDC	0.6	99.9	52.0	84.8	65.3	33.2	8.6	22.1
BSW	3.5	100.0	22.2	30.1	100.0	33.0	6.8	11.4
GUE	1.1	99.7	7.2	65.1	99.7	16.0	60.6	17.9
JER	2.2	99.9	27.6	9.2	99.4	54.2	52.1	26.1
HOL	1.0	97.4	39.1	72.5	89.8	51.6	30.1	19.7

Table 3. Mendelian error rates by breed for imputed genotypes of eight QTLs

Breed	Polled	ABCG2	β-casein	κ-casein1	κ-casein2	β-Lact	*DGAT1*	BGHR
RDC	0.01	0	0.17	0.00	0.01	0.05	0.80	0.11
BSW	0.18	0	0.10	0.12	0.00	0.12	0.51	0.03
GUE	0.00	0	0.00	0.04	0.00	0.14	0.00	0.07
JER	0.50	0	0.17	0.13	0.00	0.03	0.09	0.08
HOL	0.05	0	0.08	0.01	<0.01	0.02	0.67	0.10

Table 4. *DGAT1* imputed allele and genotype frequencies and genotypes missing in input

Breed	Tests (N)	Frequency (%)							
		Allele	Imputed genotype codes						Genotypes
		A	AA	AB	AB	A?	B?	Missing	Missing
RDC	15,110	8.6	88.11	8.84	0.07	2.85	0.06	0.07	71.6
BSW	65,172	6.8	78.14	9.27	0.45	10.38	0.86	0.90	91.0
GUE	7,620	60.6	14.00	43.24	33.23	3.49	5.66	0.38	89.0
JER	663,366	52.1	21.34	49.43	27.63	0.74	0.85	0.01	74.2
HOL	5,669,157	30.1	46.10	42.70	9.60	1.12	0.48	0.00	85.7

Table 5. Locations and effects of eight QTLs examined

Gene test	Chr:Location	Gene function	Effects in cows or in humans
Polled	1:2578598	Grow horns	Animal welfare, farm labor
ABCG2	6:36599640	Membrane transport	Yield and NM$ (biggest effect)
β-casein (a2)	6:84451299	Milk protein	More digestible? (JE protein%)
K-casein (1)	6:85656772	Milk protein	Increased cheese yield
K-casein (2)	6:85656792	Milk protein	Increased cheese yield
β-Lactoglobulin	11:103259232	Milk fat	Human allergies (BS yield & %)
DGAT1	14:611019	Fat and protein %	Fatty acid metabolism, obesity
BGHR	20:31888449	Growth hormone	Protein% (2nd biggest effect)

Table 6. Descriptive statistics for six GeneSeek chips tested for *DGAT1* calling

Chip info		Animal info		
Name	Markers	Genotyped	Discordant (N)	Discordant (%)
GGP 7K	7083	34480	239	0.69
GGP 9K	8984	452687	37417	8.27
GGP LD V4	30113	112135	327	0.29
GGP 65K	65320	95327	5578	5.85
GGP 100K	94121	30606	1676	5.48
GGP 150K	139914	36406	813	2.23

Table 7. Regression results for GGP 9K chip for *DGAT1* vs. nearby 50K SNP using 6,281 genotyped animals

	Marker P-value		Abs (marker effect)	
	50K	*DGAT1*	50K	*DGAT1*
Milk	8.9E-45	2.6E-02	70.932	11.887
Fat	1.4E-19	3.1E-02	2.062	0.546
Protein	4.9E-13	9.8E-01	0.967	0.004
Fat %	2.2E-94	3.8E-04	0.016	0.003
Protein %	4.1E-42	1.2E-04	0.003	0.001

Changes in the genome architecture of two groups of dairy bulls with marked differences in their direct genomic breeding values for production traits in the UK

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Abstract

Genomic selection has resulted in a rapid rate of genetic progress in dairy cattle in the last decade which could partly be attributed to a marked reduction in generation interval. For instance, in the UK the average age of a bull when their 100th daughter was born has decreased from over 6 years prior to the introduction of genomics to under 4 years in 2022. It is however not clearly understood if this rapid rate of genetic progress has also been accompanied by changes in the genome architecture in terms of degrees of heterozygosity, allele frequencies and linkage disequilibrium (LD) structure. This study examines changes in these parameters in 9,202 bulls born between 2009 and 2014 in the reference population for production traits and 94,204 young bulls with no daughters born between 2019 and 2023. The mean difference in direct genomic breeding values (DGV) between these reference and young bulls (YG) were 357.7, 24.5 and 16.1 kg for milk, fat and protein yields respectively. The SNP panel used in the UK evaluations consists of 79,051 SNPs and the proportion of SNPs with 0 to 1, 2 to 5, 6 to 10, 11 to 15 and greater than 15 percent change in their allele frequencies between both the reference and young bull groups were 15, 32, 29, 15 and 9% respectively. The SNPs with at least 15% change in allele frequencies accounted for 34, 43 and 39 percent of the mean difference in DGV between reference and young bulls for kg milk, fat and protein respectively, while the corresponding values for SNPs with 0 to 1 % change in allele frequencies were less than one percent for all three traits. In absolute terms the correlation between differences in mean DGV between reference and young bulls and changes in allele frequencies at the chromosome level was about 0.65 for the three traits. Thus, the rapid rate of genetic progress due to genomic selection is significantly changing allele frequencies. The rate of LD decay was similar for both groups of bulls, but tended to be higher for YG, suggesting stronger selective pressures and/or lower effective population size. The increased rate of inbreeding in shorter ROH but slower increase in longer ROH in YG seem to imply that recent inbreeding is being better controlled than very ancient inbreeding.

Keywords: genomic selection, allele frequency changes, runs of homozygosity, linkage disequilibrium

Introduction

The application of genomic selection has resulted in rapid rates of genetic gain, especially in dairy cattle, due to reduced generation intervals, higher selection intensity and higher accuracies for young genomic bulls for production and fitness traits. For an example, the annual rates of genetic gain for production traits in the USA has doubled with a Net Merit$ of $40.33 per year from 2005 to 2010 to $79.20 per year from 2016 to 2020. Consequently, in 2021 young genomic sires with no milking progeny accounted for 71% of A.I. breeding in U.S. dairy herds (CDCB 2023). The equivalent UK statistics are even higher in magnitude, where the Herdbook registered Holstein females saw an average yearly gain in £PLI (Profitable Lifetime Index) of £17.9 between 2005 to 2010, which tripled to £58.2 between 2016 and 2020. Similar to the US, over 72% of matings in 2022 were by genomic young sires. The genetic gains are bolstered by the high usage of sexed dairy semen, with 82% of all Holstein semen sold during 2022 sexed. It is however not clearly understood if this rapid rate of genetic progress has also been accompanied by changes in the genome

architecture in terms of allele frequencies, linkage disequilibrium (LD) structure and inbreeding rates. This study examines changes in allele frequencies, LD structure and inbreeding in two groups of bulls with large differences in their mean direct genomic breeding values which were in the reference and selection populations.

Materials and methods

Two groups of bulls used in this study: 9,202 bulls born between 2009 and 2014 which were in the reference (REF) population of the UK genomic prediction model for April 2023 for production traits and 94,204 young (YG) bulls with no daughters born between 2019 and 2023, which were selection candidates. The means (and standard deviations) for the direct genomic breeding values (DGV) for 9,202 in the reference population were 192.5kg (81.3),7.9kg (3.80) and 7.0kg (3.56) for milk, fat and protein yield respectively. Corresponding means and SDs for the young bulls were 550.2kg (68.08), 32.4kg (5.78) and 23.1kg (3.23) respectively. This implies that the mean difference in DGV between these reference and young bulls were 357.7, 24.5 and 16.1 kg for milk, fat and protein yields respectively.

The SNP panel used in the UK evaluations consists of 79,051 SNPs and allele frequencies were computed within each of the two groups of bulls. Using the SNP effects from the UK genomic prediction in April 2023 and the file of genotypes, the relative contribution by each SNP to the DGV, with polygenic contributions ignored, were computed and used to examine the relationship between changes in allele frequencies and contribution of each SNP to the difference between the DGVs for REF and YG bulls at chromosome and SNP levels.

Linkage disequilibrium

Linkage disequilibrium was estimated using PLINK 1.9 (Chang et al. 2015). The pairwise R-squared values were calculated between SNPs along the same chromosome and were categorised into 1 kb bins based on physical distance.

Runs of homozygosity

Runs of homozygosity (ROH) were detected with PLINK 1.9 (Chang et al. 2015). Note that for the ROH analysis, 186 REF and 1,109 YG bulls with substantial Friesian breed contribution were removed from the dataset. For this analysis, a scanning window of 10 SNPs was used, with a maximum of one heterozygote call per window. ROH were further restricted, using the following parameters: a minimum SNP count of 10; at least one SNP per 100 kb; a maximum of one heterozygous SNP per ROH; and a minimum physical length of 1 Mb. The remaining parameters were left as default.

ROH were split into six classes using physical length: 1-2 Mb; >2≤4 Mb; >4≤8 Mb; >8≤16 Mb; >16<32 Mb; and >32 Mb. Longer ROH are expected to represent regions of autozygosity from more recent inbreeding, contrastingly, shorter runs are expected to represent more ancient inbreeding due to the cumulative chance that recombination events have occurred within the ROH. The expected generational source of different ROH length classes was estimated as in Doekes et al. (2019). Briefly, common ancestors that occurred G generations ago gives rise to ROH of various length that follows an exponential distribution with mean 1/2G Morgan. A uniform recombination rate was assumed at 1 Morgan per 100 Mb.

Inbreeding coefficients (F) were calculated from ROH (FROH) using the percentage of the autosome covered by ROH in each length class for a given individual. Density distributions for each length class were compared between the REF and YG bull groups. The goodness of fit between each pair of distributions were tested using the Kolmogorov-Smirnov test. The modes and 95% confidence intervals, calculated from the 0.025th and 0.975th quantiles, were also qualitatively compared.
The data were further split by year of birth and linear regressions were calculated within and

across the REF and YG bull groups to estimate the rate of increase in inbreeding for each ROH length class. For the trends calculated by year of birth, bulls belonging to the 2015 and 2023 cohorts were removed due to small sample size (Table 1); bulls from 2023 were retained for visualization only.

Table 1. Distribution of bulls by year of birth for reference (REF) and young (YG) bulls

Reference bulls		Yong bulls	
Year	Count	Year	Count
2009	1341	2019	23319
2010	1436	2020	20341
2011	1466	2021	25244
2012	1667	2022	24001
2013	1532	2023	190
2014	1562		
2015	2		

Results and Discussion

The distribution by chromosome of SNPs with more than 5% changes in their allele frequencies between REF and YG bulls is presented in Figure 1. Of the total 75,091 SNPS, the percentage of those with 0-1, 2-5, 6-10, 11-15 and > 15 percent changes in their allele frequencies between REF and YG were 15, 32, 29, 15 and 9% respectively. The top 5 chromosomes with the highest number of SNPs with changes in their allele frequencies were BTA1, BTA3, BTA9, BTA14 and BTA30. Figure 2 presents the distribution by chromosome of SNPs with more than 15% changes in their allele frequencies between REF and YG bulls. Again, the chromosomes with the highest number of SNPs showing these changes were similar to those in Figure 1 and these were BTA1, BTA9, BTA13, BTA14 and BTA30. No previous studies were found that examined changes in allele frequencies but with about 10% out of 75,091 SNPs with more than 15% changes in allele frequencies, it would appear, substantial changes in allele frequencies have been observed in this study.

The relative contribution of individual SNPs to the overall DGV, indicates that the SNPs with more than 15% changes in their allele frequencies accounted for 34, 43 and 39 percent of the mean difference in DGV between reference and young bulls for kg milk, fat and protein respectively. The corresponding values for SNPs with 0 to 1 % change in allele frequencies were less than one percent for all three traits. The percentage contribution to the difference in DGVs between REF and YG bulls by chromosome is shown in Figure 3. The results show that chromosome BTA14 accounted for about 15% of the positive difference between REF and YG bulls for fat yield with a corresponding similar negative percentage difference between both groups of bulls for milk. This could be attributed to the presence DGAT1 on this chromosome and similar results have been reported by Sun et al, (2009). The chromosome BTA1 contributed the largest positive difference between both REF and YG bulls for milk and protein yields. In absolute terms the correlation between differences in mean DGV between reference and young bulls and changes in allele frequencies at the chromosome level was about 0.65 for the three traits. Thus, the rapid rate of genetic progress due to genomic selection has significantly changed allele frequencies.

Table 2. Summary statistics of FROH distributions across ROH length classes.

	Reference bulls					Young bulls				
Bin	Mean	SD	Mode	Q2.5	Q97.5	Mean	SD	Mode	Q2.5	Q97.5
1-2 Mb	2.31%	0.38%	2.27%	1.62%	3.09%	2.73%	0.52%	02.66%	1.91%	3.79%
2-4 Mb	2.32%	0.57%	2.23%	1.31%	3.51%	3.31%	0.79%	3.09%	1.98%	5.01%
4-8 Mb	3.18%	0.96%	2.97%	1.50%	5.28%	5.10%	1.26%	4.85%	2.74%	7.70%
8-16 Mb	3.42%	1.39%	2.87%	0.98%	6.42%	5.55%	1.80%	5.42%	2.18%	9.24%
16-37 Mb	2.29%	1.60%	1.72%	0.00%	6.04%	3.54%	2.00%	2.57%	0.00%	8.01%
>32 Mb	0.82%	1.32%	0.00%	0.00%	4.36%	1.18%	1.66%	0.00%	0.00%	5.39%

Table 3. Linear regression slope coefficients of inbreeding (FROH) change in bulls by year of birth, calculated for each ROH length class.

	Birth years	1-2 Mb	2-4 Mb	4-8 Mb	4-8 Mb	16-37 Mb	>32 Mb
All bulls	2009-2014, 2019-2022	0.044%	0.109%	0.211%	0.228%	0.135%	0.038%
REF bulls	2009-2014	0.025%	0.082%	0.159%	0.204%	0.181%	0.096%
YG bulls	2019-2022	0.032%	0.107%	0.204%	0.178%	0.101%	0.011%

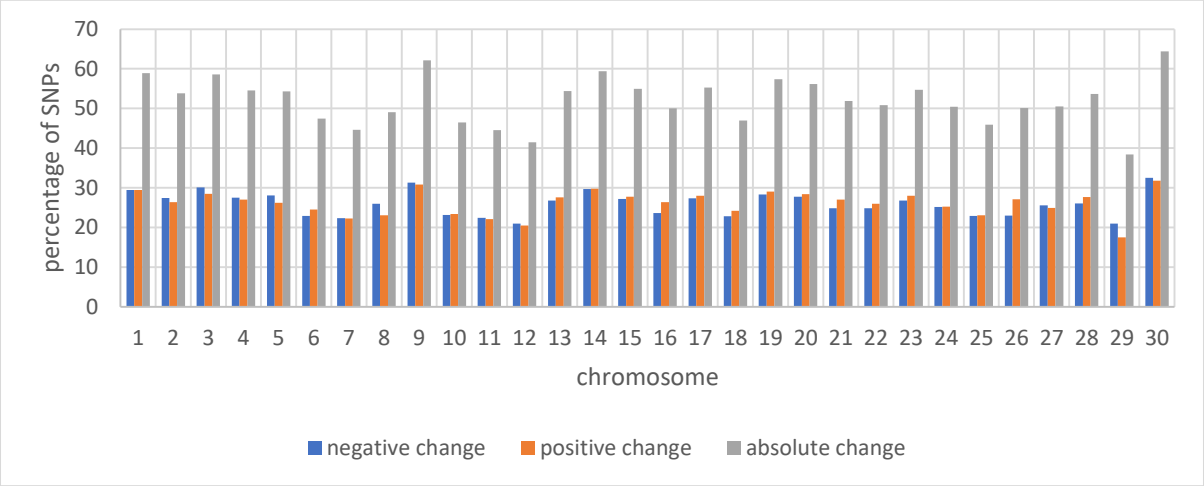

Figure 1: The distribution of SNPs with at least 5% changes in allele frequencies in young bulls relative to the reference bulls by chromosome

Linkage disequilibrium

The decay of LD follows similar curves for both the REF and YG bull groups, with a maximum R^2 at the shortest physical distance which subsequently decays by 48% to a relatively static minimum within approximately 13 kb (Figure 4). While the rate of decay was similar between groups, the magnitude differed. At the shortest physical distance between 0-1 kb, LD was greater in YG bull (R^2 = 0.87) than REF bulls (R^2 = 0.79), lowering to R^2 of 0.45 and 0.41, respectively at 13 kb. LD decay beyond this distance was at a slower rate, however, binned median R^2 values were consistently greater in YG bulls compared to REF bulls up to the maximum distance of 200 kb analysed.

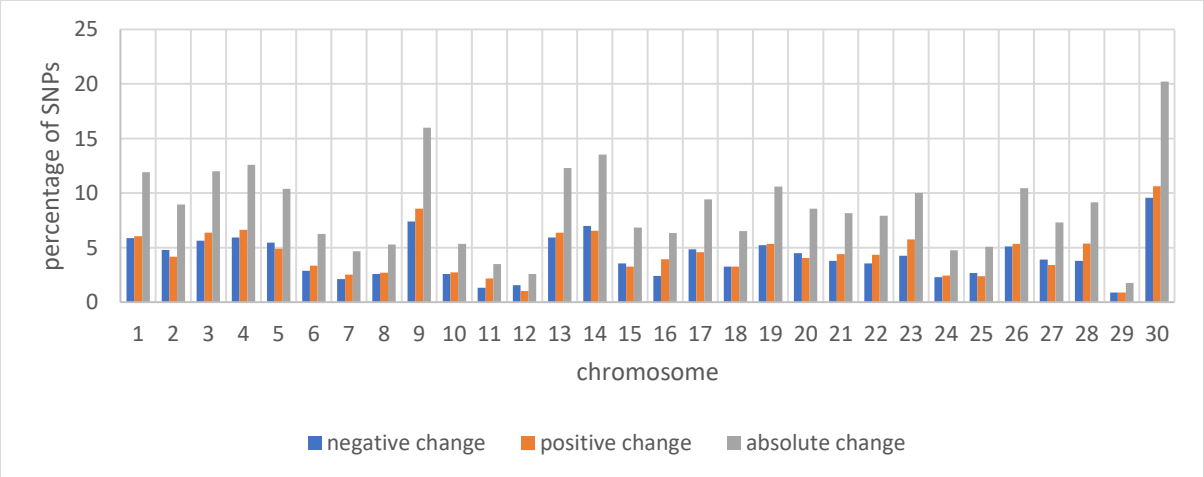

Figure 2: The distribution of SNPs with more than 125% changes in allele frequencies in young bulls relative to the reference bulls by chromosome

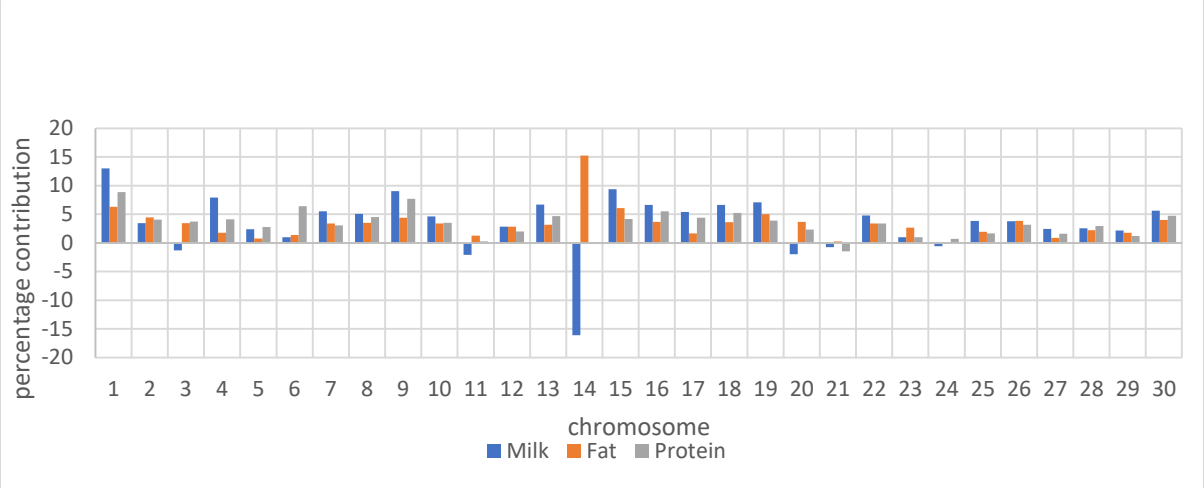

Figure 3. The percentage contribution to the difference in DGVs between reference and young bulls by chromosome

Runs of Homozygosity and Inbreeding

For each ROH size class, the Kolmogorov–Smirnov test was used to test for significant differences in the density distributions between REF and YG bull groups; each comparison was significantly different (P <0.001). For all ROH size classes except >32 Mb, YG had greater inbreeding with a mode at least 17% greater than the reference bulls. The largest proportional difference between modes was for the 5-8 Mb and 9-16 Mb classes, with a 63% and 89% increase, respectively (Table 2). While YG inbreeding has increased across all size classes, the additional increase seen between 4-16 Mb indicates a substantial increase of matings with shared ancestry in the past 10 generations (Figure 5), with 31% of ROH between 9-16 Mb likely originating from the past 2-5 generations. These increases were repeated in the upper and lower bounds of the 95% confidence intervals, with a minimum increase of 17% and the greatest increase observed for 5-8Mb and 9-16Mb (lower confidence intervals for both groups for 17-32Mb and >32Mb remained at zero, therefore no increase was observed). This further validates the overall shift of increased inbreeding between the two groups. Due to

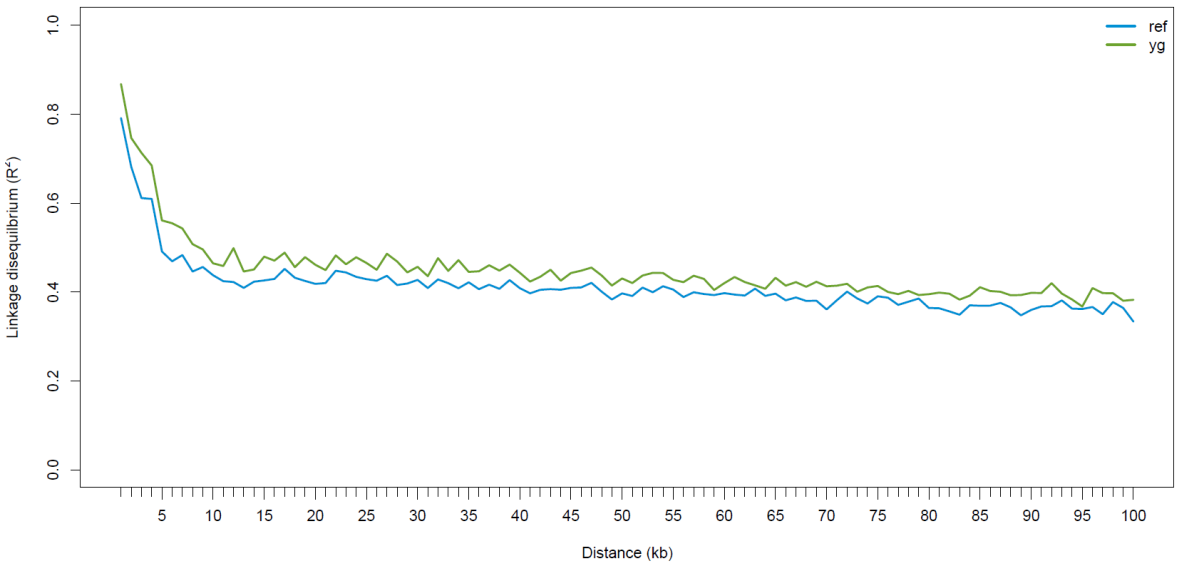

Figure 4. Linkage disequilibrium decay over physical distance in REF and YG bulls

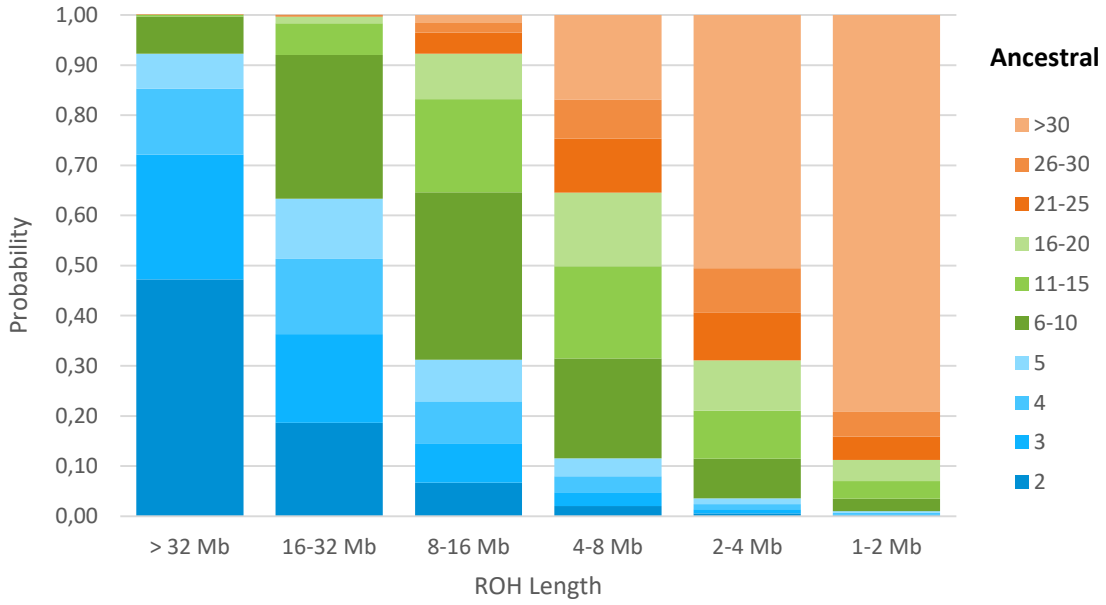

Figure 5. Expected age of ROH by length classes, based on exponential distribution with mean 1/2G Morgan and a uniform recombination rate of 1 Morgan per 100 Mb. Adapted from Doekes et al. (2019).

most individuals having zero ROH exceeding 32 Mb, the density distributions for >32 Mb formed a multimodal curve with a major mode of 0, therefore, differences between the populations were difficult to compare for this size class. The average inbreeding for >32 Mb was 50% greater in YG compared to REF populations which may indicate further inbreeding in the past 2-5 generations for YG, however, mean calculations for this size class are particularly susceptible to skewing from rare outliers due to no upper limit on maximum ROH length.

ROH class bins were further divided by year of birth to characterise trends over time – due to the small sample sizes, 2015 (n = 2) and 2023 (n = 190) cohorts were removed from trend calculations. A linear regression was calculated between year of birth and median inbreeding values, the slope coefficient therefore representing the change in genomic inbreeding per year. Inbreeding across all ROH classes is increasing over time, except for >32 Mb, where all years have a median of zero. Linear regressions were also calculated for data within the both reference and YG groups; for the shorter ROH length classes (1-2, 3-4, and 5-8 Mb) the rate of inbreeding is accelerating, with YG experiencing ~27-30% increase in the rate of increased inbreeding per year, compared to the reference group. Contrastingly, for longer ROH length classes (9-16, 17-32, and >32 Mb), more likely to arise from recent shared common ancestry (Figure 5), the yearly rate of increase is less for YG compared to that observed in the reference group (Table 3). This is potentially capturing the paradigm shift between the breeding schemes of the two groups; with the selection of more recent bulls, there is greater selection pressure on improving GEBVs while less consideration is given for ancient inbreeding allowing the relative rate of inbreeding of shorter ROH to increase.

The linear regression slope coefficient, for 17-32 Mb and >32 Mb, calculated across all bulls (all birth years) falls within the magnitude of the coefficients calculated for the REF and YG groups. This suggests that during the period that is unrepresented in our data (2015-2018, inclusive) the rate of inbreeding increase is approximately in line with the average that is observed across the two groups, and that a gradual change in reducing more recent inbreeding has occurred over time. In contrast, ROH length classes representing relatively more ancient inbreeding (1-2, 3-4, and 5-8 Mb)

the slope coefficient exceeded that of either of the two groups (Table 3). It seems that breeding decisions between 2015 and 2018, resulted in a high rate of increase between these years.

Conclusion

The marked increase in DGV of young bulls due to genomic selection has been accompanied with substantial changes in allele frequency. The SNPs with at least 15% changes in allele frequencies accounted for 34 to 43 percent of the mean difference in DGV between reference and young bulls for production traits. The percentage contribution at the chromosome level reveals the high impact of DGAT in chromosome BTA14 for fat and Milk yield. The rate of LD decay was similar for both groups of bulls, but LD tended to be higher for YG, suggesting stronger selective pressures and/or lower effective population size. Increased rate of inbreeding in shorter ROH but slower increase in longer ROH in YG seem to imply that recent inbreeding is being better controlled than very ancient inbreeding. It should be noted however that this study only considered DGV for production, but that the overall breeding goal (PLI) includes many more traits (34% of current PLI is production), which will contribute to the changes observed.

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Modeling identical twins and clones in genetic evaluations

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Abstract

Identical animals cause more complex relationships to model in genetic evaluations. The USA evaluation currently includes 4,762 pairs of natural identical twins, 1,776 split embryos, and 530 nuclear transfer clones from cells of other embryos, calves, or adults, plus seven million other genotyped animals. Genetic effects for the 7,068 animals reported to be a clone or copy of another animal were linked to the source animal, and their own effects were removed from the relationship matrix. The model retained separate permanent environmental effects for each cow. For progeny of clones, the source animals are substituted as their sires and dams. After completing the evaluation, the reverse process restored the actual sires and dams and duplicated the evaluations of source animals to their clones for publication. Pedigree inbreeding coefficients increased slightly for animals with a paternal ancestor and a maternal ancestor that were clones of each other. Genomic predictions improved by estimating just one polygenic effect instead of modelling the copies as full sibs. Milk production of adult clones was not significantly affected, but their fertility and health traits were below expected. Several AI companies now market cloned bulls. The revised model better evaluates identical twins, cloned animals, and their progeny.

Key words: Clones, nuclear transfer, relationship matrix, genomic selection

Introduction

Many elite animals have been cloned in recent years. Examples include a cow named Apple that sold for $1 million in 2008, was a Grand Champion at the World Dairy Expo in 2011 but was Reserve Grand at that show in 2013 when one of her nine clones (Apple-3) beat her to become Grand Champion (Malcolm, 2019). A heifer named Liz was Junior All-American Winter Yearling in 2001 and her clone Liz-2 was Junior Champion at World Dairy Expo in 2004 (Nauman, 2011; Figure 1). More recently, very young calves with the highest genomic predictions are being cloned.

In past generations, artificial insemination (AI) bulls were mature when selected and were mated to thousands of cows per year. With genomic selection, elite bulls are discovered at very young ages, well before they reach puberty, and new animals quickly replace even the most elite animals. In recent generations, a top young bull plus several clones born nine months later may have less direct impact than many famous bulls had in the past. In Canadian evaluations, data for identical bulls was merged since 2011 to solve for just one genetic effect as recommended by Kennedy and Schaeffer (1989) but those procedures were not implemented for identical cows.

In U.S. evaluations, identical bulls were given identical predicted transmitting ability (PTAs) and daughter counts since 2008 (VanRaden and Fok, 2008) following research by Norman et al. (2004) to verify their identical inheritance. Those methods were also not extended to cows or to monthly or weekly genomic evaluations. Clones that are genotyped get combined but not identical PTAs in current genomic evaluations. Their genotypes can be from different chips that are merged before the evaluation to ensure that all use the same genomic information, but the

marker effects in the model only account for 90% of the genetic variance. The other 10% is modelled by polygenic effects using pedigree relationships as if the clones are full sibs for that 10% portion. Those slightly different PTAs are used in parent averages for their progeny before the final published PTAs of bulls are forced to be identical.

Figure 1. Example cow Liz and her clone Liz-2.

Many identical twins have been discovered or confirmed by genomic testing, and elite bulls and cows might have 1 or several clones available for use in breeding. For daughter-proven Holstein bulls actively marketed in April 2023, the top five for lifetime net merit included two clones, and the top 20 included another clone of a different bull. The top 50 marketed young bulls also included a clone. Thus, updated methods were examined to properly model these more complex relationships.

Identical animals have been reported using pedigree format one (CDCB, 2023a) for decades. Bytes 54-70 can report either a second ID for the same animal or the ID of an identical animal. The pedigree record type in byte 88 indicates whether the second ID is a cross-reference (X) or clonal record (C). The multiple birth code (CDCB, 2023b) in byte 91 can report how the identical animal was created (embryo splitting or nuclear transfer) and can report embryo transfer, twin births, or pedigrees for genotyped embryos not born yet (Table 1).

Table 1. Multiple birth codes used for reporting twin or clone status at birth or as embryo.

Code	Description
1	Single
2	Multiple birth (not from embryo transfer)
3	Birth from embryo transfer
4	Split embryo (artificially)
5	Clone from nuclear transfer
6	Embryo pedigree (implantation date stored as birth date)

Cloning had a limited impact on livestock breeding until recently, because some reproductive technologies can result in large offspring syndrome (Center for Veterinary Medicine, 2008), and cloning remains expensive. Vegetative cloning is much simpler and is used in some plant breeding programs, where genomic prediction methods were tested for simulated cloned trees but without inbreeding in the model (Stejskal et al., 2022). Goals of the present study were to examine clone reporting methods, develop more precise modelling for clones, and apply revised programs to the national genetic evaluation of dairy cattle.

Materials and Methods

The National Cooperator Database used for April 2023 official evaluations of CDCB included 7,068 animals reported to be a clone or copy of another animal. Those include 4,762 natural identical twins, 1,776 split embryos, and 530 nuclear transfers of cells from other embryos, calves, or adults. Number of source animals was 6,625 including 5,871 females and 754 males. Copies per source animal ranged from one to 11 but averaged only 1.07. For identical twins, usually the first one in the database is considered the source animal and the other is counted as a copy. Animal names were reported for 4,416 copies and for 4,442 source animals. For nuclear transfer clones, the clone names often indicate their status by repeating the source animal's name plus a clone count suffix.

The pedigree file included 94,499,373 animals of many dairy breeds and crossbreds.

Among all animals, 88,793 were sired by copies and those sire IDs were replaced by the source animal's ID. Similarly, 7,956 reported dams were copies and were replaced by the source ID. The reduced pedigree file had 94,492,305 (94,499,373 minus 7,068) animals after also removing the IDs of copies. Producers can report identical twins without genotyping, but nearly all are discovered by genotyping and then confirmed by the producer. Nearly all nuclear transfer clones were genotyped.

Edits for cloning attempted to separate real identical animals from other cases of duplicate IDs that should instead be cross-references for the same animal. The latter cases were often caused by multiple forms of ID, typos, or reidentification of calves after export to another country. Some mistakes were easy to identify, such as those from large batches of nearly consecutive IDs with multiple birth code reported as 2 (twins) instead of 3, 4, or 5 (embryo transfer), but were not marked as twins in the name. About 80-90% of the 7,068 animals initially reported as identical twins or clones appeared to be valid.

Examples of two genotypes for the same animal but with different IDs included: many animals reported with both a USA and 840, 982, or metal ear tag number that should probably be cross-references instead of clones; calves with nearly sequential ID numbers that had two different country codes (such as CHN and USA) sent by two different companies; animals whose ID numbers were the same but with inconsistent ID format; and a few obvious typos. Some identified mistakes were changed from clones to cross-references, but not all as the owner must first agree to such changes.

Modeling for clones was improved by removing clone copies from pedigree files and by using different IDs for genetic vs. permanent environmental effects. The clone copies were removed from both the full pedigree file used in phenotypic modelling, and reduced pedigree files used in weekly or monthly predictions from subsets of genotyped animals. For progeny of clones, the source animals are substituted as their sires and dams. After completing the evaluation, the reverse process is then used to restore the actual sires and dams and duplicate the evaluations of source animals to their clones.

For females with records in each phenotypic trait group, the new code now links genetic effects of each clone to the source animal and links each permanent environment effect to the cow's own ID, recognizing that clones are different animals with different environmental effects. A previous program had merged genotypes from the source animal and its clones because they might be genotyped with different chips or have different missing loci within each genotype. The revised program now outputs only one row for the source animal instead of duplicating the merged genotype to its clones so that the model can solve for just 1 genetic effect.

All trait groups and breeds were tested to ensure a working system and measure impact. New code was developed to copy female PTAs and to do the final reporting of clone PTAs in weeklies or monthlies. The new system could be implemented for the December 2023 evaluation. To estimate if nuclear transfer clones perform as expected from their identical genotype, a regression was added to the clone model with coefficient of one for each embryo nuclear transfer (ETN) cloned cow and zero for all other cows.

Results & Discussion

Pedigree inbreeding coefficients increased slightly for some animals after removing the clone copies and listing the source animal instead as the sire or dam. The cases examined had a paternal ancestor and a maternal ancestor that were clones of each other, which increased descendant inbreeding, versus if the clones were treated as full sibs. Examples were 1) an increase from 7.0% to 7.7% for an animal whose paternal granddam and maternal 2nd-great granddam were identical, and 2) an increase from 9.8% to 10.6% for an animal

whose maternal great grandsire (MAN-O-MAN2) was a clone of his paternal 2nd-great grandsire (MAN-O-MAN).

Genetic evaluations from the pedigree model differed most for cloned animals and for bulls with daughter records if their clones also had daughter records. Reliabilities increased for those animals, as expected. Evaluations for all other animals had almost no change, and estimated genetic trends were nearly identical. Of the 6,625 source animals in the model, 3,241 had no change to their evaluation for milk and 4,725 had no change to their reliability, presumably because the copies had no phenotypes or descendants.

For source animals that did change, average difference in milk estimated breeding value (EBV) was -3.9 pounds, average absolute change in milk EBV (test - official) was 79.4 pounds, and average gain in percent reliability was +2.6. The maximum difference in EBV milk was -3052 pounds for a USA Jersey cow born in 1991 that had 11 clones. Maximum reliability difference was +50, increasing from 47% to 97% for a Holstein bull who had only 1 daughter but whose split embryo twin had 520 total progeny. For bulls, the public will not see those EBV and reliability changes because such evaluations were already superseded by data from the clone member with highest reliability.

Evaluations from the genomic model had much smaller differences because only the polygenic effects had used the full sib instead of clonal relationships, and because bull PTAs had been forced to be identical.

Ancestor discovery (Nani et al., 2020) previously did not detect and add a cloned bull or the original bull because the 1st choice was no better than the 2nd choice. The new model with revised pedigree discovered about 20,000 ancestors that were members of a clone group. The ID of the source maternal grand sire (MGS) or maternal grand grand sire (MGGS) can be automatically added to the pedigree if missing, but to make pedigrees more precise, owners can replace the discovered source ancestor with the clone ancestor if it was used in that mating.

Genomic relationships of 1.0 and singular genomic relationship matrices can cause problems in genomic BLUP algorithms. Those issues can be avoided by solving for marker effects directly (SNP-BLUP), but in both strategies the polygenic effects would remain incorrect for identical animals. Updated models and pedigree inputs to multi-step software will provide further benefits for use in single-step models.

Direct effects of nuclear transfer cloning on phenotypic performance were very small for yield traits but effects were larger and unfavorable for several other traits (Table 2). The estimated phenotypic losses were mostly in the range of one to two genetic SD which are well within normal biologic ranges but too large to justify creating whole herds of cloned cows. Compared to trait means, the unfavorable effects ranged from 27% increase in somatic cell count to 2% increase in age at first calving. However, the 0.34 effect on SCS was only 1.2 genetic standard deviation (SD) whereas the 17 days later calving date was 8.1 genetic SD. Most cloned heifers are used as embryo donors and their phenotypes should probably be edited from the age at first calving dataset.

Many countries are adopting the Cartagena Protocol on Biosafety as recommended by the United Nations. Clones and gene-edited animals are not considered “genetically modified”. Guidelines limiting cloning were proposed to the EU parliament but were not adopted. Private companies sometimes enforce cloning rules that do not exist. For example, importers may demand “clone-free” pedigrees before export. Breed associations such as Holstein USA then must provide such reports and inspect all previous generations to discover any clone. Today about 0.3% of US Holsteins have a clone in their pedigree, but >3% may in 5 generations and >50% in 10 generations (about 20 years). International exchange of breeding stock should not become limited by artificial barriers.

Conclusions

Clone modelling was improved in the national evaluation. About 67% of the 7,068 copies in the clone file were natural identical twins, 25% were split embryos, and 7% were nuclear transfer clones. The model changes were not complex but required slight revisions to many programs, which led to small positive effects for many downstream analyses. Benefits of the new model were more exact pedigree inbreeding coefficients for descendants of clones, more precise genetic evaluations for clones, identical genomic evaluations for female clones, identical evaluations for bulls in additional trait groups such as type and calving, combined progeny counts for cloned bulls instead of reporting only the daughter count of the clone with the most, and improved ancestor discovery. The new or revised programs better account for cloned animals and identical twins. Milk production of cows obtained by nuclear transfer cloning was as expected, but the clones had poorer performance than the source animals for some other traits. Many AI companies now market cloned bulls, and many dairy cattle may soon have clones in their pedigrees.

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Table 2 – Performance of nuclear transfer clones for 10 traits.

Trait	Units	Mean	Genetic SD	Clones	Effect	Effect/SD	Effect/Mean
Milk	Pounds	28,071	1134	472	+18	0.0	+0%
Fat	Pounds	1,077	50	467	-8	-0.2	-1%
Protein	Pounds	871	30	467	+7	0.2	+1%
SCS (or SCC)[1]		200k	0.28	460	+0.34	1.2	+27%
Productive life	months	25	3.4	119	-3.3	-1.0	-13%
Dtr. pregnancy rate	%	27	2.8	354	-5.0	-1.8	-19%
Heifer conception rate	%	45	2.6	37	-5.5	-2.1	-12%
Cow conception rate	%	41	3.2	123	-8.3	-2.6	-20%
Age at first calving	months	831	2.1	115	+17.0	8.1	+2%
Cow livability	%	97	3.2	423	-7.3	-2.3	-8%

[1] SCS (somatic cell score) evaluations are on log base 2 scale but were converted and compared to the SCC mean in cells / ml.

Heritability of Methane Emission in Young Norwegian Red Bulls Estimated from GreenFeed Measures at the Test Station

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Abstract

The aim of this study was to estimate heritability of methane (CH_4) emissions for young Norwegian Red bulls. Measures of CH_4 from a GreenFeed (GF) unit at Geno's test station for young bull was available. Bull calves arrive the test station 3-4 months old and are grouped in pens with on average 10 calves. Methane is measured the last 1-2 months before they leave the test station, at 11-12 months age. On average each bull had 40 days with CH_4 data. We used data recorded from September 2020 to April 2023 and the final dataset had records from a total of 76 094 GF visits from 212 bulls. The mean (standard deviation) was 218 (50) gram CH_4 per bull per day. The traits analyzed were gram CH_4 per day, measured per GF visit or computed as the average of the bull's individual visits each day. A linear animal repeatability model with fixed effects of age and group test-day, and random effects of animal and permanent environment were used to estimate variance components. The estimated heritability (standard error) was 0.24 (0.10) for CH_4 per visit and 0.56 (0.20) for CH_4 mean per day, with repeatability of 0.32 and 0.71, respectively. The predicted breeding values for bulls with phenotype varied from -37 to +60, with standard errors ranging from 12 to 15. Results so far are promising, the genetic variation for CH_4 in the Norwegian Red breed indicates that breeding for lower methane emission is feasible.

Key words: breeding values, methane, genetic evaluation, young stock, bulls

Introduction

One way of reducing environmental footprint from dairy production is by breeding. Selection for lower methane (CH_4) emission is possible (Lassen and Difford, 2020). Most of the research on CH_4 emissions in dairy cattle has been on lactating cows, and so far few genetic studies of CH_4 emissions in young stock and bulls are published. Geno, the breeding organization for Norwegian Red, has installed a GreenFeed (GF) unit at their test station for young bulls. Measures of CH_4 from this test station is now available and the aim of this study was to estimate heritability of CH_4 emissions for young Norwegian Red bulls.

Materials and Methods

Measures of CH_4 from the GF unit at Geno's test station for young bull was available. Each year the best 8 000 bull calves in the Norwegian Red population are genotyped, among these around 150 are selected and brought to the test station, and 50-60 of them will be recruited to be AI bulls. Bull calves arrive the test station 3-4 months old and are grouped in pens with on average 10 calves. Methane is measured the last 1-2 months before they leave the test station, at 11-12 months age. On average each bull had 40 days with CH_4 data. We used data recorded from September 2020 to April 2023. The number of GF visits per bull varied from 1 to 798, with an average of 356, and the number of recorded GF visits per test-day varied from 1 to 155, with mean 77. For the genetic analyses we kept records from test-day with at least 10 records, and bulls with at least 10 GF visits. The final dataset had records from a total of 76 094 GF visits from 212 bulls. The traits analyzed were gram CH_4 per day, measured per GF visit

or computed as the average of the bull's individual visits each day.

Model

Variance components were estimated with DMUAI (Madsen and Jensen, 2013) using a linear animal repeatability model with fixed effects of age and group-test-day, and random effects of animal and permanent environment. Age was the bulls age in weeks at the day of phenotyping, grouped in 23 classes where <40 weeks is the first and >60 weeks is the last class. Group test-day had 964 levels. The pedigree of the bulls was traced back 4 generations and the pedigree file included 4 233 animals.

Correlations between breeding values

To give an indication of strength and direction of genetic correlations between CH_4 and other traits, correlations between predicted breeding values were calculated for the 212 bulls with a CH_4 phenotype. Correlations between the bulls EBV for CH_4 and their indexes for all other traits included in the routine genetic evaluations of Norwegian Red were calculated. Indexes for all traits from June 2023 were provided by Geno.

Results & Discussion

The distribution of CH_4 measures are given in Figure 1. The mean (standard deviation (SD)) of CH_4 production was 218 (50) gram per bull per day. In comparison, the average for Norwegian Red dairy cows in lactations was 420 gram per day (Wethal et al., 2022). The phenotypic level of CH_4 for our young bulls was very similar to reports by Callegaro et al. (2022) who analyzed methane data from GF on 111 young Italian Holstein bulls. The average (SD) in their study was 220 (41) gram CH_4 per day.

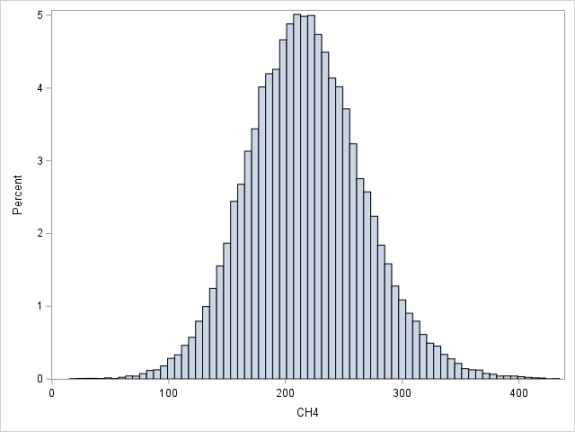

Figure 1. Distribution of phenotypic records of methane (CH_4) emissions for young Norwegian Red bulls, measured as gram per day from GreenFeed

Figure 2 shows the solutions for fixed effect of age and indicate an almost linear increase in CH_4 with increasing age. Solutions for fixed effect of group test-day in Figure 3 illustrates variation over time, seasonal variations, and group effects. The bulls stay in the pen with the GF the last 1-2 months before they leave the station. There may therefore be a large shift in mean CH_4 level from one test-day to the next, when one group leaves the station, and a new group of younger bulls start recording in the GF pen.

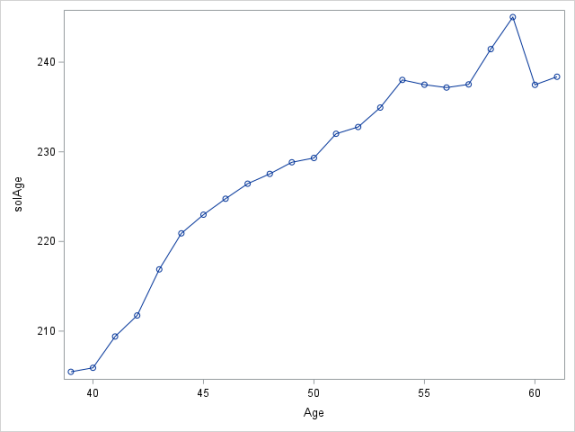

Figure 2. Fixed effect solutions for effect of age on methane emission in young bulls

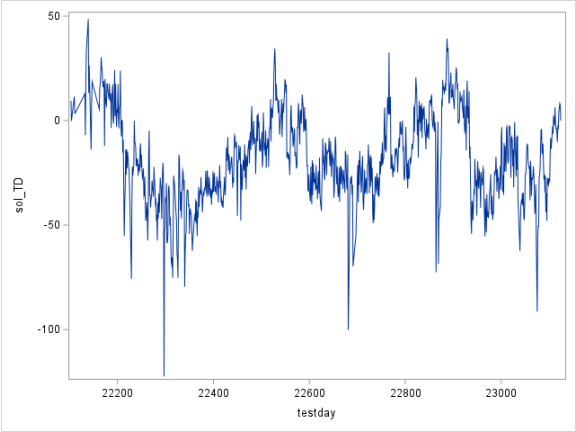

Figure 3. Fixed effect solutions for effect of group-test-day on methane emission in young bulls

Estimated variance components are given in Table 1. The corresponding heritability (standard error (se)) was 0.24 (0.10) for CH_4 per visit and 0.56 (0.20) for CH_4 mean per day, with repeatability of 0.32 and 0.71, respectively.

Table 1. Estimated permanent environment (σ^2_{pe}), animal (σ^2_a), and residual (σ^2_e) variance with standard error (se), and the corresponding heritability and repeatability for methane (CH_4), gram per day, for young Norwegian Red bulls measured per visit or computed as the average of the bull's individual visits each day.

	CH_4 per visit		CH_4 mean per day	
	Estimate	se	Estimate	se
σ^2_{pe}	153	194	141	188
σ^2_a	532	216	538	211
σ^2_e	1479	8	278	5
heritability	0.24	0.10	0.56	0.20
repeatability	0.32	0.02	0.71	0.02

The results for CH_4 per visit agree well with Wøyen Hamfjord (2022) who did the first genetic analyses on a subset of these data. Our heritability estimates correspond well with other published results, although they are not directly comparable due to differences in phenotyping equipment as well as breed. Donoghue et al. (2016) estimated heritability of CH_4 based on large scale methane measures from respiration chambers. Their study included data on 1 048 young bulls and heifers of Angus cattle in Australia. Methane production rate was measured in respiration chambers for 48 hours. Estimated heritability (se) was 0.27 (0.07). Johansen et al. (2022) estimated heritability (se) of methane concentration for beef x dairy crossbred (Belgian Blue and Holstein) slaughter calves of 0.44 (0.08) and 0.33 (0.06). They measured methane in feed boxes using an infrared gas sensor.

Figure 4 shows the distribution of EBVs for CH_4 emission for bulls with own phenotype. The EBV for CH_4 varied from - 37 to + 60, with SE ranging from 12 to 15. Here the EBVs were not standardized, the unit is gram CH_4 per day. Although the SE were relatively large this illustrates that there are significant genetic differences between bulls and potential for selection for reduced CH_4 emissions.

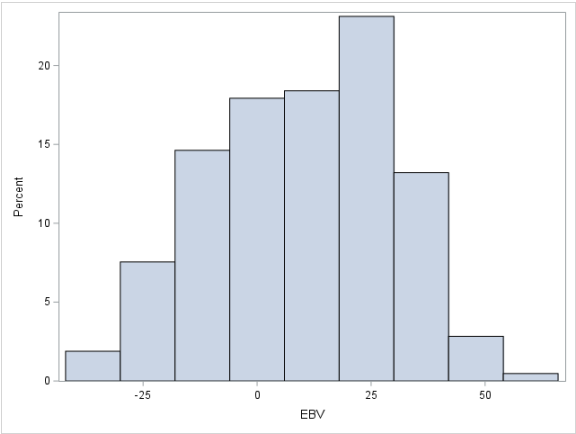

Figure 4. Distribution of predicted breeding values (EBVs) for methane for 212 young Norwegian Red bulls with phenotype. The unit of EBV is gram methane per day

Currently we do not have enough data for estimation of genetic correlations between CH_4 emissions in young bulls and other traits. Correlation between EBVs were therefore used to give an indication of strength and direction of genetic correlations between traits. The correlations between EBV for CH_4 and the bull's official indexes for all traits in routine genetic evaluation varied between -0.36 and 0.34. Many of the correlations, including the correlation to the total merit index, were close to zero. The traits with the strongest correlations to EBV (not standardized) for CH_4 are given in Table 2. The highest positive correlations were found to traits related to body size, while high

genetic merit for direct calving traits were associated with lower EBV for CH_4.

Table 2. Correlations between predicted breeding values for methane (CH_4) and indexes for other traits from the routine genetic evaluations of Norwegian Red. The traits with the strongest positive and negative correlations with CH_4, respectively, are shown.

		Correlation to CH_4
Strongest positive	Carcass weight	0.34
	Angularity[1]	0.29
	Body depth[1]	0.27
	Body total score[1]	0.26
	Stature[1]	0.25
Strongest negative	Calf size, direct[2]	-0.36
	Calving ease, direct	-0.34
	Stillbirth, direct	-0.25
	Hock quality[1]	-0.23
	Bone structure[1,3]	-0.22

[1] Trait not included in the total merit index
[2] Calf size: High score is small calf
[3] Bone structure: High score is very fine and thin bones low score for coarse bones (broad and thick)

The correlation between EBV for CH_4 and the total merit index was not significantly different from zero, suggesting that no correlated selection response should be expected. No indication of indirect selection for, or against, CH_4 emission in young bulls with the current breeding goal is further illustrated in Figure 5 where the bulls EBV for CH_4 was sorted from lowest to highest and those selected as AI bulls marked with red color.

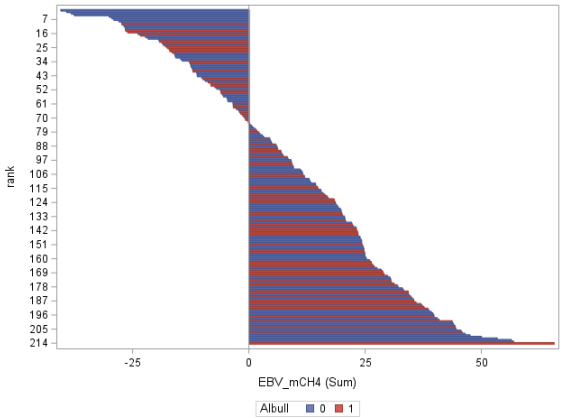

Figure 5. Predicted breeding values for methane for 212 young Norwegian Red bulls with methane phenotype, sorted from lowest to highest. Color indicates selected (red) or not (blue) as AI bull

This initial analysis of CH_4 emission in young Norwegian Red bulls shows that there is substantial genetic potential for reducing CH_4 emissions by breeding, also in young stock. It should be noted that estimates are based on few animals, standard errors of variance components are therefore large, and results should be interpreted with caution.

We need more knowledge on associations between CH_4 emissions and other important traits. In future research we will also examine whether CH_4 emission is the same trait genetically in young bulls and in lactating dairy cows.

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Implementation of Methane Efficiency Evaluations for Canadian Holsteins

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Abstract

Methane (CH_4) is a potent greenhouse gas (GHG) that warms the atmosphere at a rate 25 to 27 times more than that of carbon dioxide. The average first parity Holstein cow produces nearly 500 g of CH_4 per day or 180 kg per year, mainly due to enteric fermentation. A 30% difference above or below average can also be seen between cows, meaning two cows in the same herd can differ in their CH_4 emissions by up to 110 kg per year. As such, using genetics to select for cows with reduced CH_4 emissions is a strategy that can combat global warming and improve the efficiency of the dairy industry. In April 2023, Lactanet launched genomic evaluations for Methane Efficiency using milk mid-infrared (MIR) spectroscopy data. Previous research using artificial neural network methods determined that a cow's milk MIR spectral data can be used as a good predictor of its CH_4 emissions. Lactanet developed CH_4 predictions using CH_4 data collected from research herds in Canada through two research projects, the Efficient Dairy Genome Project and the Resilient Dairy Genome Project, and milk spectral data collected via Canadian milk recording services. Predicted CH_4 (g/d) has a genetic correlation with collected CH_4 of 0.92 and a heritability of 0.23 (0.01). Lactanet's genomic evaluation for Methane Efficiency was developed for the Holstein breed using a 4-trait Single-Step linear animal model including predicted CH_4 and milk, fat and protein yields as correlated traits. Methane Efficiency is defined as genetic Residual Methane Production in 120-185 DIM of first lactation and is genetically independent of production yields via a linear regression approach. The first genomic evaluation for Methane Efficiency included first lactation records on over 500 000 cows in Canadian milk recorded herds, of which more than 60 000 were genotyped. The average reliability of Methane Efficiency for genotyped young bulls and heifers exceeds 70%. Methane Efficiency is expressed as a Relative Breeding Value (RBV) averaging 100 and ranging from 85 to 115. For every 5-point increase in a sire's RBV for Methane Efficiency, daughters are expected to produce approximately 3 kilograms less CH_4 per year. This equates to a 1.5% reduction in CH_4 emissions per cow per year and a herd can achieve a 20% to 30% reduction by 2050 through genetic selection. Methane Efficiency does not have a significant undesirable correlation with any other trait, including LPI, Pro$, production yields and Feed Efficiency.

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

Introduction

Concerns about the effects of climate change on environmental sustainability are growing. Numerous global dairy industry stakeholders, including Dairy Farmers of Canada, have made commitments to achieve net-zero greenhouse gas (**GHG**) emissions by 2050. Methane (**CH_4**), a potent GHG, which remains in the atmosphere for about 12 years and makes up 14% of Canada's GHG emissions, has been under the spotlight as it is responsible for nearly half the net global

temperature change due to human activities in the last decade (Environment and Climate Change Canada, 2022).

To help dairy farmers in Canada contribute to achieving the industry's Dairy Net Zero 2050 goal, Lactanet has established a toolbox of genetic tools that includes Feed Efficiency (Lactanet, 2021) and Body Maintenance Requirements (Lactanet, 2023b) to reduce feed costs, as well as Methane Efficiency (Lactanet, 2023a). The focus of this paper is to describe the development and implementation of the routine genomic evaluation system for Methane Efficiency (**ME**), launched officially in Canada in April 2023 for the Holstein breed.

Materials and Methods

Data

Storage of mid-infrared (**MIR**) spectral data in Canada began in 2012 on a limited scale, and by 2018 was expanded to include all machines and laboratories. Therefore, only MIR-predicted CH_4 from milk samples analyzed since 2018 are used in the routine genomic evaluation. There have been over 18 million MIR spectra stored in the Lactanet database since the beginning of 2018. The routine editing, standardization and pretreatment of MIR spectra is the same as described in Oliveira et al. (2023). The MIR prediction model from Oliveira et al. (2023) was used to calculate MIR-predicted daily CH_4 emissions in g/d (**$CH4_{MIR}$**). The multilayer perceptron artificial neural network based on Bayesian regularization MIR prediction model was constructed subsequent to the findings and proof of concept of Shadpour et al. (2022). The model is applied to spectra recorded from first lactation Holstein cows between 120 and 185 days in milk (DIM) for inclusion in the genomic evaluation. The $CH4_{MIR}$ record is combined with the corresponding test day milk (**MY**), fat (**FY**), and protein (**PY**) yields. Animals are required to have a record for all four traits and no missing records are permitted. The April 2023 data for official genomic evaluations included 773 743 $CH4_{MIR}$, MY, FY, and PY records from 541 565 first lactation Holstein cows from 6 128 herds. Descriptive statistics are shown in Table 1 for the full April 2023 dataset.

Variance components were estimated using data from the August 2022 extract and after data editing contained 659 701 records from 462 120 cows in 5 804 herds. Because of computational demands, genetic parameter estimation was performed using five different subsets each representing 10% of the herds in the dataset. On average, the subsets contained 64 803 records from 45 137 cows.

Table 1. Descriptive statistics for MIR-predicted CH_4 production ($CH4_{MIR}$), and test day milk (MY), fat (FY), and protein (PY) yields in the complete dataset (N = 773 743 records from 541 565 cows).

Trait	Mean	SD	Min	Max
$CH4_{MIR}$, g/d	491.7	43.8	335.8	644.5
MY, kg/d	32.5	6.2	2.0	55.6
FY, kg/d	1.3	0.3	0.08	2.2
PY, kg/d	1.1	0.2	0.06	1.8

Model

The model is a four-trait linear animal model for $CH4_{MIR}$, MY, FY, and PY. The same model is used for all traits, considering the fixed effects of age at calving (nine classes), DIM, and year-season of calving, and random effects of HTD, animal additive genetic, permanent environmental (**PE**), and residual. In matrix notation, the model can be written as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{b} + \mathbf{Z}_1\boldsymbol{htd} + \mathbf{Z}_2\boldsymbol{a} + \mathbf{Z}_3\mathbf{p} + \boldsymbol{e}$$

where $\boldsymbol{y}$ is a vector of observations, $\boldsymbol{b}$ is a vector of all fixed effects, $\boldsymbol{htd}$ is a vector of is a vector of random herd-test-date effects (**HTD**), $\boldsymbol{a}$ is a vector of animal additive genetic effects, $\boldsymbol{p}$ is a vector of PE effects, **e** is a vector of residuals, and

X, $\mathbf{Z_1}$, $\mathbf{Z_2}$, and $\mathbf{Z_3}$ are the respective incidence matrices.

Assumptions are that: v(**htd**) = **I** ⊗ **HTD**, **I** is an identity matrix and **HTD** is the covariance (4x4) matrix for HY effects; v(**a**) = **H** ⊗ **G**, **H** is a combined pedigree-genotype relationship matrix, **G** is the additive genetic covariance matrix; v(**p**)= **I** ⊗ **P**, **P** is the covariance (4x4) matrix for the PE effects; v(**e**) = **I** ⊗ **R, R** is the residual covariance (4 × 4) matrix.

Variance components were estimated in AIREMLF90 using the AI-REML method (Misztal et al., 2014) with each of the subsets. The same model as described for genetic evaluation purposes above was used, but the combined pedigree-genomic relationship matrix **H** was replaced by an additive relationship matrix **A**.

Derivation of Methane Efficiency

The overall aim of ME evaluations is to select cows that produce less CH_4 at the same level of production. Methane efficiency is defined as genetic residual CH_4 production (**RCH4**), or CH_4 genetically independent of MY, FY, and PY, and derived using a recursive model operational tool (Jamrozik et al., 2017, 2021).

Let **a** = [a_1, a_2, a_3, a_4]' represent the EBV for MY, FY, PY, and $CH4_{MIR}$. A linear re-parameterization of these EBV is defined as:

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

with

$$\mathbf{\Lambda} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ -L_{41} & -L_{42} & -L_{43} & 1 \end{bmatrix},$$

such that v($\mathbf{a}^*$) = $\mathbf{G}^*$ = $\mathbf{\Lambda G \Lambda}$', with a_4^* being uncorrelated with a_1^*, a_2^*, and a_3^*. Non-zero elements of **Λ,** L_{41}, L_{42}, and L_{43} are the partial (genetic) regression coefficients of $CH4_{MIR}$ on MY, FY, and PY. The EBV of MY, FY, and PY remain unchanged, and EBV for $CH4_{MIR}$ is transformed into

$a_4^* = a_4 - L_{41}a_1 - L_{42}a_2 - L_{43}a_3$,

which is uncorrelated with EBV for MY, FY, and PY. Co-variance components involving ME can be obtained as:

$\mathbf{G}^* = \mathbf{\Lambda G \Lambda}$',

$\mathbf{P}^* = \mathbf{\Lambda P \Lambda}$',

$\mathbf{R}^* = \mathbf{\Lambda R \Lambda}$'.

The re-parameterization described above can be derived using a recursive model approach (Jamrozik et al., 2017). Let Y_1, Y_2, Y_3, and Y_4 refer to phenotypes for MY, FY, PY, and $CH4_{MIR}$, respectively, and recursive equations for the $CH4_{MIR}$ model be:

$Y_1 = fixed_1 + random_1 + e_1$

$Y_2 = fixed_2 + random_2 + e_2$

$Y_3 = fixed_3 + random_3 + e_3$

$Y_4 = L_{41}*Y_1 + L_{42}*Y_2 + L_{43}*Y_3 + fixed_4 + random_4 + e_4$,

with L_{ij} denoting a recursive coefficient parameter (effect of change in trait i caused by the phenotype of trait j). Imposing restrictions on genetic co-variances, i.e. setting $g_{14}^*=g_{24}^*=g_{34}^*=0$ of the genetic covariance matrix $\mathbf{G}^*$ of the recursive model, will lead to the same form of **Λ** and expressions of co-variance components and EBVs on a recursive scale (RCH4), as presented earlier using a simple re-parameterization of EBVs.

Genomic Evaluation

A four-trait Single-Step genomic evaluation was implemented at Lactanet Canada using MiX99 and related software (MiX99 Development Team, 2017). The April 2023 data included 134 963 genotyped animals, with 68 138 genotyped cows with records and 7 921 genotyped sires. Animals were genotyped either with 50K SNP panel or a low-density panel and imputed to 50K using FImpute (Sargolzaei al., 2014). 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 80% of the total genetic variance was explained by SNP

effects. Scaling of **G** and **A** is performed using the Christensen (2014) method. The APY algorithm for Proven and Young (Misztal et al., 2014) is applied for inversion of **G**, with the core population of 25 000 (the oldest genotyped animals in the Lactanet database). Groups for unknown parents are not included in the model. The SNP effects, to be used for calculating Genomic Estimated Breeding Values (**GEBV**) for genotyped animals not included in the single-step core analysis, are estimated from the GEBV of reference animals (as in Lourenco et al., 2015).

Reliability of GEBV is approximated by a weighted (80:20) average of Direct Genomic Value (**DGV**) and animal model reliabilities (Sullivan et al., 2005). The DGV reliabilities are calculated using SNP prediction error co-variances with the SNP-BLUP-REL software (Zaabza et al., 2020). Animal model reliabilities are calculated based on Effective Daughter Contributions (**EDC**). The EDC and reliability software of Sullivan (2023) is used.

The GEBV of $CH4_{MIR}$ are re-parameterized, giving a measure of residual CH_4 production (RCH4) that is genetically independent of Milk, Fat, and Protein, using the formula:

RCH4 = $CH4_{MIR}$ - 1.36*Milk - 156.13*Fat +204.43*Protein

The re-parameterized GEBV of $CH4_{MIR}$ are GEBV of RCH4. Reliabilities of GEBV for RCH4, being a linear function of four traits, are approximated by a selection index method (Sullivan et al., 2005.)

Relative Breeding Values

The signs of RCH4 GEBV are reversed to form the ME evaluation, such that a higher value represents a better (more desirable) methane efficiency of an animal. The ME evaluation is expressed as Relative Breeding Values (**RBV**) with a mean of 100 and SD of 5 for base bulls that for April 2023 are those born 2008-2017 and with an 'official' status. Sire evaluations are defined as 'official' for bulls with at least 20 daughters from 5 herds with $CH4_{MIR}$ records and a minimum reliability of 70%.

Genetic Correlation Between Collected and MIR-Predicted Methane

A further genetic analysis was performed to estimate the genetic correlation between the collected average CH_4 production and $CH4_{MIR}$. The collected average CH_4 production records for the cows used by Oliveira et al. (2023) for the development of the MIR prediction model were combined with the $CH4_{MIR}$ predicted for the same test day. Methane production was measured at the Ontario Dairy Research Station (Ontario, Canada) and the Dairy Research and Technology Centre (Alberta, Canada) using the GreenFeed system (C-Lock Inc., Rapid City, SD, USA). Data was recorded within the Efficient Dairy Genome Project (EDGP, https://genomedairy.ualberta.ca/) and the Resilient Dairy Genome Project (RDGP, http://www.resilientdairy.ca/) as described by Kamalanathan et al. (2023) and Liu et al. (2022). Only records between 120 and 185 DIM were considered for the genetic analysis and as a result the final dataset consisted of 442 cows after edits from the two herds with one record per cow. Descriptive statistics for these animals are shown in Table 2. Variance components for collected CH_4 production and $CH4_{MIR}$ were estimated in the DMU package (Madsen and Jensen, 2008) using AI-REML procedure for bivariate linear animal model, with the following 2-trait model:

$$\boldsymbol{y} = \mathbf{X}\,\boldsymbol{b} + \mathbf{Z}_1\,\boldsymbol{htd} + \mathbf{Z}_2\,\boldsymbol{a} + \boldsymbol{e},$$

where $\boldsymbol{y}$ is a vector of observations for collected CH_4 and $CH4_{MIR}$, $\boldsymbol{b}$ is a vector of all fixed effects (age at calving, DIM, and year-season of calving), $\boldsymbol{htd}$ is a vector of random HTD effects, $\boldsymbol{a}$ is a vector of random animal additive genetic effects, $\boldsymbol{e}$ is a vector of random residuals, and $\mathbf{X}$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ are the respective incidence matrices.

It was assumed that the random effects were normally distributed with means equal to zero.

Model assumptions were that: v(**htd**) = **I** ⊗ **HTD**, **I** is an identity matrix and **HTD** is the covariance (2x2) matrix between traits for HTD effects, v(**a**) = **A** ⊗ **G**, **A** is the additive genetic relationship matrix, **G** is the genetic covariance (2x2) matrix between traits for animal additive genetic effects, v(**e**) = **I** ⊗ **R, R** is the residual (2x2) matrix between traits.

Table 2. Descriptive statistics for the 441 cows used for the genetic correlation between collected and predicted methane emissions.

	Mean	SD
CH_4 production, g/d	494.7	78.0
$CH4_{MIR}$ g/d	493.7	49.7
Milk yield, kg/d	33.3	5.0
Fat yield, kg/d	1.3	0.2
Protein yield, kg/d	1.1	0.2
DIM, d	140.8	12.8
Age at Calving, mo	23.8	1.4

Results and Discussion

Genetic Parameters

The average genetic parameter estimates from the multi-trait analyses are given in Table 3. The heritability for $CH4_{MIR}$ was 0.23, which is similar to heritability estimates reported previously for milk MIR-predicted methane (Kandel et al., 2017) and other CH_4 traits (Lassen and Løvendahl, 2016; van Breukelen et al., 2023; Kamalanathan et al., 2023). The average heritability estimates for MY, FY, and PY were 0.38, 0.37, and 0.28, respectively. These estimates are similar to the heritabilities for the official genetic evaluation of these traits in Canada.

The genetic correlation between $CH4_{MIR}$ and FY was positive and moderate at 0.38. Kandel et al. (2017) observed positive genetic correlations between their MIR CH_4 emission trait and fat yield after 90 DIM in first lactation. Pszczola et al. (2019) also reported a positive genetic correlation of 0.21 between FY and CH_4 production. Genetic correlations between $CH4_{MIR}$ and MY and PY were slightly negative at -0.13 and -0.11, respectively. Negative genetic correlations were also reported by Kandel et al. (2017) between MY and PY with MIR predicted daily CH_4 emission.

The genetic parameter estimates after re-parametrization for RCH4 (equal to ME before the scale is reversed) are also included in Table 3. The heritability of RCH4 and therefore ME is 0.13. Genetic correlations with MY, FY, and PY are all zero. The genetic correlation between RCH4 and $CH4_{MIR}$ is 0.73 demonstrating that genetic selection to reduce RCH4 will result in lower $CH4_{MIR}$.

Table 3. Heritability (diagonal)[1], genetic correlations (above diagonal)[1] and phenotypic correlations (below diagonal) for MIR predicted methane production ($CH4_{MIR}$), test day milk (MY), fat (FY), and protein (PY) yields, and residual methane production (RCH4)

Trait	$CH4_{MIR}$	MY	FY	PY	RCH4
$CH4_{MIR}$	0.23	-0.13	0.38	-0.11	0.73
MY	-0.06	0.38	0.48	0.83	0
FY	-0.18	0.66	0.27	0.71	0
PY	0.01	0.90	0.74	0.28	0
RCH4	0.80	-0.05	-0.18	0.01	0.13

[1]Approximated SE <0.03

Genomic Evaluations

In April 2023 there were 2 142 Holstein sires with an official evaluation for ME. The ME for this group ranged from 82 to 117 and averaged 100. Average reliability of official sires was 95.9% and ranged from 72% to 99%. The average reliability was 77.2% for genotyped, young bulls born in 2020 with no daughters with records. Cows with records had an average reliability of 56.3% if not genotyped and 86.7% if genotyped. No genetic trend for ME was observed thus far, which is unsurprising given it has not been selected for and is uncorrelated with other traits including production.

Proof correlations were estimated between ME and other traits routinely evaluated using 1 763 Holstein bulls official for both ME and LPI. There were no strong relationships noted with any other trait. The greatest positive correlations were between ME and Metabolic Disease Resistance and Daughter Fertility at 0.22 and 0.15, respectively. All other proof correlations with other main traits were less than ±0.15 and are therefore deemed non-significant. Notably, the proof correlation for ME with LPI and Pro$ were 0.02 and 0.03, respectively, meaning currently selection based on either national index will not result in improved ME. Proof correlations between ME and Feed Efficiency was -0.13 and therefore selection for Feed Efficiency, another trait that is expressed independently of production yields, will not result in indirect improvement in ME.

Relationship with Collected and Predicted Methane Emissions

The genetic correlation between the collected average daily CH_4 using the GreenFeed system and $CH4_{MIR}$ was also performed to assess the utility of the MIR prediction. A genetic correlation of 0.92 (SE=0.22) between the two traits was found. This suggests that $CH4_{MIR}$ is a good indicator trait for collected CH_4 for use in genetic selection. While the prediction can still be improved, it is in its present state an efficient and cost-effective solution to begin genetic selection for reduced CH_4 emissions in Canada.

The association between collected CH_4 and cow ME evaluations were demonstrated by Oliveira et al. (2023) who showed differences in collected average daily CH_4 emissions between cows with low, average, and high ME RBV. Cows in the high RBV group had both lower collected and MIR-predicted CH_4 phenotypes.

Expression and Expected Response

The average daughter $CH4_{MIR}$ of 3 656 sires with at least 10 daughters with records were examined by sire RBV for ME. A regression of average daughter $CH4_{MIR}$ on sire RBV was performed to determine the relationship between the predicted daughter phenotype and sire RBV. The average daughter $CH4_{MIR}$ and regression is shown in Figure 1. Bulls with a higher ME evaluation have daughters with lower $CH4_{MIR}$ compared to bulls with low ME RBVs. From the linear regression, for each 5-point RBV increase for ME (1 SD), on average $CH4_{MIR}$ in their daughters will decrease by 7.55 g/d or 3 kg per year. This is approximately a 1.5% reduction in CH_4 emissions per cow per year.

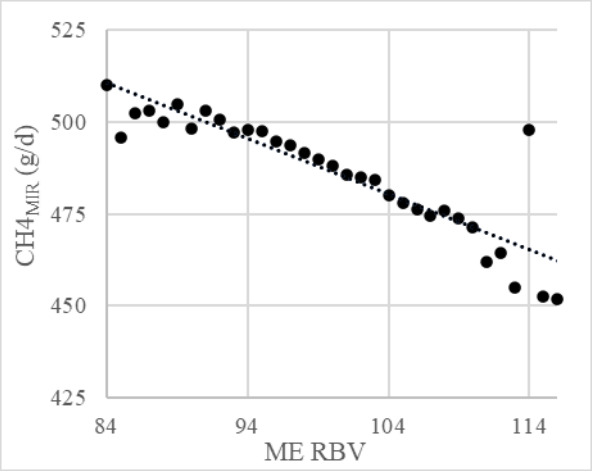

Figure 1: Daughter average $CH4_{MIR}$ averaged by sire RBV for ME

Figure 2 shows the expected response in CH_4 reduction depending on three different scenarios of selection. If top 50% ME bulls are selected we can expect a reduction of over 10% for CH_4 production by 2050. If bulls over 1 SD for ME are selected we can expect a reduction of over 20% for CH_4 production, and if bulls over 2 SD are selected we can expect a reduction of over 30% for CH_4 production by 2050.

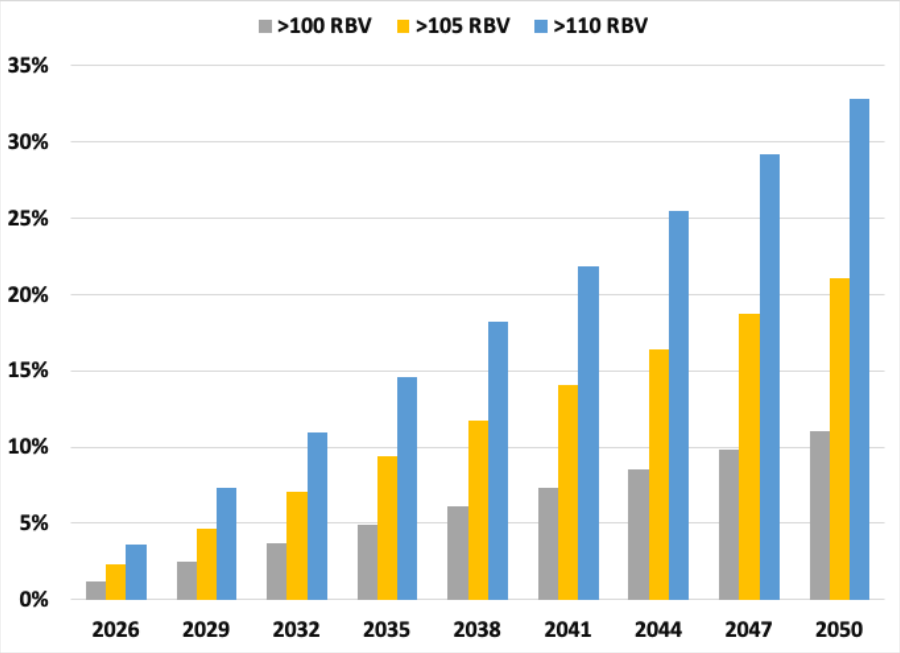

Figure 2: Expected selection response for three different scenarios: a) grey if top 50% bulls are selected; b) yellow if bulls over 1 SD for ME are selected; and c) blue if bulls over 2 SD are selected

Conclusions

The prediction of average daily CH_4 production using milk MIR spectral data is a key and rapid alternative to direct CH_4 measurements, which has permitted the development of routine genomic evaluations for ME for the Holstein breed in Canada. The genetic evaluations allow selection for reduced CH_4 emissions without affecting milk, fat, and protein production levels. The MIR prediction model will be refined in the future as the reference group of animals with collected CH_4 continues to grow and will expand into additional herds. The prediction accuracy is sufficient to begin genetic selection and help reduce the dairy industry's environmental footprint and contribute to the goal of reaching net zero GHG emissions by 2050 without impacting milk production.

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The EnviroCow index and its impact on the UK dairy industry's carbon footprint

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Abstract

Since 2021, the UK has published the EnviroCow index, derived from genetic evaluations for production traits, calf survival, cow longevity, fertility, and Feed Advantage. The goal of the index is to reduce carbon emissions per kg of product produced, and importantly reflect the lifetime environmental efficiency by incorporating survival traits. The index uses carbon emission equivalents, estimated for the traits in the index based on their feed requirements for a unit change of the trait. Feed intake in turn has been shown to affect enteric methane production. A recent analysis of lifetime efficiency was conducted on approximately 475K females by comparing their EnviroCow genetic index to their phenotypic performance for milk, fat and protein over their lifetimes, age at first calving, number of lactations, mature weight (derived from proxies as liveweight itself is not routinely recorded) and stature (where available). The data showed that each point increase in EnviroCow, on average gave animals that produced 10% less methane per kg milk, through enteric emissions, consumed 10% less feed, while producing 33% higher weight of fat and protein in their lifetime. Genetic trend data estimates that the carbon footprint per kg milk in the UK is predicted to reduce by around 1% each year due to genetic gains achieved in the population.

Key words: DMI, GHG emissions, genetic index, EnviroCow

Introduction

Recent global political events and concomitant increases in energy costs have pulled efficiency of dairy production into very sharp focus, accelerating further a previously increasing trend of increased efficiency through dilution of maintenance and improved health and longevity. Rising global temperatures and a focus on mitigation strategies has drawn attention to greenhouse gas (GHG) emissions from farmed ruminants. This has rapidly led to a shift in priorities in dairy farming towards reduced environmental impact whilst maintaining profitability, a strategy that could be described as creating sustainability. This shift is not driven by dairy farmers but by retailers, government and societal pressures. Whilst dairy farmers are not currently rewarded for reduced GHG emissions, the general expectation is that either social pressure, retailer intervention or government legislation will demand a reduction in GHG emissions and will require a tool do that and to monitor resultant changes.

Selection for reduced methane emissions has been proposed for some time (e.g. Jones et al. 2008, Wall et al, 2010) and reviewed by Pickering et al (2015), de Haas et al (2021) who describe how genetic selection can contribute to a reduction of methane emissions through appropriate phenotype collection and genotyping. Direct selection requires large numbers of methane measurements on 25,000 genotyped cattle from over 100 farms for at least 2 years (de Haas et al, 2021).

Most industrialised dairying nations have a range of genetically evaluated traits and most

combine a subset of the economically most important of these traits into an overall index either as an economic index, a desired gains index or a combination of both (Miglior et al, 2005). In the UK this index is called Profitable Lifetime Index (£PLI) expressed in monetary units as £PLI. It incorporates many traits, broadly covering production traits, survival (calf, cow), fertility, udder health, leg health, calving ability, maintenance, and feed efficiency, all weighted by their economic value per unit change in that trait. Available bull lists are ranked on this index with individual traits printed alongside allowing users to select bulls that have specifically good values for traits they are interested in out of the top bulls ranked for overall economic merit.

Wall et al. (2010) showed that selection using existing traits will reduce GHG emissions indirectly through improvements in these traits but they also pointed out that selection is also useful in breeding animals that are resilient to the inevitable changes that will occur to weather patterns such that GHG emissions from cattle are reduced and not subsequently increased by environmental changes.

To create a tool that farmers could use to explicitly reduce their GHG emissions intensity and to have a strategy that is welcomed by retailers, milk buyers and society, a new index was developed and derived from the existing £PLI that could serve most interest groups purposes, including farmers. This does not rely on direct measures of methane but uses proxy traits to demonstrate the predicted contribution of each trait to methane emissions expressed as Carbon Dioxide equivalents (CO2e). This work is aimed at providing an index that UK farmers can use to begin their journey to select for reduced methane emissions which capitalises on their historic recording activities and allows them to start selection now until direct measures of methane can be incorporated into the index in future.

Materials and Methods

Since August 2021 AHDB has published the EnviroCow genetic index to enable dairy animals to be selected for improved carbon emissions per kg product. The methodology for assigning relative carbon weights to traits was described by Amer et al. (2017) and Zhang et al. (2019) and is based on predicted feed intake requirements associated with individual trait changes. These feed intake requirements, which have shown to affect enteric methane production, can subsequently be converted to CO2 equivalents using a simple conversion factor. Gross emissions are finally converted to methane intensity values by calculating the change in product for each unit change in the traits included in the index. Similar to Amer et al. (2017) the product was determined as £ value for each trait unit compared to the value of protein.

The Predicted Transmitting Abilities (PTA) for the following traits are included in the EnviroCow index; Milk (kg), Fat (kg), Protein (kg), Lifespan (days), Calf survival (%), Non-Return rate (%), Calving Interval (days), Body Condition Score (point), and Feed Advantage (kg Dry matter).

Feed Advantage is the UK's genetic index for improving feed efficiency and is based on a combination of maintenance feed cost based on cow size, combined with genomic predictions for feed efficiency. The evaluations were described in more detail by Li et al. (2021).

The EnviroCow index is published as a carbon intensity index and standardised to a standard deviation of 1.0.

For this analysis, genetic evaluations from the April 2023 release were available along with lifetime performance data. Performance recording data was available from the milk recording organisations providing data to the UK's dairy cattle genetic evaluations (National Milk Records (NMR), Cattle Information Services (CIS), DaleFarm and Quality Milk Management Services (QMMS). Linear type

data were available for animals classified by Holstein UK.

The Holstein cows in the study had to have a death date recorded in order that their completed lifespan and lifetime yields can calculated. Therefore, cows born between 2008 and 2011 were used in the study. These years were chosen for being both the most representative of today's herd and having a recorded death date for the majority of the year cohort. Any remaining alive within this cohort – because of their long lifespans – are not included in the dataset, creating a likely small downward bias.

For each animal the following performance metrics were calculated; Age at first calving (months), total lifetime milk (kg), fat (kg), protein (kg), fat (%), protein (%), Number of calvings (lactation), age at death (days), daily lifetime yields ([kg fat + protein] / age at death (days)), Stature score (linear 1-9), estimated liveweight (kg).

Cows which had lifetime performance available were then matched up with their genetic index for EnviroCow from the official April 2023 release in order to compare their genetic index to their lifetime phenotypic performance.

For the given performance metrics, estimates for lifetime dry matter requirements were calculated, and these were used to determine CO2 equivalents (CO2e), based on the assumption that a linear relationship exists between methane emissions from enteric emissions and dry matter intake (DMI). The value of 583 grams of CO2e per kg of DMI was used, following Amer et al. (2017). The lifetime estimates for a cow in the study included both her rearing and productive life but excluded the impact of any offspring. Carbon dioxide intensity was estimated as the total CO2e divided by the total Fat and Protein corrected Milk. These were calculated as (Milk(kg) x [0.337 + 0.116 x fat% + 0.06 x protein%]).

To enable us to establish the impact of genetic selection over time, the genetic trends for Holstein females were calculated.

Results & Discussion

A total of 475,060 Holstein cows were included in the analysis, which had their completed lifetime performance available and had an EnviroCow index evaluated.

The results shown in table 1 groups cows by their genetic index for EnviroCow and then averages their recorded phenotypic performance for a range of traits. It also shows their projected CO2e intensity based on their expected enteric methane production. Note that enteric emissions represent around 46% of the total carbon footprint of a typical litre of milk in the UK, which therefore implies that the total carbon footprint estimated in this study is on average 534 grams / 0.46 = 1180 grams CO2e per litre. This total is close to those reported by Arla (1130 grams CO2) based on a more recently born group of cows (Arla, 2021).

The table shows clearly how well EnviroCow is working as the top 10% cows with the best [highest] score for EnviroCow estimated to produce the least methane for each kg of fat and protein corrected milk (FPCM). The reason they have a low environmental footprint per litre is because these higher EnviroCow index cows have, on average, higher lifetime yields of FPCM, a younger age at first calving (AFC), more lactations and longer lifespans, and so offer an excellent combination of traits required for efficient dairy production.

The fact that these cows were also of a smaller stature and lower predicted liveweight is expected, as the bigger animals would have a higher maintenance feed cost.

The bottom row in the table, showing cows with the worst 10% EnviroCow scores, is a stark demonstration of just how inefficient and polluting these are compared to the best genetics. This group has on average the tallest, heaviest cows with a late AFC and a shorter lifespan. These animals lasted just 2.57 lactations on average and are projected to produce 12% more methane than average per kg

Table 1. Relationship between EnviroCow (deviated from mean) and the lifetime performance of Age at first calving (AFC), total lifetime milk (kg), fat (%), protein (%), Number of calvings (lact.), age at death (AaD), daily lifetime yields (DLY), Stature score (ST)), estimated liveweight (LW), CO2e Intensity (CO2eI) and the deviations of Methane from the average (%). (Each row in the table represents the average of the decile group)

EnviroCow (PTA)	AFC (month)	Milk (kg)	Fat (%)	Prot (%)	Lact.	AaD (days)	DLY (kg/d)	**ST**	LW (kg)	CO2eI (gram)	Methane (%)
0.9	27.5	42 688	4.08	3.28	4.05	2 377	1.32	4.7	665	487	-9%
0.6	27.7	39 695	4.02	3.24	3.85	2 317	1.24	5.0	670	500	-6%
0.4	27.8	37 704	3.99	3.23	3.71	2 266	1.20	5.1	674	508	-5%
0.2	27.9	35 584	3.96	3.22	3.56	2 209	1.16	5.2	676	518	-3%
0.1	27.9	33 612	3.95	3.21	3.41	2 150	1.12	5.3	678	526	-2%
-0.1	28.0	31 583	3.93	3.20	3.25	2 090	1.08	5.4	680	536	0%
-0.2	28.1	30 046	3.92	3.19	3.12	2 040	1.05	5.4	682	544	2%
-0.4	28.1	28 255	3.89	3.18	3.00	1 988	1.01	5.5	685	555	4%
-0.6	28.2	26 148	3.87	3.17	2.83	1 920	0.96	5.7	688	569	7%
-1.0	28.3	22 742	3.83	3.15	2.57	1 817	0.87	5.9	694	599	12%

FPCM. Since animals which were still alive and cohorts of the analysed birth years were excluded from the data, it is highly likely that the positive links between EnviroCow and actual emissions which have been demonstrated here, are likely to be even stronger.

Genetic trends

The average yearly genetic gain for Holstein cows over the past 10 years (2012-2022) was 0.22 points EnviroCow index PTA. Over the last five years, this was slightly higher at 0.26 points. The UK dairy cattle population is achieving a favorable positive trend due to the fact that there is a strong positive correlation between the main profit index (£PLI) and EnviroCow. However, not all traits in the index show a favourable trend. Cows are increasing in size which limits the benefits from selection for other traits in the index, something which the UK and many other countries have begun to address.

Regressing the CO2e Intensity on the average EnviroCow gives a slope of 57 grams CO2e per point EnviroCow. This means that the 0.22 points gain per year is equivalent to (0.22 x 57 = 12.5gram CO2e reduction per year. Given that the average cow in our study had an estimated CO2e intensity of 534 grams, means that the percentage gain per year could be as high as 12.5/534 = 2.3%. It has to be noted however, that the relationship between EnviroCow and CO2eI in this summary review was non-linear, and this estimate is therefore a likely over-estimate.

Never-the-less, previous model estimates of the impact of genetics on the reduction of CO2e for milk were estimated at around 1% per year (Winters, 2022), providing a resemblance to these newly estimated effects of this study. With the knowledge that genetic improvements are both permanent and cumulative, the impact of genetics over time will be substantial, whichever estimate is used.

Conclusions

The analysis clearly demonstrated a strong association between improved genetics (EnviroCow), and lifetime enteric emissions (methane). Although enteric emissions of methane are the largest contributor of greenhouse gases in dairy farming – accounting for around 46% of total farm emissions – they are only part of the picture. And it is not unreasonable to assume that cows which eat less are also indirectly affecting a farm's total carbon footprint through a variety of other

factors. This includes the smaller amount of feed bought or grown which carries its own carbon footprint, whether through fertiliser, fuel or other factors, and the smaller amount of manure and its associated emissions from these higher EnviroCow rated animals.

Although many of the traits included in EnviroCow are already improving favourably over time, noticeably cows are still getting bigger which is unfavourable. The maintenance feed index was introduced in 2016 to attempt to halt this trend and is included in £PLI. Since 2021 a renewed focus on Feed Advantage (inc. Maintenance) might be more effective in achieving a reduction in cow size. The UK continues to explore how to select for reduced cow size in an effort to save more feed and reduce GHG emissions from dairy herds. However, with now close to 80% of Holstein cows being inseminated with dairy sexed semen (AHDB, 2023), there is a rapid growth in beef from the dairy herd. In order to manage the beef from dairy sustainably, the discussion of dairy cow size is gaining renewed interest. The current EnviroCow index ignores progeny impact, but this may be considered in future updates. A number of proxy traits are being developed since very few farmers weigh mature cows.

Although the EnviroCow index used in this analysis does not include a direct methane genetic index, there is substantial benefit to be had by breeding for existing traits already. In time, direct methane measured PTAs may become available, which could help to further fine-tune the EnviroCow index.

Despite the fact that there is not yet a standard agreement on how CO2e on farm is calculated and expressed, the technology to routinely record CO2e on farm is not well developed, this study provides a reassuring picture of environmental outcomes from existing practices, using existing selection traits but it is not surprising as that is exactly what the EnviroCow index was designed to deliver. However, the fact that this analysis clearly shows the extent of the benefits in practice will hopefully encourage producers and the wider industry to make sure that genetic improvements are considered as part of the process to reach Net Zero for the dairy sector.

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Breeding Values for Daily Dry Matter Intake in Norwegian Red Dairy Cows and Correlation to Other Traits

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Abstract

The current study aimed to estimate genetic parameters of feed intake in Norwegian Red (NR) dairy cows. Data from eight commercial herds with equipment for monitoring individual roughage intake was available. Our aim was also to predict breeding values (EBVs) for feed intake in lactating cows and calculate index correlations to other traits in the Total Merit index (TMI) for NR. Data on daily feed intake from roughage and concentrates together with results from weekly feed analyses were used to compute daily dry matter intake (dDMI) kg. A total of 557 NR cows with 61 321 records on feed intake from January to October 2022 were analyzed. The mean dDMI was 20.37 kg. A linear animal repeatability model was used to estimate variance components. The estimated heritability (standard error) of dDMI was 0.18 (0.04). The EBV's for dDMI varied from -3.32 to 3.65 (±1.2), with significant differences between individuals. Index correlations between cows EBV's for dDMI with other traits in the breeding goal of NR were calculated. The index correlations were in general low, ranging from -0.21 to 0.34, the strongest correlation was between 305-day protein yield and EBV for dDMI. Milk yield and body exterior traits had positive index correlations to dDMI. On the contrary, indexes for health and fertility were negatively correlated with EBV for dDMI. Although index correlations between dDMI and other traits for NR cows were not strong, our results indicate that dry matter intake are correlated with production, body exterior, fertility and health traits. We need more knowledge on the effects of selecting for novel feed intake traits, and how we best can define feed efficiency in Norwegian Red needs to be addressed. Our results so far show individual variation of feed intake capacity amongst Norwegian Red dairy cows. More phenotypes on more cows are needed to estimate genetic correlations, as well as increased knowledge on how to balance feed efficiency with production, health and fertility in the current breeding goal of Norwegian Red.

Key words: Feed intake, heritability, dairy cows, index correlations

Introduction

Feed efficiency of dairy cows is one of the most complex traits to work with in breeding programs and have gained a lot of focus for decades (Berry et al. 2007, Veerkamp 1998). The complexity lies both in the physiological processes behind utilization of gras in the rumen, as well as the fact that it is time consuming and costly to monitor individual feed intake in dairy cows. Direct measures on feed intake are necessary for establishing basic knowledge on the nature of this relative new trait in breeding context. Although challenging to measure, equipment for recording of individual feed intake exists. Data on feed intake have mainly been collected from research herds (Pryce et al. 2014), but in more recent years breeding organizations have also started collecting data from commercial herds routinely for genetic evaluation of feed efficiency (de Jong et al. 2019, Negussie et al. 2019). Equipment for monitoring actual individual feed intake in dairy cows may constitute of large mangers or feed bins where the amount of roughage or gras each cow eats during a visit are recorded, and the system provides data on kg of roughage eaten per visit in the manger. This can provide phenotypic data on feed intake throughout the lactation. Although some extra maintenance and calibration of the system are required, time consumption are manageable as

part of a daily routine for farmers. Alternative methods to predict roughage intake are also available e.g., 3D cameras, although they are less disturbing for animals in their daily routine and offers an opportunity to phenotype a large number of cows at lower costs (Lassen et al. 2023), it might be a less accurate alternative.

In order to establish a reference population of cows with direct and actual individual feed intake measures, Geno the breeding organization of Norwegian Red, have installed equipment to monitor intake of roughage and concentrates in commercial herds. Feed mangers to record daily and accurate feed intake throughout the lactation under commercial settings are now in place in fourteen herds. The feed efficiency project aims to collect phenotype and genotype data on 1000 Norwegian Red dairy cows yearly and enables Geno to implement future selection for feed efficiency in our breeding goal. This study aimed to perform the first genetic analysis of feed intake in Norwegian Red as a novel trait by using data from the first commercial dairy farms with equipment for monitoring daily roughage intake. As a secondary aim, the relationship between feed intake and traits in routine genetic evaluations was investigated.

Materials and Methods

Data

The data used in this study was extracted from the feed efficiency database and included records from eight herds with data from January to October 2022. Daily roughage intake registered in the BioControl software (CRFI) were merged with daily intake of concentrates collected from automatic milking systems (Lely or DeLaval). Feed samples on roughage were sampled by farmers every week, and we used information on dry matter content from the feed analyses. The analyzed trait was daily dry matter intake in kg (dDMI), as a sum of dry matter from roughage and concentrates eaten at a specific test-day. For the genetic analyses only Norwegian Red dairy cows with a known sire (Norwegian Red) were included. The final dataset included 557 cows, and 61 321 daily records on kg dDMI in total.

Edits of data

Restrictions on logical feed intake values were set between seven and 36 kg dDMI per test-day. Test-days with records out of this range were excluded. 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 roughage and concentrates in order to be included in the genetic analysis. Pedigree information were collected from the Norwegian dairy herd recording system, and sire and dams were traced eight generations back.

Statistical model

A mixed linear repeatability animal model was used to estimate variance components and breeding values (EBVs). Variance components were estimated with DMUAI (Madsen and Jensen, 2013). The model included fixed effects of herd, days in milk and age-parity, and random effects of animal, herd-test-day and permanent environment. Days in milk ranged from 6 to 350, and herd had eight classes. First parity cows were grouped in six groups according to their age in months at calving: $\leq$ 22, 23, 24, 25, 26 and $\geq$ 27, while second parity and third or later parities were in two separate groups (age-parity had eight classes). The random effect of herd-test-day had 1 203 levels in total. The pedigree contained 12 291 animals. The relationship matrix (A) was constructed assuming no inbreeding between animals and without genetic groups for animals with unknown parents.

Correlations between breeding values

Correlations between EBV for dDMI and other traits were calculated to give an indication of strength and direction of possible genetic correlations between traits. For the 557

Norwegian Red cows with phenotypes on feed intake, correlations between their EBV for dDMI and indexes for all other traits included in routine genetic evaluations of Norwegian Red were calculated. Indexes from the routine evaluation performed in June 2023 are provided by Geno.

Results & Discussion

The phenotypic mean dDMI was 20.37 kg per day with a standard deviation of 4.35 kg. Daily dry matter intake displayed an approximately normal distribution and large phenotypic variation amongst cows and test-days (Figure 1). These are the first results on feed intake of Norwegian Red based on data from commercial herds but are in line with what previous studies from research-facilities have reported (Salte et al. 2020, Wallén et al. 2018). Li et al. (2016) presented feed intake in Nordic Red to be close to 20 kg dry matter intake/day 24 weeks after calving in primiparous cows.

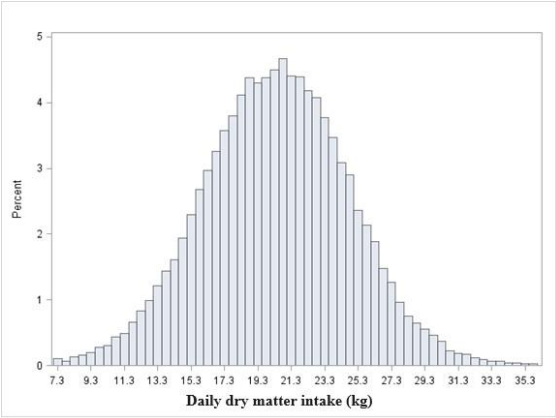

Figure 1. Phenotypic distribution of daily dry matter intake in kg for 557 Norwegian Red cows in commercial herds.

Variance components estimation

Estimated variance components for dDMI were significant different from zero (Table 1) with heritability 0.18 (0.04) and repeatability 0.34 (0.02). Our results were in the same range as a study on feed intake in Nordic Red cattle by Liinamo et al. (2012) who reported heritabilities ranging from 0.18 to -0.33 for DMI in primiparous Nordic Red cows based on data from one research herd. Li et al. (2016) analyzed DMI from 872 Nordic Red cows from four herds and estimated heritability ranged from 0.25 to 0.41 for six different periods of the first 24 lactation weeks for primiparous cows. These authors showed that heritability of DMI varies throughout the lactation, and that it should be accounted for in genetic evaluation of feed efficiency. Our results confirms that significant genetic variation in feed intake exists, despite a relatively small dataset with few animals, the standard error on our heritability estimate was low. This is promising for the further work.

Table 1. Estimated variance components for daily dry matter intake (kg) and the corresponding heritability and repeatability.

	Estimate	Standard error
Herd-test-day (σ^2_{htd})	4.28	0.18
Additive genetic (σ^2_a)	2.65	0.69
Permanent environment (σ^2_{pe})	2.40	0.57
Residual (σ^2_e)	5.62	0.03
Repeatability* (r)	0.34	0.02
Heritability** (h^2)	0.18	0.04

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

Breeding values estimation

The EBVs for dDMI ranged from -3.32 to 3.65 with standard errors of 1.2. In figure 2, EBVs for the 557 Norwegian Red cows are sorted from lowest to highest, and this illustrates that there were significant differences between the animals in the tails of the distribution. Breeding values for dDMI were not standardized but given as kg/d. Our result indicates that genetic variation for feed intake capacity exists and genetic selection for feed efficiency in Norwegian Red is possible, as dDMI are the main component trait in various definitions of feed efficiency.

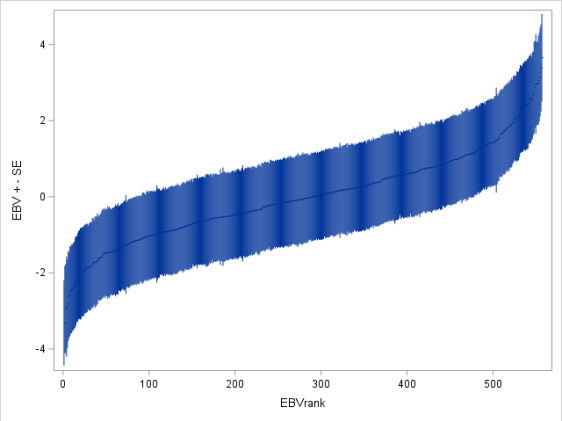

Figure 2. Breeding values (EBV) for daily dry matter intake in kg for 557 Norwegian Red cows. Standards error (SE) illustrated with blue bars. EBVs are sorted from lowest to highest rank (x-axis) and illustrated with EBV +/- SE (y-axis).

Index correlations

Correlations between indexes for all traits from routine genetic evaluations of Norwegian Red and EBVs for dDMI ranged from -0.21 to 0.34. Many correlations were low and not significantly different from zero. Only the strongest correlations are presented here. In Table 2 positive correlations are presented. Production and body exterior traits had the strongest positive index correlations with dDMI, ranging from 0.15 to 0.34, and the strongest was to 305-days protein yield. A positive index correlation means that high EBV for dDMI is associated with high index for other traits. The correlations are logical indicating a high stature, milking type cow with high milk yield will have a larger intake capacity of the rumen.

Table 2. Index correlations to daily dry matter intake (dDMI) in kg.

Trait	Correlation to EBV for dDMI
Kg protein 305-days	0.34
Kg milk 305-days	0.30
Angularity*	0.26
Kg fat 305 days	0.24
Stature body*	0.21
Rump width*	0.18
Body depth*	0.15
Foot angle*	0.15

*Trait not included in the Norwegian total merit index

The traits with the strongest negative index correlations to EBV for dDMI are given in Table 3. Here we find health and fertility traits with correlations ranging from -0.12 to -0.21. Number of inseminations and interval from calving to first insemination had the strongest negative correlations (Table 3). Clinical mastitis in different lactations had an index correlation to dDMI ranging from -0.12 to -0.15. Although the correlations were not strong, they were significantly different from zero. Top line with correlation of -0.14 indicates a weaker top line associated with higher EBV for dDMI. The correlation to calf size (direct), indicates that higher EBV for dDMI may increase calf size, and small calf size are preferable in the current index. Although these correlations are relatively weak, they are interesting and indicates the direction of the genetic correlations to dDMI.

Table 3. Index correlations to daily dry matter intake (dDMI) in kg.

Trait	Correlation to EBV for dDMI
No. of inseminations (1-4 parity)	-0.21
Interval calving to 1st ins. (1-4 parity)	-0.16
Top line [1,*]	-0.14
Calf size, direct [2] (Parity 1)	-0.14
Bone structure [3,*]	-0.13
No. of inseminations, heifers	-0.13
Clinical mastitis (1-3 parity)	-0.12 to -0.15
Silent heat (3-5 parity)	-0.12 to -0.14

*Trait not included in the Norwegian total merit index

[1] Top line: Scored from 1 to 9. 1 = weak, 9 = upward

[2] Calf size, direct: High score is small calf

[3] Bone structure: High score is very fine and thin bones, low score for coarse bones (broad and thick)

This study shows that genetic variation in feed intake in Norwegian Red exists and that dDMI is moderately heritable. These results are promising for the further work of defining feed efficiency as a novel trait. There are still many unanswered questions that must be addressed before selection for feed efficiency can be implemented. We need more knowledge on genetic correlations to other important traits in our breeding goal. Feed intake can be measured as energy or protein intake and seen in the context of energy mobilization (body condition or body weight) to capture the energy sinks in the cow. This will be part of our further work on feed efficiency in Norwegian Red dairy cattle.

Conclusions

Genetic variation of feed intake in Norwegian Red dairy cattle exists, and results from this study shows that feed intake data from commercial dairy farms can be used for genetic evaluation.

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Meta-Analysis for Heat Tolerance Traits in Holstein in France, the Netherlands and Spain

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Abstract

In the context of climate change, livestock production systems face the challenge of ensuring that, although more and more exposed to heat-stress conditions, animals will be able to remain healthy while maintaining satisfactory production, responding to consumer's demand. As part of the European project RUMIGEN, France (INRAE, Idele), Spain (INIA, IRIAF), and the Netherlands (WUR) studied the response of different dairy traits to heat-stress conditions for Holstein cattle breed. Production (milk, fat and protein yields) and udder health (somatic cell score) traits were investigated under different meteorological conditions, represented by the temperature humidity index (THI) averaged over three days on the day of recording and the two days before. A THI=50 was considered as neutral condition (i.e. no heat/cold-stress) and used as reference for level comparisons. Specific rates of changes in production/health traits at a given THI were measured as the slopes (first derivative) of the estimated reaction-norm curves for each trait. Genetic evaluations were performed by each country using test-day records of cows in their first lactation between 2010 and 2020, to estimate both levels and slopes for each trait. Estimated breeding values (EBVs) and reliabilities were obtained for sires with at least 20 daughters with test-day records. A meta-analysis was performed to estimate the genetic correlations between the three countries, using the Multiple Across Country Evaluation approach. For each country, de-regressed proofs (DRPs) and effective record contributions (ERCs) were computed using a single trait model from the EBVs, reliabilities, and variance components estimated at a national level for specific THI values. The estimated genetic correlations obtained with slopes were weak and not credible, which could be explained by the low heritability of the slopes and by the small proportion of performances recorded under heat-stress conditions. The DRPs on slopes, as they were calculated, were not able to capture the genetic (co)variability associated to these traits. Conversely, estimated genetic correlations for all level traits were high (between 0.81 and 0.97) even if they were slightly lower than under thermo-neutral conditions. This shows a very good consistency of the three national genetic evaluations under heat-stress conditions. In conclusion, valuable predictions under heat-stress conditions could be obtained through international evaluations, that would result in Northern countries benefiting from the information that already exists in the Southern countries. However, the approach for low heritable traits such as slopes should be improved if there are not enough data at high THI.

Key words: heat tolerance, dairy cattle, reaction norm model, meta-analysis

Introduction

In the context of climate change, cattle will be exposed to more frequent and more intense heat waves, inducing acute and chronic heat-stress. Their welfare, health and production will be negatively affected by this stress (West, 2003, Becker at al., 2020). Genetic selection could be a useful tool to improve heat tolerance and help dairy cattle facing future weather conditions (Carabaño et al., 2019).

As part of the European Horizon 2020 project RUMIGEN, the impact of heat-stress conditions on performances and the genetic

variability for heat tolerance in dairy cattle were studied at the national level in France, Spain and the Netherlands.

The aim of the meta-analysis was to estimate genetic correlations between countries for the Holstein breed for different traits related to heat tolerance. These correlations will be helpful to determine the potential interest of international genetic evaluations for heat tolerance, in a context where phenotypic data recorded under heat-stress conditions are more widely accessible in some countries.

Material and methods

Phenotypes

Test-day records for milk yield (MY), fat yield (FY), protein yield (PY), and somatic cell score (SCS; defined as SCS = 3 + log2(SCC/100,000), with SCC being somatic cell counts in cells/ml) of 5,753,268 French, 1,016,403 Spanish and 474,273 Dutch Holstein cows in their first lactation between 2010 and 2020, were extracted from the respective national genetic evaluation databases (BDNZ, France; CONAFE, Spain; and CRV, the Netherlands).

For each trait, different traits indicators of heat tolerance were defined as levels of production at 150 days-in-milk (DIM) under thermo-neutral and heat-stress conditions, and slopes of production decline under heat-stress conditions. In accordance with the results of previous analyses performed at the population level (Mattalia et al., 2022), thermo-neutral conditions were defined as being equal to a THI of 50 for the three countries, while heat-stress conditions were defined specifically for each country (THI equal to 65, 68, and 77, for France, the Netherlands, and Spain respectively). The slopes were defined as the response curve to increasing heat loads of each trait at the heat-stress THIs.

Weather data

Weather data were provided by the national meteorological agencies (Météo-France for France, the National Meteorological Agency (AEMET) for Spain, and the Koninklijk Nederlands Meteorologisch Instituut (KNMI) website for the Netherlands. Meteorological records were available from 1,993 Spanish and 34 Dutch weather stations distributed throughout each national territory. In France, grid weather data included various meteorological variables for each of the 9,892 8 x 8 km squares covering the whole country (Safran database). Each herd was associated to the closest weather station or square through its ZIP code.

Daily temperature-humidity indices were computed using the formula proposed by the National Research Council (1971):

$$THI = (1.8*T+32) - (0.55-0.0055*RH) * (1.8*T-26)$$

where T is the average daily temperature (in degrees Celsius) and RH is the average daily relative humidity (in percent).

The heat load measure was defined as the THI averaged over three days, including two days before test day and the test day.

Model

A genetic evaluation was performed to estimate breeding values (EBVs) and associated reliabilities at the national level using the following random regression model:

$$y=Xb+Za+Wp+e$$

where **b** is fixed effects, **a** and **p** contain additive genetic and permanent environmental random regression coefficients on THI and DIM for each animal in the pedigree and each cow with records, respectively. **Z** and **W** are matrices containing the Legendre polynomial covariates appropriate for each THI and DIM corresponding to a record. Cubic and quadratic Legendre polynomials were fit for THI and DIM, respectively, providing five EBVs for each animal in the pedigree. The fixed effects were as follows:

- France: herd-test-day of record, DIM, gestation stage and age at calving;
- Spain: THI, herd-year-season of record, age at calving, and DIM.
- The Netherlands: herd-test-day, DIM, gestation stage, age at calving-year of calving-season of calving, age at calving-year of calving-season of calving-lactation stage;

Then, the DRPs and ERCs were derived from the national EBVs and reliabilities, using a deregression approach (VanRaden et al., 2014) implemented in an INRAE program. This approach assumes that EBVs were obtained with a single-trait model. For the levels, the heritabilities used for the deregression were estimated using the afore mentioned random regression model. For the slopes, which are obtained from the estimated random regression coefficients, no proper estimates of heritability can be obtained and therefore, heritabilities were assumed to be equal to 0.10 for MY, FY and PY, and 0.03 for SCS in order to obtain DRPs and ERCs, assuming a heritability similar to that of functional traits relative to heat tolerance (e.g. heritability of rectal temperatures).

For each country and for each trait, the analyses included DRPs and ERCs of all Holstein bulls with at least 20 daughters with performances and a reliability of at least 0.25. However, no selection was performed regarding the number of daughters with performances in heat-stress conditions. For milk production levels, 7,932 French sires, 3,624 Spanish sires and 2,281 Dutch sires met these criteria, with a total of 328 common sires to the three countries. For SCS levels, 7,932 French sires, 3,607 Spanish sires and 2,257 Dutch sires met the requirements, with a total of 325 common sires. The pedigree of each bull was traced back for 3 generations.

Genetic correlations between countries were estimated using the Multiple Across Country Evaluation (MACE) approach (Schaeffer, 1994). In this study, it consisted in a pedigree-based animal model, considering each country as a separate trait. The following multiple trait model was implemented:

$$\boldsymbol{y} = \boldsymbol{Xc} + \boldsymbol{Za} + \boldsymbol{e}$$

where **y** is the vector of DRPs from each country, **c** is the vector of country of evaluation fixed effects, **a** is the vector of random additive genetic effects in all participating countries, and **e** is the vector of residual effects. It is assumed that Var(**a**) = **G**o $\otimes$ **A** and Var(**e**) = **R**o$\otimes$**D**, where **G**o and **R**o are the genetic and residual matrices of (co)variances between countries, **A** is the pedigree-based relationship matrix and **D** is a diagonal matrix with diagonal elements corresponding to the inverse of ERCs. The matrices **X** and **Z** are incidence matrices that relate phenotypes to the corresponding effects.

Variance components and solutions for a random regression model were estimated using the software WOMBAT (Meyer, 2007) for French data and using the BLUPF90 software suite (Misztal et al., 2014) for the Dutch and the Spanish ones. Reliabilities for levels and slopes were obtained with MiXBLUP (ten Napel et al., 2021) in all countries. The meta-analysis was performed with the software BLUPF90.

Results & Discussion

For all level traits, both in thermo-neutral and under heat-stress conditions, heritabilities were mostly consistent with national estimates, although lower for Spain and the Netherlands. The heritability of SCS for France was surprisingly high (0.34). In thermo-neutral conditions, the genetic correlations between the three countries were high for all level traits, with values ranging from 0.89 and 0.97 (Table 1). These estimates for level traits were consistent with those estimated by Interbull in the MACE evaluations for Holstein breed (April 2023 MACE evaluation, www.interbull.org/ib/maceev_archive. Under heat-stress conditions, a slight decrease in estimated genetic correlations was observed, although they remained high with values between 0.81 and 0.97, and in agreement with the estimates in thermo-neutral conditions (Table 2). Therefore, we consider the approach used in this meta-analysis as a valid approach

for international genetic evaluations for heat tolerance traits.

For all milk production and SCS level traits, the genetic correlations estimated between the three countries were nonetheless high (above 0.8). These results suggest that countries with limited data under heat-stress conditions could benefit from the information available in Southern countries through international evaluations.

Table 1. Heritabilities (diagonal) and genetic correlations (off-diagonal) between countries for level traits under thermo-neutral conditions for milk production and SCS. Standard deviations are within brackets.

Trait	Country	FRA	SPA	NLD
MY	FRA	0.18 (<0.01)	0.96 (0.01)	0.94 (0.02)
	SPA		0.19 (<0.01)	0.92 (0.02)
	NLD			0.25 (0.02)
FY	FRA	0.25 (<0.01)	0.97 (0.01)	0.90 (0.02)
	SPA		0.13 (<0.01)	0.89 (0.02)
	NLD			0.21 (0.01)
PY	FRA	0.15 (<0.01)	0.96 (0.01)	0.89 (0.02)
	SPA		0.12 (<0.01)	0.90 (0.02)
	NLD			0.20 (0.01)
SCS	FRA	0.34 (0.01)	0.95 (0.02)	0.88 (0.02)
	SPA		0.08 (<0.01)	0.89 (0.03)
	NLD			0.15 (<0.01)

MY: milk yield; FY: fat yield; PY: protein yield; SCS: somatic cell score.
FRA: France; SPA: Spain; NLD: the Netherlands

Several hypotheses can be drawn regarding the drop of the estimated genetic correlations from thermo-neutral conditions to heat-stress conditions. First, from a physiological point of view, a hypothesis to explain this drop is that the impact of heat-stress on gene expression differ according to the environment. The differences between farming conditions in the three countries involved in this study support this hypothesis. A second hypothesis is that this drop is due to the lower accuracy of the national EBVs estimated under heat-stress conditions. A large part of the performances used in the estimation of variance components and EBVs were recorded under thermo-neutral conditions, leading to lower accuracies for the EBVs under heat-stress conditions. These two hypotheses are non-exclusive. However, the latter seems the most likely since the genetic correlations within country were close to 1 for all level traits.

Table 2. Heritabilities (diagonal) and genetic correlations (off-diagonal) between countries for level traits under heat-stress conditions for milk production and SCS. Standard deviations are within brackets.

Trait	Country	FRA	SPA	NLD
MY	FRA	0.18 (<0.01)	0.92 (0.01)	0.89 (0.02)
	SPA		0.25 (0.01)	0.86 (0.02)
	NLD			0.25 (0.02)
FY	FRA	0.25 (<0.01)	0.97 (0.02)	0.87 (0.03)
	SPA		0.13 (<0.01)	0.85 (0.04)
	NLD			0.20 (0.02)
PY	FRA	0.17 (<0.01)	0.89 (0.01)	0.81 (0.02)
	SPA		0.17 (<0.01)	0.83 (0.02)
	NLD			0.21 (0.01)
SCS	FRA	0.30 (<0.01)	0.96 (0.02)	0.88 (0.02)
	SPA		0.09 (<0.01)	0.88 (0.03)
	NLD			0.16 (<0.01)

MY: milk yield; FY: fat yield; PY: protein yield; SCS: somatic cell score.
FRA: France; SPA: Spain; NLD: the Netherlands

For slopes, genetic parameters and genetic correlations between countries could not be estimated accurately. The estimation of heritabilities for milk production and SCS slopes resulted in unreliable results, with values ranging from 0.04 for PY in the Netherlands to 0.93 for FY in France, and for SCS from 0.003 in the Netherlands to 0.90 in Spain. The estimated genetic correlations between countries were much lower than expected and, in some cases, even negative, with values between 0.04 and 0.53 for MY, -0.17 and 0.09 for FY, 0.03 and 0.30 for PY, and between -0.87 and 0.24 for SCS.

Additional analyses were performed to evaluate the impact of the THI chosen on the genetic parameters. Large variations were found in estimated variances at different THI within countries. For example, residual variance for MY expressed in $(kg/THI)^2$ increased from 0.9 at THI 60 to 6.9 at THI 70 with French data, and in the Netherlands from 4.9 at THI 68 to 8.6 at THI 70.

Several reasons could explain these difficulties in properly estimating the genetic variability of slope traits. The slopes were defined as the derivative of the Legendre polynomial curve at a given THI. Therefore, they might be sensitive to errors in the estimation of the rate of decline under heat-stress conditions. In addition, the heritabilities of the slopes could not be estimated. Their values were guessed but could be different from the true values. Furthermore, two main issues for the deregression may have ensued from the structure of our datasets. First, the hypothesis of a single-trait model could not be satisfied. Few sires had many daughters with at least one record measured under heat-stress conditions because of the low frequency of days above heat-stress and because of the infrequent (monthly) milk recording. Therefore, the estimations of the slopes relied on phenotypes recorded at lower THI and the EBVs of most sires were indirectly predicted from the levels at lower THI. Second, EBVs for slopes were associated with low accuracies, which may have adversely affected the quality of the deregression. All bulls included in the meta-analysis were required to have a minimum reliability of 25% and at least 20 daughters with performances, but these performances were not necessarily recorded under heat-stress, but these requirements were probably not sufficient for the slopes. Bohmanova et al., (2008) encountered similar difficulties in their comparison of heat-stress EBVs of sires evaluated in the North-East and South-East of the USA. EBVs were similar in both regions only for sires having many phenotyped daughters, with correlations between EBVs increasing from 0.58 for sires with at least 100 daughters to 0.81 for sires with at least 700 daughters.

Conclusion

In conclusion, high genetic correlations were obtained for the traits based on levels, and thus even at high THI. These results show the interest in developing international collaborations to evaluate heat tolerance in Holstein. Countries of Northern part of Europe, where few performances have been recorded under heat-stress conditions so far, could benefit from the information available in Southern countries and have access to reliable predictions under heat-stress conditions through international genetic evaluations.

However, the meta-analysis showed some limits for the slopes of decay. We were not able to capture the genetic (co)variability associated to these traits with our approach. Other approaches should be investigated to better measure the decline in performances due to heat-stress.

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Meteorological data were provided by Météo-France (Safran database, downloaded via the SICLIMA platform developed by AgroClim-INRAE) for France, by the National Meteorological Agency (AEMET) for Spain, and by the Koninklijk Nederlands Meteorologisch Instituut (KNMI) for the Netherlands.

Test-day records of French, Spanish and Dutch Holstein cows were extracted from the respective genetic evaluation data bases (BDNZ, France; CONAFE, Spain; and CRV, the Netherlands).

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Inbreeding Becomes A Serious Issue

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Abstract

Analyses of single nucleotide polymorphism (SNP) data of Holstein genotypes present at Anafibj has been done to verify trends in genetic variation over time. Results show a linear decline of SNP heterozygosity in the pre-genomics era from 1990-2010, and a 5-fold larger linear decline in the genomics era from 2010-2023. This is a clear signal that the increased annual genetic progress from genomic selection goes together with a strong decrease in genetic variation. Pedigree based inbreeding rates show Italy, USA and Canada having the highest annual inbreeding rates for Holsteins among countries. Strong market competition for artificial insemination (AI) centers results in the intense selection of a smaller number of elite animals. Emphasis on short-term genetic gain might be harmful for maintaining the long-term health and diversity of the breed. In Italy, research is underway to attempt to counter the harmful effects of decrease in genetic variation as well as genetic disorders.

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

Introduction

A closed population is a population in which there is no gene flow from other populations. This means that the only source of new genetic variation in the population are mutations. Breeding in any closed population will therefore gradually increase inbreeding and hence reduce genetic variation, unless sufficient mutations occur. Selecting a small number of sires and selecting candidates that are genetically very similar will strongly increase the rate of inbreeding. Inbreeding can lead to a number of problems, including reduced fertility and fitness, increased susceptibility to disease, and decreased genetic diversity. Since the advent of genomic selection, genetic variation is declining at a strongly increased rate, due to a stronger selection intensity and shorter generation intervals. When genetic variation is reduced, populations are less likely to be able to adapt to change and may become more vulnerable to extinction.

The Holstein breed worldwide is the leading dairy breed, but the genetic base is not as large as might seem from the number of animals. Less than 10.000 animals were imported from Europe in North-America before 1890. And few lines became the leading sire lines in the <1900 period (Neptune H and Hulleman), 1920-1940 (Rag Apple and Burke), 1960-1970 (Chief and Elevation). The latter 4 foundation sires all descent from the first 2. So, in reality there are just two male lines remaining (Yue et al., 2015). And effectively there have been multiple genetic bottlenecks.

Materials and Methods

The Anafibj genomic databank was used for analysing annual trends in genetic variation of Holstein SNP genotypes. After imputation, annual average SNP heterozygosity of 88068

SNPs from animals born from 1990-2023 was computed. Animals without pedigree were excluded as well as non-genotyped animals. The year 2010 was considered as the transition point between pre-genomic and genomic selection.

The average inbreeding coefficient within a year was computed as (homozygosity this year-homozygosity first year) / (1-homozygosity first year). Average generation intervals were computed per year for males and females separately and then averaged between sexes. The relative year since 1990 was divided by the annual generation interval to estimate how many generations would be passed since 1990 at the current generation interval. Using a linear fit on SNP heterozygosity, the inbreeding coefficient **F** was computed per year. The effective population size (**Ne**) was estimated as **Ne** = 1/(2-2*((1-**F**)^(1/(number of generations)))) (Frankham, Bradshaw and Brook, 2014).

Inbreeding depression was calculated for 305d milk yield including in a linear mixed model with milk yield as response variable and the inbreeding calculated based either on Pedigree data or on Runs-Of-Homozygosity (ROH) as regressor.

Results & Discussion

Results (Table 1) from the pedigree-based annual inbreeding rates, from word Holstein Frisian federation (WHFF) show that Italy (0.26), USA (0.26) and Canada (0.23) have the highest inbreeding rates, since genomic selection. This probably results from the strong competition between AI centers within the Intercontinental Consortium.

Results of the analysis of SNP heterozygosity by birth year are shown in Figure 1. Both the pre-genomic as well as the genomic era showed a decrease in annual average SNP heterozygosity. The 1990 SNP heterozygosity was 0.3561 after which the annual decline was -0.0004. The linear regression in the pre-genomics era shown in Figure 1 has an R^2 of 0.82. From 2010 onwards SNP heterozygosity was 0.3480 and the annual decline was -0.0020. The R^2 of the linear regression in this period was 0.97. So, results show a 5-fold increase in the decline of SNP heterozygosity. If the SNP heterozygosity is 0.32 and the (linear) annual decline is 0.0020, then after 0.32 / 0.0020 = 160 years we will be at a heterozygosity of 0.00.

Table 1. Countries with highest pedigree-based inbreeding rates per year as shown by WHFF

Country	2010-2019
ITA	0.26
USA	0.26
CAN	0.23
FIN	0.20
POL	0.20
ESP	0.20
CHE	0.19
SLO	0.18
NLD	0.16
FRA	0.15
DEU	0.15
IRL	0.15

In Figure 2, the average inbreeding coefficient, annual inbreeding rate and inbreeding rate per generation are shown per birth year. The change in 2010 is evident. Genomic selection had an enormous impact on all 3 variables.

Figure 3 illustrates the decline in the effective population size of the Holstein breed. Since genomic selection started the decline has been from nearly 90 to 60. Note that FAO considers an Ne value of 50 to be a critical threshold for the long-term survival of a breed. (FAO, 1988).

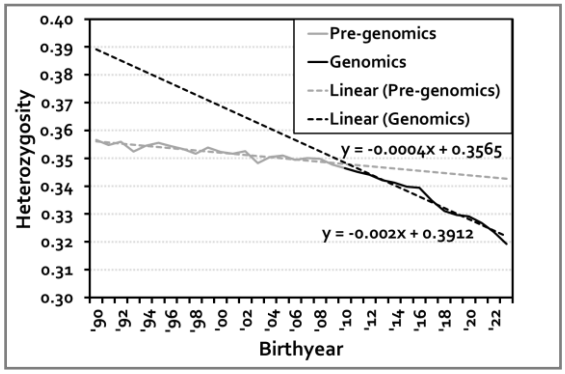

Figure 1. Pre- and post-genomic trends of SNP heterozygosity by birth year

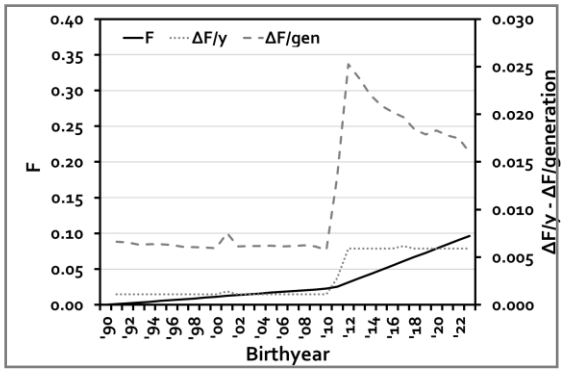

Figure 2. Inbreeding coefficient (F), annual inbreeding rate (ΔF/y) and inbreeding rate per generation (ΔF/gen) by birth year

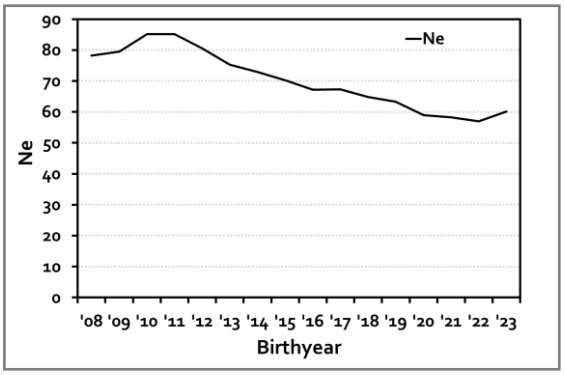

Figure 3. Decline of effective population size (Ne) during the most recent 16 birth years

In our ongoing study in Italian Holsteins, inbreeding depression resulted in declines of 44 kg and 61 kg of milk yield per % increase in pedigree and ROH inbreeding coefficients, respectively. Over the last 5 years, with a +2.35% change in inbreeding coefficient (ΔF) (Ablondi et al., 2021), this results in an inbreeding depression of 103 kg based on pedigree and 143 kg based on ROH. Considering realized genetic progress of 415 kg during this period, including the impact of inbreeding, progress without inbreeding might have been 518 kg (+103 kg based on pedigree) and 558 kg (+143 kg based on ROH). Hence 103 kg which means 20% was lost if estimated with pedigree, whereas 143 kg meaning 26% was lost based on ROH. In conclusion, the impact of inbreeding is substantial.

The decline of within breed genetic diversity, should trigger adjustments in the breeding programs to mediate harmful results. Breeders can take steps to maintain genetic diversity, such as using a wider range of bulls from different sires and dams and countries, and breeding for a variety of traits. This can help to prevent the over-use of a few elite bulls. However, this will require a change in mindsets, as breeders may need to sacrifice some short-term genetic gain in order to maintain the long-term health and diversity of the breed.

At Anafibj, research is underway to estimate genomic expected future inbreeding. Aim is to provide a premium to animals which have a lower relatedness to the expected future population, and a penalty to more related animals. Current focus is on ROH, which enables to focus on more recent inbreeding, which is considered more harmful. We aim to compute genomic expected future inbreeding coefficients, which is the probability in an autosomal segment that the haplotype transmitted from a random mate (of a reference population reflecting the future population) is identical to the transmitted haplotype of this individual, i.e. a ROH. In practice identical by state is used as if identical by descent. The importance is to lower the future inbreeding caused by the bulls rather than the own inbreeding of the bulls themselves, because own inbreeding does not pass to the descendants, given only half the chromosomes transmit to the offspring.

Conclusions

Analyses of SNP heterozygosity over time show a clear linear decline in the pre-genomics and genomics periods. A 5-fold increase in the decline of SNP-heterozygosity was found in the genomics era. It shows that the increased annual genetic progress comes at a cost of more rapid decline of genetic variation. Care has to be taken to avoid damage from the loss of genetic variation for future generations or

due to homozygosity for recessive genetic disorders.

Acknowledgments

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Approximate single step genomic prediction for Norwegian Red cattle

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Abstract

The exact single step Genomic Best Linear Unbiased Prediction (ssGBLUP) method has been used for breeding value estimation in the Geno breeding program since 2016. The number of animals with genotype information included in ssGBLUP has increased to over 210,000, making the exact inversion of the genomic relationship matrix computationally demanding. To address this, we tested two alternative approaches on ninety traits used for breeding value evaluation in the Norwegian Red cattle breed. The single step Algorithm for the Proven and Young Genomic Best Linear Unbiased Prediction (ssAPYGBLUP) approach consisted of a core dataset with 16,480 progeny-proven sires and sires of foreign origin, considering a 10% residual polygenic effect. The single step Singular Value Decomposition Genomic Best Linear Unbiased Prediction (ssSVDGBLUP) approach utilized genotypes from 5,186 progeny-proven sires, explaining 90% of genetic variation through chromosome-specific singular values. We compared estimates from these approximate methods to those from ssGBLUP for animals in the pedigree, and young genotyped animals for all the ninety traits. Correlations between ssGBLUP and ssAPYGBLUP estimates ranged from 0.976 to 1.000 for all the individuals in pedigree and from 0.940 to 0.995 for young genotyped individuals. For the ssSVDGBLUP and ssGBLUP approaches, correlations were between 0.971 and 1.000 for animals in the pedigree, and between 0.977 and 0.995 for young genotyped animals. When regressing ssGBLUP estimates to ssAPYGBLUP estimates, the linear regression coefficients were between 0.993 and 1.027 for all animals in the pedigree and between 1.005 and 1.061 for young genotyped animals. For the regression of ssGBLUP estimates to ssSVDGBLUP estimates, the linear regression coefficients were between 0.953 and 1.055 for all animals in the pedigree and between 0.866 and 0.949 for young genotyped animals. This means that predictions for young genotyped animals when using ssSVDGBLUP showed overestimation while predictions from ssAPYGBLUP were slightly underestimated.

Key words: single step genomic prediction, singular value decomposition, algorithm for proven and young, Norwegian Red cattle

Introduction

Single step genomic predictions (ssGBLUP) were implemented in routine evaluation for the estimation of genomic breeding values for Norwegian Red cattle in 2016 (Nordbø et al., 2019). In the beginning, there were about 18,000 genotypes used in the evaluation of genomic breeding values. With genotyping around 35,000 animals annually more than 210,000 genotypes were present in the middle of 2023.

The inverse of the combined pedigree and genomic relationship matrix is calculated prior to the estimation of breeding values and demands a lot of computer memory where the information is stored temporarily. The increase in the number of genotyped animals is increasing computer memory requirements quadratically. This becomes unsustainable in the long term and other solutions must be

applied. One possible solution is to remove genotype information. These could be either genotypes from animals without phenotypic information or genotypes from older animals. Increasing computer memory would be another possible solution but due to a quadratic increase in memory requirements with every genotype added this cannot be a long-term solution.

Application of approximate ssGBLUP methods eg. Algorithm for Proven and Young (ssAPYGBLUP) proposed by Misztal et al. (2014) or Singular Value Decomposition (ssSVDGBLUP) approach proposed by Ødegård et al. (2018) could represent a long-term solution when using single step genomic predictions approach on a large number of genotyped individuals. These approaches decrease computational requirements with approximations which explain only the most important part of genetic variation in the population. The difference between the ssAPYGBLUP and ssSVDGBLUP approaches is that the ssAPYGBLUP algorithm assumes that all genetic variation is explained by the additive genetic effects of the core individuals, while the ssSVDGBLUP approach assumes it is explained by haplotype blocks that segregate among core individuals (Ødegård et al., 2018).

The objective of the current study was to test the ssAPYGBLUP and ssSVDGBLUP approaches for the routine evaluation of breeding values for the Norwegian Red cattle and to compare them to the currently applied ssGBLUP method.

Materials and Methods

We used phenotypes, genotypes, and pedigree information from April 13, 2023, Geno routine evaluation. We estimated breeding values for ninety traits included in the twenty-nine single- or multi-trait mixed model equations. Genomic relationships were estimated with 206,496 genotypes imputed to the in-silico array with 121,740 SNPs and combined with the pedigree information into a single step genomic relationship.

The exact single step approach

The mixed model equations in the ssGBLUP approach combine pedigree and genomic relationship information stored in the matrix H (Christensen and Lund, 2010):

$$\begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{W} \\ \mathbf{W}'\mathbf{X} & \mathbf{W}'\mathbf{W} + \lambda\mathbf{H}^{-1} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{W}'\mathbf{y} \end{bmatrix}$$

where **X** and **W** are incidence matrices for the fixed and random effects, λ is a ratio between the error and additive genetic variances, vectors **b** and **a** are estimates for the fixed and random effects, and **y** is a vector of phenotypes. The inverse of the **H** relationship matrix calculated as:

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

where **A** is a pedigree relationship matrix, $\mathbf{G}_{\mathrm{w}}$ combines the genomic and pedigree information for genotyped animals with 10% of information coming from genotypes and 90% from pedigree, and $\mathbf{A}_{22}$ is the pedigree-based relationship matrix of the genotyped individuals. A fraction of $\mathbf{A}_{22}$ is added to **G** because the **G** matrix derived using the VanRaden 1 method is often singular while also explaining additive breeding values that cannot be described by the available markers (VanRaden, 2008).

Algorithm for Proven and Young

In the ssAPYGBLUP approach, animals are partitioned into proven (core) and young (non-core) individuals and only the inverse of genomic relationships between the animals in the core is inverted while the estimates from the non-core individuals are calculated recursively. After preliminary analysis where different core assemblies were compared the core used on all the traits contained 16,480 genotyped animals from sires with a

Norwegian herd book number, animals from foreign populations, and animals with sire from foreign populations.

Singular Value Decomposition based models

In the ssSVDGBLUP approach, core animals were used to approximate correlations between markers using the chromosome-specific singular values explaining 90% of genetic variation in the core individuals. Here the core was assembled of 5,186 genotyped sires with a Norwegian herd book number. This core definition was based on a preliminary analysis which showed that differences between various cores and the proportion of genetic variance explained were small when looking at the prediction accuracy and bias while achieving a significant decrease in computational time and memory requirements with a smaller core size and genetic variance explained.

Standardization of breeding values

The obtained estimated breeding values (EBV) from all the tree approaches were standardized (EBVs) using the following equation:

$$EBVs = 100 + k * (EBV-\overline{EBVc})/sd(EBVb))$$

where $\overline{EBVc}$ is the mean EBV of all cows born between April 13, 2015, and April 13, 2020, and sd(EBVb) is the standard deviation of the EBV from the progeny of proven bulls that were born between January 1, 2006, and December 31, 2013.

Results & Discussion

The correlations between the ssGBLUP and ssAPYGBLUP estimates ranged from 0.976 to 1.000 for all the individuals in the pedigree and from 0.940 to 0.995 for the young genotyped individuals. For the ssGBLUP and ssSVDGBLUP approaches, correlations were between 0.971 and 1.000 for animals in the pedigree, and between 0.977 and 0.995 for the young genotyped animals (Table 1).

Table 1: Mean, standard deviation (sd), minimum (min) and maximum (max) correlation between predictions from ssGBLUP and predictions from ssAPYGBLUP (APY) or ssSVDGBLUP (SVD) across ninety traits

	mean	sd	min	max
Individuals in the pedigree				
APY	0.998	0.003	0.976	1.000
SVD	0.997	0.003	0.971	1.000
Young genotyped individuals				
APY	0.983	0.013	0.940	0.995
SVD	0.990	0.004	0.977	0.995

The linear regression coefficients when regressing ssGBLUP estimates to the estimates from ssAPYGBLUP ranged from 0.993 to 1.027 for the individuals in the pedigree and from 1.005 to 1.061 for the young genotyped individuals. Linear regression coefficients when regressing ssGBLUP estimates to the estimates from ssSVDGBLUP ranged from 0.953 to 1.055 for the individuals in the pedigree and from 0.866 to 0.949 for the young genotyped individuals.

Table 2: Mean, standard deviation (sd), minimum (min) and maximum (max) linear regression coefficient when regressing predictions from ssGBLUP to predictions from ssAPYGBLUP (APY) or ssSVDGBLUP (SVD) across ninety traits

	mean	sd	min	max
Individuals in the pedigree				
APY	1.006	0.006	0.993	1.027
SVD	1.004	0.013	0.953	1.055
Young genotyped individuals				
APY	1.029	0.011	1.005	1.061
SVD	0.912	0.022	0.866	0.949

The intercept ranged from -2.929 to 0.928 for the individuals in the pedigree and from -5.848 to -0.397 for young genotyped individuals when regressing ssGBLUP estimates to the estimates from ssAPYGBLUP. When regressing ssGBLUP estimates to the estimates from ssSVDGBLUP, the linear regression coefficient ranged from -5.803 to 4.943 for the individuals in the pedigree and from 4.514 to 13.796 for the young genotyped individuals.

Table 3: Mean, standard deviation (sd), minimum (min) and maximum (max) intercept when regressing predictions from ssGBLUP to predictions from ssAPYGBLUP (APY) or ssSVDGBLUP (SVD) across ninety traits

	mean	sd	min	max
Individuals in the pedigree				
APY	-0.613	0.686	-2.929	0.928
SVD	-0.411	2.030	-5.803	4.943
Young genotyped individuals				
APY	-2.865	1.160	-5.848	-0.397
SVD	8.847	2.378	4.514	13.796

In comparison to the estimates from the ssAPYGBLUP approach, the estimates from the ssSVDGBLUP approach showed on average slightly higher correlation to the estimates from the ssGBLUP approach. This was the case when taking into account animals in the pedigree and even more when looking only at the young genotyped animals. The estimates for the young genotyped animals from the ssSVDGBLUP approach were overestimated in comparison to the estimates from the ssGBLUP approach for all the traits. Just the opposite, but to a smaller extent, was the case with the estimates from the ssAPYGBLUP approach.

The main reason for higher correlations, linear regression coefficients closer to 1 and intercept closer to 0 when analysing all the individuals in the pedigree vs. when analysing only the young genotyped animals is in the historical genetic progress. When analysing all individuals in the pedigree, genetic progress is taken into account into a much larger extent than when considering only the young genotyped animals. As selection candidates are the young genotyped individuals, it is more informative to consider only these animals when comparing different methods.

The computational time and memory requirements for the creation of $\mathbf{G}^{-1}$ were 24h 14min and 670GB, respectively, when using the ssGBLUP approach and 4h 21min and 111GB, respectively, when using the ssAPYGBLUP approach. The $\mathbf{T}_c$ matrix in the ssSVDGBLUP contained 43,917 components spread across 29 chromosomes and approximated the genotype matrix of the core individuals. Computation of $\mathbf{T}_c$ took 2h 3min and 82GB of memory.

Solving the mixed model equations across 29 single or multitrait models using the preconditioned conjugate gradient method took on average 21h 45min with the ssGBLUP approach, 2h 31min with the ssAPYGBLUP approach and 35h 6min with the ssSVDGBLUP approach. The computer memory requirements were low for all three approaches as the relationship matrices were not read into the computer memory during the iteration process.

Overall, this means that the ssAPYGBLUP approach was the fastest and used slightly more computer memory than the ssSVDGBLUP approach. On the other hand, the ssSVDGBLUP approach was slightly faster in comparison to the ssGBLUP approach and used around eight times less memory for the preprocessing of the relationship matrices than the ssGBLUP approach.

Conclusions

The two analysed approximate single step genomic prediction methods showed to be good alternatives to the exact single step genomic prediction method currently used in the Geno breeding program. Further validation studies are required to analyse if the bias observed in the young genotyped individuals is confirmed after animals are phenotyped. However, there are also other approximate single step genomic prediction approaches that need to be tested.

Acknowledgments

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Inclusion of MACE Proofs in Single-Step Genomic Analysis

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Abstract

The integration of multiple across country evaluation (MACE) proofs in single-step genomic analysis is important to provide the dairy industry with the best estimated breeding values (EBVs), especially in countries that import a major part of their genetics. The method developed earlier that uses Deregressed Proofs (DRP) that account for correlations between traits, but not relationships among MACE bulls was largely successful, but as we show here leads to large overestimations if MACE bulls are related. We developed an alternative approach that uses DRPs which take into account relationships among bulls, but still uses the old weights based on the assumption of no relationship. This proofed to be a better predictor of performance in Australia for bulls that had both a genotype and MACE proof partially based on their Australian daughters. An additional adjustment to account for that daughter information sent to Interbull proved ineffective, with the regression coefficient 0.82 in both cases. Bulls that were not expected to be affected by the singe-step procedure as they had no Australian daughters and no genotype, did in fact show large changes (regression coefficient 0.66), showing that the weights need to be in-line with the DRP estimation procedure.

Keywords: MACE, genomics, single-step

Introduction

DataGene delivers a single-step genomic evaluation for milk, fat and protein yield and Somatic Cell Count (SCC) for Red breeds, as described in Boerner et al (2022). This procedure uses deregressed MACE proofs as input data alongside test day observations for cows. The deregression method takes into account that MACE milk, fat and protein proofs are correlated and come from a multi-trait analysis in Australia and other countries. It assumes that MACE bulls are unrelated.

We observed an overestimation of breeding values for MACE bulls, especially for protein yield, and attributed this to the assumption of unrelatedness among them. This was initially resolved by setting parents of MACE bulls to missing, although this could not be done for bulls that had both local and overseas daughters. This approach no longer worked when we obtained genotypes on many MACE bulls – confirming relationships among them.

This paper describes how we have succeeded in replacing the deregression procedure with an alternative that takes into account relations among MACE bulls, though not correlations among different traits. It shows how this markedly improves EBVs for some animals but not for others.

Materials and Methods

Current Method

Our current method described by Boerner et al (2022) includes the following steps to create pseudo records and adjust the pedigree

1. Calculation of within animal residual variance
2. Adjustment of residual variance for bulls who had their EBV included in MACE (this will be referred to as 'sent')
3. Deregression of MACE proofs
4. Pedigree adjustment
5. MiX99 run

In the calculation of within animal residual variance a data point specific residual variance is modelled such that a within-animal multi-

trait mixed model equation system would yield reliabilities equal to those derived from Interbull reliabilities.

The pedigree adjustment consisted of replacing the sire and dam for a bull that had a MACE proof but no Australian daughters with a phantom group. Different phantom groups were used for sires and dams.

Alternative Method

A suit of programs tailored to the deregression of MACE proofs based on the deregression method of Jairath et al (1998) was kindly provided by Zenting Liu (VIT Germany). In here, deregress.f90 is the main program. It estimates deregressed proofs for all bulls with daughters in a MACE proof file using iteration on data and full sire-dam pedigree. A Gauss-Seidel algorithm is used to solve the equation system with pre-defined convergence criteria.

Deregressed proofs from this calculation were used to replace the DRPs calculated in step 3 above. Step 4. Pedigree adjustment was omitted.

The new DRP calculation gives one DRP per trait per animal, unlike the current procedure which calculates a DRP for each of the first 3 lactations, although they tended to be similar. We therefore tested two scenarios; one where a MACE bull only had an observation for the first lactations (the observation being the new DRP), or where it had the same observation for all three lactations.

Note that in this approach we do not make an adjustment of DRPs for bulls who had their EBV based on Australian daughters sent to Interbull (equivalent of step 2). As an alternative, we therefore further adjusted the new DRPs calculated above, by weighing them and DRPs calculated from the sent EBVs according to their Effective Daughter Contribution (EDC) as described by Pitkanen (2021).

Data

The impact of the alternative deregression method was investigated using the December 2022 Red Dairy Cattle (RDC) MACE proofs for milk, fat and protein yield. The EBVs that Australia contributed to this MACE run were based on data from the 25 October 2022, but from a special Australia-only conventional analysis (i.e. excluding MACE proofs and genomics). Breeding values from the 'current' method are those published on 6 December 2022.

The production file for RDC in December 2022 consists of 17081 bulls, of which 16721 are of breed RDC or Milking Shorthorn (MSH). Of these 836 had an Australian EBV included.

We identified 57 bulls with genomics and at least 100 test day observations per trait on their daughters and whose EBV was sent to Interbull. These 57 serve as the main validation group, and in various scenarios we remove their daughter observations, their MACE proofs or both from the data to ascertain how well the analysis predicts the Australian performance.

A second validation set consisted of the 15 556 bulls that had neither Australian daughters nor a genotype in Australia. The expectation was that a correct procedure would return EBVs and reliabilities from the single-step genomic analysis that are essentially the same as their MACE proofs and reliabilities.

Results & Discussion

As the original issue mainly showed for protein yield, most results presented below are for protein. Results for milk and fat yield are in line with these.

Figure 1, shows how various datasets predict the Australian-only conventional breeding value for 57 validation bulls for protein using the current procedure for deregression, but with full pedigree. Datasets with only genotypes but no MACE and with both genotypes and MACE perform reasonably well with slopes of 0.75 and

0.64 respectively. A dataset that includes MACE proofs but no genotypes however has a slope of only 0.35, indicating a large overestimation of true performance in Australia. Note the inverse relationship between slope and R^2.

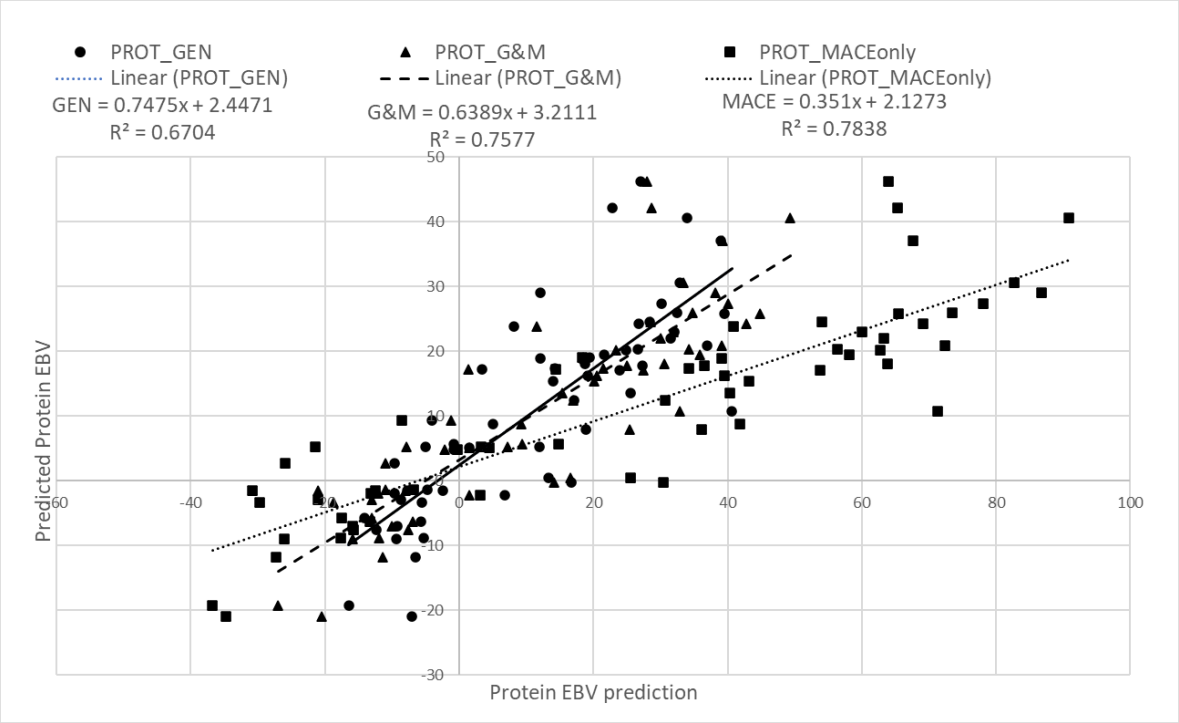

Figure 1. Australian-only Protein EBV predicted for validation bulls using the current procedure for deregression, but with full pedigree (Boerner et al. 2022), comparing predictions based on genomics only (PROT_GEN), MACE only (PROT_MACEonly) and the combination (PROT_G&M).

Figure 2 compares the Genomics & MACE from Figure 1 with the alternatives using the same data but with the new DRP calculation (referred to as ZT) and the additional adjustment for sent EBVs (referred to as ST). The ZT version is the one with the same DRP for each of 3 lactations, rather than the one with only a DRP for lactation 1, which performed slightly less. The ST version is based on 3 lactations as well. Note that ZT is not visible as ZT and ST are virtually identical for this group of bulls.

The alternative DRP calculations clearly give superior results, both in terms of slope (0.82) and R^2.

The effect of the new deregression method on the prediction bias in bulls with MACE proofs was analysed by comparing the MACE proof with the single-step breeding values for the 836 bulls that were sent to Interbull.

The adjustment for 'sent' EBV was specially meant for this group of bulls but it had minimal effect, with slopes being the same with and without the adjustment (0.93) and still showing some bias. R^2 was 0.981 for both DRPs. This may be because the adjustment is designed for a single-trait analysis, not a multi-trait.

The second validation set which had no Australian daughters and no genotype included in the analysis, showed large overestimations of protein EBVs for both the ZT and ST method when the single-step genomic EBV was regressed on the MACE proof; regression coefficients of 0.66 for EBV and 0.77 for reliability. The reason for this is most likely that while the MACE deregression accounted for relationships, the error variance did not and thereby put too much weight on the DRPs.

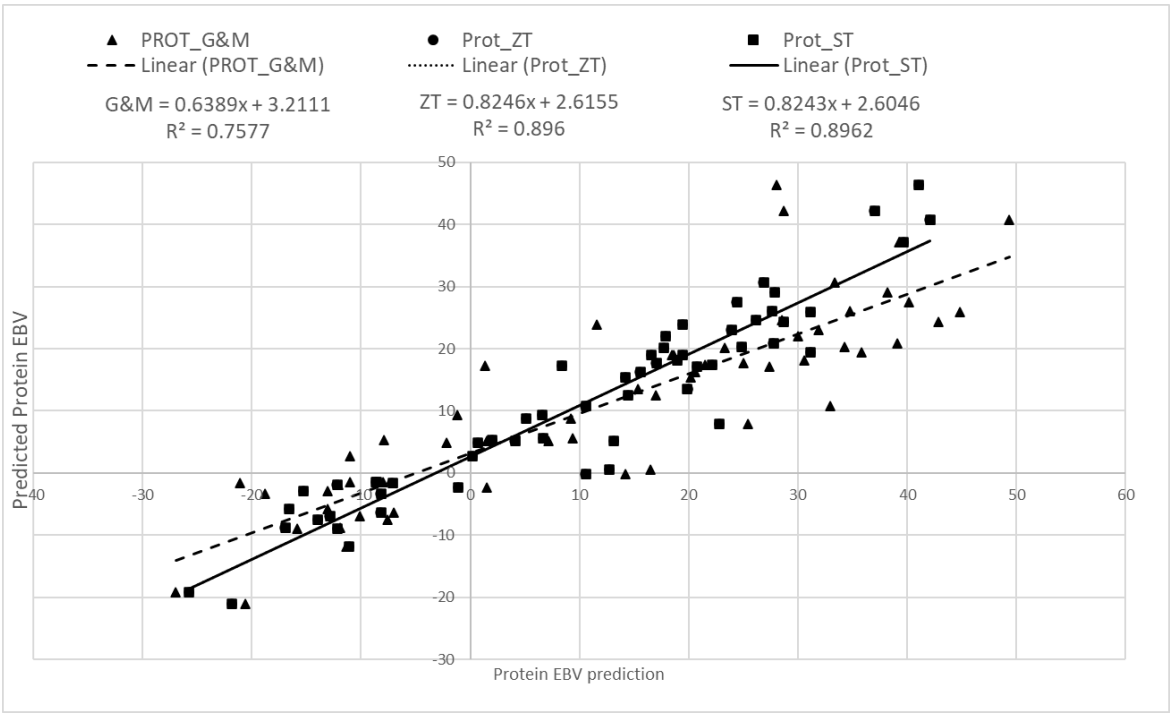

Figure 2. Australian-only Protein EBV predicted for validation bulls using different methods for deregression. a) Boerner et al 2022 (Prot_G&M); b) Zengting Liu's method (Prot_ZT), c) Deregressed using Zengting Liu's method followed by an adjustment using Pitkanen (2021, Prot_ST). Note that the last two overlap completely in the figure.

Conclusions

We have taken a pragmatic approach to try and remove a bias from single-step genomic breeding values that include MACE. We replaced the DRPs from a procedure that ignores relationships among MACE bulls with one that does, but in the process ignored lactation specific DRPs and maintained the weightings as calculated for the old procedure.

The bias in prediction of breeding values was considerably reduced, with the regression coefficient increasing from 0.64 to 0.82

An adjustment was made to the DRPs for animals who had their EBVs included in MACE. This proved to have minimal if any effect on breeding values from the genomic analysis and no effects on reliabilities at all. It appears the 'old' weights took care of this. For bulls that had no Australian daughters and no genotype included in the analysis, EBVs from the single-step genomic analysis grossly overestimated MACE proofs, while they were expected to be similar.

Acknowledgments

The authors wish to thank Zengting Liu from VIT (Germany) for providing the deregression software and assistance with implementing it, as well as Mekonnen Haile-Mariam from Agriculture Victoria (Australia) for assisting with the latter. Support from Timo Pitkanen (LUKE Finland) for providing the correction procedure for the sent EBV is also acknowledged.

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Evaluating the effect of ssGBLUP on a composite beef cattle population with limited pedigree completeness

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Abstract

To improve the accuracy of estimated breeding values (EBV), correct parentage assignment remains a cornerstone of BLUP. Genomic evaluations can alleviate constraints experienced during the assessment of young animals in large populations, especially for animals with limited pedigree depth and for traits of low heritability. South African (SA) Beefmaster (BMA) breeders mostly are prone to using multiple sires in their herd, with a low parentage verification rate resulting in a larger proportion of young animals with at least one unknown parent. Upgrading of first acceptance cows with blank pedigrees, was common in the establishment of the SA BMA breed. The completeness of a 451,009 animal pedigree, consisting of 187,448 males and 263,561 females dating back to 1937 was assessed. Records for birth weight (BW) and adjusted weights at 205, 365 and 540 days of age (WW, YW, M18W) were collated for the growth multi-trait model, while the fertility multi-trait model included records for adjusted weight at 205 days of age (WW), heifer fertility (HF) and the first three inter-calving periods (ICP). Breeding values and trait reliabilities for registered animals, were either estimated traditionally (BLUP) or with the inclusion of genomic information (ssGBLUP). Genomic profiles of 1,397 recorded animals, genotyped across five commercial single nucleotide polymorphism (SNP) arrays of varying densities, were imputed to a reference genotype of ~132,000 SNPs. Animals with varying proportions of known ancestry allowed for a comparison of genotyped animals across the herd book status of upgrading. The assessment of pedigree completeness indicated a substantial decay in pedigree depth, higher in females compared to males, after the grand-parent generational equivalent. The ssGBLUP accuracies were higher across all traits (0.01 – 0.89), with equal increases observed for animals with limited pedigree depth (only 1 or 2 generations) as to young animals with minimal to no measured phenotypes. The change between conventional and genomic breeding values decreased as the depth of pedigree increased. The results obtained indicate the knowledge of genetic relationships through ssGBLUP allow for increased reliability of predictions for foundation animals with limited or unknown pedigree structure.

Key Words: pedigree completeness, genetic evaluation, single-step GBLUP, multiple sires

Introduction

To improve the accuracy of estimated breeding values (EBV), correct parentage assignment remains a cornerstone of BLUP. Genomically enhanced breeding values (GEBVs) are increasingly being used to predict values for all animals in the pedigree using single step mixed model equations (MMEs) (Legarra et al. 2014). GEBVs are calculated using a genomic relationship matrix (GRM) in conjunction with MMEs (Taskinen et al. 2013). Genomic evaluations can alleviate constraints experienced during the assessment of young animals in large populations, especially for animals with limited pedigree depth (Clark et al. 2012; Gowane et al. 2022) and for traits of low heritability (Hayes and Goddard 2010; Kluska et al. 2018).

The South African (SA) Beefmaster (BMA) was established through the importing of live semen and live animals from Lasater's herd and purebred herds associated with the Beefmaster Breeders United (BBU) (Beefmaster SA

Website). The SA BMA was ratified as an established breed in 1987 and is currently the second largest stud beef cattle breed being serviced by the SA Stud Book and Animal Improvement Association (SASB). SA BMA breeders are distributed throughout the country, utilise a mix of extensive farming in conjunction with available crop fodder or crop residues, with average herd sizes of around 450 animals and commonly use multiple sires on their cow herds. The SA BMA has a low parentage verification rate, resulting in a larger proportion of young animals with at least one unknown parent. Breeders from a commercial background were prone to upgrading first acceptance (FA) and Section A cows with blank pedigrees alongside Stud Proper (SP) BMA bulls when establishing their SA BMA herd. These cows will also lack production and fertility related measurements themselves as they can come into the herd at any age. Progeny of Section A cows mated with SP, Section C or Section B bulls are allocated Section B herd book status. SP progeny can only arise from Section C or SP parents. The use of multiple sires introduces a high percentage of Section B calves that have an unknown sire pedigree coupled with the upgrading of cows with poor pedigree depth results in lower accuracies when predicting the genetic merit of these animals (Clark et al. 2012; Gowane et al. 2022).

The objectives of this study were to firstly assess the level of pedigree completeness across the levels of upgrading in the SA BMA and to identify any changes in breeding value estimation and accuracy of measured growth and fertility traits when using genomic data on a breed with limited pedigree completeness.

Materials and Methods

Data

The phenotypic data were acquired from the LOGIX Genetic Evaluation System (SA Stud Book / SA Stamboek). Records for birth weight (BW) and adjusted weights at 205, 365 and 540 days of age (WW, YW, M18W) as well as fertility records for heifer fertility (HF) and the first three inter-calving periods (ICP1, 2 and 3), are summarised in Table 1.

Table 1. Total number of weight and fertility records for birth weight (BW), weaning weight (WW), yearling weight (YW), weight at 18 months (M18W), heifer fertility (HF) and the first three inter-calving periods (ICPs).

Trait	Number of Male Records	Number of Female Records	Total Number of Records
BW	146,501	143,522	290,023
WW	132,022	135,323	267,345
YW	41,750	77,299	119,049
M18W	29,804	54,801	84,605
HF	-	68,089	68,089
ICP1	-	46,795	46,795
ICP2	-	33,078	33,078
ICP3	-	23,821	23,821

Pedigree information on 451,009 animals, consisting of 187,448 males and 263,561 females dating back to 01 September 1937 including the phenotypic data and herd book upgrading status, is reported in Table 2.

Table 2. Pedigree information on the South African Beefmaster based on by herd book upgrading status.

Herd Book Population	Number of Males	Number of Females	Total
Total	187,448	263,561	451,009
Stud Proper	32,339	32,149	64,488
Section C	38,728	38,316	77,044
Section B	99,836	108,575	208,411
Section A	3,281	67,971	71,252
FA	0	9,511	9,511
Pending	1,718	1,624	3,342
NFR	11,347	5,140	16,487

FA: first acceptance; NFR: not for registration.

Genomic profiles of 1,797 SA BMA animals, genotyped across five commercial single nucleotide polymorphism (SNP) arrays of varying densities, were used in this study. Much of the genomic population was initially genotyped on the GeneSeek Genomic Profiler (GGP) 150K or GGP 80K primarily through funding from the SA Beef Genomics Project (BGP). After the BGP ended in 2018, genotyping was done on commercial variants of the Illumina BovineSNP50 v.3; namely the ICBF IDB v.2, SASB 50K or the Versa 50K.

Quality control of genomic SNP data, done

in PLINK v1.9 (Purcell et al. 2007), consisted of keeping only autosomal SNPs with a known base pair position, a call rate≥0.90, a MAF≥0.10 and did not significantly deviate from Hardy-Weinberg equilibrium (p>0.001). Animals required a call rate ≥90% while individuals with ≥0.95 identical genotype were discarded. Population stratification of the post-QC genomic data allowed for the possible detection of outliers and returned a final set of 1,397 SA BMA genotypes. Genotypes were imputed alongside pedigree information with FImpute v3 (Sargolzaei et al. 2014) to a density of ~130,000 SNPs.

Models

Using R version 4.2.3 (RStudio Team 2015), the optiSel R package (Wellmann, 2019) was utilized in conjunction with Poprep (Groeneveld et al. 2009), to assess the complete generation equivalent (CGE), pedigree completeness index (PCI) and F_{PED} coefficients (Meuwissen and Luo 1992) of the total and genotyped BMA populations. The total pedigree consists of all 451,009 animals in the BMA pedigree, while the fully traced back genotyped pedigree contains 7,630 animals (1,974 males and 5,683 females) related to the core 1,397 animals in the genomic population. Grouping for the calculation of the mean (standard error) of CGE, PCI and F_{PED} occurred at a whole population level, sex level, genotyped pedigree level and herd book allocation in order to compare across levels of upgrading.

In order to predict estimated breeding values, two multi-trait animal linear models were assessed. The growth and fertility models were defined as follows:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Zu} + \boldsymbol{e},$$

where **y** is the vector of phenotypes, **b** is a vector of fixed effects, **u** is a vector representing the direct additive-genetic effects, with **u** ~ N(0,A σ_u^2), where A is the pedigree-based matrix and σ_u^2 is the direct-genetic variance, **e** represents the residual, where e ~ N(0,I σ_e^2), with σ_e^2 representing the residual variance, **I** the identity matrix while **X** and **Z** are incidence matrices for **b** and **u** respectively.

Fixed effects in **b** for the growth trait model were herd x year x season x treatment group x birth status, sex, age, dam parity (1 or >1) and linear (α) and quadratic (α^2) regression coefficients for age of dam. Fixed effects in **b** for the fertility trait model were herd x year x season x treatment group x birth status for WW which was used as an anchor trait, herd x year x season for HF and herd x year x season x previous calving group for each ICP.

Estimation of variance components for the two animal models stated above were calculated using restricted estimated maximised likelihood (REML) optimised with quasi-Newton procedure using analytical gradients in Variance Component Estimation (VCE) (Groeneveld, 2010) software. MiX99 (MiX99 Development Team, 2017) was used to predict both traditional EBVs and GEBVs using the same models in the estimation of variance components. The ssGBLUP model utilises the inverse of the joint relationship matrix $\mathbf{H^{-1}}$ (Aguilar et al. 2010; Legarra et al. 2014).

$$\boldsymbol{H}^{-1} = \boldsymbol{A}^{-1} + \begin{pmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{G}^{-1} - \boldsymbol{A}_{22}^{-1} \end{pmatrix},$$

where $\mathbf{A}^{-1}$ is the inverse of the pedigree-based matrix, $\mathbf{A}_{22}$ is the overlapping part of A for the genotyped animals and **G** is the genomic relationship matrix (GRM). The GRM was constructed among all animals using the RelaX2 HGInv program (Strandén, 2014).

Pedigree-based and genomic reliabilities were calculated utilising the program ApaX99 (Lidauer et al. 2017) implementing the Misztal and Wiggans approach (Misztal and Wiggans 1988), where the Misztal approximation method 1 (Misztal et al. 2013) accounts for full genomic information. These reliabilities were subsequently transformed into accuracies.

Results and Discussion

At a population level, 33.9% of SA BMA animals in the pedigree are demarcated as “Sire

Unknown", with a further 16.7% of animals having "Both Parents Unknown". The mean, interquartile range (IQR), and median years of birth for the whole BMA population was 2008, 1994 to 2009 and 2011, respectively and 2001, 1994 to 2009 and 2003 for the genotyped BMA population. A slightly higher pedigree depth of 16 generations for the whole BMA population was noted against the genotyped BMA populations pedigree depth of 15 generations. Assessment of pedigree depth indicated a mean pedigree completeness index (PCI) and mean complete generational equivalent (CGE) of 0.298 (SE = 0.347) and 1.975 (SE = 1.720) for the whole BMA population and 0.381 (SE = 0.350) and 2.067 (SE = 1.753) for the genotyped BMA population. Table 3 indicates the mean pedigree completeness of the genotyped pedigree to be higher than that of the whole pedigree born. Females are observed to have a shallower pedigree completeness, as SA BMA breeding bulls must have known parentage in order to upgrade cows with limited pedigree completeness.

Table 3. The mean six-generation deep pedigree completeness of the SA Beefmaster for A) the whole pedigree (451,009 animals) and B) the genotyped pedigree (7,630 animals) born within the period 2011 to 2021 and split on a sex level

	Whole		Genotyped	
GD	Male	Female	Male	Female
1	1	1	1	1
2	0.792	0.568	0.886	0.614
3	0.518	0.366	0.677	0.423
4	0.365	0.257	0.491	0.293
5	0.263	0.183	0.349	0.192
6	0.184	0.128	0.239	0.117

GD: generation depth.

The inbreeding coefficients (F_{PED}) observed ranged from 0 to 0.2995 with a mean of 0.007 for both the whole and genotyped BMA population. The CGE and PCI were seen to be lower in the whole BMA pedigree (1.975 and 0.298) in comparison to the genotyped BMA pedigree (2.067 and 0.381). Genotyped Stud Proper animals had the highest F_{PED} (0.021), CGE (4.466) and PCI (0.859), across all the levels of upgrading, with genotyped Section A animals having the lowest CGE (0.470) and PCI (0.056), respectively (Table 4).

Table 4. Pedigree statistics including the birth year range, mean (μ) and standard error (SE) for various groupings of the South African Beefmaster population which include level of inbreeding (F_{PED}), pedigree completeness index (PCI) and complete generational equivalents (CGE)

Group	Birth Year Range	F_{PED}	PCI μ (SE)	CGE μ (SE)
WP	1937–2021	0.007	0.298 (0.35)	1.975 (1.72)
GP	1956–2021	0.007	0.381 (0.35)	2.067 (1.75)
GA	1985–2021	0.012	0.603 (0.32)	3.148 (1.58)
GSP	1999–2021	0.021	0.859 (0.13)	4.466 (0.92)
GSC	1994-2021	0.012	0.660 (0.17)	3.445 (0.95)
GSB	1985-2021	0.001	0.241 (0.24)	2.095 (1.19)
GSA	1997-2013	0.002	0.056 (0.20)	0.470 (1.16)

WP: whole pedigree; GP: genotyped pedigree; GA: genotyped animals; GSP: genotyped stud proper animals; GSC: genotyped section C animals; GSB: genotyped section B animals; GSA: genotyped section A animals.

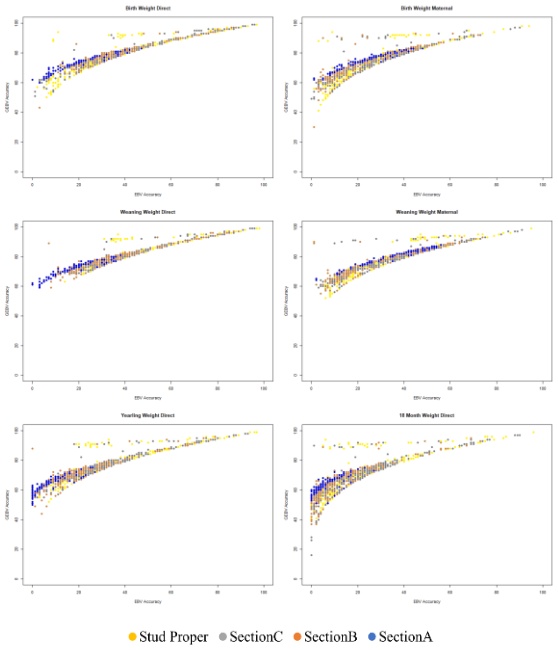
The generated solutions of the genotyped animals were extracted and compared for the various traits in the growth and fertility models. Observed coefficients of determination (R^2), between the EBVs or accuracies and their corresponding genomically enhanced solutions were lowest for Section A animals across all directly measured traits (Table 5). The biggest differences were observed for maternal traits, especially the WW_{MAT} of the genotyped SP animals ($R^2 = 0.888$), and the ICP1 ($R^2 = 0.843$) and ICP3 ($R^2 = 0.861$) of the total genotyped population.

Trait reliabilities were transformed into accuracies and plotted against their genomically enhanced counterparts. Animals were identified according to the herd book level of upgrading and compared accordingly. Increases in accuracy (0.01 – 0.89) when using genomic information were seen across all growth traits (Figures 1-6).

Table 5. The coefficient of determination (R^2) between estimated breeding values and genomically enhanced breeding values derived from the growth and fertility models for the South African Beefmaster population.

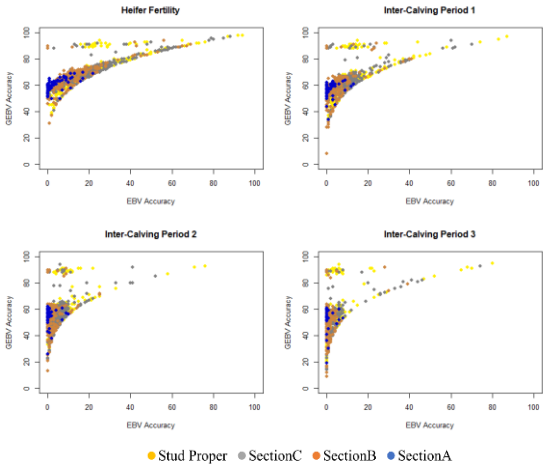
Trait	R^2				
	GA	GSP	GSC	GSB	GSA
BW_{DIR}	0.914	0.898	0.915	0.942	0.843
BW_{MAT}	0.869	0.858	0.843	0.904	0.849
WW_{DIR}	0.929	0.907	0.940	0.952	0.728
WW_{MAT}	0.907	0.888	0.907	0.930	0.928
YW	0.929	0.917	0.941	0.946	0.668
M18W	0.928	0.917	0.940	0.944	0.672
HF	0.896	0.882	0.899	0.917	0.874
ICP1	0.843	0.819	0.852	0.904	0.734
ICP2	0.879	0.842	0.898	0.929	0.844
ICP3	0.861	0.857	0.849	0.910	0.699

GA: genotyped animals; GSP: genotyped stud proper animals; GSC: genotyped section C animals; GSB: genotyped section B animals; GSA: genotyped section A animals.



Figures 1-6: The direct estimated breeding value (EBV) plotted against the direct genomically enhanced breeding value (GEBV) accuracy for the traits included in the growth model.

Higher average increases of 10% in accuracy were observed for traits included in the fertility model (Figures 7-10) in comparison to growth traits. The traits of low heritability ICP2 (0.13-0.90) and ICP3 (0.09-0.90) having the highest observed increases in accuracy.



Figures 7-10: The direct estimated breeding value (EBV) plotted against the direct genomically enhanced breeding value (GEBV) accuracy for the traits included in the fertility model.

The SA BMA had an estimated pedigree CGE of 1.975, which is similar to the CGE that were observed in local indigenous beef breeds such as the Afrikaner (2.81; Pienaar et al. 2018) and the Bonsmara (2.19; Santana et al. 2012). (Gutiérrez et al. 2003) observed low CGEs, ranging from 0.81 to 2.97, in eight Spanish beef cattle breeds, while a low CGE of 1.79 was observed in Istrian cattle (Ivanković et al. 2022). In comparison to pure and composite beef and dairy breeds with robust pedigree records such as the Lidia cattle (5.5; Cortés et al. 2019), Marchigiana cattle (4.52; Santana et al. 2012), Mexican Charolais cattle (7.86; Ríos-Utrera et al. 2021), American Brangus (6.8; Paim et al. 2020) as well as the SA Ayrshire (9.74), SA Holstein (11.70), and the SA Jersey (10.05) populations studied by Visser et al. (2023), the SA BMA showed a substantially lower mean CGE. This can firstly be attributed to the prevalent use of multiple sires in herds with low parentage verification rate, increasing the number of Section B animals with at least one unknown parent. Secondly, the upgrading process introduces foundation cows (first acceptance and Section A) with limited to no pedigree information, further contributing to a shallow pedigree depth. Stud Proper and Section C animals were observed to have a higher average CGE and PCI in comparison to Section A and B animals, which is a

consequence of these animals requiring established pedigrees through known parentage.

The low inbreeding estimates (F_{PED} = 0.007) calculated in the SA BMA, in comparison to the SA Ayrshire (F_{PED}=0.051), Holstein (F_{PED}=0.064) and Jersey (F_{PED}=0.062) populations (Visser et al. 2023) and Lidia cattle (F_{PED} = 0.13; Cortés et al. 2019), indicate an inaccurate reflection of inbreeding at a population level, that can be attributed to the observed low pedigree completeness in the SA BMA (PCI = 0.298). Similar results in other smaller populations such as Afrikaner (F_{PED} = 0.0183; Pienaar et al. 2018) the Creole Blanco Orejinegro breed (F_{PED} = 0.0132; Gallego et al. 2020), Argentinian Brangus (F_{PED} = 0.0240; Garrido et al. 2008), the SA Brangus (F_{PED} = 0.0139; Steyn et al. 2012), Bonsmara (F_{PED} = 0.0026) and Marchigiana (F_{PED} = 0.0133) (Santana et al. 2012), and F_{PED} of eight Spanish beef cattle breeds ranging from 0.0025 to 0.0313 (Gutiérrez et al. 2003) have been previously reported. These breeds have either a small population size and/or poor pedigree depth due to the behaviour of pedigree recording on a breed level, which are the two primary contributing factors to lower estimates of inbreeding (Nielsen and Slatkin 2013).

The observed changes in breeding values when including genomic information occurred at multiple levels. At a population level, the traits where genomics had the highest influence were BW_{MAT} (R^2 = 0.869) and WW_{MAT} (R^2 = 0.907) for the growth model, and ICP1 (R^2 = 0.843) and ICP3 (R^2 = 0.861) in the fertility model. Maternal traits are well-known to be lowly heritable (Olasege et al. 2021; Saatchi et al. 2012) and the accuracy of these traits traditionally increase as an animal's progeny-performance records increase. Fertility traits are sex-limited and measured later in animals' life which contributes to the lower prediction accuracies and heritability's estimated in these multi-trait models (Facy et al. 2023; Hayes et al. 2019). Progeny-performance records coupled with pedigree linkages act as a feedback mechanism that enable a more accurate prediction of a bull's or cow's genetic potential. At a herd book level, Section A animals experienced the greatest observed changes in EBV, especially as Section A animals may have no growth or fertility performance records if they are foundation cows. Interestingly, the change in WW_{MAT} for Section A animals (R^2 = 0.928) is an outlier of the previous statement and is a consequence of these foundation cow's progeny calves and great-progeny calves being measured for WW. On an individual basis, animals that can be seen as a separate bubble in Figures 1 to 10, the greatest changes were observed in Section B, C and Stud Proper bulls that were used as multiple sires but were never allocated to progeny on a known parentage basis. This resulted in these bulls never being allocated progeny-performance records. Although these multiple sires may not be linked to the broader SA BMA population through the pedigree, they are well-represented on a genetic basis through the genomic population with other genotyped animals with numerous progeny-performance records. Young Section C and Stud Proper animals also experienced similar increases in prediction accuracy and was observed to be for traits that they had yet to be measured for, were sex-limited and lowly heritable.

The assessment of pedigree completeness indicated a substantial decay in pedigree depth, higher in females compared to males, after the grand-parent generational equivalent. The ssGBLUP accuracies were higher across all traits, with equal increases observed for animals with limited pedigree depth as to young animals with minimal to no measured phenotypes. The change between conventional and genomic breeding values decreased as the depth of pedigree increased.

Conclusions

The results obtained indicate the knowledge of genetic relationships through ssGBLUP allow for increased reliability of predictions for foundation animals with limited or unknown pedigree structure.

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Investigation On the Metafounder Concept in ssGBLUP Based On a Simulated Cattle Population

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Abstract

Single-step genomic best linear unbiased prediction (ssGBLUP) has become a popular tool for genetic evaluations in dairy cattle populations. The use of the metafounder (MF) concept allows better consideration of relationships within and between founder populations and ensures correct matching of pedigree and genomic relationships. This study investigates the use of the MF concept in a simulated dairy cattle population where the base population consists of two related and inbred founder populations. The objectives are to compare genetic evaluations with and without MF and to investigate different methods of estimating MF parameters (Γ).Results show that genetic evaluations using MF are less biased and less inflated compared to evaluations using unknown parent groups or not accounting for the different founder populations. However, testing different methods to estimate Γ revealed a tendency to overestimate the relationships within and between the founder populations, leading to an overestimation of pedigree relationships compared to the genomic relationships. In summary, the MF concept in ssGBLUP is superior in this simulated scenario with two founder populations, but care must be taken when estimating Γ to ensure consistency between pedigree and genomic relationships. In general, these findings highlight the importance of considering relationships within and between founder populations in single-step genetic evaluations.

Key words: ssGBLUP, metafounder, simulation, dairy cattle

Introduction

Single-step genomic best linear unbiased prediction (ssGBLUP) uses an integrated relationship matrix (H), which combines the pedigree based relationship matrix (A) and the genomic relationship matrix (G). For this purpose, both matrices are supposed to refer to the same base population (Christensen, 2012). Without dedicated measures, this is usually not the case in cattle populations. In practice, there are several methods to match G to A (Christensen, 2012; VanRaden, 2008; Vitezica et al., 2011). Legarra et al. (2015) published the concept of metafounders (MF), which follows the idea of adapting A to G. The basic ideas are to use allele frequencies equal to 0.5 for all SNPs in the calculation of G and to assign unknown parents in the pedigree to pseudo-individuals (metafounder, MF).

Thompson (1979) and Quaas (1988) introduced the concept of unknown parent groups (UPG), which account for genetic differences within subgroups in the base populations. Since then, UPG, also known as genetic groups or phantom parents, are widely used in animal breeding, because they allow incorporating animals with missing parents and diverse genetic background in the genetic evaluation. UPG may therefore have means different from zero, but are assumed to be non-inbred and unrelated, just as the base population. MF may be seen as an extension to this concept by introducing relationships within and across UPG (Legarra et al., 2015).

For the German-Austrian-Czech Fleckvieh population, the first genomic evaluation using the ssGBLUP approach was published in April 2021 (Himmelbauer et al., 2021). To account for unknown parents, 15 UPG are presently

used for most of the fitness traits. MF is the current gold standard for ssGBLUP implementations as shown e.g. by Meyer et al. (2018) and will therefore likely be the next evolution step in the national genomic evaluation system. For reasons discussed above, the aim of this study is to test different methods for gamma estimation and to compare the difference between different genetic evaluations with and without MF for a very simple population structure with two base populations and without any unknown pedigrees.

Materials and Methods

Simulating metafounders

The basic approach for simulating the population is the same as that used and described in detail in Himmelbauer et al. (2023). The main difference, however, is that for this study not only one but two related and inbred base populations (MF) are simulated. To achieve this, the founder population is split after 2 500 generations of evolution. Both subpopulations are then selected for additional 15 generations based on the true breeding value (TBV) for trait 1, with subpopulation A selected for high and subpopulation B selected for low values of trait 1. The two subpopulations are then merged again, and a second trait (trait 2) is created with a heritability of 0.3 and a genetic correlation to trait 1 between 0.3 and 0.5. This is followed by 30 years of selection by pedigree BLUP (PBLUP) and 8 years of selection by ssGBLUP (ignoring the two separated base populations) based on trait 2 as described in Himmelbauer et al. (2023) with small adaptions: To ensure that at the end of the selection process phenotypes and genotypes of both purebred populations (A and B) and the crossbred population (AB) are available, animals are selected separately by subpopulation. Mating is controlled such that females from subpopulations A, B, and AB are mated with males from purebred populations A and B in a way that each possible combination of male and female subpopulations occurs with the same frequency in each simulated year. The schematic overview of the simulation approach is shown in Figure 1.

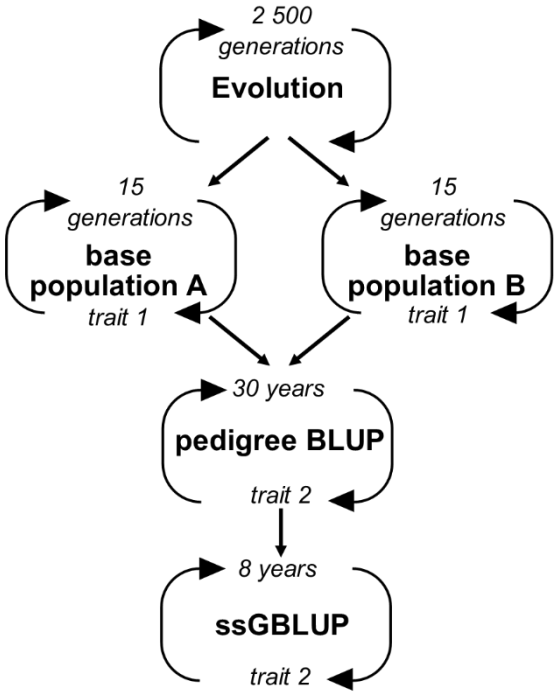

Figure 1. Schematic overview of simulation.

Dataset

The data set from the last year of the simulation serves as input for all further test runs. Basically, all females with offspring have a phenotype in the simulation. In routine datasets, phenotypes are usually not available back to the pedigree base, therefore 90% of the phenotypes from animals of the first 15 generations were randomly deleted. The final dataset consists of about 154 500 phenotypes, 204 900 genotypes and in total of about 1 105 500 animals in the pedigree.

Estimating Gamma Matrix

The true Gamma matrix ($\boldsymbol{\Gamma}$) was calculated using true allele frequencies in the base populations ($\boldsymbol{p_A}$ and $\boldsymbol{p_B}$) in the following formula derived in Garcia-Baccino et al. (2017)

$$\boldsymbol{\Gamma} = \mathbf{8} \cdot \boldsymbol{cov}(\boldsymbol{p_A}, \boldsymbol{p_B}).$$

Additionally, $\boldsymbol{\Gamma}$ was estimated using four different methods. Two methods are based on estimated base allele frequencies and equation (1). Base allele frequencies were estimated using the software Bpop (Strandén &

Mäntysaari, 2020b), which makes use of a generalized least square (GLS) method. For the first method (BFQ_pure), only genotypes of purebred animals of the two subpopulations were used to estimate $\boldsymbol{\Gamma}$. For the second method (BFQ_all), all genotypes of the final dataset, including all crossbred animals, were used. The third method (MM_pure) tested for estimating $\boldsymbol{\Gamma}$ corresponds to the method described in Legarra et al. (2015) as "Method of moments based on summary statistics for multiple pure populations" and again uses only genotypes from the purebred populations. The last method (MM_cross) is equivalent to the "Method of moments based on summary statistics for populations with crosses" and uses crossbred genotypes as described in Legarra et al. (2015).

Genetic evaluations

To evaluate the effect of inclusion of MF, several different genetic evaluations were tested with the same dataset. There are no unknown parents in the pedigree. Only the parents of the pedigree base are unknown and replaced with the true base populations. An exception is the evaluation without UPG, where the parents of the pedigree base are all set to zero.

1) PBLUP with two UPG (PED):

A simple pedigree BLUP, where the UPG were treated as random, was applied on the dataset. The evaluation was done using the commercial software package MiX99 (MiX99 Development Team, 2019).

2) ssGBLUP without UPG (no_UPG):

Breeding values were estimated based on a ssGBLUP with no UPG in the pedigree. All animals in the pedigree were traced back to one single pedigree base population. The preparation of the genomic relationship matrix (G) for ssGBLUP was done with the program HGINV (Strandén & Mäntysaari, 2020a) based on VanRaden's method 1 (VanRaden, 2008) with true base allele frequencies from the founder population and the approach for proven and young (Misztal et al., 2015). Details on the computation of the G-Matrix are the same as in Himmelbauer et al. (2023).

3) ssGBLUP with two UPG (UPG_qp):

This method is the same as no_UPG, described above, with the difference that here the true base populations were used as parents in the pedigree base. The two base populations were modeled as UPG and Quaas and Pollak (QP) transformed UPG were included in inverse G.

4) ssGBLUP with two MF and true $\boldsymbol{\Gamma}$ (MF_true):

The fourth evaluation is a ssGBLUP where the two base populations were modeled as MF. In this case the true $\boldsymbol{\Gamma}$ was used to define the relationships between the MF.

5) ssGBLUP with two MF and estimated $\boldsymbol{\Gamma}$ (MF_est):

This evaluation is equivalent to MF_true, but here an estimated $\boldsymbol{\Gamma}$ was used. The used $\boldsymbol{\Gamma}$ was estimated using strategy BFQ_all, described above.

6) ssGBLUP with two MF, true $\boldsymbol{\Gamma}$ and scaled variances (MF_sc):

This evaluation is the same as MF_true, but in this case, scaled variance components as proposed by Legarra et al. (2015) were used. The additive genetic variance was scaled using the following equation (Legarra et al., 2015):

$$\boldsymbol{\sigma^2_{related}} \approx \frac{\boldsymbol{\sigma^2_{unrelated}}}{\mathbf{1} + \frac{\overline{\boldsymbol{diag(\Gamma)}}}{\mathbf{2}} - \overline{\boldsymbol{\Gamma}}}$$

Analyzing results

All comparisons are based on 10 repetitions of the simulation described above. To evaluate the performance of the different methods to estimate Γ, the diagonal and off-diagonal values of the estimated Γ are compared to the corresponding values of the true Γ.

The comparison of the different evaluations is done using three validation measures based on the youngest animals born in the last year of the simulation. Firstly, the correlation between estimated breeding values (EBVs) and true breeding values (TBVs) is calculated. Secondly, the bias is calculated using the following formula

$$b = \overline{EBV} - \overline{TBV}.$$

Third, the regression coefficient of the following regression is used as a measurement of the dispersion:

$$TBV = b_0 + b_1 \cdot EBV + e.$$

Additionally, the estimates for the group estimators of the UPG and the MF are compared to evaluate the differences between the five evaluation methods. Because the level of the base populations varies across replicates, the estimated difference between the two base populations is compared with the true difference rather than the absolute values.

Results & Discussion

Gamma-matrix

The diagonal of $\boldsymbol{\Gamma}$ is a measure for the inbreeding in the metafounder populations. The true mean diagonal value in this study was 0.631, with values ranging between 0.622 and 0.645. There are also no systematic differences between the two MF within a replicate because both MF populations are the same size and have the same history of evolution.

Basically, all tested methods overestimate the inbreeding of MF, but the two methods based only on genotypes of purebred animals show a significantly higher overestimation (Figure 2, top).

The off-diagonal of Γ represents the relationship between the two MF. In this study, the true value is between 0.566 and 0.585 with an arithmetic mean over 10 repetitions of 0.575. Both methods based on base allele frequencies give a very good estimate of the true value, whereas the other two methods show a clear overestimation (Figure 2, bottom).

In combination, this means that the method BFQ_all is the best at estimating the true Γ in this study where two MF are simulated. This is in line with the results for one MF shown in Garcia-Baccino et al. (2017). An interesting conclusion from the comparison between BFQ_pure and BFQ_all is that genotypes from crossbred animals are very important in the estimation of base allele frequencies in this situation.

Results for UPG/MF

The mean true difference in the genetic level between the two base populations over all repetitions is 0.834 genetic standard deviations, but with a quite high variation between 0.604 and 1.051 genetic standard deviations. All metafounder evaluations slightly underestimate the difference between the base populations by about 0.025 genetic standard deviations, but also with a relatively high error variance

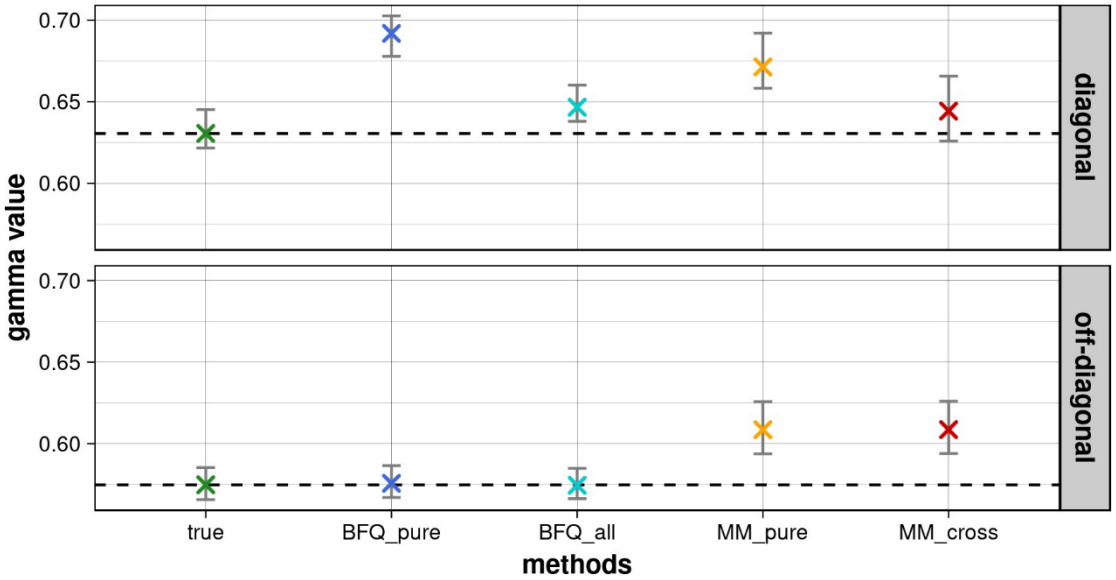

Figure 2. Comparison between true and estimated Γ for diagonal value and off-diagonal value separately. The error bars in the plot show the range from minimum to maximum and the "X" show the means over 10 repetitions. The dashed black lines indicate the true values.

between -0.16 and +0.18 (Figure 3). On average, the estimates from PED and UPG_qp are less biased, and the error variance for UPG_qp is also significantly lower than for the other estimates. This result is somehow surprising that a model with UPG can estimate the level difference of the base populations better than the MF models, one even with the true Γ matrix.

Correlation to true breeding value

The correlation of estimated breeding values (EBV) to true breeding values (TBV) for the youngest animals is more or less the same for all different evaluations (Figure 4, top). Only for breeding values from PED the correlation is substantially lower, as to be expected. Interestingly, there are hardly any differences in the correlation between no_UPG, UPG_qp and MF_true. There are already other studies on the use of MF in simulated and routine datasets and many of them report only small differences between evaluations with and without MF in terms of correlations or R^2 (Garcia-Baccino et al., 2017; Kudinov et al., 2022; Meyer, 2021).

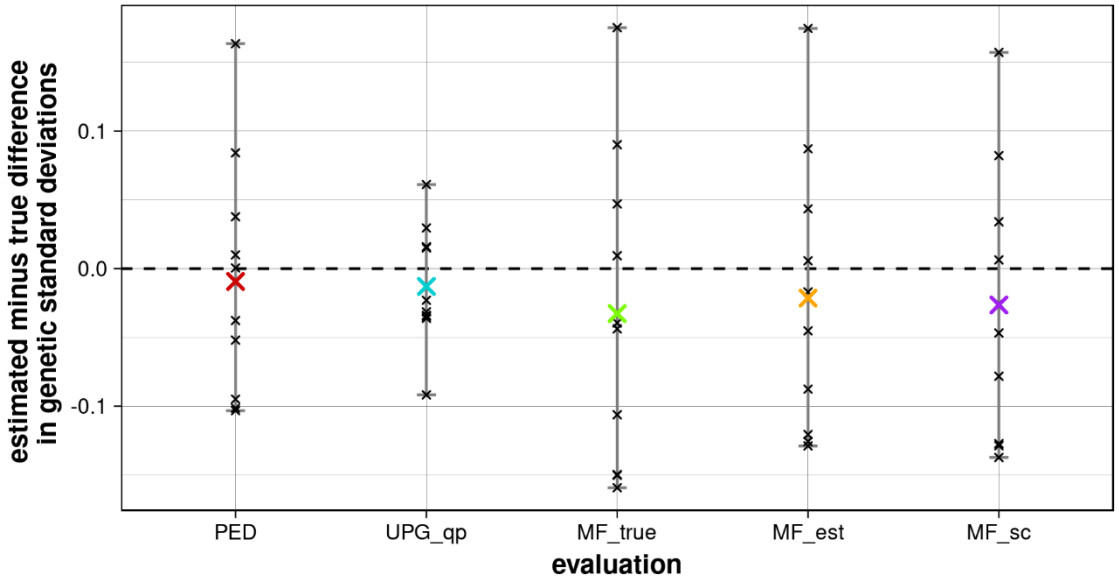

Figure 3. Estimated minus true difference between the genetic levels of the two base populations for different genetic evaluations. Results are given in genetic standard deviations. The error bars in the plot show the range from minimum to maximum and the capital colored "X" show the arithmetic means over 10 repetitions. The small "x" indicate the results for each repetition.

But unlike our findings, most studies report at least a slight improvement in correlation when using MF. This discrepancy may arise because our dataset uses MF exclusively at the pedigree base, without UPG or MF further along the pedigree. When MF are used in younger animals, the impact on correlation compared to UPG or not accounting for unknown parents in the final generation maybe becomes more pronounced than observed in our current study.

Bias

Regarding bias, the breeding values from PED show a significant downward bias of 0.627 genetic standard deviations, whereas the EBV from no_UPG and UPG_qp are on average slightly biased upwards by 0.08 and 0.04 genetic standard deviations, respectively (Figure 4, middle). The strong bias of EBV from PBLUP can be explained by the bias due to genomic preselection and was also observed in previous studies (Mäntysaari et al., 2018; Patry & Ducrocq, 2011). The EBV from MF_true and MF_est are mostly unbiased. Less biased results for evaluations with MF were also found in other publications (e.g. Garcia-Baccino et al., 2017). It is interesting to note that the breeding values from the MF model with scaled variance components are also slightly biased upward by about 0.04 genetic standard deviations.

Dispersion

Another effect of genomic preselection is the clear overdispersion of EBV from PBLUP, resulting in a regression coefficient of 0.82. Similar results have also been reported in several publications (Mäntysaari et al., 2018; Patry & Ducrocq, 2009, 2011). EBV from no_UPG and UPG_qp and also MF_sc show an overdispersion with a regression coefficient of around 0.95. There is no difference in the dispersion between EBV from MF_true and MF_est. Both evaluations lead to EBV with a regression coefficient of around 1.01, meaning that there is neither over-, nor a notable underdispersion. Other studies have also shown that the use of MF has a positive effect on dispersion and leads to less inflated breeding values (Garcia-Baccino et al., 2017; Kudinov et al., 2022; Macedo et al., 2021; Meyer, 2021).

Further simulations and analyses (results not shown) have shown that the differences between the estimates depend strongly on the difference in genetic levels between the two base populations. In simulations where the level differences between the two base populations are smaller, the positive effects of the evaluations with MF on dispersion are not so clear. In that case UPG_qp or even no_UPG give comparable or even better results with respect to dispersion than models with MF. One explanation could be that in situations with minimal or no differences in the genetic level of the base groups, MF simulates a difference that is not present at the level of causative loci.

Effects of estimated Gamma-Matrix

As there are hardly any differences in the results for MF, correlation, bias and dispersion between MF_true and MF_est, it can be concluded that the small differences between true and estimated Γ have no notable effects on the validation statistics of the evaluation in this simulated dataset. However, in the present study there are only two MF, and these only used at the pedigree base without any younger unknown parents. In more complex data sets and especially in routine data sets with multiple and also younger MF, the differences between evaluations with estimated and true Γ are likely to be larger.

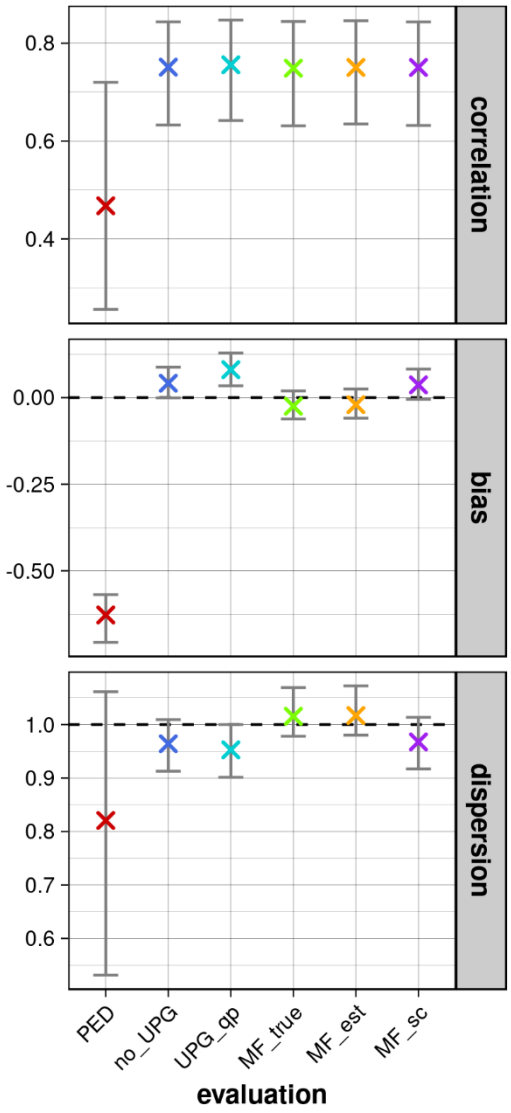

Figure 4. Results for correlation (top), bias (middle) and dispersion (bottom) for the youngest animals for different genetic evaluations. Bias (estimated minus true) is given in genetic standard deviations. The error bars in the plots show the range from minimum to maximum and the capital colored "X" show the arithmetic means over 10 repetitions.

Effects of scaling variance components

Applying the formula published in Legarra et al. (2015) on the true Γ and scaling the true variance components, results in a higher h^2. On average

$$\sigma^2_{related} \approx \frac{0.3}{0.713} = 0.421$$

resulting in $h^2 = \frac{0.421}{0.421+0.7} = 0.376$ instead of 0.3. Using the scaled variance components in

the ssGBLUP there are no remarkable differences between MF_true and MF_sc on the estimation of MF and the correlation in the validation group (Figure 3 and Figure 4, top). But compared to MF_true, scaled variance components lead to more bias and overdispersion (Figure 4, middle and bottom). These results are unexpected because it is derived in Legarra et al. (2015) that MF relatedness requires variance components to be adjusted. But there are already other authors reporting no positive or even negative effects of scaling variance components (Kudinov et al., 2022). Overall, the validation results (especially bias and dispersion) of the estimates with scaled variance components tend to show similar results to those found in other studies where the effect of an incorrect h^2 (in this case too high h^2) was investigated (Himmelbauer et al., 2023). This could be interpreted as suggesting that scaling the variance components in this case may lead to a too high h^2.

Conclusion

In summary, this study could show that already in a very simple situation with two base populations and otherwise complete pedigree, ssGBLUP with MF have significant positive effects on bias and dispersion in the youngest animal group compared to UPG. Regarding the estimation of the Γ, the method based on base allele frequencies proved to be the best method, with genotypes of crossbred animals playing an important role in the estimation of base allele frequencies. It is also interesting to note that scaling the variance components in this study did not improve the validation results, but worsened them.

But of course, it should be noted that this study uses very strong simplifications and rather optimal conditions compared to real applications. Therefore, further investigations with more MF and unknown pedigrees are necessary to be able to make statements that are more applicable to routine data.

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Beef on Dairy Genomic Evaluation for Feed Efficiency, Methane Emission and Meat Quality

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Abstract

Improving resource efficiency and meat quality as well as reducing the environmental impact from cattle industry, are important issues. Therefore, the aim of the FutureBeefCross (FBC) project is to show the genetic background of feed efficiency, methane emission and marbling score (MS) in young, crossbred calves. The aim is to implement breeding values for major beef breeds used to inseminate dairy cows. In this project, longitudinal daily dry mater intake (DDMI) and longitudinal body weight (BW) during 100 to 300 days of age were available for 4,400 crossbred animals with either Danish Blue, Charolais, or Angus sires and with Holstein dams. Feed intake is obtained by All feed system from Allflex. In addition, marbling score was obtained using image analysis. The basis was Q FOM™ images on 1,700 crossbred animals of Danish Blue sires and Holstein dams. Longitudinal daily dry matter intake and body weight were analyzed in a bivariate model with Legendre polynomials of days of age at the time of the test with first order for fixed, genetics, and permanent environment effects using Pedigree BLUP. Genetic parameters for marbling score were obtained from a univariate pedigree BLUP model. The genetic residual feed intake was measured as sum of daily dry matter intake minus the body weight gain and the mid body weight during the period of 200 to 280 days of age. The genetic residual feed intake has a moderate heritability of 0.21 and low genetic correlation of -0.12 with body weight gain and zero with mid body weight. The marbling score has low heritability of 0.15 which could be because the measured animals are young (10-12 months at slaughter). Breeding values for all traits will be implemented in 2023 and will act as a decision-making tool for artificial insemination (AI) organizations in selecting beef bulls that can improve farm profitability while meeting consumer demands.

Key words: beef on dairy, feed efficiency, genomics, heritability, meat quality

Introduction

There has been an increase in use of beef semen on dairy cattle in Denmark in the latest 10 years. In 2013 less than 5% of calves born at 2nd or later calving had a beef bull sire, but that number has risen to 34% to 46% in 2023 depending on dam breed. Since 2015 across breed estimated breeding values (EBVs) have been published for beef on dairy (BxD) bulls in Denmark. Later Nordic Cattle Genetic Evaluation (NAV) has developed a joint Nordic multibreed genetic evaluation of BxD bulls (Carlén et al., 2019).

In 2019 the FBC project started, aiming to improve economy, reducing climate impact, and improving meat quality in the production of BxD calves by improving their genetic potential. The basis is to develop new methods to phenotype 12,000 Danish BxD calves from Holstein dams and Danish blue, Angus or Charolais sires for these three traits. The aim of this project is to estimate the genetic parameter for feed efficiency during fattening period and the marbling score (MS) at slaughter. Also, we will shortly present our thought on the outline of the evaluation of

methane emission, where data is not yet available.

Materials and Methods

Data for feed intake

Daily dry mater intake (DDMI) and birth weight (BW) of 4266 crossbred animals during 100 to 300 days of age were available. The crossbred animals were from Danish Blue, Angus, or Charolais sire breeds crossed with Holstein dams.

Data for marbling score

Marbling score MS of 1686 crossbred animal from 65 Danish Blue sires and Holstein dam that were slaughtered between 240 to 360 days of age were available. Marbling score was calculated by Frontmatec by converting parameters obtained from picture of rib eye of between 5^{th} and 6^{th} ribs taken by a handheld camera device (Q FOM™).

Statistical analysis

Bivariate Random regression animal model with Legendre polynomial of the days of age on the test was used to model DDMI and BW. The model for DDMI contained fixed effects of slaughter herd by year by month of slaughter and by gender of animal interaction; sire breeds (Angus, Danish Blue, and Charolais); and start age of the test and its quadratic term. First order polynomial was fitted for fixed part of the model and the additive genetics of animal and the permanent environment effect. For BW, the fixed effects were slaughter herd by year interaction; gender; and sire breeds. The fixed effects were modeled with second order polynomials and the genetics and permanent environment with first order polynomials. For MS, a univariate animal model was used that included the fixed effects of slaughter herd by year by month interaction; gender; slaughter age; and carcass weight. The DMU software package (Madsen and Jensen, 2013) was used for genetic parameter estimation.

Genetic Residual feed intake (RFI) calculationMethodology of Esfandyari and Jensen (2021) and Shirali et al. (2018) was used to make the derivations for genetic RFI and its component traits as well as the genetic regression coefficients. Genetic RFI was obtained as the sum of daily dry matter intake (TDMI) during 200 to 280 days of age minus body weight gain (Gain) and the mid BW (MBW) in that period. Where regression coefficients for body weight gain and mid BW were obtained from the genetic (co)variance matrix.

Results & Discussion

The heritabilities were moderate for TDMI (0.24), Genetic RFI (0.21), Gain (0.21), and MBW (0.35). The genetic correlation between TDMI and RFI was substantially high at 0.82. In addition, RFI explained 72% of variation in TDMI obtained from genetic variance of RFI over the ones from TDMI (484/675). The genetic correlation between TDMI and Gain was favorable (0.43). Due to the modeling of RFI, the genetic correlation between RFI and its component traits of Gain (-0.12) and MBW (0.00) were very low and close to zero. Esfandyari and Jensen (2021) reported a heritability of 0.40 to 0.50 for feed intake and genetic RFI; and between 40% to 80% variance in daily feed intake to be explained by RFI. In literature, heritability of feed intake during growing period is reported to be moderate to high (0.25 to 0.44) (Schenkel et al., 2004; and Retallick et al., 2017).

The heritability for MS was low (0.15). In literature, higher heritabilities for marbling score were reported for example Bedhane et al. (2019), Do et al. (2016), Davis and Simmen (2000), Ríos-Utrera et al. (2005), and Nephawe et al. (2004) (0.49±0.05, 0.28±0.02, 0.27±0.17, 0.40±0.09, 0.46±0.06, respectively) reported moderate to high heritabilities. The genetic correlation between MS and Intramuscular fat

was 0.94 as both measurements are obtained from parameters in the pictures taken from rib eye captured by handheld devices.

For the methane emission trait, we are planning to use the sole ratio between CO_2 and CH_4 directly, as also suggested as a possibility by Madsen et al. (2010). Alternatively, daily methane production could be calculated, from the estimated daily CO_2 production and the ratio between CO_2 and CH_4, as also suggested by Madsen et al. (2010). However, using their method to estimate CO_2, it would require accurate information on weight gain and feed intake. Uncertainty in these data would affect the accuracy of the estimated CO_2 and thereby also the CH_4 production.

Conclusions

The one-step method for calculation of genetic RFI as measure of feed efficiency can act as a good approach to improve modelling of feed efficiency. Moreover, the genetic RFI is suitable for selection of beef bulls and can improve farm efficiency. Marbling score can be utilized to improve the quality of carcass. These new breeding values can be used as additional tools for AI organizations to select beef bulls for use on dairy cattle. The result is that farm productivity increases while consumer satisfactions are met. Adding also a breeding value to reduce methane emission and thereby lower climate impact of these BxD calves, would also increase the acceptance of beef from these BxD calves from a consumer point of view.

Acknowledgments

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Multi-breed genetic evaluation of beef bulls used in dairy herds – Emphasis on newest development

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Abstract

Since 2018, Nordic Cattle Genetic Evaluation (NAV) routinely estimates across breed breeding values for beef sires based on data from their offspring when used on dairy cows and since 2019, the Nordic farmers have access to the Nordic Beef × Dairy overall economic Index (NBDI). To fulfill requests expressed by the industry, the NAV Beef × Dairy evaluation traits portfolio has recently been expanded to include two new trait groups, youngstock survival and gestation length. The aim of this paper is to describe the current Nordic routine evaluation for beef bulls used on dairy cows with focus on the recent developments.

Key words: beef x dairy, genetic evaluation, multi-breed, youngstock survival, gestation length

Introduction

The interest in utilizing beef bulls with dairy cows has grown significantly over time. The integration of beef semen into dairy herds, particularly when paired with sexed dairy semen, has proven to be highly beneficial for farmers in terms of economic advantages. By ensuring replacements from the genetically superior animals in the herd and generating value-added beef × dairy calves from the remaining females, this practice helps improving the profitability of the dairy farm.

The selection of the appropriate beef bulls to be used on the farm is an important factor to ensure the success of this practice. Considerable across but also within beef breed genetic differences have been reported for many traits (Davis et al., 2019) which emphasizes the need for a multi-breed genetic evaluation of the beef bulls used in dairy herds.

To help farmers in their choice of the right beef sires to use, Nordic Cattle Genetic Evaluation (NAV) has developed a joint Nordic multibreed genetic evaluation of beef bulls used in dairy farms (Carlén et al., 2019).

The aim of this paper is to briefly describe the current Nordic routine evaluation for beef bulls used on dairy cows with focus on the recent developments.

The Joint Nordic Beef × Dairy Genetic Evaluation

The joint Nordic Beef × Dairy evaluation includes data from Finland, Denmark, and Sweden and crossbred calves from purebred Holstein, Jersey and Red Dairy Cattle (RDC) cows. Up to eight individual breeding values may be made publicly available for each bull, subject to compliance with publication rules.
Since its launch in 2018, the evaluation is publishing 4 breeding values for calving traits: calf survival and calving ease based on cows in 1st and later lactations, respectively and 3 combined breeding values for carcass traits: daily carcass gain, carcass conformation score and carcass fat score.

In 2019, the Nordic Beef x Dairy economic Index (NBDI) was implemented. This index assesses the economic value of beef bulls based on their genetic potential for producing crossbred beef × dairy offspring that benefit farmers economically. The NBDI currently consists of two sub-indices: one for birth traits, which includes calf survival and calving ease in later lactations and another for growth traits, including daily carcass gain, carcass conformation score, and carcass fat score. As

the rearing period's length and intensity affect the economic values of the growth traits, both the growth index and NBDI are available for short (below 550 days) and long (above 550 days) rearing periods.

Since the launch of the current evaluation, industry stakeholders have voiced their desire to introduce two additional traits into the evaluation framework: Youngstock survival (YSS) and gestation length (GL). The addition of YSS is particularly pertinent due to its far-reaching implications for both economic viability and animal welfare, thus warranting its integration into the NBDI. GL also holds significant economic importance as it plays a crucial role in effective calving pattern management on dairy farms. This importance is amplified for dairy farmers participating in beef on dairy programs, as gestation length varies across different beef breeds (Norman et al. 2009. This insight underscores the potential for managing this trait through careful selection of the beef bull used for breeding with the cow.
However, many studies such as Hansen et al., 2004 and Eaglen et al., 2013 did not support selection for shorter neither longer duration but rather opt for intermediate values which were found by many studies to be optimal for other traits like productive life and calving ease (Norman et al., 2011). Consequently, at this point, there is no plan to incorporate GL into the NBDI.To address the industry's requests, YSS was implemented in November 2022, followed by GL in May 2023.

Materials and Methods

Data

For both YSS and GL, as with the other beef × dairy traits (Davis et al., 2019), the data used in the evaluation includes crossbred calves born in the three countries from the year 2000 onward, provided they meet the following criteria:

(i) Born to a purebred dairy dam of the RDC, Holstein, or Jersey breed.
(ii) Sired by a purebred beef breed AI sire with a minimum of 50 beef-on-dairy crossbred offspring.
(iii) Born in a milk-producing herd.

Survival data from Swedish males born prior to 2008 and all Finnish data from before 2004 were omitted from the evaluation due to concerns about the completeness of information gathered for animals born before respective years. For all countries, survival data were excluded for all calves born with malformation or from multiple births/embryo transfer, those that do not survive the first 24h after calving, those that are slaughtered or exported within the considered period for survival.

Regarding GL, a similar data editing process was applied as that used for the calving traits (Fikse et al., 2019). After this editing process, data were subject to outlier removal. The Interquartile Range (IQR) statistical method (Smiti, 2020) was used to identify and discard outliers within each sire breed.

For both YSS and GL, a very limited number of records were obtained from Jersey cows in Sweden and Finland, which may pose a limitation when attempting to account for variance heterogeneity within this breed (see the "Heterogenous variance adjustment" section later in this document). Furthermore, these calves born from Jersey cows did not hold significant interest in both countries. Therefore, records collected from Jersey cows in both countries were subsequently excluded from the evaluation.

The final data set included data from 871,524 records for YSS and 1,157,256 records for GL. The data distribution per dam breed and country for both trait groups, is shown in Table 1.

Table 1. Number of survival and gestation length records per dam breed and country.

	YSS		
	Denmark	Finland	Sweden
Holstein	262335	152056	59905
RDC	28356	248622	64485
Jersey	55765		
	GL		
Holstein	361974	183625	93955
RDC	34184	305230	96969
Jersey	81319		

Trait definitions

Youngstock survival

YSS is divided on rearing period to create two separate single traits:

• Survival day 1-30 (YSS1): equal to 1 if the calf is alive at day 30, otherwise, it is set to 0

• Survival day 31-200 (YSS2): equal to 1 if the calf is alive at day 200, otherwise, it is set to zero.

In the context of beef production from dairy cattle, both male and female animals are typically raised under similar conditions and for the shared purpose of meat production. Consequently, it is reasonable to assess the YSS as one trait for both heifers and bull calves. This initial assumption was subsequently validated during the validation process, the detailed results of which are not presented here.

Gestation length

Gestation length is defined as the time interval, measured in days, from the moment of conception to the subsequent occurrence of parturition. Distinct traits are defined for heifers (GL1) and cows (GL2).

Heterogeneous variance adjustment

To account for the heterogeneity of variance of YSS across different countries, sexes, birth years and dam breeds, Snell scores were used (Snell, 1964). Groups used for the transformation are subclasses of country – sex – year - dam breed. Years with less than 1000 records were regrouped. Regarding gestation length, a simple correction for phenotypic variance was applied with respect to sex, addressing a noticeable systematic difference observed in the phenotypic standard deviation for both traits.

Genetic evaluation model

A multiple-trait linear sire model was used to evaluate YSS and GL in the Nordic beef × dairy evaluation.

Fixed effects

The fixed effects included in the model are described in Table 2. The sire beef breed effect is integrated into the model to account for systematic differences among sire breeds. However, it's important to note that this effect is subsequently added to the individual sire solutions to derive the final breeding value of a bull. Furthermore, it has to be noted that the estimated breeding values express the total genetic value meaning that they include both additive and non-additive genetic effects. The transfer effect in the model is defined as a binary variable equal to 1 if the calf was transferred during the first 100 days of its life and zero otherwise. The herd used to create the herd-year contemporary group effect for YSS2 in this case is the herd to which the calf is first transferred otherwise it is the birth herd.

Table 2. Fixed effects included in the model per trait.

	YSS	GL
Sire breed	x	x
Country – herd – year	x	x
Country – year – month	x	x
Country – year – sex	x	x
Dam breed – year	x	x
Country – parity	x	
Country – transfer*	x	
Country – age of the dam		x

* Only for YSS2

Genetic parameter estimation

The genetic parameters were estimated using the DMU software package (Madsen and Jensen 2013) with a multiple-trait model including data from all countries and breeds.

Genetic base

The genetic base is defined as 2-5 years old crossbreds born after beef breeds which can be used in all 3 countries.

Expression of breeding values

Like for the other beef × dairy traits except the NBDI (Fikse et al., 2019), YSS breeding values are presented as relative values, with a standardized mean of 100 and a genetic standard deviation of 10.

GL breeding values differ from the other traits within the NAV portfolio. They are expressed in days and as a deviation from a standard dairy gestation length average fixed at 280 days. This makes it easier to interpret by the farmers since the primary use of the GL breeding values is to help management of the calving patterns.

Results & Discussion

Survival rates

In line with what was observed in the Nordic purebred dairy YSS data (Carlén et al. 2016) and the Danish YSS beef on dairy data (Davis et al., 2020), females had a slightly higher average survival rate for both evaluation periods and survival rates for early period were slightly higher than those for later period (Table 3). Calves born from Jersey cows had a lower survival rate than Holstein and RDC for both traits (Table 4). This finding aligns with the results reported for the Danish YSS beef × dairy data by Davis et al. (2020).

Table 3. Average phenotypic survival rates per sex and trait.

	Number of calves	YSS1	YSS2
Males	429974	0.96	0.95
Females	441550	0.97	0.96

Table 4. Average phenotypic survival rates per dam breed and trait.

	Number of calves	YSS1	YSS2
Holstein	474296	0.97	0.96
RDC	55765	0.97	0.96
Jersey	341463	0.95	0.94

Gestation length data

All data combined, GL was about two days shorter on average for heifers (282 days) compared to cows (284 days) and about one day on average shorter for females (283 days) compared to males (284 days).

Phenotypic means of GL varied per sire breed with Angus and Belgian Blue having the shortest GL and Limousine and Blonde d'Aquitaine the longest (Table 5).

Table 5. Number of records (N), Mean, SD and Median of gestation length per country and sire breed.

Sire breed*	N	Mean	SD	Median
AAN	123012	280.5	5.2	280
BAQ	236749	287.4	5.6	288
BBL	289264	280.8	5.1	281
CHA	98781	283.5	5.6	284
HER	51168	281.6	5.3	282
LIM	168572	287.1	5.8	287
SIM	96720	284.5	5.6	285

*AAN: Aberdeen Angus; BAQ: Blonde d'Aquitaine; BBL: Belgian Blue; CHA: Charolais; HER: Hereford; LIM: Limousine; SIM: Simmental

Genetic parameters

The estimated heritabilities for YSS traits were low (0.01 for YSS1 and 0.015 for YSS2),

and there was a moderate genetic correlation of 0.3 between the two traits. These findings are consistent with what has been reported in other studies for YSS traits (Davis et al., 2020; Buch, 2012). The low heritabilities are expected due to the nature of survival data, where only a small proportion of animals experience mortality and the environmental variation is quite high.

In contrast, high heritabilities were estimated for GL (0.56 and 0.57 for GL1 and GL2 respectively) and a very high genetic correlation of 0.99 was estimated between both traits. These results are consistent with the literature on GL (Eaglen et al., 2013; Hansen et al., 2004; Haile-Mariam & Pryce, 2019; Amer et al 2016; Norman et al., 2009).

Relative breeding values

Figures 1 to 3 present a boxplot summarizing the breeding values per sire breed for YSS1, YSS2 and GL2 respectively. In each graph, the central box represents the interquartile range (IQR) of the data, with the horizontal line inside indicating the median breeding value. The lower and upper whiskers extend to the minimum and maximum values within 1.5 times the IQR, respectively. Data points outside this range are identified as outliers, representing sires with extreme breeding values compared to the rest of the population.

The graphs provide an insightful overview of the distribution of breeding values across different sire breeds, highlighting both the genetic variation among breeds and the variability within each breed. The results underscore the importance of a multi-breed evaluation, highlighting that breed selection alone may not suffice. Instead, farmers should pay close attention to individual bulls to make their breeding decisions.

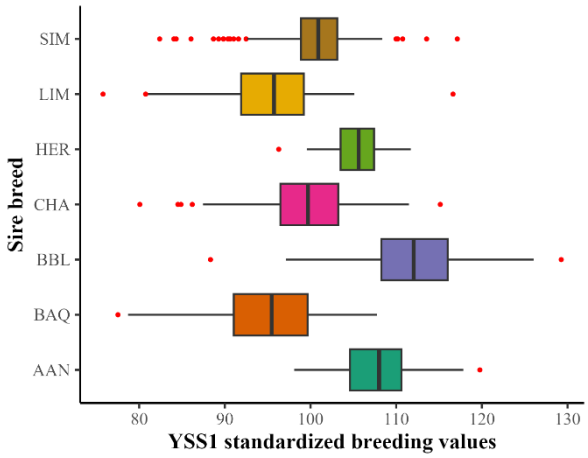

Figure 1. Box and Whisker Plot of YSS1 Relative Breeding Values by Sire Breed: AAN: Aberdeen Angus; BAQ: Blonde d'Aquitaine; BBL: Belgian Blue; CHA: Charolais; HER: Hereford; LIM: Limousine; SIM: Simmental

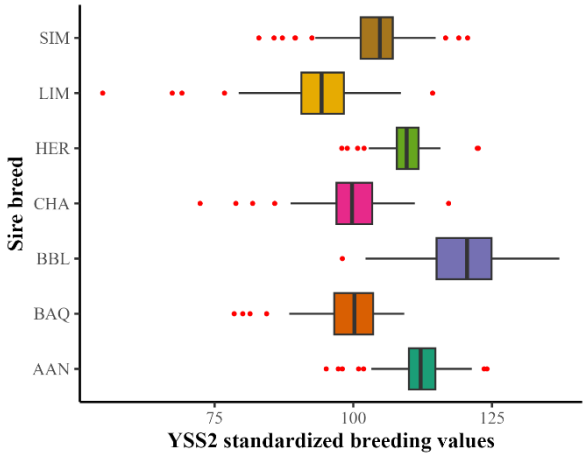

Figure 2. Box and Whisker Plot of YSS2 Relative Breeding Values by Sire Breed: AAN: Aberdeen Angus; BAQ: Blonde d'Aquitaine; BBL: Belgian Blue; CHA: Charolais; HER: Hereford; LIM: Limousine; SIM: Simmental

Due to the high genetic correlation between GL1 and GL2, suggesting that they represent the same trait, a decision has been made to exclusively publish GL2.

Both YSS1 and YSS2 breeding values, as well as a combined breeding value derived from both sources, are now made available for publication. Proper economic weights to be used for both the combined YSS index and inclusion in the NBDI are being calculated and are planned to be implemented by the end of 2023.

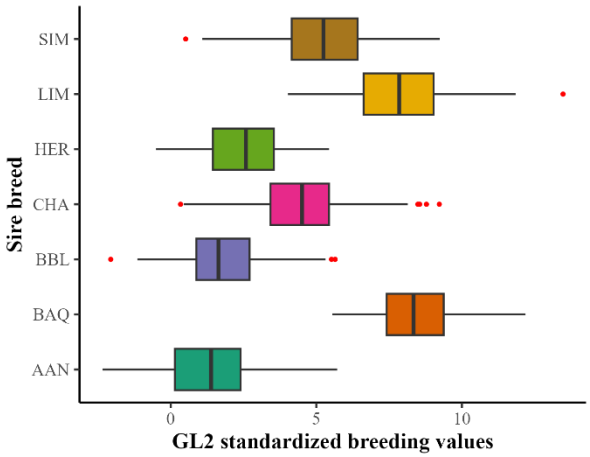

Figure 3. Box and Whisker Plot of GL2 Relative Breeding Values (expressed in days) by Sire Breed: Aberdeen Angus; BAQ: Blonde d'Aquitaine; BBL: Belgian Blue; CHA: Charolais; HER: Hereford; LIM: Limousine; SIM: Simmental

Conclusions

The study's findings reinforce the necessity of conducting multibreed evaluations when estimating breeding values for beef bulls used in dairy herds. Significant within-breed variation was observed for both YSS and GL traits, highlighting the importance of considering individual sire results rather than relying solely on specific breeds.

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Deregressed genomic breeding values from single-step evaluations of test-day traits using all genotype data

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Abstract

Single-step model has become the Golden Standard for routine genetic evaluation in dairy cattle. For various statistical analyses or genomic validation, (daughter) yield deviations or deregressed genomic breeding values may be considered as pseudo-phenotype that are more independent from early genomic prediction. The aims of this study were to assess GEBV deregression methods for cows and bulls, and to validate the deregressed GEBV via a reversibility test. A total of 13.5 million animals with phenotypic records, evaluated with a single-step model using the German genotypic and phenotypic data from April 2023, were considered in the cow GEBV deregression. Likewise, all bulls with daughters and all reference cows were included in the bull GEBV deregression. Both GEBV deregression processes used the same genotype data and pedigree file as the preceding single-step evaluation. Deregressed GEBV of the cows or the bulls were moderately or highly correlated with their GEB, respectively. For the four test-day traits, milk, fat and protein yields and somatic cell scores, the deregressed GEBV seemed to have a lower trend than their original GEBV. Equal GEBV were obtained in a special single-step evaluation using the deregressed GEBV as phenotypic data, in comparison to those GEBV from the original single-step evaluation. We obtained equal GEBV not only for the cows with test-day records and bulls with daughters but nearly equal also for young, genotyped candidates without own phenotypic records. The validation results confirmed that the GEBV deregression was a reversible process and the deregressed GEBV were proven to be correct.

Key words: Deregression, genomic breeding values, single-step model, test-day traits

Introduction

The pedigree-based deregression of estimated breeding values (EBV) by Jairath et al. (1998), also known as the matrix deregression (Calus et al. 2016), has been widely used in dairy cattle evaluations, for example, for generating deregressed bull EBV as input data in Interbull MACE evaluation. The current multi-step genomic model needed 'pseudo-phenotypic data', such as the deregressed EBV or proofs (DRP), for genomic evaluation and SNP effect estimation. Calus et al. (2016) confirmed that the matrix deregression method by Jairath et al. (1998) was more accurate than the other deregression methods. In 2020, a reversibility test was conducted on DRP of bull MACE EBV on country scale DEU and on DRP of cow national EBV for all trait groups evaluated in Germany (Liu and Masuda, 2021). We could successfully validate the correctness of the DRP for all the bulls included in MACE evaluation on DEU scale and for all the domestic cows with own phenotypic records across all the evaluated trait groups.

Liu and Masuda (2021) and Masuda et al. (2021) developed GEBV deregression methods for the single-step SNP BLUP model and the single-step GBLUP model, respectively. The aims of this study were to 1) deregress genomic estimated breeding values (GEBV) of the single-step model for four test-day traits in German Holstein separately for bulls with daughters and for cows with own test-day records, and 2) validate the deregressed GEBV for the two groups of animals with phenotypic

data as well as for all genotyped animals including young candidates.

Materials and Methods

A single-step SNP BLUP model for GEBV deregression

Prior to deregressing GEBV of cows or bulls, four test-day traits, milk yield (MKG), fat yield (FKG), protein yield (PKG), and somatic cell scores (SCS), were evaluated separately using a single-step SNP BLUP multi-lactation random regression test-day model (Alkhoder et al. 2022). For a detailed description of the single-step model, see the paper by Alkhoder et al. (2023). We applied here a special single-trait single-step model to deregress GEBV from the preceding single-step evaluation:

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{u} + \mathbf{e} \quad [1]$$

where **y** is a vector of deregressed GEBV (dGEBV) of animals with own phenotype data, **1** is a vector of 1s, μ is a general mean, **u** is a vector of GEBV for the animals with own phenotype data, and **e** is a vector of residuals. The dGEBV **y** are unknown and will be estimated in the deregression process. It is assumed that

$$[var(\mathbf{e})]^{-1} = \mathbf{D}\sigma_e^{-2} = diag\{\varphi_i\}\sigma_e^{-2} \quad [2]$$

where **D** is a diagonal matrix containing effective daughter contribution (EDC) of bulls with daughters or effective record contribution (ERC) of cows with own phenotype records on the animal-model basis, φ_i , for animal i, $i = 1, \ldots, n$, and n is the number of animals with phenotype data. σ_e^2 is residual variance. For more details about the deregression model [1], see the paper by Liu and Masuda (2021).

Phenotypic, genotypic and pedigree data were taken from the routine evaluation in April 2023 for German dairy breeds Holstein, Red Dairy Cattle, and Jersey. Table 1 describes the data sets for the single-step evaluation as well as for the following step of GEBV deregression for all cows with phenotypic records of the three breeds. All the cows with own test-day records included in the original single-step evaluation were considered in the cow GEBV deregression process, too. A total of 1,318,780 genotyped Holstein animals were included in the deregression process as in the original single-step evaluation. Consequently, the same pedigree file containing 21,850,276 animals was used in the cow GEBV deregression process as in the preceding single-step evaluation.

Table 1. Description of the data sets for the single-step evaluation and cow GEBV deregression

Frequency	Single-step evaluation	Cow GEBV deregression
Genotyped animals	1,318,780	1,318,780
Phenotyped animals	13,528,444	13,528,444
Phenotypic input data	263,673,267 test-day records	13,528,444 GEBV
Genotyped or phenotyped animals	14,402,662	14,402,662
Animals in pedigree	21,850,276	21,850,276

For deregressing GEBV of bulls with daughters, sires of the phenotyped cows were treated as animals with own phenotypic records. In addition, genotyped cows with own phenotypic data must be considered also as phenotyped animals, because the genotyped sires of the cows no longer represented the full genomic reference population when the genotyped cows were available. To guarantee the complete phenotypic and genotypic information content of the reference population to be utilized in the GEBV deregression process as the preceding single-step evaluation, all the genotyped cows with own test-day records were also added to the list of animals with phenotypic data for the deregression process of the bull GEBV. The total number of bulls with daughter phenotypic information and the genotyped cows with test-day records amounted to 664,548. To avoid double counting the reference cows' contribution to their sires, EDC of the sires was adjusted for the contribution by

their reference daughters. As in the deregression of cow GEBV process, all genotype data of 1,318,780 Holstein animals were also considered in the deregression process for GEBV of the bulls.

A reversibility test for validating the GEBV deregression

A validation study of the GEBV deregression was conducted to see if the deregression of GEBV was a reversible process or in other words if equal GEBV could be obtained from a special single-step evaluation with their dGEBV as input 'phenotypic' data. In case of the cow GEBV deregression, a single-trait single-step model [1] was applied to the dGEBV of all the cows with test-day records. The aim of this validation was to see if equal GEBV could be obtained from the special single-step evaluation as from the original single-step evaluation using the test-day records for all the cows.

To validate the GEBV deregression for the bulls with daughters, we used dGEBV of all the sires of the cows as input phenotypic data for the special single-step evaluation under Model [1]. In addition, dGEBV of the reference cows were used also in the validation process, because the genotyped bulls with daughters did not represent the complete reference population due to the high number of genotyped cows with test-day records. Like the validation of the cow GEBV, it was to be verified if equal GEBV of the bulls were obtained from the special single-step validation evaluation as from the preceding, original single-step evaluation.

Genotyped, young candidates were included both in the special single-step evaluations for validating the GEBV deregression and in the preceding, original single-step evaluation based on the test-day data. Though these candidates did not have own phenotypic records, we would like to know if they received equal GEBV from the two single-step evaluations.

Results & Discussion

The deregression of the cow or bull GEBV from the original single-step evaluation was conducted using the software suite MiX99 (Strandén and Mäntysaari, 2010). Two separate deregression processes were performed for the cows with test-day records and for the bulls with daughters. The original single-step evaluation for the four test-day traits was described already in the paper by Alkhoder et al. (2022). Overall, the GEBV deregression using the single-trait single-step model [1] required a little less computing time and less memory than the original single-step evaluation with test-day records.

Deregressed GEBV of cows with test-day records

For the cow GEBV deregression, a total of 11.8 million Holstein cows with test-day records were considered in the multi-breed evaluation system in Germany. Figure 1 shows observed correlation between dGEBV and GEBV of milk yield for the Holstein cows in green line. The number of cows born each year was shown in grey bars on the secondary Y-axis. The correlation is about 0.84 from the oldest cow birth year 2000 to 2013 and decreases gradually afterwards. As a result of incomplete or missing lactations in the last three birth years, the correlation between dGEBV and GEBV drops markedly between the single-trait model with dGEBV as input data and the original multi-lactation single-step model with test-day yields.

Figure 2 shows average dGEBV and GEBV of the Holstein cows born between 2000 to 2020 for trait milk yield. The trends in both dGEBV and GEBV are similar, with a little lower trend in dGEBV (the solid line in red) than GEBV (the dotted line in black). On average, the difference between dGEBV and GEBV changes from about 50kg in year 2000 to -50kg in birth year 2020, representing 15% genetic standard deviations over 20 years. The dGEBV of the cows have much larger (error) variance than

their GEBV, with an average within-year ratio, var(GEBV)/var(dGEBV), being 0.23.

For the 11.8 Holstein cows, similar results were obtained for the other 3 traits as for milk yield.

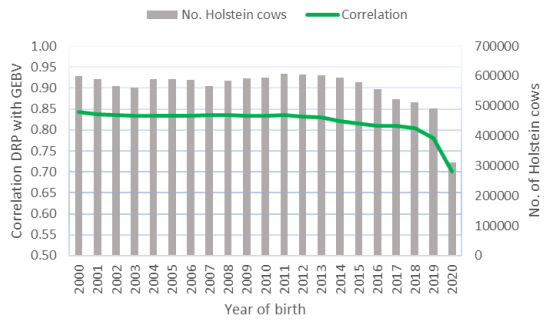

Figure 1. Correlation between deregressed GEBV and GEBV of milk yield for Holstein cows with test-day records

Deregressed GEBV of bulls with daughters

A total of 24,016 Holstein bulls that had daughters in 10 or more herds in Germany were selected for evaluating dGEBV of the bulls. Figure 3 shows the correlation between dGEBV and GEBV of the Holstein bulls born in 1998 through 2018. With the introduction of genomic selection in Germany in 2009, the correlation decreases steadily from 0.97 to 0.90. Bulls born in the last 3 years, 2016 to 2018, have much lower correlation, because their daughters have missing or incomplete lactations.

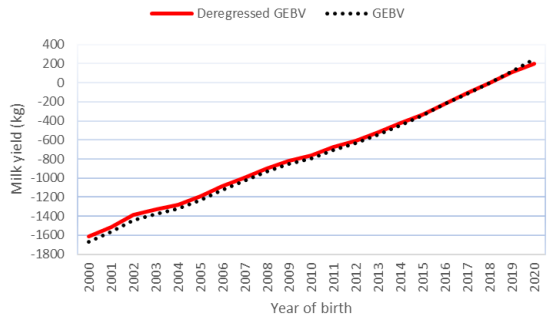

Figure 2. Averages of deregressed GEBV and GEBV of milk yield for Holstein cows with test-day records

As far as the trends are concerned, dGEBV of the bulls are shown to have almost equal averages by birth year, except the last 3 birth years (Figure 4). For the youngest bulls born in the last 3 years having daughters with missing or incomplete lactations, dGEBV of these bulls deviate evidently from their GEBV.

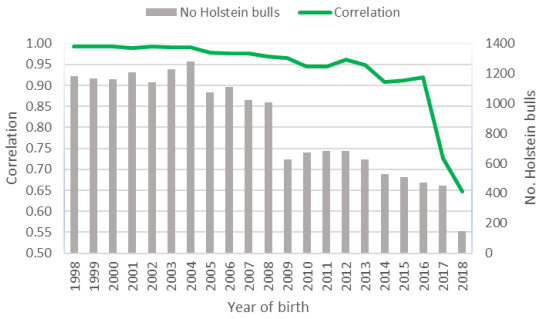

Figure 3. Correlation between deregressed GEBV and GEBV of protein yield for Holstein bulls with at least 10 herds in Germany

Deregressed GEBV of the bulls have higher (error) variance, with a ratio of standard deviation of GEBV over standard deviation of dGEBV being 0.91 for all birth years till 2016. Deregressed GEBV of the bulls born in last two years have significantly larger (error) variance than their GEBV.

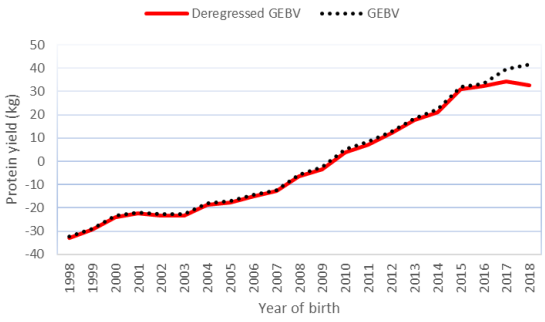

Figure 4. Averages of deregressed GEBV and GEBV of protein yield for the Holstein bulls by birth year

Validation results on deregressed GEBV of cows with test-day records

Due to different cow base populations for the Holstein breeds Black and White (B&W) and Red and White (R&W), 12.6 million B&W Holstein female animals were chosen to compare their single-step GEBV using dGEBV and test-day yields as 'phenotypic data.' The B&W female animals include all B&W cows

with test-day records, their female ancestors, and young genotyped female animals without own test-day data yet.

It can be seen in Figure 5 that the two sets of GEBV of the B&W female animals, estimated using dGEBV as 'phenotypic data' and using original test-day milk yields, are above 0.99 for all birth years, except for the birth years 2021 and later. Even the young, genotyped females without own test-day milk yields have a correlation of GEBV higher than 0.98.

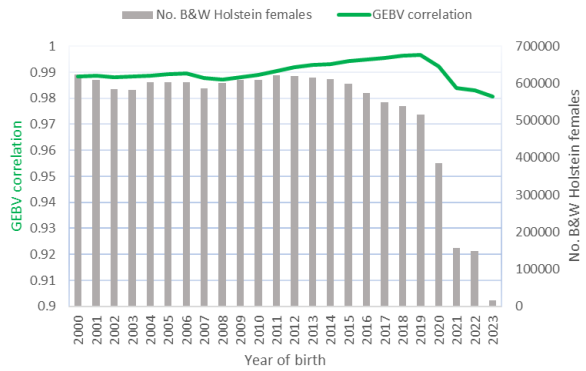

Figure 5. Correlation of single-step GEBV using deregressed GEBV and test-day yields of milk yield for all B&W Holstein females

Equal genetic trends were found in the single-step GEBV using dGEBV and test-day milk yields of the cows. In addition, the two sets of GEBV had equal variances for all birth years of the Holstein females, except that the female candidates born after 2020 had about 1% to 2% lower standard deviations of the GEBV using dGEBV as 'phenotypic records' than using test-day milk yields.

The validation results for the trait milk yield were seen also for the other remaining test-day traits. In summary, the special single-step evaluation with dGEBV of the cows as phenotypic records gave identical GEBV, as those obtained from the original single-step evaluation using test-day data, for all the Holstein females as well as for all other groups of animals in the single-step evaluation. The identical GEBV from the two single-step evaluations indicated the dGEBV of all the cows were accurately calculated.

Validation results on deregressed GEBV of bulls with daughters

As stated above, all the reference cows were considered in the bull GEBV deregression process to guarantee the complete genomic reference population being used in the deregression of GEBV of the bulls with daughters. To compare GEBV from the special single-step evaluation with dGEBV of the bulls and all the reference cows to those from the original single-step evaluation, we selected 10,770 B&W German Holstein AI bulls born from 1998 through 2022, including young genomic AI bulls born in 2019 and after.

Figure 6 shows GEBV correlation of protein yield for 10,770 B&W German AI bulls between the special single-step evaluation with dGEBV as phenotypic records and the original single-step evaluation with test-day protein yields. It can be clearly seen that the GEBV correlation is unity for all the birth years of bulls with daughters. However, for the young AI bulls born in 2019 and later the GEBV correlation is decreased to 0.985. The slightly lower GEBV correlation for the young AI bulls suggests that the multi-lactation test-day single-step model made a little different genomic prediction than the single-trait single-step model with one dGEBV as 'phenotypic records'. As far as variance of the GEBV of the two single-step models are concerned, young genomic AI bulls have slightly higher GEBV variance with dGEBV as input data of the special single-step evaluation than the original single-step evaluation (Figure 7).

From Figures 6 and 7 we can draw a conclusion that the deregression of GEBV for bulls with daughters seems to be correct.

Conclusions

Deregressed EBV or deregressed GEBV have appealing statistical properties for diverse applications. The current multi-step genomic model was relied on the deregressed conventional EBV as 'pseudo-phenotypic' data.

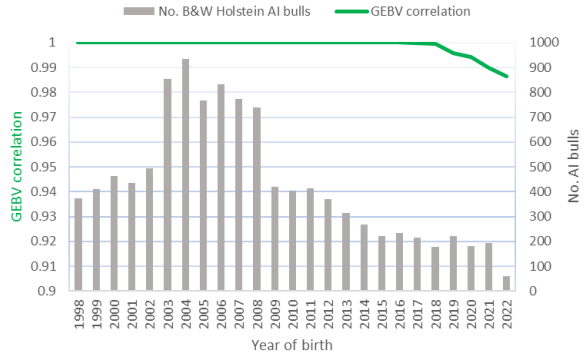

Figure 6. Correlation of single-step GEBV using deregressed GEBV and test-day yields for protein yield of all B&W German Holstein AI bulls

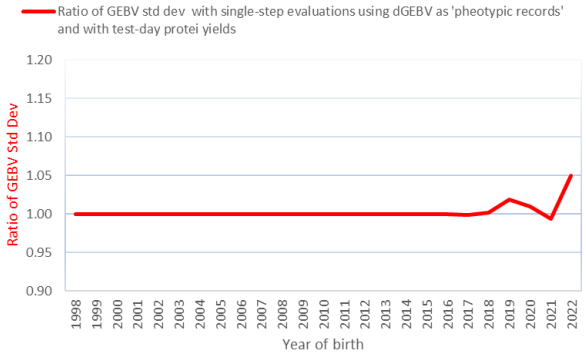

Figure 7. Ratio of standard deviations of GEBV of the B&W German Holstein AI bulls from the single-step evaluation with deregressed GEBV as input data over standard deviations of GEBV from the original single-step evaluation with test-day protein yields

We extended the matrix deregression method to deregress GEBV of the single-step evaluation. The single-step GEBV deregression method was assessed successfully for the four test-day traits in German Holstein. For all cows with test-day records, deregressed GEBV were moderately correlated with their GEBV. For bulls with daughters, degression of their GEBV must include GEBV of all genotyped cows with phenotypic records, because the genotyped bulls no longer represented the complete reference population which must be guaranteed in the bull GEBV deregression process as in the original single-step evaluation. For both cows with test-day records and bulls with daughters, deregressed GEBV had lower genetic trends, especially for cows with lactation in progress or missing lactations and for bulls having daughters with lactation in progress or missing lactations. Deregressed GEBV had higher variance than GEBV for the cows or bulls, in particular the cows' deregressed GEBV being much more variable than the bulls'. Equal GEBV were obtained from a special single-step evaluation with the deregressed GEBV as phenotypic data, compared to GEBV from the original single-step evaluation. This confirmed that the GEBV deregression was a reversible process. Not only the cows with test-day records and bulls with daughters received equal GEBV from the special single-step evaluations as from the original single-step evaluation, but also young, genotyped candidates obtained almost identical GEBV from the two single-step evaluations. In comparison to GEBV used as dependent variable in GEBV validation, the deregressed GEBV were more independent from the early GEBV of validation animals, therefore the deregressed GEBV may be more suited as the dependent variable of the GEBV test.

Acknowledgments

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Application of the Interbull genomic reliability method for single-step evaluations of test-day and conformation traits in German Holstein

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Abstract

National single-step genomic evaluation required accurate genomic reliabilities, particularly for young, genotyped animals. The Interbull genomic reliability method was tested for single-step evaluation of four test-day traits as well as for 25 conformation traits in German Holstein. Genotypic, phenotypic and pedigree data were taken from the official genomic evaluation in April 2023. More than 1.3 million genotyped animals were considered jointly with non-genotyped animals, and the genomic reference population exceeded half a million animals for the test-day traits. Selecting fewer SNP markers in reliability calculation for direct genomic values (DGV) was proven to be an efficient way of decreasing computing time or memory usage while retaining a reasonable accuracy when at least 15,000 equidistant SNP markers were chosen. Due to the extremely large reference population, the level of DGV reliabilities was very high, also for the young, genotyped candidates. Adjusting the theoretical DGV reliabilities based on the Interbull reliability method seemed to be unavoidable, especially for the large reference population. Variation in the DGV reliabilities was shown to be small among animals born in the same year, especially among the young, genotyped animals without own phenotypic records. Therefore, a constant genomic effective daughter contribution could result in reasonably accurate genomic reliability values and at the same time may provide a computationally much less demanding way for routine genomic reliability calculation with several million genotyped animals included. The single-step genomic reliability values were compared to conventional reliabilities as well as genomic reliabilities from the current multi-step genomic model for diverse groups of animals of German Holstein. The single-step genomic reliabilities of the test-day and conformation traits seemed to be consistent with the variance of genomic breeding values.

Key words: Genomic reliability, genomic breeding values, single-step model, test-day traits

Introduction

Single-step evaluation required accurate reliability values for estimated genomic breeding values (GEBV). The Interbull genomic reliability method (Liu et al., 2017) was developed for the current multi-step genomic model (MSM) as well as the single-step genomic model (SSM). The main goal of the Interbull genomic reliability method was to make national genomic reliabilities comparable across countries by applying the same reliability method in all the countries. Ideally, genomic reliability values should be consistent with the variances of GEBV. The main features of the Interbull genomic reliability method were 1) treating genotype data as an additional source of information contributing to animal's total reliability, 2) calculating exact, theoretical reliabilities of direct genomic values (DGV) for all genotyped animals under a SNP BLUP model, and 3) adjusting genomic reliabilities based on GEBV variance changes of validation bulls (VanRaden and O'Connell, 2018).

The step of calculating exact reliabilities of DGV in the Interbull genomic reliability method may take considerable computing time for countries with extremely large reference populations, even with the highly efficient software snp_blup_rel (Ben Zaabza et al. 2020).

Reducing the number of SNP markers can decrease the computing time for the calculation of DGV reliabilities. The impact of skipping this step of DGV reliability calculation in routine evaluation needed to be investigated.

The aims of this study were 1) to apply the Interbull genomic reliability method to genotypic, phenotypic and pedigree data of the German Holstein single-step evaluations for test-day and conformation traits, 2) to compare the accuracy of DGV reliabilities between scenarios using all and fewer SNP markers, and 3) to investigate the level and variation of the exact DGV reliabilities for young, genotyped candidates.

Materials and Methods

Data for single-step evaluation

Phenotypic, genotypic and pedigree data were obtained from the April 2023 routine evaluation of German dairy cattle breeds. Two groups of traits were chosen for this study: 25 conformation traits (Alkhoder et al. 2021) and four test-day traits (Alkhoder et al., 2023) including milk yield (MKG), fat yield (FKG), protein yield (PKG), and somatic cell scores (SCS). The conformation trait stature (STA) represented a linear type trait with a complete classification history, whereas the recording of locomotion (LOC) started several years later than STA. The national trait udder balance (EUB) was not included in Interbull MACE evaluation, and a new definition of angularity (ANG) was recently introduced in Germany in April 2023 with a much smaller phenotypic data set. Table 1 describes the data sets for the single-step evaluations of the test-day traits as well as the conformation traits for the German dairy breeds. The size of the bull and cow reference population for German Holstein breed is 524,187 for each of the four test-day traits or 386,062 for the conformation traits.

To validate the calculated genomic reliabilities of the test-day traits, the same truncated phenotypic data for the GEBV validation were used as in Alkhoder et al. (2023). Test-day records in last 4 years from the evaluation April 2021 were truncated for simulating an early prediction back in April 2017. In contrast to the data truncation of 4 years for the test-day traits, conformation records in last two years were removed from the full evaluation of April 2023 for simulating an early evaluation in April 2021.

Table 1. Description of the data sets for the single-step evaluations of four test-day and 25 conformation traits in April 2023

Frequency	Test-day traits	Conformation 25 traits
Genotyped animals	1,318,780 Holstein animals (1,138,039 females and 180,741 males)	
Phenotyped animals	13,528,444	3,144,366
Phenotypic records	263,673,267 test-day yields	3,144,366 type records
Genotyped or phenotyped animals	14,402,662	4,131,336
Animals in pedigree	21,850,276	10,048,593
Reference animals	524,187	386,062

Table 2 shows the data sets used for validating genomic reliabilities, including both the full and truncated evaluations. For each test-day trait, the number of reference animals decreased more than a half in the truncated evaluation, due to the rather short history of female animal genotyping in Germany. To make the genomic validation reflect more realistically a future prediction, only two years of phenotypic data were therefore deleted for the conformation traits. The number of reference animals for the conformation traits was reduced from 386,062 in the full evaluation in April 2023 to 263,252 in the truncated evaluation in April 2021. The genomic validation for the test-day traits was conducted using data from an older evaluation than the conformation traits.

Table 2. Description of the data sets for validating genomic reliabilities for the test-day and conformation traits

Frequency	Test-day 4 traits	Type 25 traits
Full evaluation	April 2021	April 2023
Truncated run	April 2017	April 2021
Genotyped Holstein animals	949,636	1,318,780
Phenotyped animals (full & truncated runs)	12,571,710 11,032,395	3,144,366 2,862,770
Animals in pedigree	20,461,400	10,048,593
Reference animals (full & truncated evaluations)	353,347 156,970	386,062 263,252

For computing the exact, theoretical reliability values of DGV for all genotyped animals, a genomic reference population comprising genotyped cows or bulls with own phenotypic data needed to be set up. Table 3 describes the composition of genomic reference population for 5 selected traits: PKG representing the test-day traits, four conformation traits STA, LOC, ANG and EUB. In Table 3 it can be seen that the test-day milk production trait PKG has more than half a million reference animals as a result of the large-scale female animal genotyping in Germany. The 4 conformation traits have a smaller reference population than the test-day trait PKG, because not all cows in milk recording program were classified for conformation. The national trait EUB has only a little lower number of reference animals than the regular type traits STA and LOC. Due to the trait definition change that was introduced in April 2023, the conformation trait ANG has the lowest number of genotyped cows with classification record according to the new definition.

Between the data sets for April 2023 and April 2021 there was a difference in genotype editing for bulls. Due to un-intentional selective genotyping of bulls in early years of genomic selection, we decided to remove genotype records of bulls born before 2005 in the single-step evaluations with the data set from April 2023. However, this genotype data editing was not implemented in the single-step evaluations with the data set from April 2021.

Table 3. Genomic reference populations of selected traits in April 2023 evaluation

Trait	Reference animals		
	Cows	Bulls	Total
Protein yield	478,588	45,591	524,179
Stature	357,365	28,635	386,000
Locomotion	349,083	27,696	376,779
Angularity	198,170	27,748	225,918
Udder balance	305,122	27,205	332,327

Scenarios of reducing SNP markers for faster calculation of DGV reliabilities

As a core component of the Interbull genomic reliability method (Liu et al. 2017), the calculation of DGV reliability values may be computationally demanding for extremely large reference populations like those in Table 3. Therefore, the impact of reducing SNP markers on the DGV reliabilities was investigated in a similar way by selecting equidistant SNP markers as by Sargolzaei et al. (2014) and Strandén and Mäntysaari (2015). Table 4 describes the test scenarios of selecting the SNP markers for faster DGV reliability calculation. The *base scenario* of using all SNP markers, RELall, has 45,613 SNP markers included in the DGV reliability calculation as in the routine genomic evaluation for German Holstein. Five additional scenarios were simulated by selecting every 2 (RELevery2), every 3 (RELevery3), every 4 (RELevery4), every 5 (RELevery5) and every 10 (RELevery10) equidistant SNP markers. When every 10 SNP markers were selected in scenario RELevery10, the number of markers was reduced to 4,562. For this specific investigation, genotypic and phenotypic data from April 2021 were used (see Table 2) and the selected trait was PKG.

Table 4. Scenarios of selecting equidistant SNP markers for faster calculation of DGV reliabilities

Scenario	No. markers
All SNP markers (RELall)	45,613
Every 2 markers (RELevery2)	22,807
Every 3 markers (RELevery3)	15,205
Every 4 markers (RELevery4)	11,404
Every 5 markers (RELevery5)	9,123
Every 10 markers (RELevery10)	4,562

Results & Discussion

All computations were done on a Linux server equipped with 42 cores and 512Gb RAM.

Impact of fewer markers on DGV reliabilities

Reducing the number of SNP markers for the DGV reliability calculation leads to significant decreases in computing time and memory usage, which can be seen clearly in Table 5.

For the base scenario of using all SNP markers, RELall, the computing time of the DGV reliability values depended mostly on the number of all genotyped animals and the number of animals in reference population. For weekly genomic evaluation by adding up to 20,000 newly genotyped animals, the DGV reliability calculation required less than 4 minutes.

Table 5. Computational requirements for the scenarios of the calculation of DGV reliabilities

Scenario	Total time (min.)	Peak RAM (Gb)
All SNP markers (RELall)	215	88
Every 2 markers (RELevery2)	96	42
Every 3 markers (RELevery3)	71	28
Every 4 markers (RELevery4)	60	21
Every 5 markers (RELevery5)	55	18
Every 10 markers (RELevery10)	47	10

Figure 1 shows average DGV reliabilities of all 949,636 genotyped Holstein animals in the April 2021 evaluation for trait PKG. The number of genotyped animals (in blue bar) increased drastically in recent years, due to the routine herd genotyping of female animals in Germany. Thanks to the higher number of reference animals, 353,347 (Table 2), DGV reliabilities for the genotyped animals have a rather high average, above 0.94 for candidates younger than 1 year old.

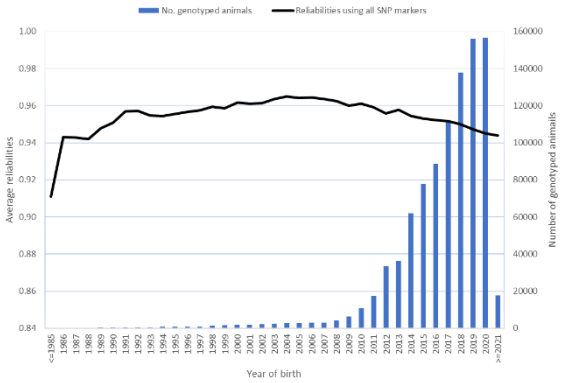

Figure 1. Average DGV reliabilities of protein yield for genotyped Holstein in April 2021 evaluation

Figure 2 shows correlation of DGV reliabilities between a scenario and the base scenario for protein yield of all the genotyped animals. Across all the birth years, the within-year correlation has an average of 0.997, 0.990, 0.980, 0.968, and 0.903 for the scenarios RELevery2, RELevery3, RELevery4, RELevery5, and RELevery10, respectively.

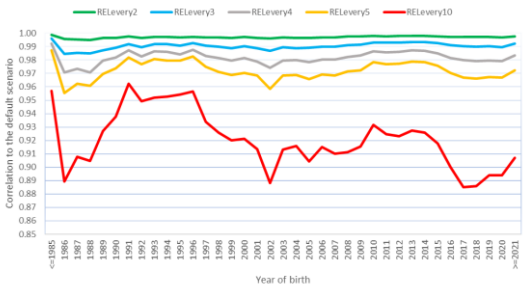

Figure 2. Correlation of DGV reliabilities between different scenarios for protein yield of all genotyped Holstein animals

Figure 3 shows average difference in DGV reliabilities of protein yield for all genotyped animals between a scenario and the base scenario. With fewer SNP markers selected, DGV reliabilities tend to be over-estimated by

comparing to the base scenario using all the SNP markers. The difference in DGV reliabilities seems to higher for the youngest or oldest genotyped animals than animals in between. It can be clearly seen that using fewer SNP markers leads to higher DGV reliability values.

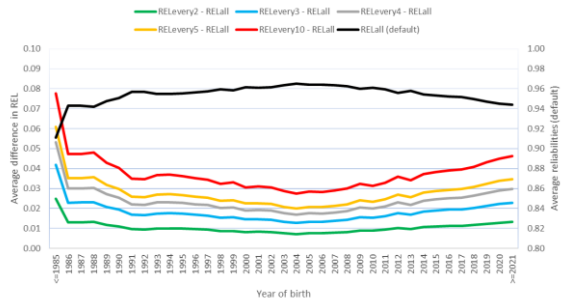

Figure 3. Average differences of DGV reliabilities of protein yield of the scenarios with the base scenario using all markers for all genotyped animals

For the youngest candidates born in 2020 and later in the April 2021 evaluation, their DGV reliabilities of the base scenario were regressed on those from each of the scenarios. Figure 4 shows that selecting every 3 equidistant markers of scenario RELevery3 gives a reasonably high correlation of DGV reliabilities with the base scenario of using all SNP markers and at the same time requires only c.a. 1/3 RAM usage and computing time (Table 5).

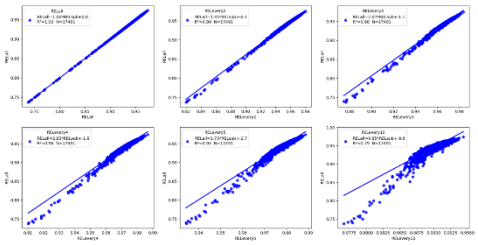

Figure 4. Regression of DGV reliabilities of youngest candidates born after 2020 from the base scenario on the other scenario for trait protein yield

Average and variance of DGV reliabilities

For 8,123 Holstein AI bulls owned by German AI studs, Figure 5 and Figure 6 show average DGV reliabilities by birth year for the test-day traits and for the four chosen conformation traits, respectively. Because of the extremely large reference populations (Table 3), the average of DGV reliabilities is very high for any of the 8 selected traits, particularly for the young genomic AI bulls born in 2020 to 2022. Trait ANG has the lowest DGV reliabilities, due to its smallest reference population. Another reason for the extremely high level of DGV reliabilities is that no residual polygenic effect be assumed in the SNP BLUP model for the DGV reliability calculation.

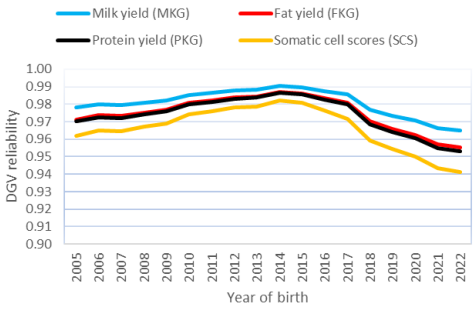

Figure 5. Average DGV reliabilities of German Holstein AI bulls for test-day traits

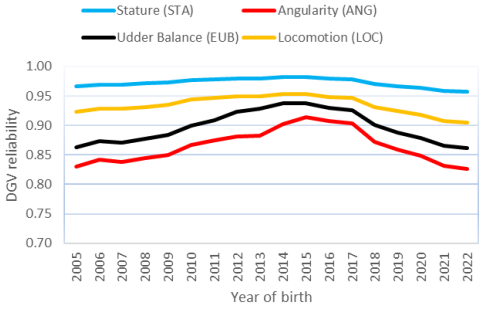

Figure 6. Average DGV reliabilities of German Holstein AI bulls for four conformation traits

Standard deviation of DGV reliabilities of the AI bulls is shown in Figure 7 for the test-day traits and in Figure 8 for the four conformation traits, respectively. It can be seen in both figures that traits with larger or more informative reference population have lower variation in DGV reliabilities. Test-day trait MKG, having the highest heritability value and thus the highest reliability among the four test-day traits and all the 8 traits, has shown to be least

variable in DGV reliabilities. In contrast, conformation trait ANG has the largest variance in DGV reliabilities due to its smallest reference population. Across all the traits, the DGV reliabilities have rather small variation, especially for young genomic AI bulls born in 2020 and later.

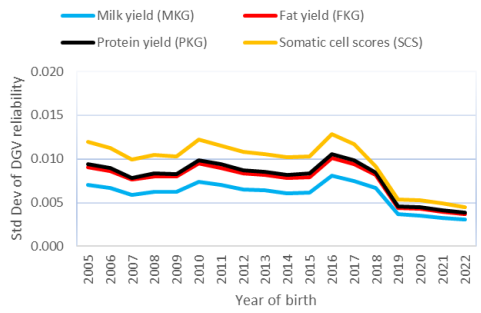

Figure 7. Standard deviations of DGV reliabilities of the German Holstein AI bulls for test-day traits

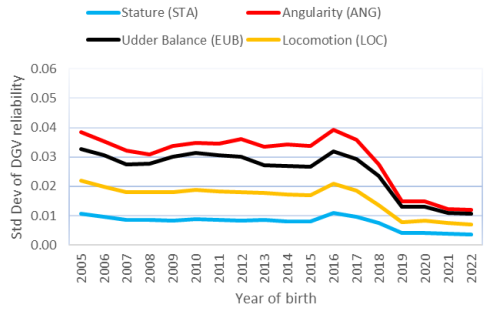

Figure 8. Standard deviations of DGV reliabilities of the German Holstein AI bulls for the four conformation traits

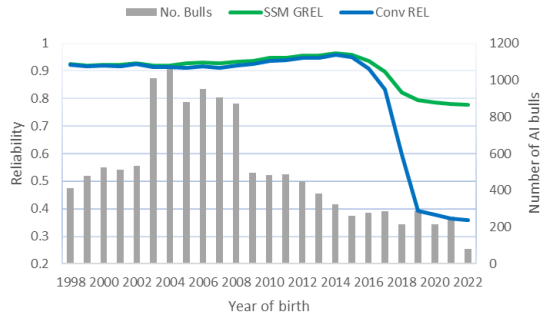

Figure 9. Genomic and conventional reliabilities of the German Holstein AI bulls for trait protein yield

Genomic and conventional reliabilities

For trait PKG, Figure 9 shows genomic and conventional reliability values of Holstein AI bulls owned by German AI studs. For bulls with complete daughter information born between 1998 and 2015, genomic and conventional reliabilities are essentially equal. However, for bulls born in 2016 and later with incomplete or no daughter information yet, genomic reliabilities are a little or significantly higher than the conventional reliabilities, respectively.

Figure 10 shows genomic and conventional reliabilities of trait ANG. Due to much less national cow data for this newly changed trait, bulls with or without daughters have always higher genomic reliabilities than conventional reliabilities.

Like trait PKG, genomic and conventional reliabilities are nearly equal for bulls with daughters and higher for young AI bulls without daughters for all the other test-day or the conformation traits, except ANG.

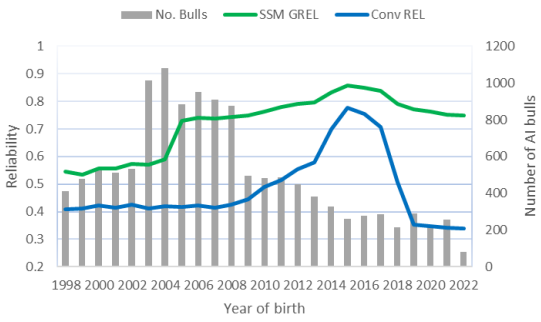

Figure 10. Genomic and conventional reliabilities of the German Holstein AI bulls for trait angularity

Single-step and multi-step genomic reliability values

For trait PKG, both single-step and multi-step genomic reliabilities are shown in Figure 11 for the German Holstein AI bulls. As a result of the removal of genotype data of bulls born before 2005, single-step reliabilities are a little bit lower than the multi-step ones for the daughter-proven bulls born between 1998 and 2004. Overall, the two sets of genomic reliabilities are nearly equal for all the bulls with daughters. The single-step genomic reliabilities are evidently

higher than the multi-step ones for the young AI bulls born in 2020 and later, because the SSM uses more phenotypic and genotypic information than the MSM.

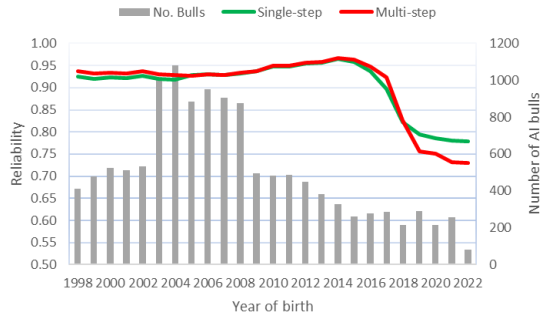

Figure 11. Single-step and multi-step genomic reliabilities of the German Holstein AI bulls for protein yield

For trait ANG with its definition changed recently, only two years of domestic cows had phenotypic records, besides the MACE data of foreign bulls. The SSM reliabilities are significantly higher than reliabilities of the MSM, as shown in Figure 12. The much lower SSM reliabilities for the AI bulls born before 2005 can be explained by the truncation of genotype data of the bulls born in 2004 and earlier. For new traits like ANG with limited phenotypic information, SSM is shown to have clearly higher genomic reliabilities than the current MSM.

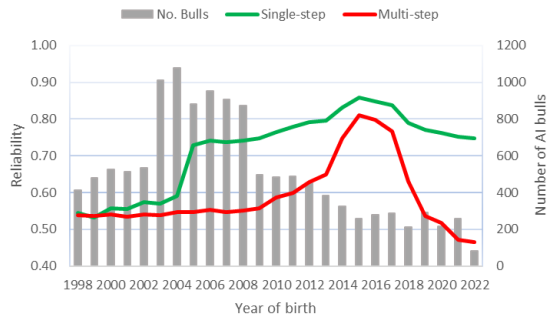

Figure 12. Single-step and multi-step genomic reliabilities of the German Holstein AI bulls for angularity

Conclusions

Interbull genomic reliability method was tested for the single-step genomic evaluation using phenotypic, genotypic and pedigree data of German dairy cattle from the April 2023 official evaluation. Calculation of exact, theoretical DGV reliability values for all genotyped animals was shown to be the most time-consuming step of the Interbull genomic reliability method. Five scenarios of reducing the number of SNP markers were conducted to investigate the computational efficiency and DGV reliability accuracy. For the extremely large reference population of German Holstein, at least 15,000 equidistant SNP markers must be chosen to achieve a reasonably high accuracy of the DGV reliabilities while significantly reducing the computing time and memory usage. Based on the genotypic and phenotypic data of four test-day traits and 25 linear conformation traits, average and variances of the DGV reliabilities for various groups of animals were calculated. The average of the DGV reliabilities for young, genotyped animals was found to be rather high, possibly caused by the size of the extremely large reference population. The very high level of DGV reliabilities suggested that an adjustment of the theoretical DGV reliabilities be necessary to guarantee the proper level of genomic reliabilities for young candidates. Meanwhile it was shown that variation of the DGV reliabilities within birth year was small, which indicated that calculating individual DGV reliabilities be less crucial for a large reference population like German Holstein. By comparing to conventional reliabilities and the current MSM genomic reliabilities, the final genomic reliabilities of the single-step model were shown to be higher for young, genotyped candidates without own phenotypic data. Based on the application of the Interbull genomic reliability method to the German dairy cattle data, guidelines for a routine implementation in national single-step evaluation will be developed.

Acknowledgments

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Interbull new services: Current and Future

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Abstract

Interbull Centre has been closely working with several working groups on different topics spacing from the improvement of the MACE model to a revision of validation methods due to genomic pre-selection to expansion of the MACE and Interbeef portfolio to new traits. The activity of such working groups has progressed so nicely that the outcome of their research has been either recently implemented or it is aimed for an implementation in the near future (within one year's time). The present article aimed at providing the reader with an overall view of such activities and the related new services they have, or are going to, generate.

Key words: International Evaluations, Interbull, Interbeef, Validation, Genomic pre-selection, New traits

Introduction

Interbull Centre is the operational unit of Interbull, a permanent sub-committee of ICAR, located in Uppsala (Sweden) and represented by a team of 10 people between geneticists and IT. In reality, though, and thanks to the world wide network available through the Interbull community, the Interbull Centre team can count on a much larger resource availability represented by the different technical working groups whose members are part of either the steering or technical committees. Interbull Centre staff works closely with such working groups providing assistance on matters like data and infrastructure availability, feedback and guidance, when needed, so to assure a smoother and timely transition of the different research areas into productions.

The activity, deliverables and/or implementations' plans for several Interbull activities as described in Fig. 1 has been reviewed in the present article.

Evaluation – Plans for a "GPS-MACE"

One of the main role for Interbull has been to provide international genetic (via MACE) and later on genomic (via GMACE, InterGenomics) evaluations to the different member countries so to facilitate the comparison of bulls' performances across countries and, in doing so, providing farmers with an independent tool to use for identifying the bulls that best would perform in their own specific environmental conditions. Over the years, however, and especially with the onset

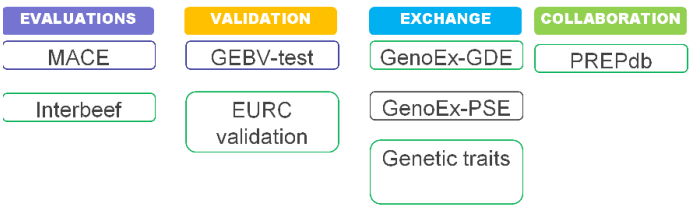

Figure 1. Graphic representation of the main activities delivered by Interbull Centre and the areas therein where new developments have been introduced.

of genomic selection, MACE international evaluations have changed their usability moving from a "comparison" tool to a way for countries to include foreign bulls' information in their national genomic evaluation. The onset of genomic selection, and its related methodologies refinements, especially single step type of evaluations, posed two main needs: 1) to assure that national input to MACE evaluations would have been kept free of genomic data, so to avoid problems with double counting of information and increased bias, 2) start investigating ways to enhance the current MACE model making it able to better account with the accumulation of genomic pre-selection bias in the data.

Before genomic selection it was reasonably correct to consider the within- family pre-selection random in EBVs models. With the onset of genomic selection, though, this assumption could no longer be considered valid, as genomics made it possible to identify above average bulls, within a family, without any need for progeny testing. The accumulation of genomic pre-selection (GPS) directly associated with this behavior altered the distributions of breeding values for AI bulls (Sullivan et al. 2023).

An Interbull working group was established back in 2018 with the double aim of understanding better the nature and source of genomic pre-selection and work towards the implementation of a "Future" MACE (GPS-MACE) model. Several reports have been produced by the working group (Sullivan et al, 2019; Sullivan et al., 2022) with the latest being the presentation, during the Interbull technical workshop, which was held in Rome on February 2023, of a new MACE model and its possible impacts on the countries data (Strandén and Mäntysaari, 2023; Jibrila et al., 2023; Sullivan et al. 2023). Further refining of the new MACE model is planned during 2024 with the aim of carrying out the first GPS-MACE pilot run in the late fall of 2024.

Evaluations – Expanding Interbull Portfolio

The need for a clear procedure to follow for identifying new possible traits suitable to the MACE or other international evaluations, has been identified as an important strategic goal during the 2020-2023 Interbull Strategic meeting which was held in Uppsala in January 2020.

In 2021 a working group was appointed with the aim of defining a clear set of steps that could assure not only the identification of all the key decision's factors for implementing of any new traits, but also could take into account the need for the required (new) infrastructure and methodology as well as the need for possible new business models, business plans and appropriate fee structure (Fig. 2).

Pivotal point of the new procedure is the usage of the latest database developed at Interbull Centre: the **P**erfomance **R**ecording

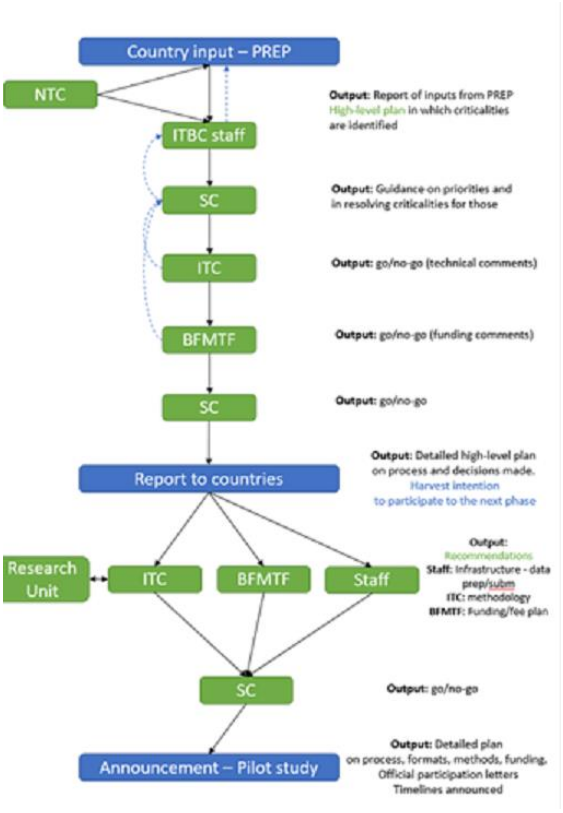

Figure 2. Interbull procedure for identification and inclusion of new traits

Evaluations and **P**ublication (**PREP**) database. PREP is equipped with ad hoc electronic forms that users can fill in to provide Interbull Centre with the information on which traits available at national level they would like to see included in an international evaluation.

By reviewing the information since then collected (Fig. 3), the working group identified three initial traits which showed some potential: Retained placenta, Hypercalcaemia/ milk fever and Gestation length.

The traits were further discussed at the Interbull technical workshop held in Rome on February 2023 where it came out how, while for the two fertility traits an international evaluation would have been considered useful the same was not entirely true for the trait gestation length which was by the majority of participants considered mostly a management tool rather than a selection trait (Haugaard et al, 2023). Figure 3 showed the amount of information collected in PREP for any new traits for which at least two countries had expressed a medium-to-high interest for an international evaluation. Two more trait groups stands out: feed efficiency and claw-health related traits.

The working group will further review all the information collected and will provide a recommendation on how to proceed to the steering committee in line with the new trait procedure as described in figure 2.

Evaluation - Expanding of Interbeef portfolio

The Interbeef portfolio, currently made of adjusted weaning weight and calving traits (including calving ease, both direct and maternal, and birth weight) has been recently expanded with the inclusion of carcass traits (carcass conformation, fat and weight). The new trait group will be estimated for all the Interbeef breeds, currently Aberdeen-Angus, Limousin, Charolais, Simmental and Hereford.

After few research and pilot runs, the results have been found satisfactory by both technical group and participating countries. The first official test run for carcass traits was performed in April 2023 and the first official routine evaluation has been performed in October 2023. (Macedo, 2023, Interbull 2023a)

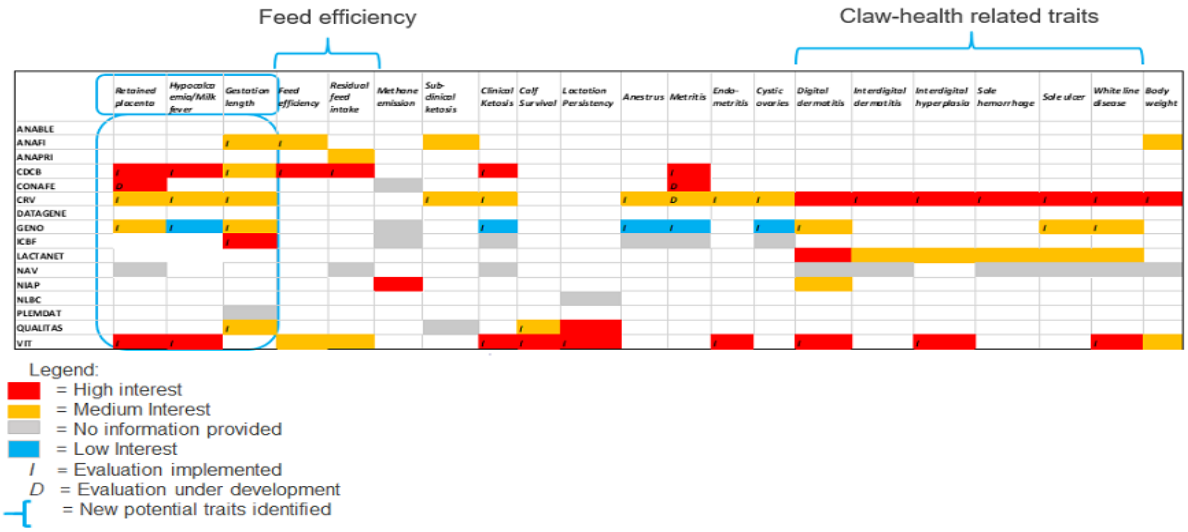

Figure 3. Overview of information on possible new traits to be considered for an international evaluation. Source: August 2023, Interbull PREP database (https://prep.interbull.org/)

Validation – Enhanced GEBV test software

Another Interbull core activity is represented by the validation of national statistical methods to assure that countries' estimates, which would then become inputs to the international evaluations, would be as unbiased as possible. Five validation methods have been developed by Interbull over the years: four dealing with conventional genetic models (trend tests I, II, III and Mendelian Sampling Variance test) and one specific for genomic models (GEBV-test). National genetic centres are requested to provide validation results a) when major changes have been introduced in their genetic/genomic models, b) when providing data for the first time for a given breed/trait evaluation, or c) when it has been more than two years since the last validation.

The fast development of genomic evaluations in many countries was the reason behind the creation of a validation working group whose aim is twofold: 1) revise the current version of the GEBV-test software as it was developed in the early stage of the genomic era when still very few countries had an evaluation for it and the amount of GPS bias was negligible, and 2) develop a new trend test III, looking at the random variation associated with new daughters' information, that could better cope with the even less number of proven bulls available.

The task of reviewing the current GEBV-test was assessed by the working group as a more urgent matter, therefore lots of activities and developments were carried out towards this aim. A presentation of an initial revised version of the program was given during the Interbull technical workshop in Rome were also feedback from countries, who had the possibility to test the software, were discussed (Sullivan, 2023; Liu et al, 2023; Mota et al., 2023; Jibrila and Eding, 2023). The version presented at the technical workshop was enhanced with several new features like: the possibility to make a base adjustment, so that the mean and variance of reduced-data evaluations would match the base of expression of full-data evaluations**;** possibility to use different validation targets than the official one (represented by de-regressed EBV) like for example EBV from full-data evaluation or GEBV from full-data evaluation. The availability of different validation targets could be useful at national level to perform further testing.

In general, the enhanced software was well perceived by the people attending the workshop. Afterwards, the software was further enhanced providing information about the power of the test in case (like for small populations) the result would be inconclusive. The output of the software has also been improved by providing additional information that would be useful for the users should they wish to perform further testing on the data.

The software is currently under its final revision and testing, and it is expected to be rolled out in production as the official Interbull GEBV-test in 2024.

Validation – EU Reference Centre (EURC) Validation

Since 1996, Interbull Centre has taken up the role of EU Reference Laboratory. Starting from 1 November 2018, Interbull Centre has taken up its duties as the EU Reference Centre for Zootechnics, to ensure continuity in this field (EU Animal Breeding Regulation, 2016).

Under the umbrella of the EURC activities, Interbull Centre launched a new service in 2022: the EURC validation, aiming at providing validation of conventional genetic models to all European breeding organisations and/or national genetic centres, regardless of their involvement with Interbull's activities. The service covers all dairy breeds and will assist European countries in the process of harmonization of models applied while at the same time providing a "quality stamp" on conventional evaluation services as required by the current EU legislation for bulls advertised in the European market.

Exchange – Genetic Traits Data Exchange

Since 2019, Interbull Centre has been involved in the collection of several genetic traits, including recessive traits, identified as important for the Holstein breed by the World Holstein Friesian Federation (WHFF). The service aims at facilitating the exchange of such information in a timely manner as well as improving the resolution of any possible conflicting information that might arise. The exchange of genetic traits data is made possible via a dedicated module of the Interbull Data Exchange Area database (IDEA).

The information that can be shared is based on direct genetic test (direct genotyping) of a well-defined list of traits (Interbull, 2023b). The service provides several benefits to its users, such as:

- One common platform to share information with other organizations taking part in the service
- Sharing of important genetic information to make better breeding decisions, avoiding mating of carriers of recessive diseases or spreading of unwanted alleles in a population.
- As the service is an extension of IDEA pedigree, the consistency of the unique international animal ID is maintained across countries.
- Allowing access to a wider set of information and assuring a smoother and more timely exchange of genetic defects' information among participating organisations
- Drastically reduce the amount of conflicting information among countries
- Cost reduction by avoiding multiple genetic tests on the same animal for the same traits

The service, started for Holstein, has been recently expanded to share genetic traits data for the Brown Swiss breed as well.

Conclusions

Important improvements of the services offered by Interbull Centre have been either implemented or are planned to be implemented during the course of next year.

Acknowledgments

Interbull Centre would like to express its gratitude towards all active working groups and working groups members, as well as to the Technical and Steering Committees for their endless support and guidance.

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Genomic Evaluations for Body Maintenance Requirements in Canadian Holsteins

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Abstract

A genomic evaluation was developed for Body Maintenance Requirements (BMR) in Canadian Holsteins, with the first official publication in April 2023. The BMR index characterizes feed requirements for maintenance based on the metabolic body weight of the animal. Body weights of lactating cows recorded through feed advisory services in Quebec since 2002 are used in the genetic evaluation. Metabolic body weight (MBW), calculated as body weight$^{0.75}$, in first, second, and third lactations are analyzed in a three-trait linear animal model as separate but correlated traits with repeated records within a lactation. Genetic parameters were estimated by MC EM REML method using a subset of the data including 373 219 records from 195 198 cows. Heritabilities for MBW in first, second, and third lactation were 0.34, 0.43, and 0.47, respectively, and repeatabilities were 0.53, 0.61, and 0.64, respectively. Genetic correlations between different lactations were strong, ranging from 0.78 to 0.86. A Single-Step genomic evaluation was implemented using the MiX99 software. The April 2023 official evaluation run had records from 540 619 cows of which 28 263 were genotyped and a total of 47 967 genotyped animals in the model. The BMR index combines genomic estimated breeding values (GEBVs) for MBW in the three lactations at equal weightings. This index is published as a relative breeding value, with a mean of 100 and standard deviation of 5 for base bulls, where the sign is reversed such that higher values represent a lower MBW and thus lower body maintenance requirements. The average reliability of BMR for young, genotyped bulls was approximately 68%. Observed phenotypic and genetic trends demonstrated that animal size has been steadily increasing over time. The BMR evaluations can be considered by producers looking to reduce or maintain cow body size in their herd as another way to reduce feed costs.

Key words: Body maintenance requirements, metabolic body weight, single-step, genomic evaluation

Introduction

While genetic selection has historically focused on increasing performance and revenue, it also has great value in reducing inputs and expenses. Feed represents the greatest expense for dairy farms and as prices continue to rise, there is increasing interest and need to improve the efficiency of feed use on dairy farms through genetic selection.

Feed efficiency can be defined in many ways, but broadly is used to describe how efficiently animals convert feed into product. Energy from the feed eaten by cows is used for milk production but also maintenance, growth, reproduction, and activity. Feed efficiency is a complex trait and there are many different expressions and indicators that can be targeted for genomic selection. Residual Feed Intake (RFI) is a popular measure of feed efficiency and can be estimated in dairy cattle by the linear regression of Dry Matter Intake (DMI) on factors representing various energy sinks, such as milk energy and body weight (Koch et al., 1963; Connor et al. 2015). Genomic selection for RFI has been shown to be feasible to breed for cows that

convert feed gross energy to net energy more efficiently without impacting production.

The other route to improve feed efficiency is to reduce maintenance requirements for a cow by decreasing body weight. Small cows will have lower maintenance requirements and require less feed to meet those needs, which is a financial benefit for producers. Breeding values for feed saved, proposed by Pryce et al. (2015), combine the reductions in feed eaten associated with RFI and the effect of body weight on feed intake as required for maintenance. Performing multi-trait selection for improved metabolic efficiency through RFI and reduced maintenance requirement can target cows that have the genetic ability to use a greater proportion of their feed intake for milk production.

In April 2021, Canada released genomic evaluations for feed efficiency which is a genetic RFI derived by using a linear function of multiple-trait evaluations for DMI and the energy sinks of energy corrected milk and Metabolic Body Weight (MBW) (Jamrozik et al., 2021, 2022). The overall aim of the Canadian feed efficiency evaluations is to enable selection of cows that use less feed at the same level of production and body size after the peak of lactation (metabolic feed efficiency).

Not included in Canadian feed efficiency is the second component for selection for reduced feed requirements, i.e. maintenance requirements. The net energy needed for maintenance is a function of MBW and establishing genetic evaluations for MBW would allow for selection for less feed required for maintenance to be used alongside feed efficiency evaluations. The focus of this paper is to describe the implementation of a routine genomic evaluation for Body Maintenance Requirements (BMR), which was launched in Canada for the Holstein breed in April 2023.

Materials and Methods

Data

Body weight (BW) data on lactating cows is collected voluntarily for feed advisory services offered by Lactanet for herds in the province of Québec. The BW measurements are estimated using a tape measuring heart girth circumference. Holstein data recorded since 2002 was considered for use in genomic evaluations. Herds determined to be consistently recording individual animal BW as a continuous measure were selected for inclusion. Body weights recorded between 0 and 305 DIM in first, second, and third lactation were converted to MBW $kg^{0.75}$. Multiple MBW measures in a lactation for an individual animal were kept if available. The average number of records per lactation per cow was 1.15. Approximately 7% of lactations in the April 2023 genetic evaluation data had multiple records (up to 11 records per lactation) and records were on average 48 days apart. Most weights were recorded within the first 60 DIM. After all, editing data used in the April 2023 evaluation for BMR consisted of 387 037, 296 604, and 198 719 records for first, second, and third lactation, respectively, from 540 619 cows.

Model

The model is a three-trait linear animal model for MBW in first, second, and third lactation with repeated records within each lactation. The same model is used for MBW in each lactation, considering the fixed effects of herd, age at calving in monthly classes, DIM class (daily DIM classes for first lactation up to 98 DIM and then weekly classes; weekly DIM classes for second and third lactation), and month of weighing (12 classes), and random effects of herd-year of calving (HY), animal additive genetic, permanent environmental (PE), and residual. In matrix notation, the model can be written as:

$$\boldsymbol{y = Xb + Z_1hy + Z_2a + Z_3p + e}$$

where y is a vector of observations (MBW in first, second, or third lactation), **b** is a vector of all fixed effects, **h** is a vector of HY effects, **a** is a vector of animal additive genetic effects, **p** is a vector of PE effects, **e** is a vector of residuals, and $\mathbf{X}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, and $\mathbf{Z_3}$ are the respective incidence matrices. Random effects were assumed to be normally distributed, with means equal to zero. Model assumptions are that: v(**h**) = **I** ⊗ **HY**, **I** is an identity matrix and **HY** is the covariance (3x3) matrix for HY effects, v(**a**) = **H** ⊗ **G**, **H** is a combined pedigree-genotype relationship matrix, **G** is the additive genetic covariance matrix, v(**p**)= **I** ⊗ **P**, **P** is the covariance matrix for the PE effects, v(**e**) = **E, E** is a diagonal matrix of residual effects.

Genetic Parameters

Co-variance components and genetic parameters were estimated by MC-EM-REML as implemented in MiX99 (MiX99 Development Team, 2017) using a subset of the data including 373 219 records from 195 198 cows. This subset of the data only included herds still recording BW within the last five years and with multiple years of recorded BW. Cows with a record in second or third lactation were required to have a record in all preceding lactations. Summary statistics for the data used for genetic parameter estimation are presented in Table 1. The same model as described for genetic evaluation purposes above was used, but the combined pedigree-genomic relationship matrix **H** was replaced by an additive relationship matrix **A**.

Genomic Evaluation

A three-trait Single-Step genomic evaluation was implemented at Lactanet Canada using MiX99 and related software (MiX99 Development Team, 2017). The April 2023 data included 47 967 genotyped animals, with 28 263 genotyped cows with records and 8 635 genotyped sires. Animals are genotyped either with 50K SNP panel or a low-density panel and imputed to 50K using F-Impute (Sargolzaei al., 2014). 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 80% of the total genetic variance was explained by SNP effects. Scaling of **G** and **A** is performed using the Christensen (2014) method. The APY algorithm for Proven and Young (Misztal et al., 2014) is applied for inversion of **G**, with the core population of 20 000 (the oldest genotyped animals in the Lactanet database). Groups for unknown parents are not included in the model. The SNP effects, to be used for calculating Genomic Estimated Breeding Values (GEBV) for genotyped animals not included in the single-step core analysis, are estimated from the GEBV of reference animals (as in Lourenco et al., 2015). Reliability of GEBV is approximated by a weighted (80:20) average of Direct Genomic Value (**DGV**) and animal model reliabilities (Sullivan et al., 2005). The DGV reliabilities are

Table 1. Descriptive statistics of the dataset used for parameter estimation.

Lactation	Records	Cows	BW (kg)		MBW ($kg^{0.75}$)	
			Ave.	SD	Ave.	SD
1	234 498	195 198	620.3	63.2	124.2	9.5
2	97 661	73 253	674.1	67.4	132.2	9.9
3	41 060	28 170	708.3	70.8	137.2	10.3

BW = body weight, MBW = metabolic body weight

calculated using SNP prediction error co-variances with the SNP-BLUP-REL software (Luke, Finland). Animal model reliabilities are calculated based on Effective Daughter Contributions (EDC). The EDC and reliability software of Sullivan (2023) is used.

Relative Breeding Values

Genetic evaluations for BMR combine the three individual MBW evaluations for first, second, and third lactation at equal weighting and it is the only value published. The sign of the combined BMR evaluation is reversed, such that the higher values represent the more desirable, lower body maintenance requirements (lower MBW). The BMR evaluation is expressed as Relative Breeding Values (RBV) with a mean of 100 and SD of 5 for base bulls that for April 2023 are those born 2008-2017 and with an 'official' status. Sire evaluations are defined as 'official' for bulls with at least 20 daughters from 5 herds with MBW data and a minimum reliability of 70%.

Results & Discussion

Phenotypic Trends

The average body weight of Holstein females in the dataset available have been increasing over time. Figure 1 shows the phenotypic trend for average BW (kg) by year of birth by lactation number, including only those weights occurring in the first seven weeks of lactations. The overall increasing trend was similar for each of the lactations. The average BW of third lactation cows has gone from roughly 671 kg for cows born in 2000 to 735 kg for 2017-born cows. Since 2010, third lactation body weights have increased 4.3 kg/year. At the same time, the age at calving for each lactation presented has been slowly decreasing.

Genetic Parameters

Heritability and genetic and phenotypic correlation estimates for MBW in first, second, and third lactation are shown in Table 2. Heritability estimates for MBW were moderately high and ranged from 0.34 for first lactation to 0.47 for third lactation. These heritabilities were slightly lower than the 0.46, 0.51, and 0.60 found by Lidauer et al. (2019) for MBW in first, second, and third lactations, respectively, but showed the same trend of increased heritability with higher lactations. The within lactation repeatability

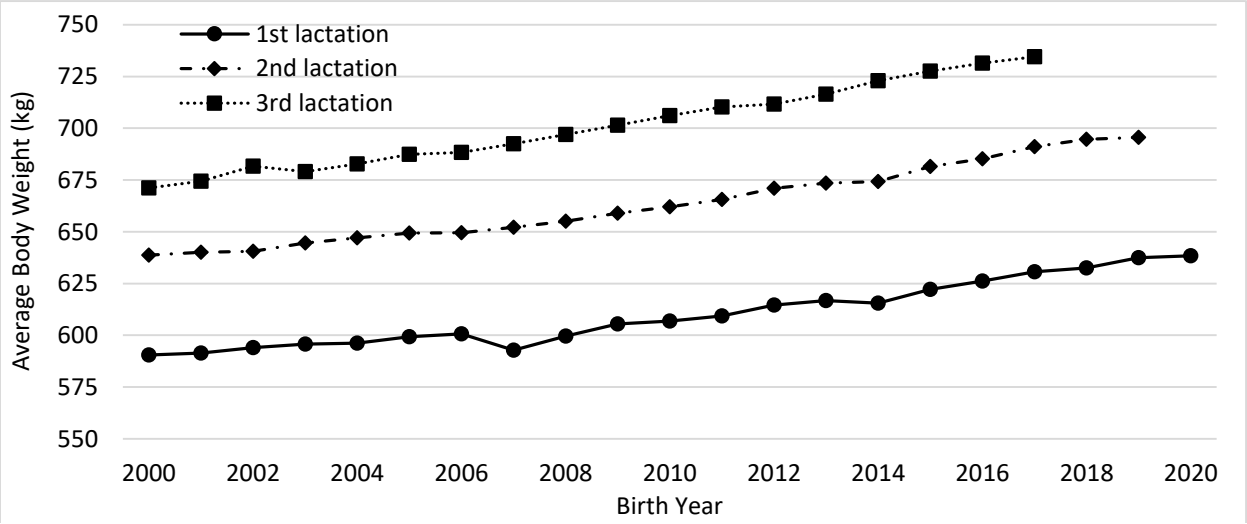

Figure 1. Phenotypic trend by year of birth for average body weight (kg) for cows in first, second and third lactation with weights recorded within the first seven weeks of the lactation.

estimates increased as parity number increased, going from 0.53 for MBW for first lactation, 0.61 for second, and 0.63 for third.

The genetic correlation between MBW in sequential lactations were similar at 0.86 for first and second and 0.85 for second and third, and a slightly lower genetic correlation of 0.78 was found between MBW in first and third lactations. The correlation between MBW in different lactations was strong but there was some variation which could be related to growth and maturity rate.

Table 2. Heritabilities with standard error in parentheses, genetic correlations (above diagonal), and phenotypic correlations (below) diagonal for metabolic body weight in first (MBW-1st), second (MBW-2nd), and third (MBW-3rd) lactation.

	MBW-1st	MBW-2nd	MBW-3rd
MBW-1st	0.34 (0.02)	0.86	0.78
MBW-2nd	0.57	0.43 (0.03)	0.85
MBW-3rd	0.60	0.61	0.47 (0.04)

Genomic Evaluations

In April 2023 there were 3 728 Holstein sires with an official BMR evaluation. The RBV for the combined BMR evaluation ranged from 85 to 121 for this group and averaged 104 as the average birth year of this group was older than the base bull group. The average reliability was 91% and ranged from 72 to 99% for official sires. The average reliability of genotyped Holstein bulls born in 2021 that were identified as being controlled by an AI organization (N=2 182) was 68%.

There has not been direct genetic selection on MBW or BW in Canada, but through indirect selection there has been a strong genetic trend observed. The genetic trend for BMR in bulls with official evaluations, as shown in Figure 2, has been in steady decline for the last 2 decades, demonstrating that genetic component for MBW and thus maintenance requirements has been increasing. In the most recent birth years, it appears that the trend may be lessening, which may be related to more awareness and a shift in selection away from larger animals and high stature. A similar genetic trend was also observed for cows, although not quite as steep.

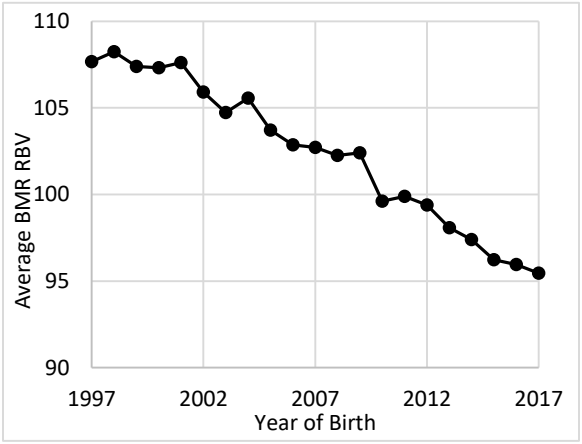

Figure 2. Genetic trend for bulls with an official body maintenance requirements (BMR) relative breeding value (RBV).

Relationships with Other Traits

Proof correlations were estimated between BMR and other routinely evaluated traits in Canada using 1 323 Holstein sires born since 2008 with an official LPI and BMR. In general, BMR had the strongest negative proof correlations with conformation traits. The major type traits of conformation, dairy strength, rump, mammary system, and feet and legs had proof correlations with BMR of -0.40, -0.49, -0.27, -0.26, and -0.06, respectively. The individual traits with the strongest proof correlation with BMR were stature (-0.73) and chest width (-0.55). Many conformation traits, especially dairy strength traits, describe various aspects of the cow's body size and structure and are often used to create proxy traits for body weight (e.g. Body Weight Composite Index, Holstein Association USA). A non-conformation trait strongly correlated with BMR was age at first service, with a negative correlation of -0.51. The group of proven sires used to estimate proof correlations spanned ten

birth years. Therefore, due to the negative genetic trend for BMR, some negative correlations that were found with traits displaying genetic improvement over this period may largely be a result of the opposite genetic trends over time. This is likely for the observed correlation of -0.29 and -0.24 with LPI and Pro$, respectively, which become slightly positive when correlations are averaged within birth year. A slight positive correlation with calving ability was also observed (0.21). As expected, no correlation was observed between BMR and Feed Efficiency evaluations.

Relationships Between Sire RBV and Daughter Phenotypes

The average daughter MBW of sires with an official BMR were averaged by sire RBV for BMR by lactation. Sires were required to have at least ten daughter records in a lactation to be included. A regression of average daughter MBW on sire RBV was conducted to determine the relationship between the observed daughter phenotype and sire RBV. The average daughter MBW and regression is shown in Figure 3. Bulls with a higher BMR evaluation have daughters with lower MBW and body maintenance requirements in all lactations compared to bulls with low BMR evaluations. The regression coefficients were similar for each of the lactations, ranging from -0.51 to -0.57 $kg^{0.75}$ per sire RBV point. As they were approximately equal, regression coefficients were averaged to form one interpretation value for interpreting BMR. For each plus five RBV points for BMR (one standard deviation) the MBW of daughters are approximately 2.75 $kg^{0.75}$ lower.

Conclusions

The genomic evaluation for BMR was first implemented in April 2023 by Lactanet for the Holstein breed. Producers can utilize BMR in their selection decisions to help reduce feed costs by decreasing cow MBW and the feed required for body maintenance. Cow size has been increasing over time and BMR can be used to help cease this trend and either maintain or decrease body size in a herd. The BMR evaluations are not correlated to genetic evaluations for Feed Efficiency, which is calculated to be genetically independent of MBW. The Canadian Feed Efficiency and BMR evaluations are published separately and not together in an index. Producers can therefore use these two tools in combination or choose to

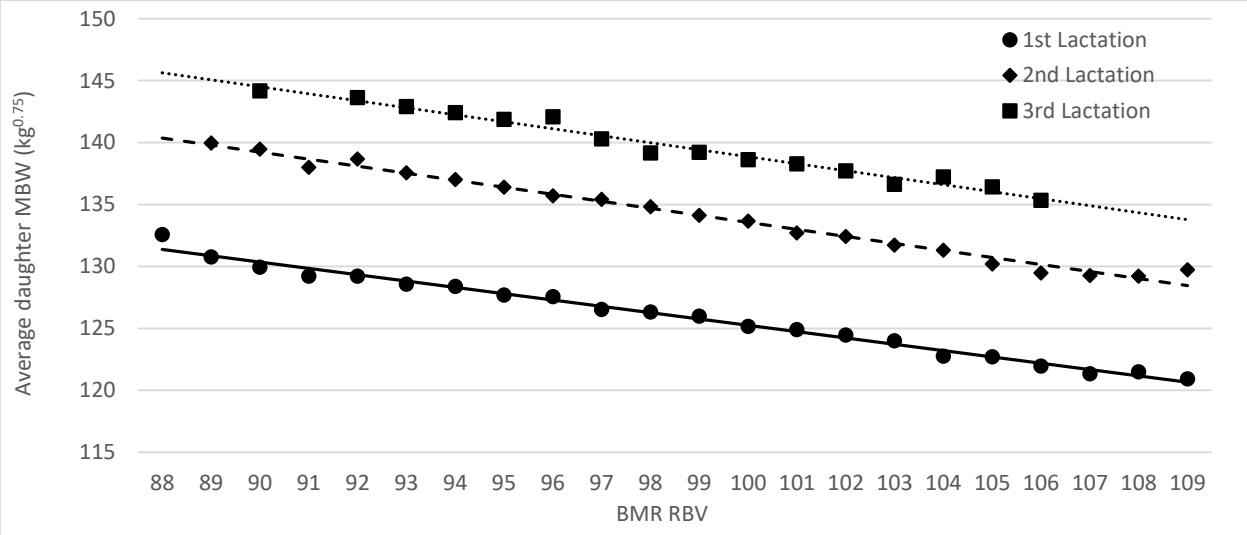

Figure 3. Average daughter metabolic body weight (MBW) in first, second, and third lactation averaged by sire Body Maintenance Requirements (BMR) RBV.

concentrate more on one to help reduce their overall feed costs.

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Genetic trends in gestation length

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Abstract

Gestation length has become increasingly important in dairy genetic evaluations worldwide. This has occurred for several reasons. The initial focus was on improving calving ease, as calves which are born earlier are typically smaller. This was generally thought to reduce the incidence of dystocia (although this has been disputed in some countries). However, the effects of gestation length on reproductive performance have since become of greater interest. Reducing gestation length has positive effects on calving interval, as cows which have greater intervals between calving and mating are more likely to be cycling and have demonstrably higher conception rates than their late-calving counterparts. However, this benefit is not without its drawbacks. Reducing gestation length does not directly improve a cow's ability to resume estrus cyclicity after calving, or to achieve fertilization after insemination. Gestation length is, rather, a trait that improves reproductive performance indirectly. Moreover, gestation length, if it is reduced too significantly, may have adverse effects on the health and survival of dairy calves, whose welfare is an increasing target of scrutiny from consumers and society in general. Genetic evaluations for gestation length are now being performed in many countries, including the United States and Australia since 2017 and 2020, respectively. This paper examines genetic trends for gestation length in these countries, with a specific focus on: 1) potential reasons for these genetic trends – for example trying to answer the question of whether selection for fertility traits could be placing indirect selection pressure on gestation length; 2) if there are differences between the countries that can be explained by seasonal or year round calving patterns; 3) how gestation length is being used as a tool to manage calving patterns, including the breeding and marketing of sires with extremely short gestation length breeding values; 4) evidence in the literature on genetic and phenotypic associations with other traits; 5) potential long term consequences of selecting for gestation length; and 6) the economic value of gestation length and its inclusion in (economic) selection indexes.

Key words: dairy, gestation length, genetic trends, genetic evaluation

Introduction

The New Zealand dairy industry is dominated by seasonal calving, where peak herd lactations (and hence, nutritional requirements) are aligned with periods of maximum pasture availability (Bowley *et al*., 2015). While this system maximizes feed utilization and reduces production costs, it also exerts significant pressure on dairy cow fertility. Cows are expected to maintain a 365-day calving interval, which, when considered in the context of a 281-day gestation period, leaves only 84 days post-calving for uterine recovery, the resumption of ovarian cyclicity and successful fertilization. This is a highly constrained window to achieve conception – one which is only exacerbated for cows calving late in the season.

For these reasons, many farmers in New Zealand and Australia routinely used calving induction to manage their calving patterns – a practice which became increasingly important as genetic merit for fertility declined. However, while well-managed calving induction did not negatively affect cows, it resulted in adverse outcomes for calf health and survival (Mansell *et al*., 2006). In response to increasing societal concerns around animal welfare and ethics, calving induction as a tool for manipulating calving patterns was phased out in 2015 and

2022 for New Zealand and Australia, respectively. However, this only intensified the pressure on cow reproductive performance.

A key strategy to addressing the dairy fertility decline has been the development of genetic evaluation for fertility traits, with the resulting EBVs incorporated into selection indices worldwide (Miglior *et al.*, 2005). In New Zealand, the current genetic evaluation for fertility relies on the Calving Season Day (CSD) phenotype, which describes the interval between planned start of calving and cow calving date. This is in line with many of the fertility traits developed worldwide which focus on continuous traits such as calving interval, days open, and calving to first service.

However, interval metrics inherently combine gestation length (GL) and conception date. GL is also considerably more heritable than most fertility traits; for example, in New Zealand estimate of heritability for GL was 0.67, which is significantly higher than the 0.02 reported for CSD (Amer *et al*., 2016). This can make it easier to influence through selective breeding. In New Zealand, there has been a consistent decline in GL over the past few decades – a pattern which seems to be gaining momentum. This not only raises animal health concerns, but also echoes the ethical issues that prompted the ban on calving induction in the first place.

New Zealand is not the only country that uses interval metrics for fertility genetic evaluations or has pursued genetic improvement in this trait. Therefore, with the cooperation of other Interbull countries, this paper aims to provide an initial exploration of global genetic trends in GL, within the context of each country's dominant breeds and systems. It also touches upon some of the genetic and phenotypic correlations that have been identified with other traits.

Materials and Methods

A request for data was sent to all member countries of Interbull who are currently evaluating GL, with responses received from the countries listed in Table 1. For those countries who supplied scaled data (for example, the Netherlands publish GL EBVs with a mean of 100 and a standard deviation of 5), additional data was obtained to convert these results to the phenotypic scale (units of days).

It is important to note that the genetic trends between countries can be difficult to directly compare due to differences in how GL EBVs are predicted. For example, some countries use pedigree-based conventional BLUP to predict EBVs, while others use two-step or single-step genomic evaluation. The publication criteria for GL evaluations can also differ from country to country, in terms of the acceptable thresholds for reliability. Other key differences, such as production system, are outlined in Table 1. It is important to note that these differences are those most relevant to the cattle populations contributing to the genetic trend data provided by each country, rather than a comprehensive description of an entire country's breed composition or calving systems.

Table 1. Interbull countries (*N* = 10) who contributed GL data, along with the system(s) associated with the population from which the data were derived.

Code	Country	System
NZL	New Zealand	Seasonal
IRL	Ireland	Mixed
POL	Poland	Year round
NLD	The Netherlands	Year round
USA	United States	Year round
CZE	Czech Republic	Year round
ITA	Italy	Year round
NOR	Norway	Year round
CHE	Switzerland	Year round
AUS	Australia	Mixed

Results

Genetic trends

Figure 1 shows overall genetic trends by country and breed, with some countries contributing multiple breed-specific trends (e.g., New Zealand, Ireland, Australia, Switzerland, and the United States). Trends

from the United States were provided separately for males and females.

Apart from Jerseys in Australia and the United States and Brown Swiss in the United States, the overall trend for GL is decreasing.

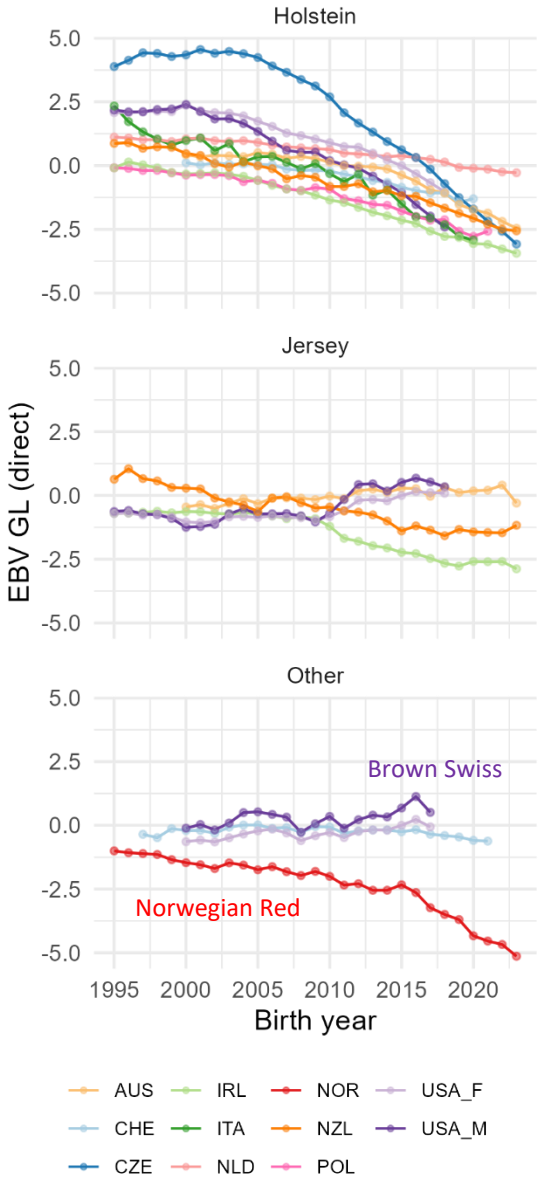

Figure 1. Genetic trends for gestation length as reported by participating countries, separated by breed.

Holsteins were the dominant breed in the provided data, with all nine contributing countries demonstrating declining genetic trends over time. Some of these countries exhibited weaker trends – such as the Netherlands, which experienced a slight decline of 0.05 days p.a. over 43 years from +1.86 in 1980 to -0.28 in 2023, while others displayed much more dramatic trends such as the Czech Republic, which peaked at +4.6 days in 2001 before dropping to -3.1 days in 2023 – a decline of 0.35 days p.a. over 22 years.

The Jersey breed had diverging trends, depending on the country of origin. Data from New Zealand and Ireland show declining GL EBVs overall, much like the Holstein and Norwegian Red populations. However, a marked split occurred in 2010, where populations from the United States (and to an earlier extent, Australia) began to experience an upward trend in GL EBVs, which occurs contrary to overall trends.

Much like the Holsteins, the Norwegian Red genetic trend shows a significant decrease over time. Data for Brown Swiss were available for two countries. In Switzerland, the trend was relatively stable over time, with a slight decrease beginning to become apparent since 2015. However, the United States Brown Swiss population exhibits a similar trend to Jerseys from the same country, with an increase in recent years.

Relationships with other traits

Selected genetic correlations between GL and other traits are shown in Table 2. These were obtained from a brief search of the scientific literature, as well as calculations on New Zealand data (data not published).

Genetic correlations between GL and fertility traits such as CSD and age at first calving (AFC) were high, as anticipated. The correlations between GL and protein yield were also high, ranging from -0.22 to -0.5, which is somewhat unexpected. However, correlations between other traits varied significantly depending on the source, with genetic correlations for longevity ranging from -0.25 to 0.09, for example, or -0.49 to 0.17 for calving ease. Whether this is due to genuine genetic differences in the populations (country, breed), or due to differences in statistical methods is difficult to say.

Table 2. Genetic correlations reported between GL and other traits.

Trait	R_g	Code	Source
CSDh[1]	0.57	NZL	Amer *et al.* (2016)
CSD[1]	0.45		
PM21[2]	-0.20		
BCS	0.02		
PR42[3]	-0.05	NZL	Unpublished data
AFC[4]	-0.42	ITA	Galluzzo *et al.* (2023)
Calving ease	-0.49	ITA	Galluzzo *et al.* (2023)
	0.17	CAN	Jamrozik *et al.* (2005)
	0.38	DNK	Hansen *et al*. (2004)
Dystocia	0.34	GBR	McGuirk *et al.* (1999)
	0.38	USA	Johanson *et al.* (2011)
Stillbirth	-0.39	ITA	Galluzzo *et al.* (2023)
	-0.11	CAN	Jamrozik *et al.* (2005)
	0.18	DNK	Hansen *et al*. (2004)
Longevity	-0.25	ITA	Galluzzo *et al.* (2023)
	0.09	GBR	Eaglen *et al.* (2013)
	-0.23	NZL	Unpublished data
Milk yield	-0.39	ITA	Galluzzo *et al.* (2023)
	-0.19	GBR	Eaglen *et al.* (2013)
	-0.25	NZL	Unpublished data
Protein yield	-0.50	ITA	Galluzzo *et al.* (2023)
	-0.22	GBR	Eaglen *et al*. (2013)
	-0.43	NZL	Unpublished data
Protein %	-0.23	NZL	Unpublished data
Overall type	-0.31	NZL	Unpublished data
Udder overall	-0.21	NZL	Unpublished data

[1]CSD: calving season day for heifers and cows; [2]PM21: 3-week submission rate; [3]PR42: 6-week in calf rate; [4]AFC: age at first calving

Discussion

Genetic trends

Differences in genetic trends by country could not be attributed to any specific factor such as dominant production system, type of genetic evaluation (i.e., genomic or conventional BLUP), or the traits used to drive genetic improvement in fertility.

This last point is of particular interest, as it could be hypothesized that the decline in GL has been due to strong selection for fertility improvement in Holsteins, which experienced the greatest historic decline (Heins *et al*., 2006). The Scandinavian dairy populations famously avoided this decline due to the early incorporation of genetic evaluations for fertility – but despite this, we still see a strong downward genetic trend for GL in the Norwegian Red breed.

The absolute difference between countries in genetic trends for GL cannot be determined from the results presented as each country's values are on different genetic scale/base. Haile-Mariam and Pryce (2019) examined differences between GL EBVs for bulls from different countries that were used in Australia, and observed that, on average, bulls that had their first proofs in Denmark, the Netherlands and New Zealand had shorter GL than bulls first tested in Australia or North America. Such results are only possible to be obtained for bulls that have already been used in each country. For importing foreign bulls with desired GL genetics, access to international genetic evaluation of GL would be of considerable value.

Relationships with other traits

Genetic associations between GL and fertility traits are high for traits that have GL embedded in, or closely related to, them (Amer *et al*., 2016; Galluzzo *et al*., 2023). This is undesirable, as the general aim of selecting for fertility traits is to address inherent infertility issues – i.e., physiological failures of reproduction in dairy cows. Arguably, achieving indirect gains in reproductive performance by decreasing GL is not true fertility improvement.

In New Zealand and Italy in particular, the relationship between GL and milk production traits is surprisingly strong. We could not find

any reported studies that would explain the source of this relationship. It is possible that admixtures of breeds or the combination of subpopulations with high milk yield and short gestation length, as well as lower milk yield and longer gestation length could cause this association. However, if there is a direct causal relationship between these two traits, the physiological mechanisms have yet to be found.

The genetic correlation between GL and calving traits varied depending on the population (see Table 2). This is especially important to monitor as shortening GL below a certain (yet unknown) threshold could have a negative impact on calf size and survival, with Norman *et al.* (2009) concluding that direct selection pressure should not be placed on GL without further research becoming available. In New Zealand, Jenkins *et al.*, (2016) concluded that slightly increased perinatal mortality rates in calves with very short GL (mean of 273 days) were likely to be offset by a reduction in calves with very long GL (mean of 291 days), which were 3 times more likely to die than calves in the short GL category. However, an appropriate lower threshold has yet to be defined.

Our findings indicate that indirect selection pressure on GL is likely to be a widespread phenomenon across various countries and dairy breeds, even though the underlying mechanisms are not yet fully understood or anticipated. Furthermore, as restrictions on calving induction and the general use of hormonal interventions increase in response to consumer concerns (Pieper *et al.*, 2016), farmers are more likely to opt for short GL sires as a tool to manage calving patterns. Although farmers are cautioned against retaining the daughters of such sires as replacement cows, these animals are still sometimes finding their way into milking herds, potentially exacerbating the decline in GL. Given this trend, the ongoing monitoring of GL is increasingly important.

GL in selection index

The economic value of GL can be substantial, especially when farmers respond to a shorter herd mean GL by delaying planned start of mating to achieve their preferred timing for seasonal calving (Ooi *et al.*, 2023). Despite not being a true fertility trait, high economic values can be derived through GL's indirect effects on fertility, with an associated improvement in milk profit, a reduction in empty rate, and a higher proportion of artificially bred calves.

This finding prompted a revision of how fertility traits are included in the national selection index for seasonal dairy cows in New Zealand. The main fertility phenotype, which previously included GL, is slated for replacement by a conception-based fertility trait that is phenotypically independent of GL (Stachowicz *et al.*, 2023). Both this new conception-based fertility trait and GL will be incorporated into New Zealand's economic selection index. This change allows the development of non-linear index functions that avoid favoring selection for excessively short GL, which could compromise the welfare, viability, and productive performance of the resulting calves (Norman *et al.*, 2009).

Conclusion

It is evident that there is a consistent downward trend in GL for almost all countries, production systems, and dairy breeds. The reasons for these trends as well as the long-term implications of them are not fully understood yet. For this reason, the authors believe that close monitoring of genetic (and phenotypic) trends of GL is important. An international genetic evaluation of GL is strongly recommended; it is needed especially for countries heavily dependent on imported semen for their genetic improvement programs.

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Genetic evaluation of persistency in extended lactations

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Abstract

In April 2023, a new breeding value for milk yield persistency was introduced for German Holsteins, Jerseys, Red Dairy Cattle, German black-and-white dual purpose, and red-and-white dual-purpose breeds. The aim is to allow selection of animals that are genetically suited for voluntarily extended lactations. The new breeding value was derived from the Random-Regression-Test-Day-Model (3 yield traits: protein-kg, fat-kg, milk-kg; 3 lactations each; 2nd order legendre polynomials in the genetic effect) that is used for routine genetic evaluations. The new trait is defined as the slope of the genetic Legendre polynomial between DIM 150 and 305 for each yield trait in each lactation. EBVs for fat and protein persistency are then combined according to their economic impact, using the same weights as for the overall yield index RZM: fat-kg:protein-kg weighted 1:2. The newly defined persistency index has a cumulative heritability of 0.34. Correlations of genomic breeding values to the traits that are included in the total merit index RZG are close to zero, except for RZM (0.24) and longevity RZN (0.18). Reliabilities for youngest genotyped animals without performance observations are approx. 0.60, which is lower than for the RZM. We observe a small positive genetic trend in the newly defined persistency trait in German Holsteins, which is expected, given the small but positive correlation to RZG that stems from the correlations to RZM and RZN.

Key words: milk yield, persistency, Holstein, extended lactations, Germany

Introduction

In recent years, the interest of farmers to extend the lactations of their cows has increased. Main reasons are: 1) low calf prizes; 2) each calving exposes cows to severe risks in their health and even survival; 3) reduce the portion of dry periods, where cows are not productive; 4) expectation of higher successful insemination rates (Römer et al. 2021, Van Knegsel 2022). This voluntary increase of lactation lengths puts new focus on the persistency of milk production and promises benefits in contact to animal health, economics and management (Lehmann et al. 2014; Do et al. 2017; Sehested et al. 2019). Common definitions of persistency consider a fixed period from lactation peak up to a certain day in milk (DIM) in the second half of the lactatation (Van Doormaal 2007, Biassus et al. 2010, Fürst et al. 2021, Aamand 2022). Our interest is persistency in extended lactations that go well beyond 305 days (Sehested et al. 2019). The aim is therefore to provide farmers with a breeding value that allows for the selection of animals that are genetically suited to maintain their production in extended lactations: the RZPersistenz.

Materials and Methods

Data was taken from the German routine genetic evaluation system of milk production traits for Holsteins in August 2022:

- EBVs for the Legendre coefficients from the conventional pedigree-based Random-Regression Test-Day Model (RRTDM).
- Genotype data used in the German Holstein genomic evaluations.
- Raw phenotypic data to validate the results.

The EBV data set from the RRTDM, which is described in Liu et al. (2000), consisted of

about 17 million females and 200 000 bulls. The raw phenotypic test-day-data included about 19 million lactations and was used to assess the impact of the selection on the new EBV for persistency on the phenotypic scale in extended lactations. For this purpose, the following selections were applied:

- Data from black-and-white Holsteins only
- lactations from 2012 onward
- minimum calving interval of 550 days
- only use days in milk (DIM) up to 400

These filters were applied to achieve a best-possible representative data set of long lactations without an impact of gestation to the shape, which was described in previous works (Grossman and Koops 2003; Muir et al. 2004). After this step, approx. 7% of phenotypic data were used for validation.

The genetic deviation curve of the RRTDM for German dairy cattle is modelled using 2nd order Legendre polynomials, which gives the following function 1:

$$f(dim) = \frac{\sqrt{5}(3(\frac{dim-155}{150})^2 - 1)}{2} \quad a_2 + \sqrt{3}(\frac{dim - 155}{150}) \quad a_1 + a_0 \qquad [1]$$

Where a_0, a_1, a_2 are the EBVs for the Legendre regression coefficients (index denotes the order), and *dim* is the time, measured as days in milk.

Legendre polynomials in the RRTDM are defined from DIM 5 to DIM 305. We defined lactation persistency as the slope of the curve between DIM 150 and DIM 305. The first point was chosen, because we do not want to affect the lactation peak region and the latter is simply the end of the parameter range. The weights for the EBVs of the Legendre coefficients can then be computed from the following formula 2, where the subscripts *1* (earlier) and *2* (later) of *dim* represent the start and end DIM during lactation.

$$\frac{f(dim_2) - f(dim_1)}{dim_2 - dim_1} = \frac{3\sqrt{5}}{2}(\frac{dim_2^2 - dim_1^2}{150^2} - \frac{310(dim_2 - dim_1)}{150^2}) \quad a_2 + \frac{\sqrt{3}}{150}(dim_2 - dim_1) \quad a_1 \qquad [2]$$

From this calculation, we get for each trait and lactation a persistency breeding value. These were then combined in the same way as the milk production index RZM is combined: weighting the first three lactations equally and afterwards combine fat-kg and protein-kg with a ratio of 1:2, which represents their economic weights. This results in the RZPersistenz.

To compute the h^2 of the RZPersistenz, we used the estimated variants component Tables per trait for genetic (G), permanent environment (PE) and residual (R) effects used in RRTDM with the resulting weights (w) for a_1 and a_2, while a_0 get zero weight. The formula is: $h^2 = w'Gw / (w'Gw + w'Pew + w'Rw)$. For the cumulative h^2 of RZPersistenz, the same weighting as for the EBV between lactations and traits are applied.

The aim of the new breeding value is to identify the genetic ability of animals to maintain their production level in extended lactations that go well beyond DIM 305. Therefore, we conducted a validation with phenotypic data up to DIM 400 to assess that our definition, which goes only to DIM 305, also works well for extended lactations. For this, approx. 1000 black-and-white Holstein AI-bulls born from 2013 to 2016 were grouped into 25%-quantiles based on their EBVs for persistency. Mean phenotypic daughter performance was then compared between daughters of top and bottom bulls.

Genomic breeding values were estimated with the same method as described in Liu et. al. (2011). Deregressed EBVs of RZPersistenz were used as phenotypes for the reference population. The training set based on German animals with a minimum of 8 phenotypic test day records (for bulls, at least 10 daughters were required that fulfilled this requirement) to

cover enough phenotypic information in the relevant interval of the lactation.

Results & Discussion

In Figure 1 and 2, we see the mean phenotypic lactation curves in milk-kg from daughters of AI bulls in different lactations. These results for milk-kg are also largely valid for fat- and protein-kg. In general, we observe differences between the first and higher lactations, with a lower absolute level, but flatter course from primiparous cows. These findings were expected and are also described in Schutz et al. (1990) and López et al. (2015). The daughters of the top 25% bulls with regard to RZPersistenz show the same mean phenotypic curve in early lactation as the daughters of the bottom 25% bulls. The curves start to differentiate in the second half of the first lactation and after the first third of higher lactations. The difference is a flatter slope of the top 25% bulls' daughters.

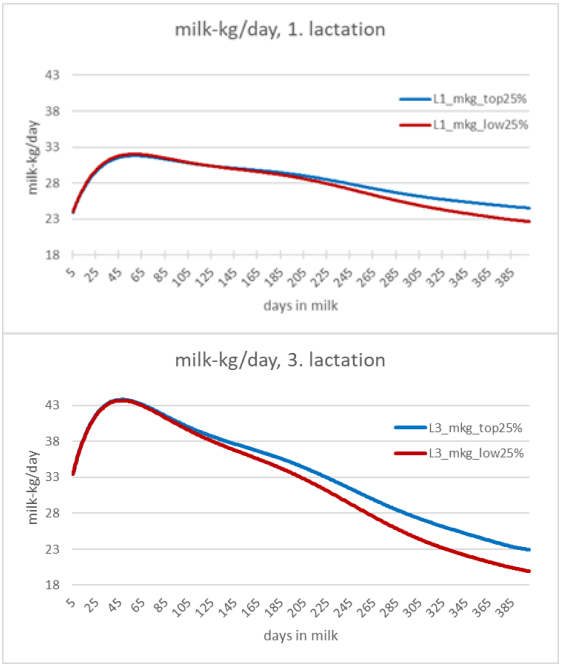

Figure 1 and 2. Phenotypic lactation curves for milk-kg from daughters of AI-bulls divided in best and worst 25% for RZPersistenz.

In the mean phenotypic curves of third lactation, the yield of 23kg is reached approx. 70 days later in for daughters of top bulls compared to bottom ones. In first lactation the difference on 25kg is approx. 60 days. These results show that the RZPersistenz extrapolates well up to DIM 400, although the lactation curve information used in RZPersistenz includes only DIM up to 305.

With a cumulative heritability of 0.34 for RZPersistenz we observe that this is lower than the h^2 of RZM. One reason most likely is the definition of the RZPersistenz that includes also less information than in RZM (only 2 of 3 regression coefficients). The mean genomic reliability of young, genotyped animals is 0.6. This is less than for gRZM (0.74), which results from the lower h^2 on the one hand and a smaller training set for the genomic evaluation on the other hand. Reasons for the latter are:

- We only include bulls with a minimum of 10 daughters with at least 8 test-day records (compared to 6 test-day records for RZM), in order to have enough phenotypic information in the relevant second half of the lactation.
- Only German information can be used in RZPersistenz. For RZM, we can use MACE results to also get foreign information. This does not work for RZPersistenz, because MACE gives only single-trait results and no information on single regression coefficients.

gEBV correlations calculated from 105 557 young female black-and-white Holsteins from herd genotyping to most other EBVs in RZG (total merit index) are close to zero. RZM (0.24) and RZN (0.18) show a small positive correlation. We observe also a small positive genetic trend also for recent years in the newly defined RZPersistenz in German Holsteins, which is likely caused by these small but positive correlations. In contrast to previous studies for persistency like Harder et al. (2006a) or Appuhamy et al. (2007), who report undesired genetic and phenotypic connections to metabolic diseases or udder health, we do not observe such negative correlations. The reason might be that our definition does not include the lactation peak period. This point may also explain why we found only few and small positive correlations and close-to-zero correlations to, e.g., reproduction traits.

Different studies on lactation persistency expect benefits in reproduction traits or less metabolic challenges early in lactation. Among others because of a decrease of peak yield (Jakobsen et al. 2002, Muir et al. 2004, Harder et al. 2006a, Harder et al. 2006b, Van Knegsel 2022). With our consideration of the period after peak, we increase the focus on extend lactations, without an impact to peak yield (Figure 1 and 2). In herds with voluntarily extended lactations, a positive effect on conception rates might be expected, because at a later insemination time point, the energy balance of the animals is more favorable, which aids conception (Van Knegsel 2022). Therefore, in the context of extended lactations, fertility traits need some re-consideration, e.g., the interval from calving to first insemination is no longer as relevant as it is currently in the RZR, the over-all fertility index (Vit 2023).

Future developments will include the extension of the RRTDM beyond DIM 305, which will give the opportunity to directly use information on genetic curves from longer lactations to further increase the accuracy of RZPersistenz.

Conclusions

The RZPersistenz provides breeders with information to support the effective selection of animals that are genetically suited to perform lactations with flatter curves, without an influence on the peak period. This can be used in extended lactations. Based on gEBV correlations no negative side-effects on other traits are expected from selection on the RZPersistenz.

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International evaluations for clinical mastitis in Brown Swiss

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Abstract

The SNP training for clinical mastitis (STCM) trait was introduced by the Interbull Centre in April 2021. Previously, the mastitis trait within the udder trait group allowed for a wide range of trait definitions: direct clinical mastitis, somatic cell score, or a combination of clinical and subclinical mastitis. Since the introduction of STCM, the Council on Dairy Cattle Breeding (CDCB) has participated with data for Holsteins and Jerseys, aiming to enhance the domestic SNP reference population with foreign evaluations. As expected, for Holstein and Jersey the inclusion of Multiple Across Country Evaluation (MACE) evaluations had a minor impact on the US evaluation due to the dominance of US animals in the reference population. In the August 2022 evaluation, Brown Swiss (BSW) animals began receiving domestic health evaluations in the US, which were also incorporated into the Net Merit Index. During the January 2023 test run, the BSW STCM successfully completed Trend Methods I and III validation at Interbull. Effective as of the April 2023 evaluation and going forward, BSW foreign evaluations are included in the United States of America (US) clinical mastitis evaluations. The initial expectation was that the impact of this inclusion would be limited, as only two other foreign countries (France and Switzerland) contribute to the Interbull clinical mastitis evaluation. However, the observed impact on the evaluations was noticeable. Correlations between April 2023 and March 2023, triannual genomic evaluations were as low as 0.73 for reference animals and 0.56 for young animals. Along with the significant variation in Genomic Estimated Breeding Values (GEBV), the mean genomic reliabilities (GREL) for young animals increased from 24% in March 2023 to 30% in April 2023. These results can be explained by two main factors: i) the contribution of foreign bulls from France and Switzerland in the SNP reference population for US BSW made the inclusion of MACE evaluations more relevant; ii) a large number of US BSW clinical mastitis records became available prior to the April 2023 evaluation and were added to the national cooperator database. Large changes in GEBV and GREL resulting from the initial inclusion of foreign data are not expected in subsequent evaluations unless more countries participate.

Key words: foreign information, participating countries, PTA variation, reliability, gain, young bulls

Introduction

The United States of America (US) has ongoing health trait evaluations for three breeds: Holsteins (HOL; Parker Gaddis et al., 2017), Jerseys (JER; Parker Gaddis et al., 2020), and Brown Swiss (BSW; CDCB Connection, 2022), commencing in 2018, 2020, and 2022, respectively. Health traits evaluations consists of six disease resistance traits: milk fever, displaced abomasum, ketosis, mastitis, metritis and retained placenta. All six traits are incorporated into Net merit index (NM$) with a sub-index Health dollars, accounting for a total weight of 2%. (VanRaden et al., 2021). The same pipeline for all three breeds (HOL, JER and BSW), and the predicted transmitting abilities (PTA) are presented as percentage points of event resistance above or below breeds average (Mota et al, 2021).

As the most common and costly trait among the six traits, mastitis has a significant impact on the dairy cattle sector. Mota et al. (2021) noted the rapid increase in mastitis phenotypes,

with an increase from 1.8 to 5.1 million records in three years. Two years later, the national cooperator database managed by the Council on Dairy Cattle Breeding (CDCB) accounts for 7.1 million records, including 5.7 million HOL, 840k JER, 19k BSW, and half a million from other breeds where health trait evaluations have not yet been implemented.

The SNP training for clinical mastitis (STCM) trait was introduced by the Interbull Centre in April 2021. Previously, the mastitis trait within the udder trait group allowed for a wide range of trait definitions: direct clinical mastitis, somatic cell score, or a combination of clinical and subclinical mastitis. Since the introduction of Multiple Across Country Evaluation (MACE) evaluations for STCM, CDCB has participated with data for HOL and JER, aiming to enhance the domestic SNP reference population with foreign evaluations. In the case of BSW, this is even more crucial since over 50% of the reference population originates from Switzerland (CHE) and France (FRA). The percentage of bulls with MACE in more than 10 herds are 31% from FRA and 27% from CHE. The US has only 22% of its reference population consisting of domestic animals.

To assess the impact of adding foreign information to the clinical mastitis evaluation for BSW, during the January 2023 test run, the BSW STCM successfully completed Trend Methods I and III validation at Interbull (Interbull Centre, 2018). Effective as of April 2023, BSW foreign evaluations are included in the US clinical mastitis evaluations. As expected, for HOL and JER, the inclusion of MACE evaluations had a minor impact on the US evaluation due to the dominance of US animals in the reference population. However, even though the initial expectation is that the impact of this inclusion will be limited, as only two other foreign countries (FRA and CHE) contribute to the CDCB clinical mastitis evaluation, the non-US dominating reference population may suggest otherwise.

Materials and Methods

The data used in this study were BSW MACE values provided by the Interbull Centre, Uppsala, Sweden (Interbull Centre, 2020).

To assess the impact of including clinical mastitis MACE information in BSW, PTA means and standard deviations were calculated, as well as correlations among three different scenarios: i) 2303_D, the previous March run with domestic information only; ii) 2304_D, the current April run with domestic information only; iii) 2304_F, the current April run including MACE information.

The Pearson correlations were calculated as follows:

$$rg = \frac{\sigma_{ab}}{\sqrt{\sigma_a^2 * \sigma_b^2}}$$

where r_g is the genetic correlation, and a and b can be either of the investigated runs (2303_D, 2304_D or 2304_F).

The statistical analyses were done by using SAS software (Statistical Analysis System, Version 9.4, 2023).

Finally, the investigation was conducted for the reference population and prediction animals divided in five different groups: i) all animals; ii) all bulls; iii) all cows; iv) bulls with REL > 50%; v) cows with REL > 50%.

Results and Discussion

The total number of animals within each group is presented in Table 1. Please note that the GEBV and GREL means, and standard deviations may vary for the same evaluation. This variation is due to the different number of animals whose GEBV is affected by MACE when compared to a specific evaluation.

Table 1. Number of bulls in common between evaluation scenarios.

Group	2303_F vs. 2304_D	2303_F vs. 2304_F	2304_D vs. 2304_F
Reference Population			
All	1,165	1,354	1,354
Bulls	561	902	902
Cows	604	452	452
Bulls GREL >50%	168	164	168
Cows GREL >50%	21	20	21
Prediction Population			
All	62,168	61,979	62,301
Bulls	45,361	45,020	45,066
Cows	16,807	16,959	17,235
Bulls GREL >50%	-	-	-
Cows GREL >50%	-	-	-

2303_F: foreign March 2023 run; 2304_D: domestic April 2023 run; 2304_F: foreign April 2023 run

The GEBV and GREL means, standard deviations, as well as correlations among three evaluation scenarios (2303_D, 2304_D, or $2304_{F)}$ are presented in Table 2 for the reference population and in Table 3 for the prediction population.

In Table 2, which represents the reference population, GEBV means and standard deviations were very similar when domestic data was the sole source of information. However, when MACE information was included, there was a notable increase in GEBV variability. This had a pronounced impact on the correlations, particularly for non-reliable animals. The GEBV correlations exhibited a slight drop, ranging from 0.01 to 0.03 for bulls or cows with REL > 50%, but a drastic drop occurred in the group of all bulls, decreasing to 0.69 when compared to the March run and to 0.68 within the same April run.

Despite this substantial GEBV variability, a significant increase in GREL was observed for the reference population, with an increase of approximately 38% for all animals and 47% for bulls. It is worth noting that both groups exhibited noticeable drops in correlations for both GEBV and GREL. As expected, the effects on bulls were much more pronounced than on cows.

Table 3 reveals an even greater GEBV variability in the prediction population, amounting to approximately 52% increase in GEBV variability between the April evaluations with (2304F) and without (2304D) MACE information. Regarding GEBV correlations, the observed decrease was more significant compared to the reference population, plummeting from 0.95 (all groups) to a range of 0.55-0.56 for all animals and 0.45-0.49 for the group of all bulls.

Conversely, the increase in GREL was also quite notable, with a 25% increase for all animals and a 30% increase for the group of all bulls. These results highlight a substantial impact on both traditional and genomic evaluations in the US. Despite the substantial GEBV variability, the gain in GREL suggests that it is worthwhile to incorporate foreign information into the US mastitis evaluation. In general, these initially unexpected results, which have a noticeable impact on US evaluations, can be explained by several factors. First, the US BSW population is relatively small, with approximately 50% of the reference population originating from either CHE or FRA. Furthermore, all foreign BSW bulls in the reference population have genotypes in the national cooperator database, a situation different from that of HOL and JER breeds, where the impact was less pronounced due to the predominance of US animals in the reference population.

Table 2. GEBV and GREL means (standard deviations), and correlations between evaluations for five groups of reference population animals.

BSW Reference Population						
2303_F vs. 2304_D						
Group	GEBV Mean (SD)		r	GREL Mean (SD)		r
All	0.56 (2.14)	0.50 (2.23)	0.97	41 (11)	41 (11)	0.99
Bulls	0.55 (2.33)	0.49 (2.40)	0.98	46 (13)	46 (13)	0.99
Cows	0.57 (1.95)	0.51 (2.07)	0.97	36 (6)	36 (6)	0.99
Bulls GREL >50%	0.61 (2.92)	0.57 (3.00)	0.99	63 (10)	63 (10)	0.99
Cows GREL >50%	0.53 (2.33)	0.54 (2.39)	0.99	55 (5)	55 (5)	0.99
2303_F vs. 2304_F						
All	0.73 (2.00)	-0.09 (2.96)	0.73	37 (13)	51 (14)	0.33
Bulls	0.76 (2.00)	-0.14 (3.20)	0.69	38 (15)	56 (15)	0.29
Cows	0.65 (2.00)	-0.01 (2.42)	0.86	36 (7)	41 (7)	0.93
Bulls GREL >50%	0.61 (2.92)	-0.06 (3.29)	0.92	63 (10)	69 (12)	0.78
Cows GREL >50%	0.53 (2.33)	0.26 (2.53)	0.93	55 (5)	58 (5)	0.93
2304_D vs. 2304_F						
All	0.62 (2.07)	-0.09 (2.96)	0.73	37 (13)	51 (14)	0.33
Bulls	0.66 (2.05)	-0.14 (3.20)	0.68	38 (15)	56 (15)	0.29
Cows	0.54 (2.12)	-0.01 (2.42)	0.88	36 (7)	41 (7)	0.93
Bulls GREL >50%	0.53 (2.99)	-0.11 (3.30)	0.92	63 (10)	68 (12)	0.79
Cows GREL >50%	0.46 (2.36)	0.16 (2.50)	0.94	55 (5)	59 (5)	0.94

GEBV: genomic estimated breeding value; GREL: genomic reliability; 2303_F: foreign March 2023 run; 2304_D: domestic April 2023 run; 2304F: foreign April 2023 run

Table 3. GEBV and GREL means (standard deviations), and correlations between evaluations for five groups of prediction population animals*.

BSW Prediction Population						
2303_F vs. 2304_D						
Group	GEBV Mean (SD)		r	GREL Mean (SD)		**r**
All	0.81 (1.24)	0.61 (1.31)	0.95	24 (5)	24 (5)	0.99
Bulls	1.00 (1.08)	0.77 (1.14)	0.95	23 (5)	23 (5)	0.99
Cows	0.32 (1.49)	0.18 (1.61)	0.95	27 (6)	27 (6)	0.99
2303_F vs. 2304_F						
All	0.81 (1.24)	0.15 (1.99)	0.56	24 (6)	30 (6)	0.64
Bulls	1.00 (1.08)	0.30 (1.97)	0.49	23 (5)	30 (6)	0.62
Cows	0.32 (1.49)	-0.24 (1.99)	0.70	27 (6)	31 (6)	0.77
2304_D vs. 2304_F						
All	0.61 (1.31)	0.15 (1.99)	0.55	24 (6)	30 (6)	0.64
Bulls	0.77 (1.14)	0.30 (1.97)	0.45	23 (5)	30 (6)	0.62
Cows	0.18 (1.61)	-0.24 (1.99)	0.72	27 (6)	31 (6)	0.77

GEBV: genomic estimated breeding value; GREL: genomic reliability; 2303_F: foreign March 2023 run; 2304_D: domestic April 2023 run; 2304F: foreign April 2023 run; *No young animals with GREL >50%

Additionally, the clinical mastitis data for BSW only has three contributors: CHE, FRA, and the US (https://interbull.org/ib/geforms). The volume of BSW information provided by the US is relatively small compared to the other two countries. In the most recent April 2023 evaluation, the US submitted only 82 estimated breeding values (EBV), while FRA and CHE combined contributed a total of 1,167 EBV. Finally, the impact on evaluations can also be attributed to the fact that the CDCB received a substantial number of US records that became available after the December 2022 evaluation

and were incorporated into the system for the April 2023 evaluation.

In summary, while these results were initially unexpected, they can be reasonably explained by the factors mentioned above. With data stability, we do not anticipate such a significant impact in future runs, unless substantial changes occur in terms of data availability and the participation of countries.

Conclusions

The use of foreign information has enabled an increase in the reference population, but the US still has a long way to go to build a strong reference population for BSW. There has been a noticeable impact in both traditional and genomic evaluations, with a 52% increase in GEBV standard deviations, which is more than expected from the prediction GREL gain (30% vs. 24%). No impact is expected in subsequent evaluations unless more countries participate. The authors hope that these results serve as inspiration to facilitate the exchange of such traits, particularly to assist small population countries like the US.

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Implementation of A Routine Genetic Evaluation of Milk Coagulation Properties in Italian Holstein Using a Mixed Reference Population of Bulls and Cows

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Abstract

Cheese production is one of the most important segments of the agrifood sector in Italy and milk coagulation properties (MCP) are a key factor for an efficient cheesemaking process. Milk coagulation properties, referred as rennet coagulation time (RCT), curd firmness (a30) and curd-firming time (k20), are available from mid-infrared spectroscopy (MIRS) prediction models implemented within the official national milk recording system (LEO project, PSRN mis 16.2, AIA, 2023). Aim of this study was to assess the possibility to genetically improve MCP in Italian Holstein population and to develop a routine genetic evaluation for such traits. A multiple-trait repeatability linear animal model was employed, with RCT, a30, k20 and casein percentage (CAS) as outcome variables. Fixed effects were the interaction between year and season of recording, between parity (1,2+), year and age at calving class (7 classes) and between parity, year and days in milk class (10 classes). Random effects were contemporary groups, animal permanent environment, animal additive genetic and residuals. The models for RCT, a_{30} and k_{20} accounted also for somatic cell score as covariate. A total of 64,720 records from 150 herds, randomly sampled from the full dataset of 4,001,769 observations after edits, were used for variance components estimation using THRGIBBS1F90. The pedigree was traced back to 4 generations and was composed of 59,124 individuals. Convergence was assessed using R package BOA. The posterior mean (PM) for heritability was 0.33 for CAS, 0.11 for RCT, 0.16 for a30, and 0.15 for k20. The genetic correlation between RCT and a30 was -0.87, highlighting their antagonistic relationship; the same conclusion can be drawn from the correlation between k20 and a30 (-0.98). RCT and K20 were positively correlated (0.77). CAS was negatively genetically correlated to both RCT and k20 (-0.04 and -0.76, respectively), and positively to a30 (0.51). A SNPBLUP model was employed for estimating genomic breeding values (GEBV) using two distinct training populations: solely bulls and both bulls and cows (mixed reference population). The validation of GEBVs, conducted with complete and partial datasets (with a three-year back cutoff date for phenotypes), consistently demonstrated that employing a mixed training population results in reduced dispersion and heightened reliability for these traits. These results showed the feasibility of selecting for MCP improvement within the Italian Holstein population. Furthermore, they establish the foundation for implementing a routine genetic evaluation aimed at enhancing cheese production, utilizing a mixed reference population for SNP effects estimation.

Key words: cheesemaking, dairy cattle, genomic selection, mixed reference population, mid-infrared spectroscopy, genetic parameters

Introduction

In Italy, 77% of the milk is used for cheese production, the 55% of which is specifically utilized for crafting the 56 geographical indications and traditional specialties officially recognized by the European Union (ISMEA, 2022). In this scenario, the significance of dairy production in the Italian agrifood sector is readily evident. The importance of milk coagulation properties (MCP), referred as rennet coagulation time (RCT, minutes), curd firming time (k20, minutes) and curd firmness (a30, millimeters), for an efficient cheesemaking process has been widely discussed (Riddel-Laurence et al, 1989; Wedholm et al, 2006; Pretto et al, 2012). Moreover, MCP were demonstrated to be moderately heritable, indicating the potential for improvement through genetic selection (Cassandro et al, 2008; Visentin et al, 2017). Milk coagulation properties are now available at the National Breeders Association of Italian Holstein, Brown Swiss and Jersey (ANAFIBJ) from mid-infrared spectroscopy (MIRS) prediction models implemented within the official national milk recording system (LEO project, PSRN mis 16.2, AIA, 2023). Considering the aforementioned, the aim of this study was the implementation of a routine genetic and genomic evaluation of MCP for the Italian Holstein breed. Furthermore, given the substantial availability of genotypes of Italian Holstein cows, the feasibility of incorporating them into the training population for the estimation of the SNP effects was explored.

Materials and Methods

Data editing

The input dataset was composed of 6.7 million records from 2017 onwards. Only records from regions that provided a consistent data flow were kept (10 regions out of 20). Records from parity 1 to parity 5 and from 5 to 405 days in milk (DIM) were considered. Regarding MCP traits, accepted range of values for RCT, k20 and a30 where five to 60 minutes, one to 20 minutes and five to 60 millimeters respectively: all records out of these ranges were removed as obvious errors. In order to detect laboratory measurement anomalies, isolation forest algorithm implemented in the python module Scikit-learn was employed (Pedregosa et al, 2011). Briefly, reference values from Visentin et al, 2015 were used for the phenotypic correlations between the three traits: -0.73 for RCT-a30, 0.80 for rct-k20 and -0.79 for k20-a30. Phenotypic correlations within herd-year-test-day (HTD) groups were calculated: all milk samples collected from the same herd in the same day are processed in the same laboratory. All the HTD groups with an anomalous value compared to the reference have been excluded. All test-day observations had to have a record for casein percentage (CAS) too to be included in the analysis. Finally, only herd-year-season of recording groups with at least 20 contemporaries were kept. Pedigree was traced back to four generations.

Statistical model

A multiple trait repeatability linear animal model was used, with CAS, RCT, a30 and k20 as correlated dependent variables.
The model for CAS was the following:

$$CAS_{ijklmnop} = hys_i + S_j{*}Y_k + DIM_l{*}PARC_m{*}Y_k + AGEC\_PAR_n{*}Y_k + a_o + pe_o + e_{ijklmnop}$$

with $CAS_{ijklmnop}$ as the p*th* phenotypic observation of casein percentage. Fixed effects were $S_j{*}Y_k$ as the crossed effect of season j by year k, $DIM_l{*}PARC_m{*}Y_k$ as the l*th* days in milk class (10 classes of 40 days) by parity class m (3 classes: 1, 2, 3+) and year k, $AGEC\_PAR_n{*}Y_k$ as the n*th* age at calving by parity class (9 classes: 3 age at calving classes for every parity class) by year k. Random effects were hys_i as the i*th* contemporary group for herd-year-season of recording, a_o as the additive genetic effect of the o*th* animal,

pe_o as the permanent environmental effect of the o*th* animal and $e_{ijklmnop}$ as the residual of observation p. The same model with the addition of the fixed linear regression of somatic cell score was applied to MCP traits.

Variance components estimation, genetic and genomic evaluation, approximate genetic correlations

Variance components estimation was performed with the software THRGIBBS1F90 (Misztal et al, 2002) on a sample of 64,720 animals (150 herds). Convergence was assessed with R package BOA, Bayesian output analysis (Smith, 2007). Conventional estimated breeding values (EBVs) were estimated with MiX99 software (MiX99 development team, 2012). Genomic evaluation was performed with a SNPBLUP model using GS3 software (Legarra et al, 2007). 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, 2011). Approximate genetic correlations were calculated as Pearson correlation coefficients between genomic estimated breeding values (GEBVs) of 87,569 heifers born after 2016.

Genomic validation

Genomic validation was performed as 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 2308 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 4,001,769 records: phenotypes averaged 2.72%, 25.40 minutes, 20.73 millimeters and 7.29 minutes for CAS, RCT, a30 and k20 respectively. The results of variance components estimation are listed in Table 1. Posterior mean for heritability was moderate to high for CAS and moderate for MCP traits: genetic correlations were high for all combinations of traits except for CAS and RCT.

Table 1. – Results of variance components estimation.

	CAS	RCT	a30	k20
CAS	0.33(0.01)	-0.04	0.51	-0.67
RCT		0.11(0.01)	-0.87	0.77
a30			0.16(0.01)	-0.98
k20				0.15(0.01)

Posterior means of heritability on diagonal with posterior standard deviations in parentheses, genetic correlations above diagonal.

The results of the genomic validation are listed in Table 2. Adding the females, the training population increased by 40,478 individuals. The mixed training population performed better than the one composed by only bulls for both the parameters considered, dispersion coefficient and reliability of the model.

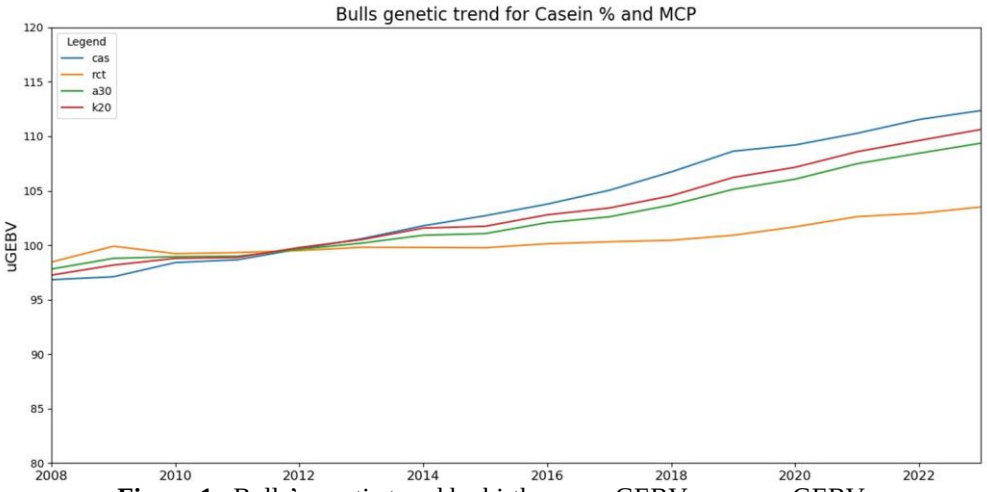

Figure 1. Bulls' genetic trend by birth year. µGEBV=average GEBV.

Regarding dispersion, a mean coefficient of 0.91, compared to a mean of 1.28 for the bulls only, was detected for the mixed reference population; regarding reliability, a mean reliability of 0.48 was detected for the bulls' reference population while a mean of 0.76 resulted for the mixed one. These evidences suggest that the inclusion of females in the reference population would be beneficial for MCP traits.

Based on these results, the mixed reference population was chosen for the subsequent analyses.

Table 2. Results of genomic validation.

	Training	Animals	b	r^2
CAS	B	3,276	1.205	0.452
	M	43,754	0.898	0.790
RCT	B	3,276	1.359	0.421
	M	43,754	0.925	0.737
a30	B	3,276	1.319	0.478
	M	43,754	0.911	0.767
k20	B	3,276	1.246	0.459
	M	43,754	0.895	0.763

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

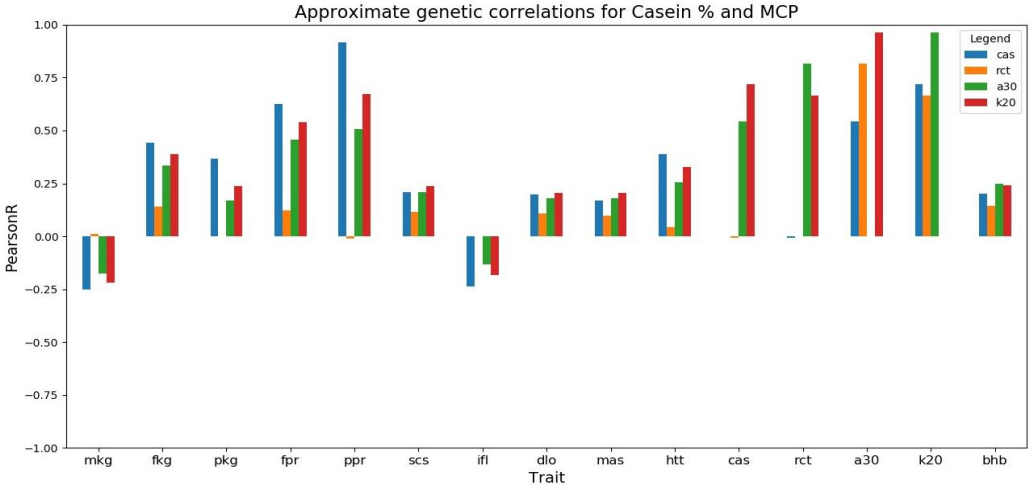

Figure 2. Approximate genetic correlations for MCP traits. Mkg=milk yield, fkg=fat yield,pkg=protein yield, fpr=fat percentage, ppr=protein percentage, scs=somatic cell score, ifl=interval first-last insemination, dlo=direct longevity, mas=mastitis, htt=heat tolerance, cas=casein percentage, rct=rennet coagulation time, a30=curd firmness, k20=curd firming time, bhb=ketosis.

The genetic trend of bulls' GEBVs by birth year is represented in Figure 1: an increasing trend is evident for CAS, a30 and k20. The trend for RCT is increasing too, but in a milder way compared to the other traits.

The approximate genetic correlations are represented in Figure 2. For all the four traits analyzed, this study revealed a null or favorable approximate genetic correlation with all the traits considered. The only exception is milk yield: for this trait, a negative and unfavorable correlation was detected with CAS, a30 and k20. The strong favorable genetic correlations of CAS, a30 and k20 with protein yield and percentage may explain their increasing genetic trend. In contrast, the milder correlations calculated for RCT can be the motivation of its less pronounced trend.

Conclusions

In conclusion, this study increased the knowledge about the genetic aspects of MCP in the Italian Holstein population, revealed the possibility to genetically improve the breed for these traits and highlighted the benefits of including females in the reference population for SNP effects estimation. A routine genetic evaluation of CAS and MCP traits will be soon implemented in Italy for the Italian Holstein breed.

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Evaluating Male Fertility in Brown Swiss Cattle Combining Multiple Sources of Information

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Abstract

Improving reproductive performance remains one of the major goals for the dairy industry worldwide. Bull fertility has been recognized as an important factor influencing reproductive success in dairy cattle. In this study, we investigated bull fertility in the Italian Brown Swiss dairy cattle population based on extensive records. The data set included a total of 397,926 breeding records from 1,228 bulls and 129,858 lactating cows between first and fifth lactation from 2000 to 2019, and all bulls have a genomic analysis on 454k single nucleotide polymorphisms (SNP). We estimated sire conception rate using only factors related to the bulls, our analyses revealed that there is a substantial variation in conception rate among Brown Swiss bulls, with more than 20% conception rate difference between high-fertility and low-fertility bulls and cross-validation analyses achieved predictive correlations equal to 0.30 for sire conception rate. The analysis included alternative whole-genome scans and gene-set analyses identified two genomic regions, located on BTA6 and BTA26 that showed marked non-additive effects. These regions harbor genes, such as *WDR19* and *ADGRA1*, that are directly involved in male fertility, including sperm motility, acrosome reaction, and embryonic development. The analysis to evaluate association between runs of homozygosity (ROH) and male fertility showed four different ROH regions located on chromosomes 6, 10, 11, and 24 were significantly overrepresented in low-fertility bulls. The predictive performance of the linear kernel-based regression models fitting the entire set of SNP markers exhibited predictive correlations around 0.19. Interestingly, the inclusion of two major non-additive markers as fixed effects achieved predictive correlations around 0.32. Moreover, including in the estimation also a new knowledge on the effect of ROH on the male fertility could improve reliability of prediction.

Key words: Bull fertility, Genomic analysis, Genomic Inbreeding, Brown Swiss cattle

Introduction

Fertility is a critical factor for profitable dairy farming, but challenges persist in achieving optimal reproductive performance in dairy herds, leading to economic losses (Abdollahi-Arpanahi et al., 2017). While female reproductive traits have been a focus in breeding programs, male fertility, which also plays a significant role in pregnancy success, has been somewhat overlooked (García-Ruiz et al., 2016; Toledo-Alvarado et al., 2017). Traditional laboratory methods are used to assess bull fertility based on semen characteristics. However, these methods often fall short in accurately predicting a bull's true fertility, which can be better estimated using field records, such as cow insemination and pregnancy data. Some national evaluations exist for female fertility traits, but male fertility assessments are usually conducted by individual breeding organizations and may not be widely available (Stahlhammar et al., 1994; Kastelic and Thundathil, 2008, Han and Peñagaricano, 2016). The Brown Swiss breed, with a substantial global presence, holds great importance in the dairy industry. This study aims to investigate bull fertility in the Italian Brown Swiss dairy cattle population by examining cow field records and evaluating statistical models for pregnancy success and sire conception rate (SCR), focusing on factors related to the bull (Kuhn and Hutchison, 2008; Kuhn et al., 2008). The study also assesses model predictive ability through cross-validation techniques.

Materials and Methods

We meticulously analyzed a comprehensive dataset, encompassing 397,926 breeding records from 1,228 bulls and 129,858 lactating cows over a two-decade period (2000-2019). This extensive dataset provided a valuable resource for our investigation. Moreover, for all bulls the genotype based on 454k SNPs ware available. The fundamental metric we employed to gauge bull fertility was the Sire Conception Rate (SCR).

Sire conception rate was estimated using different factors closely related to the fertility of the bull. These factors included the age of the bull, inbreeding levels, mating inbreeding, the AI company involved, and the year of insemination (Kuhn and Hutchison, 2008a; Kuhn et al., 2008b). Additionally, our model incorporated genetic components, both additive and non-additive, and other influential variables. These variables encompassed the number of lactations, days in milk, AI company-specific effects, and the effects associated with different herd-year-season conditions:

$$SCR = 100 \times \left(\begin{array}{c} \widehat{\beta}_5 \times Age_{Bull} + \widehat{\beta}_6 \times Age^2_{Bull} + \widehat{\beta}_9 \\ \times Inbreeding_{Bull} + \widehat{\beta}_{10} \times Inbreeding^2_{Bull} \\ + AIcompany\_\widehat{\quad\quad}\_year + \widehat{Bull} \end{array} \right)$$

The solutions for the linear and quadratic effects for age and inbreeding of the bull, namely $\hat{\beta}5$, $\hat{\beta}6$, $\hat{\beta}9$ and $\hat{\beta}10$, and the solutions for the random effects AI company_year were all obtained from the model used to evaluate cow pregnancy success. The proportion of the total variation in SCR due to additive genetic effects was estimated using a classical animal model with SCR as response variable and the kinship matrix constructed using pedigree information. Finally, male fertility evaluations were assessed using the Spearman's rank correlation coefficient on the cross-validation test (Pacheco et al., 2021).

Whole-genome sequencing for additive and no additive effect were conducted to assess the impact of additive and non-additive effects on service sire fertility at a genomic level. A two-step mixed-model approach was employed, involving a model with fixed and random effects for SCR records and the evaluation of individual SNP effects for various genetic effects using a regression approach (Nicolini et al., 2018; Pacheco et al., 2022).

We evaluated the feasibility of predicting bull fertility in Brown Swiss using genomic data under different scenarios. To assess the predictive power of the entire high-density SNP dataset, a whole-genome prediction model was employed using two model one with just a polygenic effect and the second including two SNPs (identify with genome scan on the non-additive effect) were coded as 0 or 1, to represent the effect of having at least one or two copies of the B allele and were fitted as fixed effects in an alternative whole-genome prediction models. The predictive ability of two models was assessed by 5-fold cross-validation. Runs of homozygosity (ROH) analysis was conducted using PLINK software (Chang et al., 2015). The genome was scanned for consecutive homozygous SNPs. Various characteristics of ROH segments were calculated, including the total number of segments, average and maximum segment length, and the number of SNPs within each ROH (Pacheco et al., 2023). The bull population was divided into low- and high-fertility groups based on SCR (Sire Conception Rate). The level of homozygosity, measured as total ROH length, was compared between these groups. To explore potential genetic factors related to male fertility, genomic regions with overlapping ROH segments were examined.

Results & Discussion

The study unveiled a myriad of insights into the factors influencing male fertility in Brown Swiss cattle.

Sire Conception Rate

The Sire Conception Rate (SCR) reflects male fertility, with a 1-point difference

representing a 1% change in Conception Rate (CR). SCR values varied significantly among the 1,228 Italian Brown Swiss bulls, with a 20% difference between high and low-fertility bulls. Notably, 20% of this variation was attributed to additive genetic effects. A positive correlation of +0.35 (**P< 0.01) was observed between Italian and American SCR values for 44 bulls evaluated in both countries.

We then assessed the predictive performance of different models for SCR, revealing correlations between SCR and bull's CR ranging from 28.2% to 30%. Variables like milk yield (MY), AI company within a specific year (AI company_year) and inbreeding of the potential embryo (Inbreeding mating) influenced these correlations.

Genomic Non-Additive Effects

Whole-genome scans divulged the presence of two genomic regions on BTA6 and BTA26 with substantial non-additive effects on male fertility. These regions encompassed genes like *WD Repeat Domain 19* (*WDR19*) and *Adhesion G protein-coupled receptor A1* (*ADGRA1*), with known direct implications for key aspects of male fertility, such as sperm motility, acrosome reaction, and embryonic development (Figure 1).

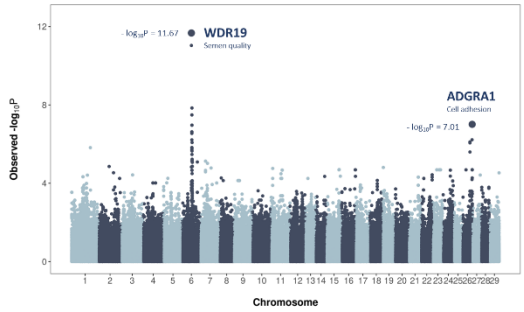

Figure 1. The Manhattan plot illustrates the significance of recessive effects and their relevance to male fertility in Italian Brown Swiss cattle.

Enhancing Genomic Predictive Accuracy

Figure 2 shows the predictive ability of linear kernel-based regression models fitting the whole-genome model, 'IT: Polygenic", and the 'IT: Polygenic + Major Markers' model that includes two significant recessive SNPs fitted as fixed effects. The 'IT: Polygenic' model exhibited an average correlation between observed and predicted SCR values of 0.19, and a mean-squared error of prediction (MSEP) equal to 22.11. The 'IT: Polygenic + Major Markers' model delivered an average predictive correlation equal to 0.32 and MSEP equal to 20.34. Notably, the model predictive ability was largely improved by including the two markers with large effect, representing an increase in predictive correlation of about 68%. Pacheco et al. (2022) reported that these significant non-additive markers are near genes directly involved in male fertility, including sperm motility, acrosome reaction, and embryonic development.

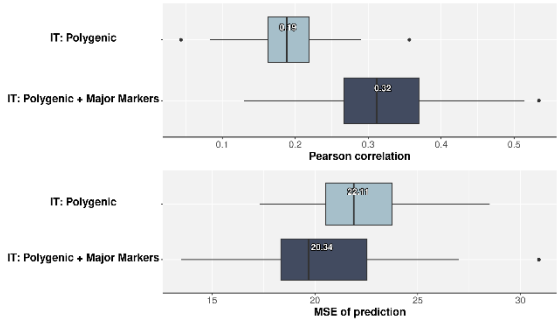

Figure 2. Genomic predictions within the Italian Brown Swiss population using alternative whole-genome predictive models. Predictive correlation (top) and mean squared error of prediction (bottom) were calculated using 5-fold cross-validation with 10 replicates. Light blue boxes represent the 'IT: Polygenic' model that includes the whole SNP dataset (481,839 SNPs). Dark blue boxes represent the 'IT: Polygenic + Major SNPs' model that includes two major SNP markers fitted as fixed effects.

Runs of Homozygosity

The study highlighted the detrimental impact of inbreeding on male fertility in Brown Swiss cattle. Four regions of homozygosity located on chromosomes 6, 10, 11, and 24 were significantly overrepresented in low-fertility bulls. The results underscored the complexity of factors influencing male fertility and the potential of combining multiple sources of

information to enhance our understanding of this critical aspect of cattle breeding.

Conclusions

This study delves into the male fertility of Italian Brown Swiss dairy bulls using extensive cow field records and genomic data. It uncovers substantial variability in sire conception rates and successfully demonstrates the potential of assessing bull fertility directly from confirmed pregnancy records. The research reveals insights into genetic and genomic factors influencing male fertility, highlighting non-additive genetic effects, relevant genomic regions, and the impact of inbreeding. Ultimately, this study provides a foundation for refining management and selection strategies in the dairy industry and offers valuable contributions to understanding male fertility in Brown Swiss cattle, with potential for future enhancements in this field.

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International beef evaluation for Carcass traits

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Abstract

Since the early 1990s, different initiatives have emerged worldwide to establish beef across-countries genetic evaluations. The experiences involving Ireland, France and the UK served as a precursor for today's Interbeef Working Group (WG). Interbeef is a WG of ICAR and offers, through the Interbull Centre, international beef cattle evaluation services for 12 countries, five breeds and three trait groups including adjusted weaning weight (aww), calving (calv), and since 2023, also carcass (carc). The evaluation of carcass traits has been an objective of Interbeef WG since its origin. The results of a survey carried out in 2014 verifying the status of this group of traits at the European level, determined that the main traits to be included in the evaluation would be carcass weight, fat and conformation. The first data call was carried out in 2018, and after several research evaluations and modifications to the model, a final pilot evaluation was performed in December 2022. The results were considered satisfactory, and the evaluation of carcass traits became a routine service run starting in October 2023.

Key words: beef cattle, across countries genetic evaluations, carcass traits

Introduction

Interbeef is a Working Group (WG) of the International Committee for Animal Recording (ICAR), whose main objective is to develop and promote genetic and genomic evaluations of beef cattle at the national and international levels. In this regard, the Interbeef WG acts as a worldwide network for the improvement of beef cattle, in permanent dialogue with the different sectors of the industry, and develops international evaluation systems. On the other hand, it also has a role in coordinating and collaborating in scientific research related to beef cattle breeding (ICAR, 2022).

Through the Interbull Centre, Interbeef offers international services to participating countries for five different breeds, including Aberdeen Angus, Charolais, Hereford, Limousin and Simmental, and three trait groups including adjusted weaning weight (adww), calving (calv) and, since 2023, carcass (carc). Female fertility is an important trait group currently under development and is intended to be introduced as a service in the coming years.

All trait group services follow specific steps for their development, which may vary subtly given the requirements of the different traits. This article summarises the development process of the carcass trait service, from its idealisation to the recent first routine evaluation.

Brief history of Interbeef

The collaborative way of service development within Interbeef is related to its history and conception.

The ambition to perform joint beef cattle evaluations for groups of countries dates back to the early 1990s. Since then, several projects have been conducted in different regions worldwide (Bullock et al., 2003; Reverter et al., 2002; Journaux et al., 1996).

In 1999, a collaborative research project brought institutions together from three European countries: the Irish Cattle Breeder Federation (ICBF) from Ireland, the Institut de l'Elevage (IDELE) from France and the Meat Livestock Commission (MLC) from the United Kingdom. The main objective of the project, called European International Beef Evaluation (EUBEEVAL), was to develop methodologies to obtain estimated breeding values (EBV) between European countries, accounting for the differences between production systems, and, in the other hand, to study the best way to compare

EBVs obtained in different systems (Journaux et al., 2006).

One of the most important results of the project was the determination of the best model for international across-countries evaluations. In beef cattle production systems, the use of artificial insemination (AI) is limited; consequently, the connection between countries is also weak. In this context, it was determined that the best model to apply in beef international genetic evaluations is an Animal Model accounting for Across Countries Interactions (AMACI) (Phocas et al. 2004). Following this system, phenotypes, different models for each country and across-countries covariance are used.

The efforts made in the framework of this project laid the foundation for the formation of Interbeef WG. The Interbull Centre team carries out the services offered by the WG, which currently consists of beef international evaluations for adjusted weaning weight, calving traits (calving ease and birth weight) and carcass traits (weight, fat and conformation) with the participation of 12 countries.

Carcass traits service

There are several stages in introducing a set of traits into the routine evaluations performed by the Interbull Centre.

When community interest in a set of traits is identified, a working group will conduct the investigation on their possible inclusion as a service.

Although the latter stages have to be done with the resources of the Interbull Centre, part of the development and the estimation of the variance components are carried out in national genetic centers. This was the case with adjusted weaning weight developed mainly by ICBF in Ireland, calving traits at the Institute of Animal Science, Czech Republic, and fertility traits under development at Vereinigte Informationssysteme Tierhaltung (vit), Germany. Adjusted weaning weight and calving traits have been part of routine evaluations since 2013 and 2016, respectively.

Carcass traits have been an Interbeef WG's goal since the beginning, and studies of this group of traits began at the initiative of Scotland's Rural College (SRUC) and then continued through the ICBF and Interbull Centre. Figure 1 presents a timeline from the first steps until the carcass traits routine evaluation.

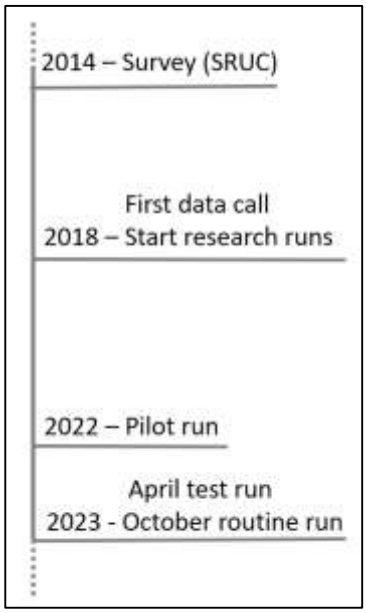

Figure 1. Timeline of the development of the Carcass traits service.

As a first step, SRUC surveyed the participating countries to diagnose the status of national evaluations for carcass traits. The survey covered different aspects, such as proxy traits measured on the live animal, traits measured directly on the carcass, and breeds and models of the evaluations in the different countries. It identified carcass weight and EUROP grades of carcass conformation and fat as the most recorded traits (Table 1). Another important finding was the high number of crossbred individuals recorded in several countries, and some countries commented on the importance of carcass traits in beef from dairy systems.

Table 1. Carcass traits recorded by countries. Adapted from SRUC survey.

	CHE	CZE	DNK	FIN	SWE	FRA	GBR	IRL
C. Weight	✓	✓	✓	✓	✓	✓	✓	✓
C. Conformation	✓	✓	✓	✓	✓	✓	✓	✓
C. Fat	✓	✓	✓	✓	✓	✓	✓	✓
Individual Primal Cuts							✓	✓
Total meat yield								✓
Tenderness								✓
Age at slaughter						✓		

CHE = Switzerland, CZE = Czech Republic, DNK = Denmark, FIN = Finland, SWE = Sweden, FRA = France, GBR = Great Britain, IRL = Ireland.

Given the importance of the participation of crossbred individuals in this trait group, the WG postponed the data call until the conclusion of studies to incorporate this type of information in the international evaluations of Interbeef.

The data call was finally presented in September 2018 for the three recommended traits, carcass weight, fat and conformation, including three breeds: Charolais, Limousin and Simmental.

The first research evaluations were performed on data from 4 populations: Ireland (IRL), Great Britain (GBR), Switzerland (CHE), and Denmark, Finland and Sweden (DFS) with a set of (co)variance components estimated by ICBF (Ireland). In 2021, new data was collected via the Interbull Data Exchange Area (IDEA) and new research runs were conducted within the Interbull Centre. Finally, in December 2022, a new set of (co)variance components was prepared by ICBF, and the carcass traits pilot run was performed with acceptable results.

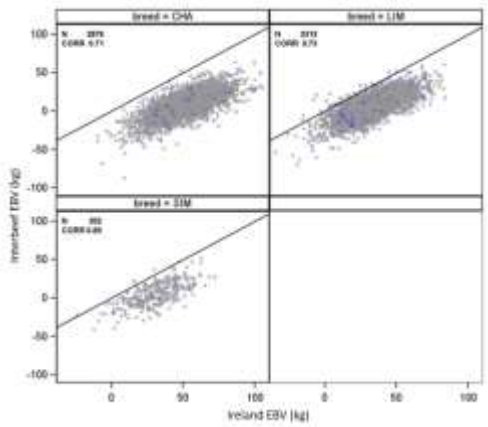

Figure 2. Comparison of carcass weight EVBs from bulls with reliability >0.7 from Interbeef pilot and Irish evaluations. Courtesy of Thierry Pabiou, ICBF.

For example, Figure 2 presents a comparison of carcass weight EBVs of bulls with reliabilities over 0.7 from the Interbeef pilot evaluation and the Irish domestic evaluation for the three evaluated breeds. The correlation between the EBVs from each evaluation was approximately 0.7 in all of the breeds. Therefore, the Interbeef WG decided to include this trait group in the April 2023 Interbeef test run.

The 2023 test and routine runs

Even though four populations participated until the pilot run, for different reasons, only Ireland and Great Britain went ahead with the April 2023 test evaluation. The countries submitted a total of 1,620,279 records for Charolaise, 1,619,571 for Limousin and 450,708 for Simmental (Table 2). A total of 1,232,423, 1,276,613, and 361,419 individuals for Charolais, Limousin and Simmental, respectively, were included in the evaluation. The test and posterior routine evaluations used the set of (co)variance components estimated for the pilot run. Table 3 shows the correlations across Ireland and Great Britain for the three traits included in the evaluation. In general, the correlations are in a medium range, between 0.56 and 0.75, with the highest ($\geq$ 0.7) for carcass fat and the lowest ($\leq$ 0.62) for carcass conformation.

Table 2. Number of records submitted to the April 2023 test evaluation.

		Number of records per trait
CHA	IRL	388,500
	GBR	151,593
LIM	IRL	386,257
	GBR	153,600
SIM	IRL	48,230
	GBR	102,006

Table 3. Carcass genetic correlations between Ireland and Great Britain.

	CHA	LIM	SIM
C. Weight	0.61	0.65	0.69
C. Conformation	0.60	0.62	0.56
C. Fat	0.70	0.75	0.72

The October routine evaluation counted with the participation of both countries, and an increase of 1.6% in the number of performance submissions was observed.

Future developments

Regarding future developments of the carcass evaluations, it is expected that more countries will be interested in participating in the evaluation, given the importance of this trait group for beef cattle breeding.

It is also possible to expand the evaluation to Aberdeen Angus and Hereford when requested by the participating countries.

Concerning potential new carcass traits to include in the evaluation, in the framework of the European GenTORE project, some research has been carried out on the evaluation of age at slaughter. This trait of interest can also be included in the future after a short research and pilot evaluation.

On the other hand, Interbeef WG is currently discussing the development of international genomic evaluations. When discussions conclude on how to carry out the genomic evaluation, all current trait groups under evaluation are expected to be included.

As previously mentioned, the SRUC survey identified many countries linking carcass traits to beef on dairy systems. Given the economic potential it represents this is also a topic of great interest within the beef cattle industry. Recently, a study conducted by Jo Newton (2023) under the ICAR Brian Wickham Young Person Exchange Program, identified the opportunity for international evaluation of beef on dairy through the Interbull Centre, whereby carcass traits could be the first trait group to be tested in a beef on dairy system.

Conclusion

After many years of development and testing different models, the carcass trait Interbeef evaluation has been successfully introduced as a service in routine evaluations.

In the near future, more countries are expected to participate, as well as expansion of the evaluation to more breeds.

The international carcass traits evaluation has great potential for the development of an international evaluation of beef on dairy.

Acknowledgements

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Inheritance of A New Mutation Affecting Muscle Weakness Within a Common Haplotype in Holsteins

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Abstract

A haplotype associated with calf recumbency and mortality having a recessive effect but apparent incomplete penetrance was previously linked to the end of chromosome 16 (78.7 to 80.7Mbp). Genotype analysis of 5.6 million Holsteins indicated that the haplotype was common and traced back to 1952, with a key ancestor born in 1984 (HOUSA1964484, Southwind) identified from chip genotypes as homozygous for the suspect haplotype. Sequence data from Southwind, an affected calf, and the sire of the affected calf were scanned for candidate mutations. A mutation in the *CACNA1s* gene causing symptoms of recumbency (lately termed Holstein Early Onset Muscle Weakness; HMW) was homozygous in the affected calf and heterozygous in the calf's sire and Southwind. Improved methods for using pedigree to track new mutations within existing haplotypes were developed, and gene tests for the mutation were also included. For new mutations within existing common haplotypes, determining carrier status without gene tests is difficult, even with accurate pedigrees when the original haplotype has a high frequency.

Key words: HMW, CACNAS1, Recessive, Recumbency

Introduction

Computational genomic tools allow for monitoring known genetic diseases in dairy cattle, predict the rise of novel markers (causal/associated), and link them to newly identified disorders of a genetic nature (https://www.ars.usda.gov/ARSUserFiles/80420530/Publications/ARR/Haplotype%20tests_ARR-Genomic5.pdf). Recently, Holstein newborns exhibited higher death rates due to inability to stand and neuropathological symptoms described and termed as recumbency by Dechow et al. (2022). They identified the disorder using genotypes and pedigrees of affected calves that shared a haplotype on chromosome 16 (78.7 to 80.7Mbp on ARS-UCD1 map) and ancestral links traced back to a bull born in 2008.

The condition leading to calf recumbency is currently known as "Early Onset Muscle Weakness" (**MW**). Despite its clinical significance, official recognition of this disorder as a recessive trait is still in progress, and the nomenclature remains subject to finalization.

Carriers of recessive inheritance genes are usually identified and tracked using a haplotype-based tests and, once enough research data supports the identification of the actual causal variation, a gene test is developed as a diagnostic tool with high accuracy. Precisely monitoring the rise in frequencies of inherited haplotypes of recessive conditions is essential, and constant improvement of the haplotype-based test is critical. One such example is Holstein Haplotype for Cholesterol Deficiency (HCD), where a new mutation occurred in a commonly and highly frequent haplotype (VanRaden and Null, 2015).

The current study investigates the muscle weakness haplotype (**HMW**) using US data, reports a new mutation within it, and validates improved accuracy of the haplotype-based test by resolving status using pedigrees and gene test results.

Materials and Methods

The affected calves investigated by Dechow et al. (2022) were found to have a common ancestor born in 2008 (HOUSA64966739 Roylane Socra Robust-ET). By using a larger pool of chip-genotyped animals (over 5.5 million animals), the anticipated associated haplotype of the affected calves was found in further older ancestors (HOUSA1964484, Southwind Bell of Bar-Lee) born in 1984. Southwind carried the suspect haplotype and was also an ancestor of some recumbent calves that did not trace to Robust. Disregarding the association with the MW affected calves, the haplotype was common and was identified in further genotyped animals where the oldest (HOUSA1189870 Osborndale Ivanhoe) was born in 1952.

Sequence data for Southwind, an affected calf, and the sire of the affected calf were examined and to identify a potential causal mutation. *CACNA1s* mutation at position 79,613,592 was identified as the most likely variant with high association concordance. Sorting Intolerant From Tolerant (SIFT) software (https://sift.bii.a-star.edu.sg/) utilized to predict the deleterious consequence of this mutation.

A commercially developed gene test using the CACNA1s mutation was used to identify carrier status for 4,416 animals and reported by the Holstein Association USA (HAUSA). The method by VanRaden and Null, 2015 identifies recessively inherited haplotypes with a harmful effect was further improved and validated to identify the MW haplotype, and then sort genotyped animals that are carriers of the *CACNA1s* mutated MW haplotype. The revised algorithm also requires pedigree relatedness to the common ancestor of the affected calves. Then, it categorizes the haplotypes into the different genotype status groups (0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous).

As of May 2023, Select Sires, ABS Global, Genex, and Semex companies utilized the MW gene test and publicly reported results for 2,609 tested animals. The reported results were examined, compared to the haplotype tests, and included in the haplotype determination algorithm to improve the accuracy of future calling of the MW carrier status.

Results & Discussion

Sequence data analysis confirmed the hypothesis of a new mutation. Southwind was homozygous for all sequence variants within the suspect region as expected but was heterozygous for the mutation at location 79,613,592 bp in the CACNA1s gene and was the earliest known carrier of the mutated haplotype (HMW). Thus, Southwind, or an ancestor between him and Ivanhoe was the source of a new mutation. The identified causal mutation was then utilized to develop a muscle weakness (MW) gene test.

Haplotype test results from the newly adopted method were further updated by matching haplotype data to the gene test results to rule out ancestors that inherited the common haplotype but not the mutation. Genotyped animals with unknown haplotype status may be resolved using gene tests for their ancestors (Table 1). This test examined 424,109 HO males (Table 2), and 5.9 million HO females (Table 3) genotyped as of July 2023. Before including 4,416 gene tests from HAUSA indirectly in the pedigree, 93.58% of males and 88.35% of females had certain haplotype codes (0, 1, or 2), and the other 4.96% of males and 11.11% of females had uncertain haplotype codes (3 or 4). That sex difference is because commercial females often have incomplete pedigrees and non-genotyped dams, whereas nearly all males have complete pedigrees and both parents are genotyped.

For animals with uncertain haplotype status, the inclusion of gene tests changed the haplotype status for 22% of the males but only 5% of the females. For example, of the 19,283 males with uncertain status, 3,574 resolved to noncarrier and 1,845 to carrier status. Very few (513) of the 5.6 million animals that previously

had certain status (< 0.1%) changed after including gene tests in the pedigree. The conducted research affirmed the accuracy of the newly adopted haplotype test method and a high concordance achieved in identifying actual noncarriers, carriers, and affected Holstein calves.

Popularity of Robust was responsible for the rapidly increasing trend of the mutation. The improved haplotype prediction method requiring a pedigree association to Robust and incorporating the CACNA1s mutation gene tests predicted only 220 HMW carriers by the end of 2009. The number of HMW carriers increased significantly over the next ten years to 66,432 carriers, of which 59,243 HMW carriers were animals genotyped in the last four years (2020 – present). Holstein Association USA and the dairy cattle industry are following our recommended preventative measurement by identifying muscle weakness disorder gene carriers and reporting affected animals using the gene test.

Another observation for recessively inherited diseases such as MW with incomplete penetrance and/or semi-lethal nature is that under-reported death losses by farmers are negatively affecting estimated effects calculated for mating carrier sires and maternal grandsires (data not shown in this study). Accuracy of reporting death losses from such inherited diseases could influence other similar phenotypes such as longevity traits (heifer livability, stillbirth. etc.) (Wiggans and Carrillo, 2022).

Conclusions

Haplotype-based testing methods for recessively inherited diseases such as MW are essential but are complex when new mutations occur. The accuracy of identifying carriers can be greatly improved by using pedigrees to track the haplotype source and incorporating gene test results when they are available. Farmers are encouraged to accurately report newborn death losses and/or cases of calves' early onset inherited disability as these accurately reported data could be correlated to infer confirmational estimates of the carriers of such recessive haplotypes.

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Table 1. Carrier status (count and %) before and after adding gene test results to the pedigree and haplotype information (males and females). Status 3 reports unsure heterozygote and 4 reports unsure homozygote.

Status before (rows) vs. after (columns)	0	1	2	3	4	Total (%)
0	5,497,696 (86.59)	513 (0.01)	1 (0)	91 (0)	0 (0)	5,498,301 (86.6)
1	31 (0)	133,105 (2.1)	9 (0)	13 (0)	0 (0)	133,158 (2.1)
2	0 (0)	0 (0)	796 (0.01)	0 (0)	0 (0)	796 (0.01)
3	27,271 (0.43)	7,866 (0.12)	0 (0)	634,717 (10)	0 (0)	669,854 (10.55)
4	1,048 (0.02)	1,058 (0.02)	221 (0)	0 (0)	44,470 (0.7)	46,797 (0.74)
Total (%)	5,526,046 (87.04)	142,542 (2.25)	1,027 (0.02)	63,4821 (10)	44,470 (0.7)	6,348,906 (100)

*Genotype status: 0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous.

Table 2. Improved predictions are achieved when gene test results are incorporated into the population pedigree information (male data).

(old vs. new) gene code (%)	0	1	2	3	4	Total (%)
0	381,409 (89.93)	228 (0.05)	0 (0)	41 (0.01)	0 (0)	381,678 (90)
1	10 (0)	15,321 (3.61)	4 (0)	5 (0)	0 (0)	15,340 (3.62)
2	0 (0)	0 (0)	181 (0.04)	0 (0)	0 (0)	181 (0.04)
3	3,574 (0.84)	1,845 (0.44)	0 (0)	19,283 (4.55)	0 (0)	24,702 (5.82)
4	148 (0.03)	232 (0.05)	91 (0.02)	0 (0)	1,737 (0.41)	2,208 (0.52)
Total (%)	385,141 (90.81)	17,626 (4.16)	276 (0.07)	19,329 (4.56)	1,737 (0.41)	424,109 (100)

*Genotype status: 0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous.

Table 3. Improved predictions are achieved when gene test results are incorporated into the population pedigree information (female data).

(old vs. new) gene code (%)	0	1	2	3	4	Total (%)
0	5,116,287 (86.35)	285 (0)	1 (0)	50 (0)	0 (0)	5,116,623 (86.36)
1	21 (0)	117,784 (1.99)	5 (0)	8 (0)	0 (0)	117,818 (1.99)
2	0 (0)	0 (0)	615 (0.01)	0 (0)	0 (0)	615 (0.01)
3	23,697 (0.04)	6,021 (0.1)	0 (0)	615,434 (10.39)	0 (0)	645,152 (10.89)
4	900 (0.02)	826 (0.01)	130 (0)	0 (0)	42,733 (0.72)	44,589 (0.75))
Total (%)	5,140,905 (86.77)	124,916 (2.11)	751 (0.01)	615,492 (10.39)	42,733 (0.72)	5,924,797 (100)

*Genotype status: 0 = noncarrier, 1 = carrier, 2 = homozygous defect, 3 = suspect carrier, 4 = suspect homozygous.

A Single-Step Genomic Evaluation of Claw Health Traits in French Holstein, Montbéliarde and Normande Breeds

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Abstract

Claw lesions are the third most important health issue in dairy cattle, after mastitis and fertility issues. 21 lesions defined according to ICAR standards are recorded by trimmers on touch pad since the early 2010s. Seven of these lesions (Digital Dermatitis (DD), Heel Horn Erosion (HHE), Interdigital Hyperplasia (IH), Sole Hemorrhage Circumscribed (SHC), Sole Hemorrhage Diffused (SHD), Sole Ulcer (SU) and White Line Fissure (WLF)), which have a prevalence of more than 10%, and/or may be responsible for lameness, were studied in the Holstein, Normande and Montbéliarde breeds. Breed specificities have also led to study Toe Necrosis (TN) and Corkscrew Claw (CSC). In summer 2022 dataset, more than 440,000 Holstein trimmings (respectively 80 000 Montbeliarde and 62 000 Normande) from 250 000 cows (respectively 44 000 Montbeliarde and 35 000 Normande), including 35,000 genotyped cows (respectively 15 000 Montbeliarde and 10 000 Normande) were available for the development of the genetic evaluation model. 40% of the cows were trimmed more than once, but only 20% were trimmed in different lactations. Estimated heritabilities ranged from 0.01 and 0.22 depending on the trait. Genetic correlations showed two groups of traits that were highly correlated: 1) within the infectious traits (DD, HHE and IH), in particular with high correlations between DD and IH (between 0.65 and 0.80 according to the breed); 2) within the non-infectious traits (SHC, SHD, WLF and SU) with genetic correlations between 0.40 and 0.89 in Holstein; TN and CSC being relatively independent from the other traits. Multiple trait single-step genomic evaluations have been developed for each group of traits to limit computational times, with a negligible effect on estimated genetic values compared to a nine traits genetic evaluation. Implementation of routine evaluation is planned for April 2024.

Key words: claw health, single-step, genomic evaluation, genetic correlations

Introduction

Claw health are a major welfare problem in dairy farming, often causing pain and lameness in cows. In France, it is the third most costly disorder and is responsible for a fifth of culling after mastitis and fertility trouble. Lameness usually has a multifactorial origin. 24 claw health traits as described in ICAR Atlas (ICAR, 2020) can be registered, and 11 of them are mandatory.

Until now, the development of claw health genetic evaluation in France has been private initiatives directly led by breeding companies (Leclerc et al., 2019). A need to harmonise the different initiatives and to bring the Holstein closer to the Eurogenomics golden standards, led to initiate a national project in summer 2022. With data currently available, it is now possible to set up a multiple trait model that takes into account successive trimmings using a single step methodology, and to include new traits of interest as required.

Based on data collected since 2012 in the 3 main breeds (Holstein, Normande and Montbéliarde), the objective of this study was: 1) to select traits of interest from the 24 claw heath traits; 2) to estimate genetic relationships between those traits; 3) to develop a multiple trait model that takes into account successive trimmings using a Single Step Genetic Evaluation.

Materials and Methods

The claw health database

Breeding organizations (Evolution/Synetics, Genes Diffusion, Origen Normande, Umotest) gathered information on 642 540 claw trimming animals (Table 1 with breed distribution), collected by 220 professional trimmers on touch-pad from 2012 to 2022 in 9 091 herds.

Data comes from a limited number of herd, because not all of them are using trimming service, and a large part part of the trimming service is carried out by independent trimmers who do not have the touch-pad to collect claw health data. In addition, the breeder chooses which cows to trim. Therefore, we have non-exhaustive data within herd.

To ensure the quality of the data, only data from herds enrolled in official milk recording, having a lactation rank of one to five5 and a lactation stage of one to 550 are considered. The cows must have at least the two rear claws trimmed and a minimum recovery period of four months after the previous lesion to be considered as a new lesion as mentioned in EuroGenomics Golden Standard (S. De Roo, 2022 - personal comm.).

Analysis of herds with exhaustive trimming has shown that only 12% (Normande) to 21% (Holstein) of cows have no lesions, so we decided to not assume a healthy status / absence of claw disorders of untrimmed cows by default.

were selected: Digital and interdigital Dermatis (DD), Interdigital Hyperplasia (IH), Heel Horn Erosion (HHE), White Line Disease (WDL), Sole Hemorrhage Diffused (SHD) and Circumscribed (SHC), Sole Ulcer (SU), Toe ulcer and Necrosis (TN) and CorkScrew Claw (CSC). These traits present a prevalence of at least 10% in one of the three studied breeds, or for TN an increasing frequency and a large economic impact, culling being in most cases inevitable.

Due to infectious status of Digital Dermatitis, only cows from a herd with affected contemporaries are considered healthy.

Model

Bivariate and Multivariate linear animal models were fitted using REML procedure from the Wombat software (Meyer, 2007), based on selected data described in Table 1. A minimum of 10% of the herd trimmed per year is required to select data for genetic evaluation, but this minimum is increased to 15% with two annual visits for genetic parameter estimation (and 50% in Holstein).

The following linear animal model, with repeated observations within and across lactations, was applied:

$$\boldsymbol{y}=\boldsymbol{X\beta}+\boldsymbol{Za}+\boldsymbol{Zp}+\boldsymbol{e}$$

where **y** is the vector of severity scores for the traits (from 0 = healthy to 3=severe lesion except for TN and CSC which are treated as a binary traits 0/1); **β** the vector of fixed effects consisting of a herd × trimming date effect (with minimum five cows in

Table 1. Description of the datasets used for the differents step of the study

		Holstein	Normande	Montbéliarde
Database	#trimmed cows	451 322	61 975	79 371
Genetic parameters estimation	#trimmed cows	89 930	25 551	38 148
	#trimmed data	142 090	41 017	64 471
	#herd x trimming date	4 258	3 298	3 682
	#animal in pedigree file	190 212	70 719	95 243
Single Step Genetic Evaluation	#trimmed cows	299 679	44 268	45 878
	#trimmed data	532 712	82 265	76 672
	#herd x trimming date	26 228	6 854	4 707
	#animals (♂+♀) in reference pop	46 072	13 291	12 817

Nine claw health traits from the 24 ICAR Atlas

Holstein and four in the other breeds), trimmer × year effect, age of calving × parity effect, calving month × year effect, parity × lactation month × 3 year period effect ; **a** the vector of additive gene
tic effect ~$\mathbf{N}(0,\mathbf{A}\sigma^2_g)$, **p** the vector of random effect of permanent environment ~$\mathbf{N}(0,\mathbf{I}\sigma^2_{pe})$. **X** and **Z** are incidence matrices.

A Single-Step genetic evaluation using HSSGBLUP software (Tribout et al., 2020) using multivariate model similar to variance component estimation was performed on the 9 traits and then split in two groups of traits: a group of three infectious traits (DD, IH and HHE) and 6 traits (SHC, SHD, SU, WLD, CSC and TN) to limit memory requirements and computational time by two to three.

Results & Discussion

The prevalence of the traits is not similar from one breed to another one. In Holstein (Table 2) (Normande & Montbéliarde breed are in Annex), DD and HHE have a higher prevalence than on other breeds. In Montbeliarde breed, it is mainly the prevalence of WLD and CSC that distinguishes it, while in the Normande breed, many traits show higher prevalence than in the other breeds (DD, IH, TN, WL, SU).

In Holstein (Table 2), heritabilities are quite low, between 2% and 10% (between 2 and 8%, in Normande except for interdigital hyperplasia with a moderate heritability of 22% (Table 5 in Annex) and between 4 and 9% in Montbéliarde (Table 6 in Annex), but within the range of similar studies (CRV, 2022 ; Johansson et al., 2011). The repeatability trend is similar between breed, with some traits with moderate repeatability ranging from 0.17 to 0.23 for digital dermatitis, white line disease and sole ulcer and quite high for interdigital hyperplasia and toe ulcer and necrosis ranging from 0.34 to 0.47, illustrating how difficult it is to treat for this lesions in the long term.

The estimated genetic correlations tend to show the existence of 2 groups of traits: A first group of infectious traits with DD, HHE and IH and a second group with mechanical/physical lesions with SHC, SHD, WL, SU, TN and CSC. The genetic correlations within group are high: for instance, between 0.50 and 0.71 between the 3 infectious traits in Holstein. Within group of mechanical lesion, genetic correlations are usually moderate (generally in the range from 0.25 to 0.50), except high correlations between SHC and SU with 0.89, 0.78 and 0.84

Table 2. Holstein genetic parameters estimates (Prevalence of the traits (%), heritabily on diagonal, Genetic correlations (rg) above diagonal – standard error of heritability and range of standard error of genetic correlations, and repeatability) for claw health traits (Digital Dermatis (DD), Interdigital Hyperplasia (IH), Heel Horn Erosion (HHE), White Line Disease (WDL), Sole Hemorrhage Diffused (SHD) and Circumscribed (SHC), Sole Ulcer (SU), Toe ulcer and Necrosis (TN) and CorkScrew Claw (CSC)).

Holstein	**Preval.**	**DD**	**HHE**	**IH**	**TN**	**SHC**	**SHD**	**WL**	**SU**	**CSC**	**repeat.**
DD	**35%**	**0.08**	0.68	0.71	-0.06	-0.08	-0.17	-0.14	0.01	0.00	**0.18**
HHE	**39%**		**0.04**	0.50	-0.12	0.22	-0.10	-0.05	0.28	0.18	**0.09**
IH	14%			**0.10**	-0.10	-0.02	-0.09	-0.06	0.06	0.02	**0.41**
TN	3%				**0.01**	0.48	0.55	0.50	0.58	0.06	**0.34**
SHC	16%					**0.04**	0.44	0.47	0.89	0.19	**0.08**
SHD	25%						**0.02**	0.43	0.40	0.32	**0.05**
WL	17%							**0.05**	0.63	0.20	**0.17**
SU	13%								**0.06**	0.09	**0.17**
CSC	5%									**0.02**	**0.11**
σ_{error} $\mathbf{h^2}$		0.005	0.004	0.006	0.002	0.003	0.003	0.004	0.004	0.003	
σ_{error} $\mathbf{r_g}$	**Min**	0.03	0.04	0.03	0.08	0.03	0.06	0.04	0.04	0.06	
	Max	0.10	0.11	0.10	0.13	0.10	0.11	0.10	0.09	0.13	

respectively for Holstein, Normande and Montbéliarde breed. This suggests that SHD may be a precursor to SU. In the 3 breeds, the correlation between the two different sole haemorrhages (SHC and SHD) is moderate with values between 0.26 and 0.50, clearly showing that they are two different traits.

Impact of splitting the nine traits into two groups of traits is negligible. Correlations between GEBV obtained in a nine traits sets vs a 3+6 traits are over 0.99 for all traits in the three breeds (except for DD and HHE in Montbéliarde breed > 0.984)

More than 1,6 millions of animals were evaluated based on the 500 thousand trimmed data and 46 thousand animals in the Holstein reference population. GEBV are expressed in genetic standard deviation unit. Analysis of the risk factor (% of animals affected) as a function of GEBV shows, for instance in the Normande breed, that number of animals with IH drops from 73% for an index of -1 to 25% for an index of 0, and from 19% of animals with TN to only 1% for similar index than previously.

Composite indexes have been defined for each breed to optimize their uses and to improve the genetic level of the population, taking into account prevalence and estimated incidence costs (Table 3) (synthesis from Dolechek and Bewley, 2018 & 2019; Whay and Shearer, 2017; Willshire and Bell, 2009; Bruijnis et al, 2010; Charfeddine and Perez-Cabal, 2017; and discussion with French veterinarians R. Guatteo and A. Waché – personal comm.).

For infectious traits, the composite SLI (Table 4) has the same weighting for the three breeds. For mechanical traits, breeds specificities have been taken into account (Table 4) by including toe necrosis in selection index in Normande breed, and corkscrew claw in Montbéliarde breed as well as increasing weight on white line disease for this breed.

Table 3. Estimated claw disorders costs in €uro.

	Estimated Cost		
	Direct	Indirect	Total
DD	50€	150€	200€
HHE	25€	0€	25€
IH	50€	50€	100€
SHC	25€	0€	25€
SHD	25€	0€	25€
WD	30€	100€	130€
SU*	50€	200-300€	300€
TN*	50€	300-1000€	450€
CSC	25€	0€	25€

⁂ High culling risk for high severity levels

A claw health index gathers the SLI and SLM, with a balanced weight in Montbéliarde, whereas Holstein and Normande give 60% on SLI and 40% on SLM.

Conclusions

From the nine claw health traits studied, two groups of traits emerge which are more or less genetically independent of each other, and which make it is possible to evaluate them in two sets of 3+6 traits.

Breed-specific composite for claw health have been decided in concertation between breed societies and will be included in future revisions of the Total Merit Index.

The first routine genetic evaluation is currently implemented at GenEval and the official release is scheduled in April 24.

Table 4. Composite of claw health traits: Infectious traits index (SLI), Mechanical trait index (SLM), and Claw Health index (STPI).

	Infectious Traits index (SLI)			Mechanical Traits index (SLM)						Claw Health index (STPI)	
Traits	DD	IH	HHE	SHC	SHD	WL	SU	TN	CSC	SLI	SLM
Holstein	0.60	+0.30	+0.10	0.10	+0.10	+0.40	+0.40			0.60	+0.40
Normande	0.60	+0.30	+0.10	0.05	+0.05	+0.25	+0.40	+0.25		0.60	+0.40
Montbéliarde	0.60	+0.30	+0.10	0.10	+0.10	+0.45	+0.30		+0.10	0.50	+0.50

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Annex 1. Normande and Montbéliarde genetic parameters estimates

Table 5. Normande genetic parameters estimates (Prevalence of the traits (%), heritabily on diagonal, Genetic correlations (rg) above diagonal – standard error of heritability and range of standard error of genetic correlations, and repeatability) for claw health traits (Digital Dermatis (DD), Interdigital Hyperplasia (IH), Heel Horn Erosion (HHE), White Line Disease (WDL), Sole Hemorrhage Diffused (SHD) and Circumscribed (SHC), Sole Ulcer (SU), Toe ulcer and Necrosis (TN) and CorkScrew Claw (CSC)).

Normande	Preval.	DD	HHE	IH	TN	SHC	SHD	WL	SU	CSC	repeat.
DD	43%	0.08	0.37	0.80	-0.45	-0.25	-0.26	-0.39	-0.27	-0.19	0.18
HHE	33%		0.02	0.18	-0.29	0.11	-0.01	-0.22	0.31	0.00	0.04
IH	30%			0.22	-0.29	-0.24	-0.23	-0.16	-0.22	-0.15	0.47
TN	5%				0.03	0.23	0.27	0.43	0.22	0.15	0.39
SHC	14%					0.03	0.50	0.31	0.78	0.20	0.06
SHD	29%						0.03	0.45	0.30	0.36	0.06
WL	28%							0.07	0.25	0.36	0.23
SU	18%								0.07	0.10	0.23
CSC	3%									0.05	0.19
σ_{error} h^2		0.010	0.004	0.017	0.008	0.005	0.006	0.010	0.009	0.010	
	Min	0.04	0.12	0.04	0.10	0.06	0.09	0.08	0.08	0.09	
σ_{error} r_g	Max	0.12	0.17	0.12	0.17	0.16	0.16	0.14	0.13	0.16	

Table 6. Montbéliarde genetic parameters estimates (Prevalence of the traits (%), heritabily on diagonal, Genetic correlations (rg) above diagonal – standard error of heritability and range of standard error of genetic correlations, and repeatability) for claw health traits (Digital Dermatis (DD), Interdigital Hyperplasia (IH), Heel Horn Erosion (HHE), White Line Disease (WDL), Sole Hemorrhage Diffused (SHD) and Circumscribed (SHC), Sole Ulcer (SU) and CorkScrew Claw (CSC)).

Montbél.	Preval.	DD	HHE	IH	TN	SHC	SHD	WL	SU	CSC	repeat.
DD	24%	0.04	0.58	0.65		0.26	-0.10	-0.02	0.32	-0.20	0.13
HHE	34%		0.04	0.34		0.44	-0.03	0.14	0.44	0.11	0.06
IH	13%			0.09		-0.01	0.04	-0.02	0.06	-0.08	0.39
TN	2%										
SHC	14%					0.04	0.26	0.36	0.84	0.36	0.08
SHD	33%						0.04	0.17	0.32	0.46	0.06
WL	33%							0.08	0.49	-0.02	0.18
SU	10%								0.05	0.21	0.19
CSC	15%									0.07	0.17
σ_{error} h^2		0.006	0.005	0.009		0.005	0.005	0.008	0.006	0.008	
	Min	0.07	0.08	0.07		0.05	0.08	0.07	0.07	0.08	
σ_{error} r_g	Max	0.12	0.11	0.10		0.11	0.11	0.09	0.11	0.10	

Genomic Evaluation for Foot and Claw Disorders in Czech Holstein

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Abstract

Genomic breeding values (GEBV) for resistance to foot and claw disorders (CD) have been estimated using a multi-trait model comprising linear type traits and single-step GBLUP. Infectious digital disorders (IDD) included dermatitis digitalis and interdigitalis, interdigital phlegmon, and heel horn erosion; claw horn lesions (CHL) included ulcers, white line disease, horn fissure, and double sole; overall claw disorders (OCD) comprised all the recorded CD. Datasets for IDD, CHL and OCD included 40,859; 25,143; 57,567 Holstein cows and 71,219; 44,265; 100,903 lactations with a lactation incidence rate of 13.3%; 12.5%; 17.0%, respectively. Cows calved between 2017 and 2021 in 46 (IDD), 30 (CHL), and 64 (OCD) herds, respectively. CD traits were binary with 0 (no CD) and 1 (at least one CD) during lactation. Linear type traits were foot angle (IDD, CHL), rear leg set (side view) (CHL), feet & legs score (CHL, OCD), and locomotion (IDD, CHL, OCD). Linear model equations included the random additive genetic effect of animal, and for CD traits, fixed effects of parity and age at calving class, herd-year-season of calving and random effect of the permanent environmental effect of a cow; for linear type traits included fixed effects of herd-year-season of scoring, classifier and linear and quadratic regression on the age at calving and the days of scoring. Pedigree involved 102,862, 72,921, and 130,354 animals. Number of genotyped animals was 12,959; effective SNP 36,520; effective animals 12,672. Variance and covariances in the multi-trait model prediction yielded heritabilities 0.09 foot angle, 0.12 rear leg set (side view), 0.09 feet & legs score, 0.11 locomotion, 0.07 IDD, 0.08 CHL, 0.04 OCD; genetic correlations between IDD and foot angle 0.23, locomotion -0.30, between CHL and foot angle -0.33, locomotion -0.22, rear leg set (side view) 0.21, feet & legs score -0.28, between CHL and locomotion -0.42, feet & legs score -0.27. For young genomic bulls (n=186), average reliability of GEBV: for IDD 0.24 (0.14 to 0.34); for CHL 0.20 (0.10 to 0.27); for OCD 0.26 (0.15 to 0.35).

Keywords: Genomic breeding values, multi-trait linear model, single-step genomic evaluation, foot and claw disorders, exterior, Holstein cow

Introduction

Foot and claw disorders are a foremost welfare problem in dairy cattle (Krpálková et al. 2019). They often caused pain, lameness, decreased production, and reduced reproduction (Charfeddine & Pérez-Cabal 2017). Not surprisingly, they are associated with high costs and have been identified as the third most costly disease in dairy farming after mastitis and fertility problems (Green et al. 2002).

Although improving claw health can be achieved through better herd management, the most important is to change the genotype of cows through selection because it is a permanent solution lasting over generations.

Until recently, the selection for improving claw health in the Czech Republic was attended indirectly by feet and leg type traits in selection indices (Krupová et al. 2019). However, it has been shown that there are low correlations between exterior traits and foot and claw disorders (Van der Waaij et al. 2005), which, therefore, do not allow effective and optimal selection progress in claw health

The direct selection, generally more effective, for claw health traits was enabled because a source of information on the phenotypes of claw diseases appeared in Czechia. In 2017, the national cattle health

monitoring system "The Diary of Diseases and Medication" web application was implemented (Kašná et al. 2017). This recording system consists of farmers' online health recording form and a key of diagnoses based on ICAR recommendations. The arising databases are usable in genetic evaluation for several cattle health traits.

Multi-trait linear mixed models are often employed to estimate genomic breeding value for claw disorders, possibly combining the multiple disorders in one multi-trait analysis (Machioldi et al. 2020). The single-step genomic method proved successful in genetic evaluation (Misztal et al. 2020). In the Czech Republic, it is used in the routine evaluation of many traits in Holstein cattle (Přibyl et al. 2012). We also suggest using it as a proven method for the health traits.

This study aimed to present the genomic breeding value estimation method for foot and claw disorders in Czech Holstein cattle that employed a multi-trait linear model and the single-step genomic BLUP.

Materials and Methods

Data

Datasets for IDD, CHL and OCD consist of 40,859; 25,143; 57,567 Holstein cows and 71,219; 44,265; 100,903 lactations with a lactation incidence rate of 13.3%; 12.5%; 17.0%, respectively. Cows calved between 2017 and 2021 in 46 (IDD), 30 (CHL), and 64 (OCD) herds, respectively.

Holstein Cattle Breeders Association of the Czech Republic and the Czech and Moravian Breeding Corporation provided health traits, linear type traits, and genomic data, including pedigree.

The foot and claw data

Foot and claw disorders (CD) records were gathered by farmers and registered voluntarily in the national cattle health monitoring system "The Diary of Diseases and Medication". The health records are unified with ICAR diagnoses. Three group traits of CD were defined according to the aetiology of disorders: infectious digital disorders (IDD), including digital and interdigital dermatitis; interdigital phlegmon and heel horn erosion; claw horn lesions (CHL), including ulcers, white line disease, horn fissures, and double sole; and overall claw disorders (OCD) comprising all the recorded disorders. Separate analyses were made for each of these CD group traits. Similarly, Buch et al. (2011) analysed the CD disorders according to aetiology.

The linear type

The linear type trait datasets included foot angle, rear leg set (side view), locomotion as scored traits (1 to 9 points), and feet & legs in %. Cows were scored for the exterior in the first parity between the 30^{th} and 210^{th} day in milk. The linear type traits were chosen for adding to the multi-trait genomic evaluation of the specific CD group trait according to the values of genetic correlation to the CD group trait: foot angle (IDD, CHL), rear leg set (side view) (CHL), feet & legs score (CHL, OCD), and locomotion (IDD, CHL, OCD).

Genetic parameters

Genetic parameters for CD and linear type traits have been estimated in separate analyses preceding genomic evaluation.

First, the genetic correlations have been set between linear type traits and CD group traits by bivariate analyses.

Table 1. Heritability and repeatability for CD group traits and linear type traits.

Trait	Heritability	Repeatability
Infectious digital disorders	0.07	0.14
Claw horn lesions	0.08	0.16
Overall claw disorders	0.04	0.22
Rear leg set (side view)	0.12	
Foot angle	0.09	
Locomotion	0.11	
Feet & legs score	0.09	

The variance-covariance matrices were estimated by multi-trait animal model analysis, each formed by one of CD group traits and chosen linear type traits. The heritability and repeatability of analysed traits employed in the genomic analysis are in Table 1. The estimated genetic correlations between CD group traits and linear type traits are in Table 2.

Table 2. Genetic correlations between CD group traits and linear type traits.

Trait	Infectious digital disorders	Claw horn lesions	Overall claw disorders
Rear leg set (side view)		0.21	
Foot angle	0.23	-0.33	
Locomotion	-0.22	-0.30	-0.42
Feet & legs score		-0.28	-0.27

The genomic data and method

Animals were genotyped using the Illumina BovineSNP50 Bead chip (Illumina, San Diego, CA, USA).

For the prediction of genomic breeding values, a single-step procedure was applied (Aguilar et al. 2010; Christensen & Lund, 2010) with 12,959 genomic animals: 5,374 bulls and 5,856 (IDD); 5,439 (CHL); 7,354 (OCD) cows with CD phenotype; a total number of effective SNPs used in the calculation of G matrix was 36,520; effective animals 12,672.

Description of model equations

The following linear animal model was used to estimate genetic parameters and genomic breeding values for CD group traits in multi-trait genomic analysis:

y_{ijklm} = parity_agegroup$_i$ + herd_year_season$_j$ + PE_k + A_l + e_{ijkl},

where y_{ijkl} is the CD group trait: IDD, CHL, OCD, 0/1 occurrence per lactation; parity_agegroup$_i$ is the effect of parity combined with age at calving class (15 levels: first, second, third, fourth, and five and higher parity; 3 classes of age at calving per parity); herd_year_season$_j$ is the combined effect of herd (46 (IDD), 30 (CHL), and 64 (OCD) levels); of calving year (2017-2021 levels) and calving season four levels: January–March, April–June, July–September, and October–December); PE_k is the random permanent environmental effect of cow across parity (40,856 (IDD); 25,143 (CHL); 57,567 (OCD)); A_l is the random additive genetic effect of animal (number of animals in pedigree: 102,862 (IDD); 72,921 (CHL); 130,354 (OCD)), and e_{ijkl} is the random residual effect.

The following linear animal model was used to estimate genetic parameters and genomic breeding values for linear type traits in multi-trait genomic analysis:

y_{ijkl}= herd_year_season$_i$ + classifier$_j$ + β_1 age$_k$ + β_2 age$_k$ + γ_1 dim$_l$ + γ_2 dim$_l$ + A_k + e_{ijkl},

where y_{ijkl} is analysed linear type trait (foot angle, rear leg set (side view), locomotion as scored traits (one to nine points), and feet & legs in %.); herd_year_season$_i$ is the fixed combined effect of herd, year and season of scoring; classifier$_j$ the fixed effect of the classifier. The model included the linear and quadratic regressions on age at calving $\beta_1$age$_k$; $\beta_2$age$_k$ and the linear and quadratic regressions on days in milk at scoring $\gamma_1$dim$_l$; $\gamma_2$dim$_l$; A_k is the random additive genetic effect of animal and e_{ijkl} is the random residual effect.

Software employed

The basic editing and preparation of datasets, processing of results and basic statistical evaluation were carried out by the SAS 9.4 programme) (SAS, 2016. Program package BLUPF90 (Misztal et al. 2018) was used to estimate genetic parameters and genomic breeding values.

Results & Discussion

The heritability of the analysed traits, see Table 1, including their genetic correlations with the type traits, correspond to commonly published values (Heringstad et al. 2018). The heritability of health traits was lower than those chosen linear type traits. The high values of repeatability occurred for the analysed health traits. These findings are following Van der Waaij et al. (2005).

The linear type traits chosen to be included in the multi-trait genomic evaluation with the CD group trait showed at least a genetic correlation over 0.2, see Table 2. The estimated genetic correlations were different between the CD group traits, confirming the etiological differences between the CD group traits. While locomotion was an important indicator for all CD group traits, foot angle showed an opposite relationship with IDD or CHL. These results agree with Chapinal et al. (2013). Genetic correlation between foot angle and IDD indicated that the higher genetic predisposition for IDD is connected with a genetic disposition for steep foot angle, while the higher genetic predisposition for CHL is connected with a genetic predisposition for low foot angle. As Pérez-Cabal and Charfeddine (2016) stated, infectious foot and claw disorders, for example, digital dermatitis, are not strongly affected by the foot exterior. However, our analysis found low to moderate genetic correlations between CD group traits and the type traits, similar to what Chapinal et al. (2013) estimated.

The mean values of breeding values for CD traits and their standard deviation are shown in Table 3. For all analysed CD group traits, the means for young genomic bulls are lower than those for all genomic bulls, which could indicate a positive genetic trend in bulls. The mean values of accuracy of breeding values and their standard deviations are shown in Table 4. The highest accuracy occurred for genomic bulls and cows with health records highlighting the importance of knowing the phenotype. In Figure 1, the accuracy is presented for genotyped animals. The accuracy is higher for genotyped cows than for genotyped bulls, probably due to more information available for cows including the phenotype. The most important is GEBV and its accuracy for young genomic bulls (n=186). Their average reliability of GEBV was for IDD 0.24 (0.14 to 0.34), for CHL 0.20 (0.10 to 0.27), and 0.26 (0.15 to 0.35). If comparing the accuracy of GEBV from the single-trait and multi-trait models, the increase is about two p.p. due to adding the type traits in the multi-trait model. The low effect of the type traits on the increase of the breeding value accuracy agrees with the findings of the small genetic correlations between CD traits and type traits and agrees with Ødegård et al. (2015).

Table 3. Average genomic breeding values for multi-trait model.

Category	No.	Mean	SD
Infectious digital disorders			
All	102,862	0.0033	0.03
Genomic bulls	5,374	-0.0022	0.05
Cows with health phenotype	40,859	0.0047	0.04
Young bulls	186	-0.0133	0.05
Claw horn lesions			
All	72,921	0.0033	0.04
Genomic bulls	5,374	0.0011	0.05
Cows with health phenotype	25,143	-0.0037	0.04
Young bulls	186	-0.0431	0.03
Overall claw disorders			
All	130,354	0.0037	0.03
Genomic bulls	5,374	0.0012	0.04
Cows with health phenotype	57,567	0.0019	0.03
Young bulls	186	-0.0190	0.04

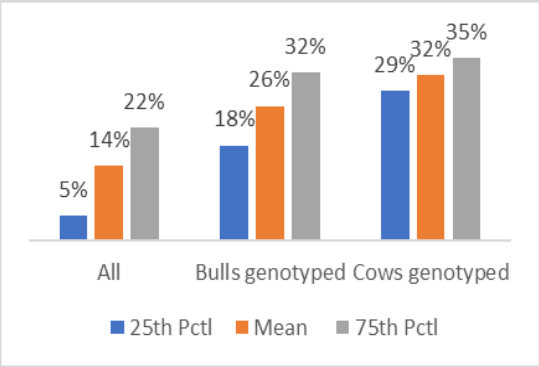

Figure 1. The accuracy of the breeding values for infectious claw disorders

However, we welcome any increase in accuracy of GEBV because the datasets of health phenotypes are small.

Table 4. Average accuracy of genomic breeding values for multi-trait model.

Category	No.	Mean	SD
Infectious digital disorders			
All	102,862	0.14	0.11
Genomic bulls	5,374	0.26	0.12
Cows with health phenotype	40,859	0.26	0.12
Young bulls	186	0.24	0.04
Claw horn lesions			
All	72,921	0.12	0.10
Genomic bulls	5,374	0.21	0.10
Cows with health phenotype	25,143	0.17	0.08
Young bulls	186	0.20	0.03
Overall claw disorders			
All	130,354	0.15	0.11
Genomic bulls	574	0.26	0.12
Cows with health phenotype	57, 567	0.21	0.08
Young bulls	186	0.26	0.04

Conclusions

We conclude that the presented method for genomic evaluation of the foot and claw disorder traits for the Holstein breed in the Czech Republic employing the multi-trait model and single-step BLUP method is feasible for genomic selection for the claw health of cows. The employed method depends closely on the structure and size of the datasets available. We assume the procedure will be adjusted depending on increasing herds and cows with foot and claw disorder records.

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